

ABSTRACTS

1. **International Perspectives on Today's Key Issues for the Classroom Teaching of Developmental Biology.** George M. Malacinski. Department of Biology, Indiana University.

The need for more effective classroom experiences for undergraduates has never been greater, as documented in various national studies. Historically, the balanced model which involves connections between faculty research, undergraduate teaching, and intrinsic student interest in the discipline has worked well. Currently, however, in the tug-of-war between research and teaching priorities, course innovation is often neglected. In addition, as reductionist research strategies come to dominate the discipline, undergraduate interest is waning, for to undergraduates what appear as seemingly esoteric/abstract considerations of the arrangements of a four-letter alphabet (e.g., data mining) are much less engaging than an emphasis on living creatures (e.g., gastrulation). Recall, it is the diversity of the biological kingdom and its myriad physiological processes which usually attracts students to the study of biology. Several suggestions for enhancing the learning experience in the classroom will be offered, including remarks on the need for more outreach programs, anti-information flood comments, a plea for formal assessment of outcomes, and thoughts on increased emphasis on the use of live material in laboratory exercises. Those points will serve as a prelude to an international perspective provided by a review of specific innovations from professors from five different countries.

2. **Doing It Right: An Intense 2-day Multifaceted Developmental Biology Laboratory Course for Third-Year Undergraduates at the University of Bordeaux, France.** Nadine Thézé. Université Victor Segalen Bordeaux 2, Unité INSERM 441, Avenue du Haut-Livique 33600 Pessac, France.

Students are stimulated to inquire by working with live *Xenopus* material, which covers a spectrum of experimental approaches ranging from the physiology/histology of oogenesis and fertilization to mesodermal and neural induction assays (in animal cap explants). They then observe slides that depict various stages of oogenesis and organogenesis. This is all accomplished over two consecutive days (15 hours). The goal is to introduce students to a single model system that permits inquiry across a broad range of approaches, including biochemical, cellular, molecular, physiological, and histological approaches. During this course, students learn how to make the transition from simply learning about hypotheses and theories to designing experiments for the purpose of collecting data to validate present conceptions and ideas. The lecture will focus on the following topics: (1) the background of the students; (2) the teachers and how well the staff is organized; (3) the details of the schedule, including the following planned activities: alternative manipulations and slide shows, interspersed exercises (morning/afternoon), and microscope with screen projection; (4) the list of manipulations and techniques performed by the students; (5) the evaluation of the student's performance; and (6) the cost of the laboratory course and who pays for it.

3. **Beginning at the End: A Developmental Biology Course at the University of Pisa (Italy), Which Begins with *Drosophila* Molecular Genetics.** Robert Vignali. Università di Pisa, Dipartimento di Fisiologia e Biochimica, Laboratori di Biologia Cellulare e dello Sviluppo, via G. Carducci 13, 56010 Ghezano (Pisa), Italy.

Our developmental biology course is taught in conjunction with a molecular biology course to third-year undergraduates. We begin the course with a short review of molecular mechanisms such as promoters, transcription factors, and transactivation domains. Since the focus is on "causal" issues (such as why one end of an embryo makes a head and the other makes a tail) rather than descriptive ones (such as morphological changes in the progression from a single-celled egg through gastrulation and organogenesis), it is necessary to employ an approach which relies heavily on choice of model organisms, genetics, and molecular tools. We think that *Drosophila* provides the student with the perfect synthesis between descriptive and experimental embryology, genetics, and molecular biology, the main disciplines from whose close interaction modern developmental biology originates; therefore, we begin the course with fruit fly development. Eventually, however, more traditional topics such as mosaic and regulative development and inductive interactions in amphibian embryogenesis are reviewed. In discussing all these aspects, it is essential for the students to keep an open mind to possible unorthodox views and to the importance of the experimental approach.

4. **Novel Ways Student Engagement Is Achieved in the Developmental Biology Classroom at the University of Queensland (Australia).** Victor Nurcombe and Brian Key. School of Biomedical Sciences, University of Queensland, St Lucia, Australia 4072.

We have engineered a novel, multiyear undergraduate developmental biology program that is student-oriented, ensuring that students are continually exposed to the underlying principles and philosophy of this discipline throughout their undergraduate career. To change the classroom mode from passive listening to engaged learning, we developed a set of student activities, such as games (e.g., the growth cone game), which, as students play them out in the space of the classroom floor, enhance learning with these powerful human metaphors. Guided by an instructor, who monitors student (cellular) movements, and the scent of air freshener (chemotropic agent) and arm movements (filopodia), a metaphorical reenactment of growth cone development ensues. These activities will be illustrated with slides and film clips. The intrinsic interest of the students has been found to overcome any shyness in the subjects, and we can stop at any time and vary conditions to stimulate further interest. The exercise has proven so powerful that it has been exported into other cell biology courses. The "three-dimensionality" of the experiment is a powerful symbol; several students have fed back that it was the most educative experience they had as an undergraduate.

5. **Respecting the Personal Sensitivities of Students in Modern Biology Courses in Izmir, Turkey.** Irfan Yilmaz. Dokuz Eylul University, Department of Biology Education, Buca Faculty of Education, Buca-Izmir, Turkey.

At Dokuz Eylul University's School of Education (one of the leading teacher education institutions in Turkey), biology is taught in a student-centered (vs authority-driven) manner. In our animal embryology course (35 students) a flexible attitude is taken with regard to philosophical and metaphysical issues on which principles of evolution and theories of creationism are in conflict. This approach permits students who have opposing views on, for example, evolution and creationism to engage in constructive discussion with one another. Intelligent design and Darwinian evolution are both considered, and it is explained that students can seek an understanding of biological processes in a manner which is devoid of both atheistic and antiscience biases. The predispositions students bring to class, considering that Turkey has, historically, been an Islamic society, will be reviewed. Then specific examples of developmental/embryological phenomena, which are taught from either one, or the other, or both perspectives, will be reviewed. In most Western countries discussion of intelligent design theories is deliberately omitted from university biology courses. Current trends in Turkey will be reviewed.

6. **Developmental Biology Teaching as Part of the Strategy of Singapore to Achieve World-Class Status as a Biotechnology Center.** Tit-Meng Lim. Department of Biological Sciences, National University of Singapore.

As part of the nation's strategic move toward a knowledge-based economy with life sciences poised as a new engine for economic growth, Singapore has embraced life sciences as an important discipline to be emphasized in schools and universities. In life sciences, the area of developmental biology is of prime interest since there is much in developmental biology that can be of biotechnological implication. At different levels in the Singapore educational system, students are exposed to various aspects of developmental biology. The very nature of how a complex organism develops from a seemingly simple cell is intriguing and through the journey of development students learn about the amazing cell and molecular machineries that drive the process. Such knowledge is useful for the understanding and application in biotechnology-related activities, especially when misconceptions easily prevail, resulting in public apprehension on biosafety and bioethical issues. Besides making life sciences an important component in the revised school curriculum, with input from specialized teachers and professors, the general public and policymakers are also enlightened through seminars or life science crash courses conducted by university professors. In such outreach endeavors to align, to update, and to prepare the nation for the biotechnology revolution, developmental biology related topics often serve as a platform for demonstration and discussion on the hope and hype of biotechnology. An informed and educated nation can then move as one to develop Singapore into a world-class biotechnology center.

7. **Embryonic Problems: Science, the Public, and Ethics.** Lewis Wolpert. Department of Anatomy, University College, London, United Kingdom.

Many aspects of how the embryo develops are quite well understood. What questions would developmental biologists like

to ask the good fairy of the embryo? These might include polarity, how positional values are specified and interpreted, the downstream targets of HOX genes that lead to morphology, the molecular basis of left/right asymmetry, the genetics and mechanics of morphogenesis, the robustness of development against noise, and the evolution of the embryo. All of this is poorly understood by the public, particularly how similar we are to flies and how universal many developmental processes are. Ethical issues about the status of the very early human embryo have arisen, particularly in relation to cloning and stem cells. It will be suggested that while human reproductive cloning raises no new ethical issues, it should also be prevented because of likely damage to the child. The early embryo is not a human being and so stem cells from it raise no ethical issues that are not similar to assisted reproduction by in vitro fertilization, as both result in loss of early embryos. Developmental biologists should have as much direct contact with the public as possible and school education on these issues should be improved. This could help clarify these issues.

8. **Breathing New Life Into the Life Sciences: Teaching Hands-On Developmental Biology with Axolotl Embryos.** Jill Gresens, B.S., ALAT. Indiana University Axolotl Colony.

The Indiana University Axolotl Colony has been supplying the scientific research community with Mexican axolotls (*Ambystoma mexicanum*) since 1957. We send adult axolotls and their embryos worldwide to researchers in almost every biological science specialty. Because of this unique salamander's importance to the research and educational communities, our work has been funded entirely by the National Science Foundation since 1969. Axolotl embryos are large and can be easily seen through a dissecting microscope; they are easy to handle and hold up well to microsurgery and other manipulation. The characteristics that make axolotls so appealing to research have also made them popular in undergraduate education. Their hardness helps them withstand damage from inexperienced hands, making them excellent tools for teaching introductory developmental biology, and for practicing basic microsurgical techniques. Here we describe axolotl embryos and some of the appealing characteristics that allow educators to guide undergraduate students through their exploration of developmental biology.

9. **Using Human Adult Mesenchymal Stem Cells in an Undergraduate Teaching Laboratory.** J. Doctor. Department of Biology, Duquesne University, Pittsburgh, Pennsylvania (doctor@duq.edu).

The current excitement and promise of stem cell research was brought directly into an undergraduate lab course at Duquesne University. Commercially available human adult mesenchymal stem cells (hAMSC) were the focus of a 6-week-long module in an advanced (junior/senior level) lab course in Cellular and Molecular Biology. The lab meets twice a week for 3-4 hours per session and once a week for a 1-hour recitation. The 29 total students in the course during the springs of 2002 and 2003 learned the basics of cell culture and aseptic technique and set up experiments to examine the developmental potential of hAMSC. Cell culture was a component of all 12 lab sessions over the 6-week period. The hAMSC (from Cambrex) are readily passaged in medium provided by the supplier. Several experiments by small teams of students evaluated hAMSC morphology, proliferation, and osteo-

genic differentiation via fluorescent microscopy, cell counting, MTT vital dye staining/colorimetric quantitation, alkaline phosphatase histochemistry, and chemical determination/alizarin red staining to assess calcium deposition. The students in the lab also applied these methods in a set of preliminary experiments on the attachment, proliferation, and differentiation of hAMSC on novel bone tissue engineering scaffold materials. Several recitation sessions were devoted to seminal publications in stem cell research and journal club discussions. The students analyzed and presented their research results in written papers and at a poster session for the lab course.

10. Abstract #10 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

11. **Undergraduate Curriculum Reform: Integrative Biology and Thematic, Inquiry-Based Laboratories.** Carol A. Hurney. James Madison University.

Curriculum reform at JMU resulted in the implementation of four introductory courses focused on the following: (1) Fundamental Processes of Organisms; (2) Evolution and Ecology; (3) Cell and Molecular Biology; and (4) Genetics and Development. Laboratory components of each course contain thematic, inquiry-based exercises that incorporate the study of microbes, plants, and animals. Three themes using microbes, plants, and animals were developed to span the introductory course series. The microbe theme is on antibiotic resistance; the plant theme is on *Arabidopsis* linking responses to the environment, acid phosphatase, and genomics, and the animal theme is on amphibian morphology and development. These themes were selected primarily on the basis of faculty expertise, the ease with which the organisms could be used in inquiry-based labs, and the incorporation of topics related to faculty research interests. I will present information regarding the faculty-driven process of curriculum reform, the successful funding of an NSF-CCLI grant, and unique aspects of select inquiry-based laboratory activities. Another unique aspect of our new curriculum is the pedagogical approach utilized to provide students with an integrative biological experience. To highlight this approach, I will explore the integration of topics in the Organisms course, where students begin a comparative study of the processes of life, focusing on fundamental issues such as form, function, multicellularity, and evolutionary relationships. Since assessment of educational outcomes is a critical component of our reform process, I will conclude by outlining our multifaceted assessment strategy.

12. **The More Inclusive Course Proposed in 2002 and Executed in 2003.** Judith E. Heady. University of Michigan-Dearborn.

In 2002 I reported about my plans to teach development using three books, current literature, and the usual group original projects for laboratory. I made the decision to use books in addition to current literature after two classes where students had trouble connecting the concepts discussed generally with the research as reported. The three books previewed in 2002 were all helpful both as readings and as resources to accompany the papers. In the current course I used 11 papers, mostly primary research, along with 13 text assignments. Each class period students came prepared by answering questions about the current reading and singly or in small groups by writing their answers on

the board. I checked the answers; students read what they wrote, and we discussed each paper section or text topic. Many times I added additional material from other sources that explained the work or extended the research with information from other publications. I collected their written critiques each class period and returned them with comments. I will be reporting on student reactions to the teaching, pre- and posttest results, my analysis of the course, and more details on several topics. The SDB-Education page has a link to my web site.

13. **Vade Mecum2: Educational Multimedia for Developmental Biology.** M. S. Tyler and R. N. Kozlowski. Department of Biological Science, University of Maine.

Multimedia offers an inexpensive and dynamic method for augmenting courseware in developmental biology. To this end, we have created an interactive CD-ROM/web hybrid, *Vade Mecum2: An Interactive Guide to Developmental Biology*, which takes the student through the developmental cycles of a number of model organisms and illustrates techniques used in studying these organisms. It includes chapters on the following: Dictyostelium discoideum; planarian; sea urchin; *Drosophila melanogaster*; chick; amphibian; and zebrafish. Techniques chapters include the following: microscope, mudissecting tools, and histological techniques. New in this second edition of *Vade Mecum* are the addition of the chapter on zebrafish, emphasizing the effects of teratogens; a glossary, that is searchable and also reveals definitions on rollover; sets of study questions for each chapter, in printable PDF format; *iVade Mecum*, a feature connecting users to interactive puzzles, web sites, and personal bookmarks; a web site that augments the CD-ROM, including recipes from the lab book, glossaries, developmental staging series, addresses of suppliers, and news stories in science. Also new, and included on the CD-ROM, is an electronic version of the lab manual, *Developmental Biology: A Guide for Experimental Study*, 3rd ed., by M. S. Tyler. It is in printable PDF format and has hyperlinks for glossary terms and contents. Each chapter has a corresponding chapter on *Vade Mecum*, and the two together offer an integrated package allowing students to fully prepare for and gain the most from their lab experience. Publ: Sinauer. Support: NSF-DUE-CCLI Grant 0087657.

14. **Expanding the Definition of "Good Science": Incorporating Discussion of Ethics and Public Policy into the Standard Science Curriculum.** Mary K. Montgomery. Macalester College, St. Paul, Minnesota.

Undergraduate science courses appropriately focus on teaching students the fundamentals of the discipline, both content and methodology. As an additional objective, we may also want to help them to place the discipline into a social, historical, and ethical context. I have begun formalizing assignments on controversial issues such as eugenics, GMOs, and human cloning in my genetics and developmental biology courses. I assign several articles on an issue and have students write thesis-governed essays in response to the readings; students then share their informed opinions during a classroom discussion. Students have found these assignments very engaging, as it allows them to connect what they are learning in the classroom to the world at large, as well as to make transparent the relevancy of the discipline to their lives. In an effort to get students to even more fully appreciate the complexities of these issues, and perhaps to

view them in a more pragmatic as opposed to ideological light, I also have them form "advisory panels" modeled after the National Bioethics Advisory Commission; each group produces a public policy report, which includes a summary statement and specific recommendations. Because students view this type of assignment as a form of public scholarship that will be shared with their classmates and the larger campus community, they are motivated to learn the science, dig deeper into the issues, to fully debate them, and to eventually reach consensus to draft a workable policy. The objective of such activities is to encourage students, whether they become scientists or not, to develop into well-informed engaged citizens.

15. **Current Events in Biology: A Nonmajors Course Taught by Postdoctoral and Graduate Students.** Sarah Bondos. Department of Biochemistry and Cell Biology, Rice University, Houston, Texas 77005.

Public opinion and understanding of science influences decisions regarding scientific research, funding, regulations, and applications. Thus, the challenge in educating the general public is threefold: to teach basic principles that underlie active areas of research, to clearly explain methods and ramifications of scientific research that is "in the news," and to develop a long-term interest in science. To this end, a group of postdoctoral associates and graduate students at Rice University have developed a biology course specifically geared toward nonmajors that emphasizes a hands-on approach to current topics in biological research. The course covers biological topics relevant to human life, each of which are taught in the three following parts: (1) the basic principles and research methods; (2) human health problems associated with that topic (if relevant); and (3) applications of these methods and status of current research in that field. In-class demonstrations and many laboratory tours involve the students in each topic. Reading assignments were derived from popular science essays, an introductory biology textbook, and research articles aimed at a general audience (e.g., from *Science*). This course format also provides teaching experience for postdoctoral associates and graduate students interested in academic careers. Each lecturer was responsible for one or more topics in an area directly related to their research, including assigning reading, homework questions, test questions, and grading. In addition, I coordinated the effort to keep the lecturers informed of class progress, to keep the teaching styles and level of detail consistent, to assemble the test questions into exams, and to arrange the lab tours. Our approach toward collaborative teaching and details on the course material are described, along with specific challenges and recommendations for others using this procedure.

16. **Teaching Scientists to Teach: Lessons from the FIRST Program.** Andrea R. Morris, Douglas Eaton, Arri Eisen, Robert Gunn, and J. K. Haynes. Emory University School of Medicine and Morehouse College, Atlanta, Georgia.

One of the keys to the expansion and success of basic biomedical research is to ensure that new talent is continuously introduced to the field. Meeting this challenge depends on having science educators who excel in both research and teaching. Since the postdoctorate is the final stage of formal "training" a scientist usually receives before becoming a faculty member and most junior faculty teach at least one course during their first year, it seems logical that training postdocs in aspects of teaching along

with research will contribute greatly to their success. At Emory University and the Atlanta University Center (Morehouse, Spelman, and Morris Brown Colleges, Clark Atlanta University, and the Morehouse School of Medicine), a 3-year training program sponsored by the Minority Opportunities in Research (MORE) division of the NIH, known as the Fellowships in Research and Science Teaching (FIRST) program, is completing its first cycle. The goals of FIRST are to support extensive primary research, expose postdoctoral fellows to a wide variety of teaching and learning techniques, give full-time teaching experience within undergraduate courses, and give guidance in balancing teaching and research responsibilities. Along with mentors who have excelled in teaching, the fellows are able to bring new ways of teaching and learning into college science classrooms at historically minority-serving institutions. This opportunity also allows FIRST fellows to serve as role models and potential mentors to undergraduate students in an effort to help increase the representation of minority students in higher science education.

17. **Thomas Jefferson University's Science Outreach Program: Bringing Live Science to the Community.** Jamie R. Schaefer and Steven A. Farber, Ph.D. Thomas Jefferson University, Philadelphia, Pennsylvania.

We have created a Science Outreach Program incorporating life science and laboratory education using live zebrafish larvae. The program has two main components to teach students science literacy, genetics, the experimental method, and the cardiovascular system, as follows: (1) educating students through interactive tours of the Zebrafish Facility, and (2) bringing live zebrafish into grade school classrooms throughout Pennsylvania and New Jersey. The goals of the hands-on experiments are to instill in children a love for science, with the hope that this exposure will lead students to productive careers in science. Students participating in their classrooms mate zebrafish and learn how genetics plays a role in the offspring's development. Students touring the facility view different stages of embryo development, observe the digestive tract after fish have consumed fluorescent lipids, and watch a robotic pipette processor in action. In both components, the students learn about the role of zebrafish in genetic research. Since December 2002, the program has reached over 700 students and 75 teachers with the classroom unit, facility tours, and science teacher seminars. Ninety-one percent of the 130 students participating in the tours answered that the tour has exposed them to something in medical research that they were not aware of before this experience and 76% of them rated it excellent in comparison to other tours or demonstrations they have seen. Eighty-five percent of all students rated working with the zebrafish as an excellent experience. In recent years there has been heightened sensitivity regarding the ethical use of animals for teaching. The optically clear zebrafish larvae are possibly one of the most easily available model organisms that allow students to see organ systems similar to their own without harming the larvae.

18. **Gametes, Fertilization, and Embryogenesis Come Alive—Biology: Exploring Life Promises True Interactivity.** Jacqueline McLaughlin and Robin Heyden. The Pennsylvania State University, Berks-Lehigh Valley College.

It's a Different Animal! Biology: Exploring Life (Prentice Hall, 2003) integrates a short, concepts-oriented textbook, a large Web site that allows students to interact with science instead of interacting with the computer, and inquiry-based labs into a dynamic and balanced biology program. High school students read the text, go interactive to explore and do activities, and then go to the lab to experiment. Key concepts in human reproduction and development presented in this product include the anatomy of the reproductive organs, gamete production, hormonal regulation, ovarian and menstrual cycles, fertilization, embryogenesis, and fetal development. Online activities reinforce the textbook and support these concepts by animating key processes and promoting active and collaborative learning environments. The National Science Foundation (NSF) funded a 3-year evaluation study of the program. The goals of that study were to determine its fit to the national standards and to what degree the program was inquiry and activity-based and to examine the teacher's use of technology in his or her teaching. Teachers from all over the United States are raving over the ease of use, pedagogy, program performance, and depth of content. Exploring Life materials include teacher resource materials that facilitate professional development for high school teachers such as chapter planning guides and Teacher Wraparound Notes. Jacqueline McLaughlin (content expert) and Robin Heyden (author) herein present an overview on the use of Biology: Exploring Life to teach reproduction and development through a group of instructional materials aimed at improving teaching and learning.

19. **Cell—Cell Interactions in the Patterning of the Rostral Segments in the Leech.** Dian-Han Kuo and Marty Shankland. Section of MCDB, University of Texas at Austin, Austin, Texas.

In the leech, a segmental repeat is derived from a defined set of blast cells budded off from five bilateral pairs of teloblasts. Each blast cell clone displays a stereotypic cell lineage pattern and gives rise to a defined set of pattern elements. Among the five teloblastic lineages, the specification of the O and P lineages requires cell—cell interactions, while the other three lineages appear to be specified autonomously. In most segmental repeats, a pair of equipotent o/p blast cells are specified to take on distinct O and P fates by a series of cell—cell interactions. However, in the rostral segments, a single “op” blast cell takes the place of the pair of o/p blast cells found in midbody segments. The OP pattern elements in a rostral segment are serially homologous to the sum of O and P pattern elements in a midbody segment. Laser ablation experiments were used to detect cell—cell interactions in the specification of OP sublineages. The two granddaughter cells of a op blast cell that normally express O fates are specified independently of the other OP sublineages. In contrast, the two granddaughter cells which normally only express fates express both O and P fates if the adjacent O-type cells are ablated. We also found that the O-type cells play a role in repressing fate in the P-type cells of the OP lineage. These results differ from the results of comparable experiments performed on midbody segments and suggest that a distinct set of cell—cell interactions may be involved in the specification of the serially homologous cell fates in rostral and midbody segments of the leech.

20. **Anterior—Posterior Axis Specification in Insects. Is It Chiseled in Stone?** Vladimir Zhurov and Miodrag Grbic. University of Western Ontario, Department of Biology, London, Ontario N6A 5B7, Canada.

At the morphological level, development of various insect species falls into the “developmental hourglass scheme”: a wide variety of early development modes are followed by the highly conserved stage of extended germ band, which develops into extremely diverse adult morphologies. Does the genetic machinery that underlies such morphological changes follow the same “hourglass” pattern? Formation of the anterior—posterior axial pattern in insects is thought to be regulated by a cascade of genetic factors that subdivide the egg into a segmented embryo. It is still uncertain whether this process, well studied in *Drosophila* and conserved among Diptera, is conserved among other insects. Recent studies show that early steps of the patterning cascade may be conserved between insect species as distant as grasshopper and *Drosophila*. Here we present a study on the endoparasitic wasp *Aphidius ervi* that suggests that such conservation is not universal across insects. Using *in situ* hybridization and antibody staining, we have investigated how anterior—posterior patterning cascade has evolved in the derived mode of development of *Aphidius*. We have analyzed the expression pattern of maternal-coordinate, gap, pair-rule, and segment-polarity genes. Our results suggest that while late elements of patterning cascade are retained in *Aphidius*, early stages of patterning are modified, reflecting dramatic changes in the early embryogenesis.

21. **Functional Comparative Analyses of *otd/Otx* Genes in Anterior Development.** Ernst A. Wimmer, Bernhard G. M. Schmid, Kerstin Meier, and Joachim Reischl. Lehrstuhl Genetik, University Bayreuth, 95447 Bayreuth, Germany.

The *otd/Otx* gene family encodes homeodomain transcription factors that are involved in the determination of the anterior region (cephalization), in the formation of certain head segments, in neurogenesis, and in photoreceptor differentiation. We tested phylogenetically diverged homologues of orthodenticle (*otd*) in *in vivo* assay systems in *Drosophila*. Within triploblastic bilaterians, *otd/Otx* genes are functionally conserved, whereas a diploblastic *otd* homologue is not able to carry out these developmental functions. By testing deletion variants of OTD, two functional domains besides the homeodomain could be identified. The C-terminal domain of OTD acts as a transcriptional activator domain. The molecular function of a required N-terminal domain is still unknown. This domain, however, is sufficient to confer triploblastic developmental functions to a diploblastic OTD homologue, which suggests that this domain has evolved in order for the OTD/OTX proteins to fulfill the required functions in triploblastic organisms. Further functional analyses of this N-terminal domain are currently carried out. When investigating the cephalizing activity of OTD by using *Drosophila* embryos without Bicoid (BCD) activity, we can show that OTD is capable of both repressing posterior and inducing anterior development. BCD, the anterior determinant of *Drosophila*, seems to be a phylogenetically young acquisition of higher dipterans, whereas OTD is a highly conserved anterior determinant. To further investigate this ancestral role of OTD, we have started to functionally characterize *otd* homologues in a series of diverse arthropod species.

22. **The Molecular Mechanisms Underlying the Establishment of the Dorsal/Ventral Axis in the Chaetognath, *Flaccisagitta***

enflata. David Q. Matus and Mark Q. Martindale. University of Hawaii at Manoa, Honolulu, Hawaii.

We are interested in the evolution of the dorsal/ventral axis in the Metazoa. Using PCR, we have isolated two developmental regulatory genes from the chaetognath, *Flaccisagitta enflata*, that have homology to the dorsal marker, decapentaplegic (*dpp*)/bone morphogenetic protein 2/4 (*BMP2/4*), and to the ventral marker short of gastrulation (*sog*)/chordin. Chaetognaths, or arrow worms, are marine animals that are ubiquitous and abundant members of oceanic zooplankton communities, yet their phylogenetic position within the Metazoa has remained obscure. While they possess an array of morphological and developmental characteristics that make resolving their relationships to the rest of Metazoa difficult, recent molecular evidence from structural and developmental regulatory genes suggests that they may occupy a basal position within the protostomes. However, their many deuterostome-like characteristics (holoblastic radial cleavage, enterocoely, a tripartite coelomic arrangement, a postanal tail, and a presumed posterior fate of the blastopore) suggest that their origins may lie prior to the protostome/deuterostome divergence. The expression of *dpp* and *sog* is being studied by in situ hybridization to investigate both conserved and novel patterns of expression. The recovery of a *sog*-like gene from a chaetognath represents the first identification of this potential ventral marker in protostomes outside of the arthropods and suggests that the interaction between dorsal and ventral molecular markers may be an ancient one.

23. **Segments and Joints: Patterning the Segmenting Vertebrate Skeleton.** Patricia L. Crotwell and Paula M. Mabee. Department of Biology, University of South Dakota, Vermillion, South Dakota.

What is the evolutionary origin of the tetrapod joint? What genes are involved in joint development, and how do they interact to induce segmentation of a cartilage rod into two or more segments? A clearer picture of the interactions of a number of genes such as *Wnt14*, *Bmp2/4*, *Sox9*, *noggin*, and *Gdf5*, among others involved in tetrapod joint development, is beginning to emerge. Yet this understanding does not address the evolutionary origin of the segmentation process. Fishes exhibit segmentation in their developing gill arches and median and paired fin skeletons. The dorsal and anal fins are supported by cartilaginous rods, the radials, that undergo segmentation during development, yet they do not have the joints that are characteristic of tetrapod limbs. We have hypothesized that the mechanisms underlying radial segmentation in fishes were co-opted over several million years of evolution to give rise to tetrapod synovial joints. To test this hypothesis, we are using in situ hybridization methods to examine, in zebrafish median fin radials, expression patterns of genes known to be critical in synovial joint development. Among the genes examined to date are *Wnt14*, *Gdf5*, *noggin*, *chordin*, *Collagen II*, *Bapx1*, *Bmp2a*, *Bmp2b*, and *Bmp4*. Expression patterns are dynamic, changing in both extent and intensity during development and segmentation of the radials. Nevertheless, these genes are expressed in patterns that are strikingly similar to those described for tetrapod joint development, and thus, serve as data supporting our hypothesis. Testing the functional interactions of these genes in zebrafish is the current research focus of our lab.

24. **Patterning a Feather: A Tale of Two Keratins.** Paul Maderson. Brooklyn College of CUNY, Brooklyn, New York.

Baden and Maderson's (1970) *Vertical Alternation of Keratinization* (VAK) denoted where epidermal tissues comprising β - and α -keratins are produced in cyclical vertical sequence. VAK distinguishes adult lepidosaurian skin from that of most other sauropsid amniotes wherein the two keratins alternate horizontally over the body (HAK). Mammals, with only α -keratin, show a Homogeneous pattern (H) also seen in some turtles. Histology does not reliably identify keratin distribution in sauropsid epidermis, but evaluating classical studies in light of Rudall's (1947) X-ray diffraction data permits distinguishing α - and β -keratogenic tissues. Appendages may comprise either α - (in mammalian claws, hair) or both (sauropsid claws, feathers) keratins. VAK is a sauropsid synapomorphy as studies of skin development in representatives of all taxa show that initially the entire epidermis manifests it. Thus, when HAK and/or H occur in sauropsids, whether over the entire body or regionally in tissues or appendages, such are secondary, postnatal states. Rudall showed that pulp epithelia inside a mature feather's tubular, β -keratogenic calamus are α -keratogenic; TEM confirms this. Although old accounts of feather development described external pulp caps, both this, and the superior umbilicus (SU) of mature feathers, are ignored in recent papers. Final barb maturation occurs around a scaffold of a column of external caps; the SU is where caps are internalized at the onset of calamus formation. Feather anatomy and development are understandable only within the context of VAK. Data concerning two keratins and derived tissues must be considered in studying patterning mechanisms, interpreting putative fossil protofeathers, and posing evolutionary models.

25. **Pleiotropic Tradeoff during Blind Cavefish Evolution and Development.** Y. Yamamoto and W. R. Jeffery. Department of Biology, University of Maryland, College Park, Maryland, USA.

The question of why cave animals have lost their eyes is currently unresolved. We study this problem in the blind cavefish, *Astyanax Mexicanus*. Cavefish have evolved regressive characters, such as a degenerate eye and less pigment, and constructive characters, such as a large jaw and additional teeth, cranial neuromasts, and taste buds. Although functional eyes are lacking in adults, cavefish embryos form a small eye primordium, which later arrests in development and degenerates. Lens apoptosis plays a major role in eye degeneration. We have shown that enhanced midline signaling by sonic hedgehog (*shh*) controls lens apoptosis and eye degeneration. At the pharyngula stage, *shh* is also expressed at the edge of the oral cavity and in developing taste buds. To test the hypothesis that *shh* also controls jaw size and taste bud development, *shh* antisense morpholinos were injected into cavefish embryos. Inhibition of *shh* expression resulted in a smaller jaw and a reduced number of taste buds. We also injected *shh* mRNA into surface fish embryos to increase *shh* expression, which resulted in an increased number of taste buds. The results indicate that *shh* is sufficient and necessary for modification of jaw size and taste bud number. We propose a tradeoff based on the pleiotropic effects of *shh*, in which eyes were sacrificed to allow for the development of a larger jaw and extra taste buds. This tradeoff would be possible in the cave environment because selection for vision has been relaxed. (Supported by NSF Grant IBN-0110275 and NIH Grant EY014619.)

26. **Using a Comparative Approach to Study Eye Growth in the Blind Cavefish *Astyanax mexicanus*.** Allen G. Strickler and William R. Jeffery. Department of Biology, University of Maryland, College Park, Maryland 20742.

We are interested in the evolutionary processes involved in eye development and growth. To study this, we use the characid teleost *Astyanax mexicanus* as a model system. This species consists of an eyed surface-dwelling form (surface fish) and a cave-dwelling form (cavefish). Although cavefish form an eye primordium, the embryonic eye ceases to grow, degenerates, and fails to form a functional adult eye. We compared gene expression patterns, programmed cell death (PCD), and cell proliferation in the cavefish and surface fish retina to begin to understand the underlying causes of why cavefish eye growth is arrested. The homeobox genes *Rx1* and *Vsx2* were expressed normally in the proliferative zone of the cavefish retina. PCNA expression in this region also verified the occurrence of active stem cell proliferation in the cavefish retina. However, in striking contrast to surface fish retina, regions of dying cells appeared in the cavefish retina, as shown by TUNEL and acridine orange assays. Furthermore, BRDU pulse/chase experiments indicated that some cells of the proliferative zone of the cavefish retina are lost, presumably by PCD, as development proceeds. We conclude that cell proliferation appears to be normal in the cavefish retina. However, some new cells formed in the retinal proliferative zone appear to be eliminated by PCD. We conclude that this altered balance of cell proliferation and PCD in the retina is a contributing factor to modification of the eye phenotype during cavefish evolution. (Supported by NSF Grant IBN-0110275 and NIH Grant EY014619.)

27. **Hsp90 Regulates Lens Apoptosis during Cavefish Eye Degeneration.** Thomas A. Hooven, Yoshiyuki Yamamoto, and William R. Jeffery. University of Maryland, College Park, Maryland.

The teleost *Astyanax mexicanus* exhibits two morphotypes: an eyed surface-dwelling form (surface fish) and a blind cave-dwelling form (cavefish). Although adult cavefish lack eyes, optic primordia initially develop in cavefish embryos and later degenerate and sink into the orbits. Lens apoptosis plays a major role in eye regression. Since some of the heat shock proteins are known to promote or prevent apoptosis, we studied the activities of heat shock protein 70 (hsp70), hsp90 α , and hsp90 β in the cavefish lens. Hsp90 β was not expressed in the lens of either surface fish or cavefish embryos, whereas hsp70 was expressed in the lens of both forms, suggesting that neither is directly involved in cavefish lens apoptosis. In contrast, hsp90 α was expressed strongly in the cavefish lens, but not in the surface fish lens. Hsp90 α expression was prominent between 24 and 48 hpf, shortly before the peak of lens apoptosis. To test the possible role of hsp90 α in lens cell death, cavefish embryos were exposed to two chemical inhibitors of hsp90 α protein function. Hsp90 α inhibitor treatment reduced or abolished lens apoptosis and increased the size and structural complexity of the lens relative to untreated controls. The results indicate that hsp90 α expression is necessary for lens apoptosis and is an important factor in the evolution of cavefish eye degeneration. Functional experiments are currently underway to determine whether hsp90 α activation can induce lens apoptosis and eye degeneration in surface fish. (NSF Grant IBN-0110275 and NIH Grant EY014619.)

28. **Evolution of *Dlx* Genes and the Teleost Fish Dentition.** David W. Stock and William R. Jackman. University of Colorado, Boulder, Colorado 80309-0334.

Evolutionary reduction of the dentition is common in teleost fishes, as illustrated by the zebrafish, which has lost teeth in the oral cavity and retains them only on the last gill arch. To investigate the mechanism of this loss, we compared the expression of orthologs of mammalian *Dlx2*, a marker of the early dental epithelium, in the zebrafish and a relative retaining oral teeth, the Mexican tetra, *Astyanax mexicanus*. *Dlx2* is the pro-ortholog of the duplicates *dlx2* and *dlx5* in the zebrafish. We found that both genes are expressed in the epithelium of pharyngeal tooth germs in each species. However, while these genes are also expressed in the oral epithelium of *A. mexicanus*, neither is expressed in this region in the zebrafish. This result is consistent with an early arrest of oral tooth development in the zebrafish, but two lines of evidence suggest that loss of *dlx2* and *dlx5* expression was not the direct cause of oral tooth loss. This expression loss is more parsimoniously interpreted as a change in a common *trans*-acting regulator than as changes in the *cis*-regulatory regions of each gene. In addition, injection of morpholino antisense oligonucleotides targeting *dlx2* and *dlx5* singly or in combination did not affect zebrafish pharyngeal tooth development. In testing the generality of *dlx2* and *dlx5* expression in teleost oral teeth, we found both genes to be expressed in this location in the catfish *Synodontis multipunctatus*, but detected little or no *dlx2* expression in oral teeth of the medaka. We are investigating the possibility that the lack of a requirement for *dlx2* and *dlx5* in zebrafish pharyngeal teeth and *dlx2* in medaka oral teeth is the result of redundancy with other *Dlx* genes.

29. **Oral Expression of *Bmp2* and *Bmp4* Orthologs in Dentally Divergent Fishes.** Sarah B. Wise and David W. Stock. University of Colorado, Boulder, Colorado 80309-0334.

To investigate the developmental genetic mechanism of oral tooth loss in the zebrafish, we compared its gene expression patterns to those of a related species retaining oral teeth, the Mexican tetra, *Astyanax mexicanus*. We previously observed that *dlx2* and *dlx5* are expressed in the oral epithelium of *A. mexicanus*, but not in the oral epithelium of the zebrafish. These genes are semiorthologs of mammalian *Dlx2*, a marker of the dental epithelium which is known to be regulated by planar *Bmp4* signaling. As *Bmp2* is related to *Bmp4* by a gene duplication and also expressed in mouse dental epithelia, we cloned *bmp4*, *bmp2a*, and *bmp2b* genes from *A. mexicanus* and compared their oral expression patterns with those of their zebrafish orthologs. *bmp2a* is not expressed in the oral epithelium or mesenchyme of either fish, but *bmp2b* expression is present in species-specific patterns. In *A. mexicanus*, *bmp2b* is first localized to the epithelium of oral tooth germs, coincident with observed *dlx2* and *dlx5* expression, while zebrafish *bmp2b* is expressed in a widespread pattern in the oral epithelium. In contrast to the condition in mice, *bmp4* is absent from the oral epithelium of both fishes at all stages examined. Later, *bmp2b* and *bmp4* are expressed in the mandibular mesenchyme of *A. mexicanus*, but are absent from corresponding regions in the zebrafish. These observations point to *bmp2b* as a candidate inducer of *Dlx* expression in the dental epithelium of *A. mexicanus*. Contrary to the observed pattern in the mouse, *A. mexicanus* *bmp4* appears involved only in later stages of oral

tooth morphogenesis. Future work will test the functional consequence and genetic basis of these expression differences.

- 30. FGF Signaling Is Required for Zebrafish Pharyngeal Tooth Development.** William R. Jackman and David W. Stock. University of Colorado, Boulder, Colorado 80309-0334.

We are investigating molecular interactions involved in zebrafish oral and pharyngeal patterning in the hope of identifying the evolutionary cause or causes of oral tooth loss in cypriniform fishes. Teeth develop via reciprocal epithelial-mesenchymal signaling, and mouse studies have identified fibroblast growth factors (FGFs), including Fgf8, as essential to this process. We have examined the role of FGFs in zebrafish tooth development with antisense oligonucleotide inhibitors of fgf8 expression (morpholinos), fgf8 mutant fish (ace), and the FGF receptor inhibiting compound Su5402. We find that fgf8 mutant and fgf8 morpholino-treated fish develop normal-looking pharyngeal teeth. They also exhibit normal expression of some of the genes known to interact with FGFs during mouse tooth development, including the epithelial marker pitx2, as well as lhx6 and pax9 in the mesenchyme. In contrast, fish treated with Su5402 prior to 45 hours do not form visible teeth. While expression of pitx2 appears normal, lhx6 and pax9 are down-regulated in the oral and pharyngeal mesenchyme relative to other areas. From these data we hypothesize that as in mammals, an FGF signal is required for the activation of genes in the zebrafish dental mesenchyme, but that a ligand other than fgf8 is fulfilling this role. Consistent with this hypothesis, we have found no evidence of fgf8 expression near wild-type pharyngeal teeth and are working to identify the FGF ligand(s) necessary for zebrafish tooth patterning. Finally, the expression of fgf8, pax9, and lhx6 in zebrafish oral mesenchyme suggests that cypriniform oral teeth were lost due to changes downstream of, or parallel to, early FGF signaling.

- 31. FGF9 Signaling in Mouse Gut Development.** Xiuqin Zhang, Thaddeus S Stappenbeck, Andrew C. White, Kory J Lavine, Jeffrey I Gorgon, and David M. Ornitz. Department of Molecular Biology and Pharmacology, Washington University Medical School, St. Louis, Missouri 63110, USA.

Fibroblast growth factors (FGFs) and FGF receptors (FGFRs) signaling is important in mediating limb and lung development. However, there is little information about FGF/FGFR signaling in gut development. Here we show one of the FGF family members, FGF9, is expressed in the outer lining of the foregut and is strongly expressed in the luminal epithelia of the intestine in E14.5-E16.5 mouse embryos. FGFR2 is one of the high-affinity receptors for FGF9 and is also strongly expressed in stomach serosa and intestinal epithelium. Analysis of FGF9 null-mutant embryos showed that the stomach was smaller, that the small intestine was short, and that the cecum failed to develop. Histological analysis showed that the FGF9^{-/-} embryonic stomach wall was thick and epithelial tissue architecture had defects compared to wild-type embryonic stomach. Cell proliferation was significantly reduced in the stomach serosa in FGF9^{-/-} embryos. Immunohistological studies using intestinal fatty binding protein (i-FABP) and ileal lipid binding protein (ILBP), proximal and distal intestinal epithelial cell maturation markers, respectively, showed that i-FABP expression in FGF9^{-/-} E18.5 embryonic intestine was significantly reduced and that the ex-

pression of ILBP was absent in distal intestine. This analysis showed that intestine maturation is significantly delayed in FGF9^{-/-} embryos. Taken together, these results suggested that FGF9 signaling is required to regulate a morphogenetic wave of differentiation along the cephalocaudal axis and the organ size of the stomach.

- 32. A Fresh Look at a Developmental Enamel Defect in Humans, Mutant Mice, and Fossil Giraffes: A Contribution to Evo Devo.** Tamara Franz-Odenaal. Zoology Department, University Cape Town, South Africa.

An elaborate series of developmental events is required for enamel formation by ameloblasts. It is therefore not surprising that a variety of enamel malformations arise from defects in matrix synthesis, secretion, and extracellular processing. These processes can be affected by a variety of hereditary and environmental conditions. Enamel hypoplasia (EH), a developmental defect, affects the laying down of enamel by ameloblasts in great apes, humans, and many ungulates. If EH develops as a result of nutritional and/or environmental stress, then it only affects those teeth developing during stress events, whereas EH resulting from genetic disorders (such as amelogenesis imperfecta in humans) affects all teeth. This poster outlines the current understanding of the etiology of EH in humans and discusses mice mutants with similar phenotypes. A large assemblage of fossil giraffes (5 MYA) with EH exist in South Africa. Histological and SEM analyses of defective enamel were conducted. Both the temporal and the spatial distribution of EH in all tooth types, on both lingual and buccal surfaces, were documented and contribute to understanding this defect. Key questions are whether or not single populations of cells (such as ameloblasts) can accumulate stress signals and how much stress ameloblasts can tolerate before they are permanently damaged. What protects adjacent ameloblasts from disruption at a particular time during development? This study shows how analysis of a developmental defect in modern animals sheds light on the evolutionary aspects of the disorder in fossils and how an assessment in fossils sheds light on the disorder in modern populations.

- 33. Fundamental Regulation of Chordate Heart Development.** B. J. Davidson and M. Levine. University of California, Berkeley, California.

We are investigating the function and regulation of genes involved in tunicate heart development. Research on the genetics of vertebrate heart development has been hindered by the presence of paralogous genes and by large and complex regulatory regions. The tunicate, *Ciona intestinalis*, is an excellent organism in which to explore conserved chordate heart development. In contrast to the situation in vertebrates, the *Ciona intestinalis* genome has only one ortholog for many of the major heart development regulatory genes, including *Nkx2-5*, *Mef-2*, and *d/e-Hand*. Our preliminary research indicates that the *Ciona* *Nkx2-5* ortholog, *Ci-Nkx*, is expressed in early heart precursor cells. Additionally, we have demonstrated that a 1.3-kb piece of DNA upstream of the *Ci-Nkx* promoter is sufficient to drive reporter expression in early heart precursor cells. Our characterization of *Ci-Nkx* regulation demonstrates the advantages of working on *Ciona*, where compact regulatory regions and high-

throughput transformation through electroporation allow for rapid progress.

34. **Cardiomyocyte Apoptosis as a Cause of Death in Mice Lacking Cardiac Muscle α -Actin.** Eltyeb Abdelwahid,*†, Lauri J. Pelliniemi,‡ John C. Szucsik,§ James L. Lessard,§ and Eero Jokinen¶. *Department of Pediatrics, †MediCity Research Laboratories, and ‡Laboratory of Electron Microscopy, University of Turku, FIN-20520 Turku, Finland; §Children's Hospital Medical Center, University of Cincinnati, Cincinnati, Ohio 45229-3039, USA; and ¶Department of Pediatrics, University of Helsinki, FIN-00290 Helsinki, Finland.

Mice lacking cardiac muscle α -actin die during the perinatal period. About 56% of mice that are homozygous null ($-/-$) for a functional cardiac α -actin gene do not survive to term and the remainder generally die within 2 weeks of birth. We found that there were neither morphological differences nor differences in the extent of apoptosis between the mutant and normal hearts on embryonic day (E) 12 and E14 of development. However, apoptosis was greater in the hearts of homozygous null mice on E17 and postnatal day 1 when compared with wild-type hearts. The growth of the prenatal and postnatal hearts of the cardiac α -actin-deficient mice was retarded and the cytoplasmic filaments were disorganized. While apoptotic cells were observed in both the atria and the ventricles in the hearts of the homozygous null animals, the frequency was greater in the ventricles than in the atria. Our results indicate that the functional and structural disturbances in the mice with a homozygous lack of cardiac α -actin are apparently due to disorganized development of actomyosin filaments in the affected cardiomyocytes. Other actin isoforms cannot compensate for the lack of cardiac α -actin and this apparently induces apoptosis in defective cardiac myocytes, which are not able to cope with the increased workload in the perinatal phase.

35. **A Comparative Approach to the Analysis of Novel Genes in Vascular Development.** A. Mukhopadhyay, K. Siva, D. Das, and M. S. Inamdar. Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore 560 065, India.

We have undertaken a comparative analysis of the origins and functions of circulatory systems in vertebrate and invertebrate models. These studies will provide tools to analyze the large variety of human cardiovascular disorders. Pivotal to our studies is the analysis of two novel, evolutionarily conserved genes, *asrij* and *rudhira*. Both genes express in undifferentiated ES cells and are later regulated in a stage- and tissue-specific manner to show predominant vascular expression. *Asrij* is a novel endosomal protein with an asymmetric subcellular localization. *Asrij* expression in mouse embryonic mesodermal clusters precedes that of the earliest vascular precursor marker Flk-1 (VEGFR2). *Asrij* isoforms show tissue-specific expression in the vasculature, brain, and other tissues. *Drosophila asrij* expression is initiated in blood precursors before the expression of the earliest known fly hematopoietic transcription factors and continues in embryonic blood and trachea. *Rudhira* is a WD40 domain protein that is also expressed in blood vessels and their precursors. Human *rudhira* is down-regulated in cancer cell lines and maps to breakpoints of hematological neoplasms. *Drosophila rudhira* also shows hematopoietic expression. We are elucidating the functional signifi-

cance of *Asrij* and *Rudhira* expression and analyzing their interactions with known and novel pathways of vascular development. Recent data on functional analysis of the two genes will be presented.

36. **Comparative Limb Morphogenesis in Mice and Bats.** Chris J. Cretekos, John J. Rasweiler IV, and Richard R. Behringer. UT M.D. Anderson Cancer Center, Department of Molecular Genetics.

The basis of species-specific morphogenesis has been a topic of speculation for centuries. One idea that fits well with comparative and molecular data is that morphological divergence arises from genetic differences in timing, level, and spatial pattern of developmental gene expression between species. We are testing this idea using a functional genetic approach comparing limb development between mouse and *Carollia perspicillata*, the short-tailed fruit bat. Limb morphogenesis is a classic comparative biology and bat limbs are highly modified relative to mouse. We hypothesize that the divergence in limb morphology between these species is reflected in meaningful differences in gene expression. *Prx1* encodes a paired homeobox protein required in mice for distal limb development. An enhancer directing limb expression in a *Prx1*-specific pattern in mouse has been defined. We have isolated genomic clones of bat *Prx1* and identified a sequence element similar to the mouse *Prx1* enhancer in a conserved genomic position. Bat and mouse *Prx1* limb enhancers direct reporter gene expression in similar overall patterns in transgenic mice, but there are some subtle differences. We have also replaced the mouse *Prx1* limb enhancer with that of the bat by gene targeting in mouse ES cells, with the goal of generating mice that express the endogenous *Prx1* gene under control of the bat enhancer. If there has been meaningful divergence between the mouse and bat regulatory sequences, the gene will be expressed in a bat-specific pattern in the limbs of targeted mouse embryos. If the divergence is functionally significant, this will result in mice with altered limb morphology.

37. **Beetle Wing Development: A Model System to Analyze Insect Diversity.** Y. Tomoyasu,* S. R. Wheeler,† T. D. Shippy,* and R. E. Denell*. *Division of Biology, Kansas State University, Manhattan, Kansas; †Department of Genetics, Washington University, St. Louis, Missouri.

To understand how changes in patterning mechanisms have contributed to the evolution of morphological diversity, we are analyzing the regulation of wing morphogenesis in *Tribolium* and comparing it to that of *Drosophila*. In *Drosophila*, the T2 wing is used for flight, while the T3 haltere is highly reduced and used only for balance. In *Tribolium*, however, the flight wings are located on T3, while T2 bears highly sclerotized elytra that are used for body cover. Also, morphologies of the wings (such as vein pattern and the positioning of sensory organs) are different from those of *Drosophila*. To identify the changes in developmental programs that have contributed to these morphological diversities, we have analyzed the expression patterns of genes that might be important for wing development in *Tribolium*. Many genes we examined are expressed similarly in T2 elytra and in T3 wing, and these expression patterns are similar to those in *Drosophila* wing disc. However, several genes such as *Ubx*, *spalt*, and *achaete* are expressed differently in elytra and wing. This differential expression suggests that these genes might have important roles for

divergent fore/hind wing differentiation in *Tribolium*. These changes might also contribute to morphological differences in the wings of flies and beetles.

38. **Conserved and Divergent Functions of *Drosophila atonal*, Amphibian, and Mammalian *Ath5* Genes.** Nadean L. Brown,* Shami L. Kanekar,† Monica L. Vetter,† Sharon Gorski,‡ Yuh-Nung Jan,§ Tom Glaser,¶ and Yan Sun||. *Divisions of Developmental Biology and Ophthalmology, Children's Hospital Research Foundation; †Department of Neurobiology and Anatomy, University of Utah; ‡Department of Molecular Biology and Pharmacology, Washington University School of Medicine; §HHMI and Departments of Physiology and Biochemistry, University of California, San Francisco, California; ¶Departments of Internal Medicine and Human Genetics, University of Michigan Medical School; ||Institute of Molecular Pathology, Vienna, Austria.

Insect and vertebrate eyes differ in their formation, cellular composition, neural connectivity, and visual function. Despite this diversity, *atonal* and its vertebrate orthologue in the eye, *Ath5*, each regulate determination of the first retinal neuron class—R8 photoreceptors and retinal ganglion cells (RGCs)—in their respective organisms. We have performed a cross-species functional comparison of these genes. In *atol* mutant *Drosophila*, ectopic *Xenopus Ath5*, *Xath5*, rescues photoreceptor cell development comparably to *atonal*. In contrast, mouse *Ath5*, *Math5*, induces formation of very few ommatidia and most of these lack R8 cells. In the developing frog eye, ectopic *atonal*, similar to *Xath5*, promotes the differentiation of RGCs. Despite strong conservation of *atonal*, *Xath5*, and *Math5* structure and function, other factors must contribute to the species specificity of retinal neuron determination. These observations suggest that the *atonal* family may occupy a position in a gene hierarchy where differences in gene regulation or function can be correlated with evolutionary diversity of eye development.

39. **An Evolutionarily Conserved Tyrosine-Rich Domain Is Required for *Nkx2-5* Function.** Mark Solloway, David Elliott, Owen Prall, and Richard Harvey. The Victor Chang Cardiac Research Institute, Darlinghurst, NSW Australia.

The vertebrate homeobox gene *Nkx2-5* sits high in a genetic regulatory hierarchy controlling cardiac morphogenesis. However, little is known of the functional domains within *Nkx2-5* and how they regulate cardiogenesis in vivo. The C-terminus of *Nkx2-5* has been shown to positively influence transcription, so we searched for evolutionarily conserved amino acids within this region in an effort to further define functionally important domains. A novel tyrosine-rich region (TRR) was identified in addition to two previously described motifs: the GIRAW sequence and the *Nkx2-5*. The TRR has been conserved in cardiac NK2 class homeodomain proteins from cephalopods to humans, separated by over 500 million years of evolution, but has been lost in the *Drosophila* homolog tinman. The relative positions of individual tyrosines have also been preserved, and these tyrosines are essential for transcriptional activation in heterologous assays in fibroblasts and cardiac cells. To further define the function of the TRR in vivo, we replaced the tyrosine residues with alanine (*Nkx2-5^{Y-A}*) and targeted this allele into heterozygous *Nkx2-5^{GFP/+}* ES cells. Analyses in embryoid bodies demonstrate that the

modified allele is correctly expressed, and *Nkx2-5* target genes are downregulated in cardiomyocytes as measured by RT-PCR and microarrays. Furthermore, high-level *Nkx2-5^{GFP/Y-A}* chimeras undergo a block in cardiogenesis analogous to that seen in *Nkx2-5* null embryos. Together, our results indicate that the evolutionarily conserved tyrosines in the TRR are critical for maintaining *Nkx2-5* function and may serve as a regulatory target.

40. **Esx1-Dosage Effect and Cellular Function in the Placenta.** Laurel E. Fohn and Richard R. Behringer. MD Anderson Cancer Center, Houston, Texas.

Female eutherian mammals achieve dosage compensation in somatic tissues by random X-chromosome inactivation; however, in mouse placental tissues, the paternally derived X is preferentially inactivated. *Esx1* is a mouse paired-like homeobox gene on the X chromosome. *Esx1* expression is limited to extraembryonic tissues and testicular germ cells. By targeted deletion, *Esx1* was shown to be an X chromosome imprinted regulator of placental development and fetal growth. This study examines the importance of maintaining wild-type *Esx1* expression levels and the function of *Esx1* at the cellular level. The purpose of imprinted X inactivation within the placenta is unclear, but in mouse, increased maternal X chromosome dosage or loss of imprinted paternal X inactivation leads to defects in extraembryonic development and subsequent embryonic lethality. In humans, duplication of the *Esx1* syntenic region leads to defects in growth and gonadogenesis. *Esx1* is a candidate for a role in these abnormalities based on its imprinted placental expression, its impact on fetal growth, and its expression in male germ cells. To test the importance of maintaining *Esx1* dosage at wild-type levels and of imprinted *Esx1* expression in the placenta, we have created *Esx1* BAC transgenic animals. *ESX1* contains a homeodomain, suggestive of a role as a transcription factor. To examine the cellular function of *Esx1*, *Esx1* null α wild-type chimeric embryos have been generated. Analysis of the behavior of mutant cells in the presence of wild-type cells will allow determination of whether *Esx1* acts cell autonomously. These studies should help define the molecular and cellular roles of *Esx1* in placental development.

41. **Role of *lin-41* in Developmental Timing of Vertebrates.** Betsy R. Schulman and Frank J. Slack. Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut.

The heterochronic pathway in *C. elegans* is involved in the temporal regulation of developmental events. *lin-41* is regulated by a small RNA, *let-7*, a late acting gene in this pathway, and together these genes regulate the timing of proliferation versus differentiation decision by skin cells in *C. elegans*. Both *lin-41* and *let-7* are conserved across phylogeny in such organisms as *Drosophila*, Zebrafish, humans, and mice. The protein encoded by *lin-41* is an oncogenic-like ring finger B-box Coiled Coil (RBCC) protein. This family of proteins plays many significant roles in vertebrate development and health. We are interested in determining the significance of the *lin-41* and *let-7* genes in mammals as well as further characterizing their interactions. Using in situ hybridization experiments on mouse embryos, we have determined the stage and tissues that *lin-41* is expressed in the developing mouse embryo. *Mlin-41* is expressed temporally in limb buds as well as other tissues. We are using gene trap

technology to make a mutant *lin-41* mouse and coinjection approaches to study its overexpression. We hope to gain insight into the timing of human development in our use of the mouse as a model organism for these experiments.

42. Abstract #42 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

43. **Generation of Primate:Mouse Interspecific Chimeras.** Guy S. Eakin, Norio Nakatsuji, and Richard R. Behringer. Baylor College of Medicine Program in Developmental Biology, Kyoto University, and M.D. Anderson Cancer Center.

At the initiation of gastrulation primates display distinct morphological differences from similarly staged embryos of the mouse or rat. The most overt of these differences is the topological dissimilarity of the gross embryo. While the primate embryo develops as a planar epiblast within a spheroidal cytotrophoblastic shell, the rodent epiblast develops in a cupped shape, surrounded principally by a cylindrical shell of hypoblast. Additionally, the appearance of primate mesoderm is precocious with respect to that of rodents. Primate extraembryonic mesoderm is evident prior to the establishment of a primitive streak. In rodents, mesoderm appears only after establishment of this structure. To understand the molecular reasons for these differences, we are developing models of nonhuman primate and rat biology using interspecific chimeras produced using tetraploid mouse blastocysts. We have injected marked tetraploid blastocysts with outgrowths of rats or GFP expressing macaque embryonic stem cells. This technique segregates the extraembryonic tissues of the mouse from the developing embryonic structures of the second species. Comparisons between mouse embryos and our interspecific chimeric models will shed light on the molecular nature of the morphological differences between these mammals.

44. **Regulation of Molting and Reproduction by Conserved Nuclear Receptor Genes in *Caenorhabditis elegans*.** Chris R. Gissendanner,* Ann Sluder,† and Claude Maina*. *New England Biolabs, Beverly, Massachusetts; †Cambria Biosciences, Woburn, Massachusetts.

The *C. elegans* genome encodes >280 nuclear receptor (NR) genes. Among these are genes broadly conserved among the metazoa. Five genes are homologs or orthologs of NR genes that function during molting and metamorphosis in insects. Analysis using RNAi has established that one of these genes, *nhr-25*, the *C. elegans* Ftz-f1 ortholog, is required for ecdysis. The *nhr-25* RNAi molting phenotypes are similar to those observed for *nhr-23*, the DHR3 ortholog that has been previously described (Kostrouchova, 2001). Orthologs of DHR38(*nhr-6*), DHR78(*nhr-41*), and the putative ortholog of E75(*nhr-85*) were not implicated in molting by RNAi. However, *nhr-23*, *nhr-25*, *nhr-41*, and *nhr-85* all appear to be required for the proper formation of diapausing dauer larvae. The temporal expression patterns for the mRNA isoforms of these genes have been determined using RT-PCR analysis. Most of these genes have an oscillating pattern with peaks of expression occurring at precise times during each intermolt. These data suggest a temporal expression hierarchy of NR genes that may regulate events of the nematode molting cycle. *nhr-6*, the ortholog of DHR38/NGFI-B, does not appear to func-

tion during larval development. However, *nhr-6* is required for reproduction. Genetic analysis has demonstrated that *nhr-6* is required for somatic gonad development and the proper ovulation of oocytes. We are also performing a comparative analysis of *nhr-6* and its homolog from the filarid *D. immitis*, *dinhr-2*. NHR-6 and DINHR-2 both have similar biochemical properties and we are currently assessing whether they also share a similar pattern of expression.

45. **The Identification of the Phylotypic Stage in Nematode Embryos.** Wouter Houthoofd, Maxime Willems, Sandra Vangestel, Clarinda Mertens, Gaëtan Borgonie. Ghent University, Department of Biology, K.L. Ledeganckstraat 35, 9000 Gent, Belgium.

In every phylum the body plan is represented in development by the phylotypic stage, a stage where all members show a maximum degree of similarity. For nematodes, Slack et al. (1993) defined the phylotypic stage in nematodes as the moment after the completion of most embryonic divisions. Until recently, however, only a detailed description of the embryonic development of the model organism *Caenorhabditis elegans* was established by Sulston et al. (1983). Now, we recorded the embryonic development of three additional nematode species, *Pellioditis marina*, *Rhabditophanes* sp., and *Halicephalobus* sp., with a 4D microscope and established their complete embryonic cell lineages. Based on the comparison of the individual positions of all cells in all embryonic stages of the four species, we defined the phylotypic stage between the 8th and 9th division round (176 cell stage), before the completion of most embryonic divisions. This stage is located between a variable early embryonic development and a variable later embryonic development (egg timer model). In the early development, founder cells are formed in different sequences and configurations. These configurations converge before gastrulation to an identical configuration. After the phylotypic stage, tissues get their final species-specific configuration in the morphogenesis phase of embryonic development, where the embryo gets its worm-like form.

46. Abstract #46 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

47. **Sterile Caste in the Polyembryonic Wasp *Copidosoma floridanum*.** Tomislav Terxin, Vladimir Zhurov, and Miodrag Grbic. Department of Biology, University of Western Ontario, London N6A 5B7, Canada.

The existence of a sterile worker caste is a principal feature of eusocial organization in insects. The polyembryonic parasitic wasp *Copidosoma floridanum* produces two larval morphs that may be equivalent to the castes in traditional eusocial wasps (ants and bees). Both larval morphs, named precocious and reproductive, develop clonally from the same zygote during the course of polyembryonic development. Precocious larvae perform worker's tasks, including defence and sex ratio distortion and have morphology that is different from their reproductive siblings. It is believed that these larvae are also sterile because they have never been observed to molt and reproduce. Here we report, using molecular markers, that their patterning shows profound differences compared to reproductive morph, suggesting novel regula-

tory interactions that create divergent morphologies from the same zygote.

48. **The Role of APETALA3 and PISTILLATA Homologs in the Production of Novel Floral Morphologies.** E. M. Kramer and M. A. Jaramillo. Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts.

Understanding the generation of the enormous diversity of floral morphology found in the Angiosperms has long been a major goal of botanists. Due to the characterization of the genetic basis for the control of floral organ identity, comparative developmental genetics has become a major component of this process. In particular, the B class genes, which control petal and stamen identity, have been the target of much research due to the especially plastic nature of petal evolution. We have been studying the evolution of homologs of the B class genes APETALA3 and PISTILLATA in several families of flowering plants which are characterized by novel petaloid organ morphologies. In the lower eudicot family Ranunculaceae, gene duplications appear to have provided raw material to support the evolution of distinct types of petal identity programs. In contrast, in the Magnoliid family Aristolochiaceae, a type of co-option may be responsible for the generation of highly modified sepals. The implications of these findings for broader patterns of petal diversification within the Angiosperms will be discussed.

49. **Sequences Determining RNA Movement.** Thanh T. Dinh, Suzanne Gerttula, Cristina Ubach, and Neelima R. Sinha. University of California, Davis, California; Monsanto, St. Louis, Missouri.

The discovery of long distance RNA movement in plants has been a unique and interesting phenomenon. Recent studies done by Kim et al. (2001), using grafting experiments with *Xanthophyll* (*Xa*) scions and Mouse Ears (*Me*) stocks have demonstrated that a fusion RNA containing the the homeobox gene *LeT6* can be translocated long distances via the phloem and functions in developmental regulation. However, it is unknown what part of the *LeT6* gene is needed for movement. The goal of this project is to determine which RNA sequences facilitate RNA movement and lead to RNA localization. To determine which sequences are responsible for movement, *AGAMOUS* (*AG*), a floral homeotic gene, was chosen because it does not display RNA movement and produces a visible phenotype in flowers. *AG* was fused to the 5' 3' untranslated region of *LeT6* and constructs were made using a Chlorophyll AB Binding Protein (*CAB*) to express *AG* in leaves and Cauliflower Mosaic Virus (*CaMV*) 35S promoter for constitutive expression. These constructs were put in *Agrobacterium tumefaciens* and used in tobacco transformations to determine if the added *LeT6* gene would cause *AG* to move and produce a phenotypic change. Preliminary results using phenotypic analyses and grafting experiments on the tobacco transgenics have indicated that the *LeT6* 5' and 3' untranslated regions are responsible for movement. Molecular analyses, such as PCR and in situ RT-PCR, will be used to confirm the hypothesis by showing where the RNA is localized. Future experiments will be aimed at deleting various regions of the RNA and utilizing a similar transgenic and grafting approach.

50. **Functional Dominance Among Hox Genes: The Genetic Requirements.** Melissa E. Williams and Jeffrey W. Innis. Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48109.

Hox genes encode highly conserved transcription factors that are expressed in precise domains during development. Posterior HOX proteins expressed ectopically in more anterior or proximal domains generally exert functional dominance over endogenous *Hox* gene products and give rise to malformations or "posterior" transformations. We are exploring the genetic requirements for posterior prevalence in developing mouse limbs using a transgenic approach employing the *Prx-1* promoter. Experiments expressing *Hoxa13* or *Hoxd13* individually throughout the mesenchyme of the developing limbs show that each is sufficient to cause similar shortening of the zeugopods and stylopods characteristic of posterior prevalence, while expression of the more anterior gene *Hoxa9* does not. Chimeric *Hox* constructs are being tested to determine precise regions/residues of the posterior HOX proteins that are both necessary and sufficient for functional dominance. These experiments will advance our understanding of the contribution of protein sequence evolution in HOX functional divergence.

51. **Evolution of the Hoxa2 Gene: Genomic and Expression Comparisons.** Jean-Luc Scemama, Kerri Augustino, and Edmund Stellwag. Department of Biology, East Carolina University, Greenville, North Carolina 27858.

Hox genes are an evolutionary conserved family of transcription factors that confer identity along the primary body axis of animals. Their clustered organization, which has been maintained during evolutionary history, belies a considerable lability in the composition and organization of the *Hox* clusters, particularly between the lobe and ray-finned fish lineages. Our examination of the *Hox* gene organization and expression from *Morone saxatilis*, the striped bass, a representative of a relatively recently evolved ray-finned fish lineage, the *Acanthopterygii*, underscores the dynamic nature of *Hox* gene evolution and suggests that the evolutionary divergence of this developmentally pivotal gene family corresponds to the evolution of morphological differences reflected among the divergent animal lineages. Detailed comparative genomic analysis between striped bass and mouse *Hoxa2* genes, the only vertebrates for which a *Hoxa2a* ortholog has been investigated, revealed the partial conservation of rhombomeric and neural crest enhancers but also the presence of lineage-specific regulatory elements. Interestingly, comparative expression studies between these two species throughout development show strong similarities, including expression in the hindbrain and in neural crest migrating into the second branchial arch. Given the differences in regulatory element structures, the similarities in expression pattern are surprising and we argue that either a more detailed analysis of the expression patterns will reveal differences between these two species or that regulatory element composition does not determine the pattern of expression for this gene.

52. **A Global Control Region Defines Regulatory Landscapes Containing the HoxD Complex and Activates Gene Expression in Developing Digits.** François Spitz, Federico Gonzalez, and Denis Duboule. Department of Zoology and Animal Biology and NCCR "Frontiers in Genetics", University of

Geneva, Sciences III, Quai Ansermet 30, 1211 Geneva 4, Switzerland.

The formation of digits required the coordinated expression of several genes of the *HoxD* cluster, suggesting that they are controlled by a shared global enhancer. At 90 kb upstream of the *HoxD* cluster, we found a novel gene, *Lunapark*, which shared expression domains in limbs and CNS with *HoxD* genes and *Evx2*, a gene located nearby *Hoxd13*. We developed a locus-targeted enhancer-trap approach to look for enhancer sequences in this region and identified a DNA fragment, lying further upstream, which was able to direct expression of a reporter gene in both limbs and CNS, following the *Lnp*, *Evx2*, and posterior *Hoxd* gene-activation patterns. This Global Control Region (GCR) showed an unusual interspecies degree of sequence conservation, including with the teleost fish counterparts. However, while the pufferfish GCR was able to activate a reporter gene in the CNS, similarly to the tetrapod GCR, it failed to trigger any expression in digits. The importance of the GCR was further verified by the molecular resolution of the *Ulnaless* mutation, a balanced inversion which altered the position of *HoxD* and *Evx2* relative to the GCR and, consequently, their expression in digits and CNS. Altogether, these results show that the GCR controls the expression of several unrelated genes within a large genomic locus, thus defining "regulatory landscapes." These findings are discussed in terms of gene regulation at the level of whole genomic loci, as well as from an evolutionary perspective.

53. **The Significance of Precise Temporal Regulation of Hoxc8 Expression at Early Embryonic Stages in Pattern Formation.** Aster H. Juan, and Frank H. Ruddle. Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut.

The proper temporal expression of the Hox genes during embryogenesis is essential for normal development, yet the control mechanisms involved are poorly understood. We have identified a phylogenetically conserved Hoxc8 regulatory sequence, the Hoxc8 early enhancer (EE), that is required for the activation of Hoxc8 at early developmental stages. The EE contains a minimum of seven putative transcription factor-binding motifs including two CDX binding sites. It has also been shown that the EE is involved in chromatin remodeling. To further define the timing function of the EE, we have deleted it endogenously using ES cell technology. Analysis of Hoxc8 expression patterns in the mutant embryos by whole-mount in situ hybridization at different developmental stages shows that expression of Hoxc8 is delayed at 8 dpc but subsequently is resumed with the appropriate expression domain later. However, this slight expression delay of Hoxc8 is sufficient to induce high penetrance of skeleton transformations in the trunk region in mutant mice. This data suggest the transitory deficit in the Hox gene product in presomitic mesoderm is enough to induce morphological alterations. We also show that a second domain similar to the EE in both structure and function resides in the *Hoxd* cluster, indicating the possible existence of a concerted system regulating the temporal regulation of the Hox genes. This study provides direct knowledge of enhancer behavior in the context of the intact Hox cluster and illustrates the importance of precise temporal regulation of Hox genes through *cis*-regulatory elements in pattern formation.

54. **Antp-Class Hox and non-Hox Sequences in a Basal Metazoan: The Marine Sponge *Haliclona loosanoffi*.** April L. Hill, Jeff Tetraault, Marc Bartman, and Malcolm S. Hill. Fairfield University, Fairfield, Connecticut.

In contrast to some well-known animal models, very little is known of the developmental control of growth in sponges. This lack of information has limited our understanding of important events in the course of animal evolution. Therefore, the broad goal of this research is to understand the genetic constituency of potentially important genes in sponge development and the functionality of morphogenetic regulatory programs in sponges. We used both degenerate PCR and library screens to isolate Antp-class homeobox sequences from sponge genomes. We report here the isolation of potential central class *Hox* gene from a Poriferan genome. Phylogenetic analysis consistently placed the Hox3H1 sequence in the PG-3 group. The Hox3H1 sequence has family-specific PG-3 amino acid residues at several positions. This finding is surprising given that only anterior and posterior class Hoxgenes have been found in cnidarians. Additionally, we have isolated and characterized other Antp-class non-Hox genes from sponges. We used RT-PCR analysis to examine the expression profile of these genes in developing sponge tissues. For one of those genes, a BarH1-like Antp-class homeobox, we piloted in situ hybridization protocols to examine where the gene is expressed in adult tissue (housing developing embryos) and developing larvae. Future studies aimed at elucidating the roles of these genes during sponge development will give us additional insight into the evolution of animal body plans.

55. **Functional Analysis of Maternal Hro-nos, A Nanos Homolog in the Leech *Helobdella robusta*.** Sara J. Agee and David A. Weisblat. Dept. of Molecular and Cell Biology, 385 LSA, University of California, Berkeley, California 94720-3200.

A major problem in studying the evolution of developmental mechanisms is that so little is known about Lophotrochozoa, the clade that arguably comprises most extant bilaterian phyla. In particular, functional studies of molecular mechanisms are nearly absent. For example, previous studies have demonstrated the existence of both maternal and zygotic expression of a nanos-class gene (Hro-nos) in the leech *Helobdella robusta* (Lophotrochozoa: Annelida). Hro-nos is expressed zygotically in primordial germ cells, consistent with an ancient function for nanos-class genes in formation of the germ line (Kang et al., 2002), but the significance of the abundant maternal Hro-nos transcripts remains unclear. Maternally driven expression of HRO-NOS protein peaks at fourth cleavage, specifically in cells DM (2D) and DNOPQ (2d). These cells go on to form mesodermal and ectodermal stem cells (teloblasts) of the posterior growth zone, from which segments arise (Pilon and Weisblat, 1997). To investigate the function of maternal Hro-nos, we injected antisense morpholino oligonucleotides into freshly laid zygotes. Immunostaining showed that expression of HRO-NOS is partially knocked down in both DM and DNOPQ. Lineage tracing revealed that DM and DNOPQ make their normal complements of mesodermal and ectodermal teloblasts, respectively. These produce distinct mesodermal and ectodermal layers of segmental founder cells (blast cells), but the morphology and bilateral organization of these tissues is abnormal. Also, stem cell divisions of the mesodermal teloblasts terminate prematurely, resulting in fewer segmental founder clones. These results suggest that maternally

inherited Hro-nos is important for organization and maintenance of the posterior growth zone of the leech embryo.

56. **Proper Differentiation and Function of the *Drosophila* Insulin-Producing Cells Is Dependent on the Pax6 Homolog *eyeless*.** Jason S. Clements,* Yuan Yuan Kang,* Rick Kollman,* Eric J. Rulifson,† and Patrick Callaerts*. *University of Houston, Department of Biology and Biochemistry, Houston Texas; †Stanford University, Department of Developmental Biology, Stanford, California.

Pax6, a transcription factor containing two highly conserved DNA-binding domains, a homeodomain and a paired domain, has a major role in eye and brain development of both vertebrates and invertebrates. In *Drosophila*, the Pax6 homolog Eyeless is required during brain development for normal differentiation of major neuropils including the optic lobes, the mushroom bodies, and the central complex. Loss of *eyeless* in these structures results in severe morphological defects, with prominent defects in axon outgrowth, guidance, and branching. We have identified a requirement for *eyeless* in development of neurosecretory cells in the *pars intercerebralis*. Specifically, we have demonstrated that Eyeless is expressed in the Insulin-Producing Cells (IPCs) via colocalization with the *Drosophila* insulin-like peptide Dilp2. We also show that in homozygous *eyeless* animals, Dilp2 is normally expressed, but the IPCs fail to project their axons to their normal targets, resulting in animals with phenotypes similar to those previously associated with disrupted insulin signaling. Our data show that Eyeless function is required in Dilp2-expressing IPCs for normal differentiation. Remarkably, a role for Pax6 in the differentiation of insulin-producing pancreatic β cells has been previously documented. The evolutionary implications of this possibly conserved link between Pax6 and normal differentiation of insulin-producing cells will be discussed.

57. **Comment on Brain Developmental Profile Study.** K. K. Leung, M. C. Fung, and S. M. Ngai. The Chinese University of Hong Kong, Hong Kong.

It is a moot point if mouse experimental results can be applied to human. Yet, there is a trend of using two-dimensional gel electrophoresis equipped with mass spectrometry (2DE-MS) on studying mouse model. In an investigation on the developmental profile analysis on mouse brain, one of our 2DE-MS results agrees with Yang's gene trap based observation [1]. Prtb matches a human homologue DAZAP2. However, there is a missing link between human male fertility and mouse brain development. 2DE-MS analysis employed in my case of studies is often limited by the inability to detect low abundance proteins that may only be present at a certain stage during the brain developmental process; regulatory proteins that with extreme pI values (e.g., pI less than 3 or above 10) are not included. 2D gel randomized spot migration is also one of the major sources of errors in my data interpretation. Besides, the fact that both human [2] and mouse [3] brains differ in early developmental stages with different sexes is worthy of notice. High-throughput and robotized approaches become crucial for such 2DE-MS experiment in order to capture the nuances of protein expression. If one appreciates the complex developmental process, then making strenuous efforts to retrieve a nondelusive picture should become logical. Our movie to rouse student interest in human development can be found at: <http://www.cs.cuhk.edu.hk/~kkleung/m.htm>;

Dev Dyn 1999, 215: 108-116; Sci Am 1992, 267(3): 118-127; Biochem J 1989, 261: 769-773.

58. **The UNC-39/Six5 Homeodomain Transcription Factor Affects Anterior Neuronal Development in *C. elegans*.** Judith Yanowitz,* Afaq Shakir,† Ed Hedgecock,‡ Andrew Fire,* and Erik Lundquist†. *Carnegie Institution of Washington, Baltimore, Maryland 21210; †Department of Molecular Biosciences, University of Kansas, Lawrence, Kansas 66045; ‡Department of Biology, Johns Hopkins University, Baltimore, Maryland 21210.

Six-class homeodomain transcription factors specify anterior ectodermal development and mesodermal development throughout metazoan phylogeny. We found that UNC-39 in *C. elegans* is a Six-class homeodomain transcription factor most similar to human Six5. *unc-39* mutants displayed defects in anterior neuronal development specifically and had defects in many aspects of mesodermal development, indicating that the role of this protein family is conserved in nematodes. A full-length *unc-39::gfp* fusion construct rescued *unc-39* mutants. Expression of this fusion was first seen in early embryos in 10-12 anterior nuclei whose positions were consistent with anterior ectoblasts and mesoblasts. Later, expression was observed in posterior mesodermal nuclei. We found that *unc-39* mutants were partially rescued by human Six5, suggesting these genes are functional homologs and that DNA binding specificity and possibly protein:protein interaction specificity of these molecules are conserved. Indeed, Six5 disruption in humans is thought to contribute to type I myotonic dystrophy, an inherited disease characterized by anterior ectoderm defects (e.g., mental impairment and cataracts) and mesoderm defects (e.g., gonad dysgenesis and fragile musculature), a phenotypic spectrum similar to *unc-39* mutants. Studies are ongoing to determine if upstream regulators and downstream targets of *unc-39* are also conserved throughout metazoan evolution.

59. **Ventral Patterning of the CNS in Flies and Fish: The Role of Nkx6 Proteins.** S. E. Cheesman,* T. Von Ohlen,† C. Q. Doe, and J. S. Eisen. *Institute of Neuroscience, University of Oregon, Eugene, Oregon; †Kansas State University, Kansas.

Recent evidence suggests the *nkx/gsh/msx* cassette of transcription factors is important in patterning ventral-to-dorsal domains in the central nervous system (CNS) of fruit flies and mice, members of two distantly related phyla. However, in zebrafish the known *nkx* homologs are expressed too late to pattern early-developing ventral neurons. In a search for other *nkx* family members we have identified zebrafish *nkx6.1*, which is expressed in many ventral neural tube cells including early-developing 1° motoneurons (mns) and later-developing 2° mns. Using mutants and morpholino oligonucleotides, we show that Hedgehog signaling is required for *nkx6.1* expression in zebrafish and in the absence of Nkx6.1 protein there are fewer 2° mns, although the number of 1° mns is normal. Intriguingly, ectopic expression of *nkx6.1* is sufficient to generate supernumerary 1° mns in the zebrafish CNS, suggesting there may be additional *nkx6* genes functioning in the ventral neural tube. Flies also possess an *nkx6* gene that is expressed early in neurogenesis in a group of anterior ventral neuroblasts and later in neurons (Uhler et al., 2002). Ectopic expression of either fly or zebrafish *nkx6* within the fly nerve cord creates extra mns and ins in the CNS.

Conversely, ectopic expression of fly *nkx6* RNA is sufficient to induce supernumerary 1° mns within the zebrafish CNS. Collectively these data suggest that *nkx6* genes are ancient regulators of ventral CNS fate.

60. Differences in Midline Kinetics of Forebrain Commissural Axons in vivo. Magdalena Bak and Scott E. Fraser. California Institute of Technology, Pasadena, California.

Neuronal scaffold studies in both vertebrates and invertebrates suggest that early arising neurons and their axons serve as guides for later neurons and their processes. While this might aid axon navigation, the specific interactions and their consequences are ill-defined in vertebrate systems. To attack this research problem, we have employed a transgenic zebrafish line (*gata2::GFP*) with GFP expression in the earliest differentiating neurons. Confocal microscopy and double labeling with acetylated alpha-tubulin confirms that the GFP positive cells in the zebrafish forebrain give rise to neurons of the ventro-rostral cluster. Previous time-lapse studies have shown that the earliest spinal commissural axons slow down at the midline; however, our studies reveal that it is only the first axon to cross the midline that slows. Later axons fasciculate with the leader and display no slowing unless the leader is ablated. A single leader axon across the midline is sufficient to protect both the contralateral leader growth cone as well as the follower growth cones from slowing at the midline. Quantitative analysis demonstrates that axon kinetic differences and growth cone morphology are highly correlated, with all followers showing a simple, narrow morphology. This suggests a simple model: the morphology of the growth cone changes the relative levels of exposure to midline cues and to direct axon interactions, reducing the influence of the midline cues on followers. These results set the context for future molecular studies of midline commissural axon guidance in vivo. Our current experiments are aimed at dissecting the molecular basis of these interactions using dynamic imaging.

61. Abstract #61 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

62. Changes in Activities of Developmental Genes and Genetic Networks Underlying Reproductive Flexibility in the *Cnidarian Nematostella*. P. Burton,* J. Ryan,* K. Pang†, M. Eggen,* S. Schaus,* M. Martindale,† and J. Finnerty. *Boston University, Boston, Massachusetts; †University of Hawaii, Hawaii.

The sea anemone *Nematostella vectensis*, similar to many invertebrates, exhibits a range of reproductive plasticity not seen in developmental model systems such as fruit flies and mice. *Nematostella* develops in three different life history contexts: embryogenesis, asexual reproduction, and regeneration following injury. In each context, the same phenotype is produced, but the developmental starting points are quite different. How does the genome of *Nematostella* encode this flexibility, and by extension, why are our standard models unable to exhibit such flexibility? Our work has concentrated on illuminating the spatial and temporal expression and regulatory interactions of *Nematostella* developmental genes, specifically those involved in patterning the body axis. In situ hybridization experiments indicate that Hox genes are important for patterning *Nematostella* during both embryogenesis and regeneration, as in *Bilateria*. However, the

role of individual Hox genes is distinct between modes of development. For example, expression of *anthox1*, a posterior Hox gene, is observed in the extreme end of the future aboral ectoderm of sexually developing anemones. During regeneration, *anthox1* expression is restricted to the endodermal mesenteries of the aboral pole of developing head tissue. We have also developed an oligonucleotide microarray chip, which in conjunction with RNAi experiments, is being used to identify regulatory connections among *Nematostella* developmental genes. Using this system, we are investigating the conservation and divergence of genetic regulatory networks between developmental contexts.

63. Abstract #63 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

64. The Role of BMP4 in Early Prostate Development. G. Almahbobi,* S. Hedwards,* G. Fricout,† D. Jeulin,† L. A. Cullen-McEwen,‡ J. F. Bertram,‡ and G. P. Risbrider*. *Monash Institute of Reproduction and Development and †Department of Anatomy and Cell Biology, Monash University, Melbourne, Australia, ‡Centre de Morphologie Mathématique, Ecole des Mines de Paris, France.

Branching morphogenesis (BM) in the mammalian prostate is accelerated during the neonatal period, determining the 3D structure of the gland. The quantification of this complex structure has not been mapped in a spatial and temporal manner during the neonatal period, due to the lack of suitable methods. Using new methods of image analysis developed for this purpose, we report a quantitative description of the 3D patterns of the normal AP and VP that show significant asymmetry during the first 4 days after birth. Since altered prostate growth was reported in adult BMP4 haploinsufficient mice, the methods were applied to study the mechanism of action of BMP4 on early BM. Initial budding of the main prostatic ducts is altered in VP, but not AP, so that the number of main ducts is significantly increased in BMP4 mutant mice. In parallel, there is a significant increase in the total volume and length of VP over the same period in mutant mice compared to wild-type littermates, without significant change in the number of branches, branching points, or tips. In contrast, there is no change in initial budding in AP but the significant increase in volume over 4 days is due to significant increase in the number of branches and branching points and not branch length. The detection and quantification of aberrant BM in mutant animals within the first 4 days of neonatal life demonstrates the lobe-specific action of BMP4 that predicts the prostate pathology, known to occur in adulthood.

65. Understanding the Cambrian Explosion by Estimating Ontogenetic Depth. Paul A. Nelson and Marcus R. Ross. Discovery Institute and the University of Rhode Island, Kingston, Rhode Island.

Various attempts have been made to assess the increase in biological complexity exhibited by metazoans across the Neoproterozoic-Cambrian boundary. These include such metrics as genome size, cell type (Valentine et al., 1994), and several complexity measures (e.g., McShea, 1996). We develop a measure of *ontogenetic depth*, which estimates the distance, in terms of cell division and differentiation, between a unicellular condition and a macroscopic adult metazoan capable of reproduction (gen-

eration of gametes). We apply this metric to the radiative events that occurred during the Cambrian Explosion and evaluate the evolutionary mechanisms that may explain the increases in ontogenetic depth at the origin of the phyla.

66. **Computer Modeling of Phosphoinositide Signaling: Combining Structure, Electrostatics, and Bioinformatics to Understand How the Binding of Proteins to Membrane Surfaces is Regulated.** Diana Murray. Weill Medical College of Cornell University, Ithaca, New York.

The reversible binding of proteins to membranes is crucial to many biological processes and is often accomplished through lipid-interacting protein domains. Although there are many ways in which membrane binding can be achieved and controlled, lipid-interacting domains appear to share common features with respect to the physical basis of their membrane association. Our research suggests that various combinations of two physical factors—electrostatics and hydrophobicity—are major determinants of membrane binding. Calculations of the interaction of peripheral proteins with realistic models of phospholipid membranes provides information unobtainable through structural analyses of the proteins alone, and the application of bioinformatics tools allows for the extension of detailed calculations on individual proteins to whole families of membrane targeting domains. The finite difference Poisson—Boltzmann (FDPB) method has proved extremely accurate in its ability to account for many of the experimentally determined electrostatic properties of protein/membrane systems. This work focuses on the recent application of the FDPB method to structures and homology models of phosphoinositide binding domains, i.e., PH, FYVE, PX, and ENTH domains. Our calculations complement experimental studies and show how the binding of these domains to polyphosphoinositides at membrane interfaces dramatically alter their electrostatic properties. These electrostatic changes have implications for the penetration of these domains into membrane interfaces as well as their lateral redistribution at membrane surfaces.

67. **Genetic Interactions of the *Dictyostelium discoideum* E3 Ubiquitin Ligase Component FbxA with cAMP Metabolism and a Histidine Kinase Signaling Pathway.** David I. Ratner,*†, Turgay Tekinay,* Herbert L. Ennis,* Mary Y. Wu,* Margaret K. Nelson,* and Richard H. Kessin*. *Department of Anatomy and Cell Biology, Columbia University, New York, New York; †Department of Biology, Amherst College, Amherst, Massachusetts.

Dictyostelium amoebae altered in a gene called *fbxA*, thought to encode a component of an SCF E3 ubiquitin ligase, have defective regulation of cell-type proportionality. In chimeras with wild-type cells, the mutant amoebae form mainly spores, leaving the construction of stalk to wild-type cells. Because *fbxA* cells are developmentally deficient in pure culture, we were able to select suppressor mutations that promote sporulation of the original mutant. One suppressor arose by disruption of the gene *dhkA*, encoding a “two component” histidine kinase known previously to influence *Dictyostelium* development. A second suppressor mutation resides within the gene *regA*, which encodes a cyclic AMP phosphodiesterase linked to an activating “response regulator” domain subject to phosphorylation. *RegA* appears precociously and in greater amounts in the *fbxA* mutant, arguing that

RegA is a normal target of FbxA-mediated ubiquitination and proteolysis. In the *fbxA/dhkA* double mutant, *RegA* is restored to wild-type levels. Because the basis of *regA* suppression might involve alterations in cAMP levels during development, the cAMP profile was determined in all strains. Cyclic AMP levels are significantly lowered in *dhkA* mutants, restored to near normal levels by *fbxA* mutation, and unexpectedly normal in the *regA* background. We continue to explore the interplay between these and other signal transducing components.

68. **Sightless Encodes a Transmembrane Acyltransferase Required for Hedgehog Signaling.** Grant I. Miura, Jeffrey D. Lee, and Jessica E. Treisman. Skirball Institute for Biomolecular Medicine and Department of Cell Biology, New York University School of Medicine, New York University, New York, New York.

Hedgehog (Hh) signaling is required for many processes during development. The Hedgehog protein undergoes autocatalytic cleavage accompanied by the addition of a cholesterol moiety to the C-terminus. In a genetic mosaic screen, we identified mutations in *sightless* (*sit*), which is required for the activity of Hh in eye and wing imaginal discs and in the embryo. It was found to act in the Hh producing cells rather than the receiving cells in the wing disc. *sit* encodes a transmembrane acyltransferase that is likely to catalyze the palmitoylation of the N-terminal cysteine of the mature signaling domain of Hh. Furthermore, mutation of this cysteine to serine in Hh caused it to act as a dominant negative protein when overexpressed, suggesting that Hh needs to be acylated by Sit for proper function. Loss of *sit* causes additional phenotypes unlikely to be due to defects in Hh function. We are currently investigating other potential substrates for Sit.

69. **Smoothed Translates Different Levels of Hedgehog into Distinct Responses.** Joan Hooper. UCHSC.

Hedgehog (Hh) acts as a morphogen to pattern a variety of tissues through varying the levels of the activator (CiA) and repressor (CiR) forms of the transcription factor, Ci. In the *Drosophila* wing Hh directs at least three levels of response; OFF where CiR predominates, LOW where both CiR and CiA are low, and HIGH where CiA predominates. Costal (Cos), Fused (Fu), and Sufu form a complex with Ci that regulates its fate. Patched, the cell-surface receptor for Hh, is a repressor of the serpentine receptor, Smoothed (Smo). Smo modulates the Ci regulatory complex by unknown mechanism(s) that are presumed to involve second messengers. We have undertaken a genetic analysis of truncated and chimeric forms of Smo to address its signal transduction mechanisms. Smo lacking its cytoplasmic tail is dominant negative for HIGH signaling, similar to classII *fu* mutants, and is sensitive to dosages of Smo and Fu but not Cos. This suggests that dimeric or oligomeric Smo transduce HIGH Hh and that nonproductive dimers prevent Fu from activating Ci. The cytoplasmic tail of Smo drives cells to the LOW state, regardless of Hh, similar to *cos* mutants. This LOW signaling requires endogenous Smo and is sensitive to dosage of Smo and Cos. This suggests that LOW signaling is normally achieved by stoichiometric inactivation of Cos by the cytoplasmic tail of Smo. Coimmunoprecipitations show (1) that Smo is oligomeric, at least when signaling is active, (2) that transmembrane regions of Smo are necessary for oligomers, and (3) that Fu and Cos associate with Smo, at least when signaling is active. This

strongly supports a model of Smo signal transduction where there are no second messengers and where activation is relayed by interactions of Cos and Fu with Smo.

70. **Subcellular Localization of rab23, a Negative Regulator of the Hedgehog Signaling Pathway.** Timothy M. Evans, Brandon J. Wainwright, Robert G. Parton, and Carol A. Wicking. Institute for Molecular Bioscience, The University of Queensland, Australia.

The hedgehog signaling pathway is pivotal to growth and patterning during embryonic development as well as tumorigenesis. Intracellular trafficking events are believed to play an important role in the transduction and regulation of the hedgehog signaling pathway. This was exemplified in the recent finding that rab23, a member of the rab-GTPase family, was implicated as a negative regulator of the hedgehog signaling. As specific rab proteins regulate the fusion and docking of membranes between specific vesicles and organelles, it was hypothesized that the study of rab23 localization would clarify trafficking events important in the regulation of hedgehog signaling. To understand these events more clearly, we first sought to determine the subcellular localization of members of the pathway. Using over-expression of GFP-tagged rab23 constructs in mammalian cells, we have subsequently investigated the subcellular distribution of rab23 with respect to members of the hedgehog signaling pathway.

71. **Genetic Dissection of Notch Functions in Rostrocaudal Patterning of Somites.** Yu Takahashi,* Tohru Inoue,* Achim Gossler,† and Yumiko Saga‡. *Cellular and Molecular Toxicology Division, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagayaku, Tokyo 158-8501, Japan; †Institut für Molekularbiologie, MHH, 30625 Hannover, Germany; ‡Division of Mammalian Development, National Institute of Genetics, Yata 1111, Mishima 411-8540, Japan.

Elaborate metamerism in vertebrate somitogenesis is based on the segmental gene expression in the anterior presomitic mesoderm (PSM). Notch signaling with ligands Dll1 and Dll3, and the transcription factor Mesp2, are implicated in the rostrocaudal patterning of the somite. We have analyzed genetic interactions among Dll1, Dll3, Mesp2 and the Notch signal mediator Psen1, and elucidated the roles of Dll1- and Dll3-Notch pathways, with or without Psen1, in rostrocaudal patterning. The positive and negative feedback loops of Dll1 and Mesp2, and their coordination by Dll3, appear to be critical for the patterning. Psen1 is required for Dll1-Notch signaling for activation of Dll1, while the Dll3-Notch pathway may counteract the Psen1-dependent Dll1-Notch pathway. Mesp2 functions as a central mediator of such Notch pathways.

72. Abstract #72 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.
73. **Canoe: Linking Signaling Pathways.** A. Carmena and M. Baylies. Sloan-Kettering Institute. New York, New York.

Cross-talk among signaling pathways is essential for cells to integrate multiple stimuli and achieve unique responses. The PDZ-containing protein Canoe (Cno) binds Ras and genetically

interacts with Notch (N) during *Drosophila* development. We previously showed that Wingless (Wg), N, and Ras widely cross-communicate in this process. Now we present Cno as a crucial regulator of all, Wg, N, and Ras throughout progenitor specification. Cno is expressed in the mesoderm at the moment progenitors are specified. *cno* loss and gain of function displays a similar phenotype, reminiscent to that shown in embryos in which activated forms of N, Ras, and Wg are expressed simultaneously in the mesoderm. Genetic interactions between *cno* and both the distal Ras-effector Pointed and the DER-inhibitor Argos support a Ras-repressing action of Cno. However, cases of diPMAPK expansion in *cno* gof mutants suggest that Cno is not merely inhibiting Ras. In fact, *cno* strongly interacts genetically with different components of N and Wg signaling pathways during progenitor specification. Most significantly, Cno not only binds Ras but also physically interacts with the N-inhibitor protein Dishevelled (Dsh)—a key factor in Wg pathway. Coexpression of Dsh, Cno, and N in muscle/heart progenitors further supports the relevance of this interaction. A working model will be presented in which Cno would act as a repressor of all: Ras, by direct binding, Wg canonical pathway, by “sequestering” Dsh, and N, by contributing to Dsh localization at the membrane where Dsh can bind and inhibit N. Altogether, our data reveal Cno as a critical linker and modulator of major signaling pathways.

74. **Regulation of Src64 Activity during *Drosophila* Oogenesis.** A. Ballew,† A. O'Reilly,† H. Stocker,* E. Hafen,* and M. Simont. *Department of Zoology, Universität Zürich; and †Department of Biological Sciences, Stanford University, Stanford, California.

To understand the dynamic regulation of Src family tyrosine kinase (SFK) activity in vivo, we analyzed the effects of expressing a constitutively activated form of the *Drosophila* SFK Src64 (Src64^{Y547F}) during oogenesis. Expression of Src64^{Y547F} lead to disruptions in ring canal stability and in packaging of germline cysts into individual egg chambers. These effects resembled loss of function *src64* phenotypes, demonstrating that Src64 activity is dynamically regulated during oogenesis. To further investigate this regulation, we cloned the *Drosophila* homolog of the protein tyrosine kinase C-terminal Src Kinase (CSK) and obtained mutations in *dCSK* for analysis. CSK negatively regulates SFK activity in other systems by phosphorylating the C-terminal tail of SFKs. Defects in ovaries that were homozygous mutants for *dCSK* mimicked those in ovaries that expressed Src64^{Y547F}. Additionally, removing one copy of *dCSK* in a hypomorphic *src64* mutant background dramatically suppressed the *src64* phenotypes, confirming that dCSK functions to down-regulate Src64 activity during oogenesis. Interestingly, ring canal growth, which requires Src64 activity, was unaffected by either loss of dCSK or expression of constitutively active Src64. This indicates that there are different modes of SFK regulation and highlights the importance of identifying other regulators of Src64 activity in this system.

75. **Mutational Analysis of LRP6 in Wnt Signal Transduction.** Keiko Tamai, Xin Zeng, and Xi He. Division of Neuroscience, Children's Hospital, Department of Neurology, Harvard Medical School, Cambridge, Massachusetts.

The Wnt family of secreted ligands play important roles in embryonic development and tumorigenesis. LRP5/6 (LDLR-related protein 5/6) is a single transmembrane protein, identified

as a coreceptor in Wnt signaling. While it was demonstrated that the scaffolding protein Axin interacts with the intracellular domain of LRP5/6, the mechanism of Wnt signal transduction by LRP5/6 remains unknown. Here we investigate the role of intracellular domain of LRP6. We show a membrane tethered cytoplasmic domain of LRP6 is sufficient to induce complete double axis in *Xenopus* and is more active than the wild-type protein, which only induces partial secondary axis. We have generated a series of amino acid substitutions into cytoplasmic domain of LRP6 and identify important residues for signal transduction. These studies provide crucial insights into the mechanism of LRP5/6 in transducing Wnt signaling.

76. **Identification of Wnt Target Genes in the Neural Crest.** Lisa Taneyhill Ziemer and Marianne Bronner-Fraser. California Institute of Technology, Pasadena, California.

The vertebrate neural crest is a population of migratory cells, arising from an interaction between the neural plate and adjacent epidermal tissue, which gives rise to most of the peripheral nervous system, melanocytes, and the craniofacial skeleton. Recently our laboratory has demonstrated a role for Wnts in mediating this tissue interaction. Although a Wnt signal functions as the initial inducer of this interaction, the molecular nature of the downstream targets of Wnt signaling are not known. To discern whether genes expressed in the dorsal neural tube are targets of Wnt signaling, and thus may play a role in crest induction, we have performed quantitative polymerase chain reaction (QPCR) using gene-specific primers and intermediate neural plate tissue cultured in collagen in the presence of either Wingless (Wg) or control conditioned medium as a template. The fold response of these potential targets to Wnt signaling was assessed after 18, 24, and 36 hours of culture. The results indicate that Wnt signaling up-regulates several genes (*slug*, *Pax3*, *Precrest-1*, *Msx1*) at the times tested. Expression of *rhoB*, however, is unchanged or even decreased in the presence of Wg CM. Future experiments will involve assessing the dose response of these targets to Wg CM to generate a temporal and concentration-dependent induction profile, as well as determining whether these genes are direct or indirect targets of β -catenin-mediated Wnt signaling. Novel Wnt response genes will also be identified by screening a chick macroarray cDNA library. Through this research we hope to understand how Wnt signaling, and the genes it activates, regulate neural crest induction.

77. **Regulation of Cell and Tissue Polarity by Daam and Wnt Signaling.** Masa-aki Nakaya,* Raymond Habas,† Xi He,† and Terry P. Yamaguchi*. *Cancer and Developmental Biology Laboratory, NCI-Frederick, Frederick, Maryland 21702, USA; †Division of Neuroscience, Harvard Medical School, Boston, Massachusetts 02115, USA.

We are interested in understanding the molecular mechanisms underlying the relationship between cell polarity and morphogenesis during mouse embryogenesis. Orienting cell polarity along a body axis in groups of cells (i.e., tissues) is critical for the cell—cell interactions that occur in specialized cell movements such as convergent extension. Members of the Wnt family of secreted signaling molecules are highly conserved in metazoans and regulate numerous cellular and developmental processes including proliferation, differentiation, cell fate, cell polarity, and convergent extension. However, little is known about the mo-

lecular mechanisms underlying the control of cell and tissue polarity by vertebrate Wnts. We have identified mouse homologues of Daam (Dsh-associated activator of morphogenesis), a molecule that contains Formin homology domains and which interacts directly with Dsh and Rho to control convergent extension in *Xenopus* embryogenesis (Habas et al., 2001, 2003). mDaam1 and mDaam2 are expressed in gastrulation and organogenesis stage mouse embryos in a tissue-specific fashion. Interestingly, these expression patterns overlap with Wnt5a, consistent with the Daam molecules signaling downstream of Wnt5a in the Wnt-PCP pathway. We have also identified nematode and fly Daam genes, indicating evolutionary conservation. We are taking both gain and loss of function approaches in vivo to understand how the Daam proteins control the Wnt/PCP pathway to regulate cell and tissue polarity during mouse embryogenesis.

78. Abstract #78 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

79. **Evidence for a GPI-Anchored Coreceptor in Bone Morphogenetic Protein Signaling.** J. A. Sánchez-Duran, E. Kanakubo, L. Pham, A. Kumbasar, M. Stigson, and A. D. Lander. Department of Developmental Cell Biology, University of California, Irvine, California 92697.

Bone Morphogenetic Proteins (BMPs), members of the TGF-beta family of growth factors, regulate the proliferation of neural progenitors, the differentiation of neurons and glia and, in some cases, synaptic homeostasis. TGF-beta-related growth factors signal through transmembrane receptors that then phosphorylate cytoplasmic proteins known as Smads. Several TGF-beta family members also use cell-surface coreceptors to enhance or refine the specificity of signaling. Although no BMP-specific coreceptors have been described, we found that pretreating PC12 cells with phosphoinositide-specific phospholipase C (PIPLC), which removes GPI-anchored proteins, significantly reduces responses to BMP-2 or -4. PIPLC dramatically, yet reversibly, decreases BMP-induced phosphorylation and nuclear translocation of Smad1, as well as BMP-dependent transcription. These effects reflect a substantial decrease in the binding of labeled BMP4 to BMP receptors (which are not themselves GPI-anchored). Although PIPLC inhibits formation of BMP-receptor complexes, it does not affect their stability; these and other data point to the existence of a coreceptor that catalyzes, rather than stabilizes, BMP-receptor binding. Candidates for the GPI-anchored coreceptor include glypicans—heparan sulfate proteoglycans that enhance BMP signaling in some *Drosophila* tissues—and the EGF-CFC proteins Cripto and Cryptic, which are coreceptors for Nodal, also a TGF-beta family member. Experiments with mutant and engineered forms of glypican-1, cripto, and cryptic suggest, however, that the BMP coreceptor on PC12 cells is a distinct and potentially novel protein. (Supported by R01-HD37861.)

80. **Par-1 and Par-4 (XEEK1, LKB1) Function in *Xenopus* embryos.** Olga N. Ossipova and Jeremy B. A. Green. Dana Farber Cancer Institute, Harvard Medical School Genetics Department, 44 Binney Street, Boston, Massachusetts 02115, USA.

The *C. elegans* and *Drosophila* Par (partitioning defective) polarity proteins have polarity functions during oogenesis, early embryogenesis, and later formation of polarized epithelia. Some

vertebrate Par proteins have recently been shown to have conserved polarity functions. To better understand their molecular roles in polarity during embryogenesis, we have investigated the properties and embryonic functions of *Xenopus* Par-1 and Par-4 (XEEK1/XLKB1). Par-1 has previously been identified as a Wnt-dependent Dishevelled kinase and we have identified effects of Par-1 interference on both canonical and noncanonical Wnt functions in *Xenopus* embryos, including the planar cell polarity functions of gastrulation. We have found that Par-4 (XEEK1/XLKB1) also impinges on the Wnt pathway, but at a different level from that of Par-1. Functional interference and antisense knock-down show that Par-4 (XEEK1) acts in the canonical pathway downstream of Dishevelled and upstream of beta-catenin. It appears to exert its effects on Wnt signaling by inhibitory phosphorylation of GSK3b, either directly or through other Par proteins and associated protein kinases. This has implications for the possibly membrane-associated localization of signal transduction events normally associated with signaling the nucleus.

81. **Inhibition of PDGF Signaling Results in Apoptosis of Mesoderm Cells During Gastrulation in *Xenopus laevis*.** Melanie Van Stry,* Kelly McLaughlin,† and Karen Symes*. *Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts; †Department of Biology, Tufts University, Medford, Massachusetts.

Gastrulation is the stage during development in which the primary germ layers are first established in the vertebrate embryo. Using frogs (*Xenopus laevis*) as a model system, our laboratory has previously shown that PDGF signaling is required for normal gastrulation. The PDGF- α receptor is expressed in the involuting mesoderm, while PDGF-AA is expressed in the blastocoel roof. Injection of dominant negative PDGF α receptor (PDGFR-37) mRNA results in gastrulation defects, including incomplete closure of the blastopore, spina bifida, and reduced anterior structures. In addition, the involuting mesodermal cells do not adhere to the blastocoel roof. In embryos coinjected with mRNA for PDGFR-37 and β -galactosidase with a nuclear localization signal, the involuting mesoderm cells are enlarged and have diffuse β -Gal staining when compared to controls. These cells are positive for TUNEL staining, suggesting that they are undergoing apoptosis. Coinjection of Bcl-2 mRNA or caspase inhibitors rescues this cell death, but not the gastrulation defects. Moreover, embryos injected with PDGFR-37 mRNA have a higher caspase-3 activity than control embryos. These data suggest that PDGF signaling is required for mesoderm cell adhesion to the blastocoel roof, cell survival, and cell migration.

82. **IGF-I Receptor Signaling in Zebrafish Development.** Jian Wang, Joanne Chan, and Thomas Roberts. Department of Cancer Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115.

The type I insulin-like growth factor (IGF-I) plays a critical role in regulation of cell growth, differentiation, and protection from apoptosis. Newborn mice homozygous of a targeted disruption of Igf1r exhibit growth retardation and general organ hypoplasia. Unexpected from the previous mouse work, knocking down the IGF-I receptor (IGF-IR) by RNA interference induced severe defects in zebrafish brain and eye development. This indicates that IGF-IR signaling is essential for brain and eye formation during embryogenesis. In addition, down-regulating the IGF-IR

signaling caused cell migrating defects in hatching gland cells and other cell types. To investigate the role of IGF-IR signaling in zebrafish development, we first cloned the key molecules in the pathway and characterize the expression pattern. The two IGF-IRs and the two ligands, IGF-I and IGF-II, are ubiquitously expressed up to 18-somite stage. At 24 hpf, the receptor and ligands are specifically expressed in brain, eye, notochord, somite, and tail bud. Knocking down the IGF-IR by RNA interference caused similar phenotypes in zebrafish as in Igf1r(-/-) mice. In addition, the morpholino-injected embryos also display disrupted brain structure, notochord vacuolation defect, and U-shaped muscles. Interestingly, ectopic expression human IGF-IR can partially rescue the phenotypes. Up-regulating the IGF-IR signaling by overexpressing zebrafish IGF-I also disrupted zebrafish development from early gastrulation stage. To further dissect the roles of IGF-IR in development, we use standard zebrafish markers as well as TUNEL assay, phosphohistone-H3, to analyze the defects.

83. **Dissecting the Role of Vascular Endothelial Growth Factor during Arterial Development and Differentiation.** Nathan Lawson, Laurence Covassin, Brigid Diamond, Joshua Mugford, and Brant Weinstein. Program in Gene Function and Expression, UMass Medical School, Worcester, Massachusetts and Laboratory of Molecular Genetics, NIH, NICHD, Bethesda, Maryland.

The structural features that distinguish arteries and veins were long thought to be a result of differences in blood flow and pressure. However, we have found that a complex genetic pathway drives differentiation of arterial and venous endothelial cells before circulation in the vertebrate embryo. In zebrafish, we find that *vascular endothelial growth factor (vegf)* is required for arterial differentiation downstream of Sonic hedgehog and upstream of the Notch pathway. To further define signaling components of the Vegf pathway that are required for artery development, we have performed a genetic screen using transgenic zebrafish with fluorescently labeled blood vessels. We have found a number of mutants that fail to form segmental vessels, a phenotype that results from loss of Vegf function. Five of these are unique mutations in *phospholipase C gamma-1 (plcg1)*, an important downstream component of receptor tyrosine kinases. We find that the *plcg1*^{y10} allele is a splice acceptor mutation and causes loss of artery formation and defects in the differentiation of arterial endothelial cells. We can phenocopy the *plcg1*^{y10} defects using a morpholino directed against *plcg1*, while we can rescue mutants by injecting mRNA encoding wild-type Plcg1. Finally, we show that embryos mutant for *plcg1*^{y10} fail to respond to exogenous Vegf. These results underscore the importance of the Vegf signaling pathway for arterial development and demonstrate the utility of the zebrafish for the dissection of this pathway.

84. **An Allelic Series of Mutants in Phospholipase C-gamma-1 Reveals Its Requirement for Arterial Development in Zebrafish.** Laurence Covassin, Michele Bakis, Brant Weinstein, and Nathan Lawson. Program in Gene Function and Expression, UMass Medical School and Laboratory of Molecular Genetics, NICHD, NIH.

Phospholipase C-gamma-1 (Plcg1) is a target of numerous receptor tyrosine kinases and is required for vertebrate develop-

ment. In the zebrafish we have found that *Plcg1* is required specifically for the development of arteries and differentiation of arterial endothelial cells. Here we describe and compare the phenotypes of embryos from five zebrafish lines that bear unique mutations in *plcg1*. We have previously shown that the *plcg1*^{y10} mutant is a splice acceptor mutation and causes a fully penetrant loss of segmental blood vessels (SeV) and circulation and a semi-penetrant loss of artery-specific gene expression. We find that *y13*, *y15*, and *y18* are mutations in the catalytic domain of *Plcg1* that is required for the generation of diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3). These mutants display fully penetrant loss of SeV and circulation similar to *y10* mutant embryos. We find that the *y16* mutant harbors a point mutation in the N-terminal SH2 domain of *Plcg1* which is important for interaction with receptor tyrosine kinases. *y16* mutant embryos display a less severe loss of SeV and partial recovery of circulation suggesting that this mutant may be a hypomorph. We are currently analyzing these alleles for the loss of artery-specific gene expression and the penetrance of this phenotype and these data will be presented as well. These alleles provide valuable insight on the functional requirement for *plcg1* during arterial development and will help identify downstream factors involved in this process.

85. **Differential Signaling by ErbB Family Ligands in Fetal Mouse Submandibular Glands.** Masanori Kashimata, Noriko Koyama, and Edward W Gresik. Pharmacology, Asahi University School of Dentistry, Gifu, Japan (MK,NK); Cell Biology and Anatomical Sciences, CUNY Medical School, New York, New York.

Branching morphogenesis of fetal mouse submandibular glands (SMGs) is stimulated by epidermal growth factor (EGF) and retarded by inhibition of the EGF receptor (EGFR). EGFR is a member of the ErbB family, containing four isoforms (ErbB 1-4). In addition to EGF, several ErbB ligands bind homo- and heterodimers of ErbB receptor isoforms, differentially activating signaling cascades. RT-PCR detected mRNAs of all four ErbB receptor isoforms, and for several ErbB ligands: EGF, TGF- α , HB-EGF, amphiregulin (AR), betacellulin (BTC), epregrulin (EPR), and two neuregulins. Levels of these mRNAs show age-specific variation. We hypothesized that different ligands promote formation of different homo- and heterodimers of ErbB receptor isoforms, leading to different activation of signaling pathways dependent on this system of receptors. We focused on the capacity of the above ErbB ligands to stimulate phosphorylation in E16 SMGs. After 5- and 30-min stimulation, complex ligand-specific patterns of phosphorylation of ErbB1, ErbB2, ERK-1/2, and PK-B(Akt) were revealed by Western blotting. These findings show that levels of signaling via ERK-1/2 and Akt in the fetal SMG vary for different ErbB ligands. (Supported by Ministry of Education, Science and Culture, Japan to M.K.; NIH DE 10858 to E.W.G. and M.K.)

86. Abstract #86 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

87. **MEK Signaling Regulates Cartilage-Specific Gene Expression in Embryonic Limb Mesenchyme.** Brent E. Bobick and William M. Kulyk. Department of Anatomy and Cell Biology, University of Saskatchewan, Saskatoon, Canada S7N5E5.

It was recently proposed that signaling through the ERK mitogen-activated protein kinase pathway may function as a negative regulator of embryonic limb cartilage differentiation (Oh et al., 2000, J. Biol. Chem. 275: 5613). To critically examine this hypothesis, we tested the effects of two pharmacological inhibitors of MEK, the upstream activator of ERK, on chondrocyte differentiation in micromass cultures of embryonic chick limb mesenchyme cells. We found that both the PD98059 and the U0126 MEK inhibitors promoted increased accumulation of cartilage-specific mRNAs for type II collagen, aggrecan, and the transcription factor, Sox9. PD98059 treatment also stimulated increased deposition of sulfated glycosaminoglycan (GAG) into both cartilage matrix and the surrounding culture medium, while U0126 elevated GAG secretion into the medium fraction alone. Conversely, transfection of limb mesenchyme cells with a constitutively active MEK plasmid resulted in a marked decrease in expression of a cotransfected collagen II enhancer-luciferase reporter gene. Cumulatively, these findings support the hypothesis that signaling through the MEK/ERK kinase cascade may be an important inhibitory regulator of embryonic chondrogenesis.

88. **RAW Restricts DJNK Signaling during Dorsal Closure in *Drosophila*.** Katherine L. Bates and Anthea Letsou. University of Utah, Salt Lake City, Utah.

We have cloned and characterized the dorsal-open group gene *raw*. At the molecular level, we have shown that the *raw* gene product represents the prototype for a new family of proteins. The *raw* gene codes for a "pioneer protein," and its deduced polypeptide sequence is most notable for excluding, rather than suggesting, mechanisms of RAW function. In addition to its unique sequence, *raw* is notable in representing the first of the dorsal-open group mutants to show gross defects in dorsal closure that are attributable to a gain in DJNK signaling rather than to a loss of DJNK signaling. To establish a regulatory link between *raw* and components of the DJNK cascade, we determined their epistatic relationships. Embryos doubly mutant for *raw* and *Jra* were scored for the appearance of alternative *dpp* expression phenotypes (either missing from leading edge epidermal cells as in *Jra* mutants or ectopic epidermal expression as in *raw* mutants). Our finding that *dpp* is not expressed in leading edge epidermal cells in *raw Jra* double mutants defines *Jra* as epistatic to *raw* and confirms our hypothesis that *raw* functions upstream of the DJNK signaling pathway. More importantly, these studies have led us consider two models of RAW function: First, RAW could be required to restrict the range of an as yet undefined amnioserosal DJNK activator. Alternatively, RAW could function to directly repress DJNK signaling in lateral epidermal cells. Our data are consistent with the latter model of RAW function, and here we present a summary of our studies documenting RAW-mediated restriction of DJNK signaling in a tissue autonomous fashion via the epidermis.

89. **Neuroglial/Central Brain Deranged Signals through Small GTPases during Axon Growth, Guidance, and Branching in the Developing Mushroom Bodies.** P. Callaerts, N. Sidhu, P. R. Hiesinger,* R. Islam,† M. Hortsch, and Y. Y. Kang. Department of Biology and Biochemistry, University of Houston, Houston, Texas; HHMI, Baylor College of Medicine; and †Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, Michigan.

Correct wiring of the nervous system depends on axon growth, guidance, and branching and involves cell adhesion molecules and guidance cues. However, the mechanisms by which these signals are integrated and result in controlled remodeling of the actin cytoskeleton are not well understood. We are addressing this fundamental question by studying the role of the L1-CAM homolog *neuroglian* (*nrg*) in development of the *Drosophila* mushroom bodies, lobed neuropils with an important role in olfactory learning and memory. Neuroglian is expressed at high levels in the mushroom bodies and is localized preferentially on the axon tracts. We showed that the mutation *central brain deranged* is allelic to *nrg*. A molecular-genetic analysis of three *nrg/ceb* alleles revealed distinct roles for neuroglian in axon growth, guidance, and branching, that at least in part depend on homophilic interactions. We observed that the *nrg/ceb* phenotypes display very strong similarities with mushroom body phenotypes described for *Drosophila* Rac GTPases and were then able to reveal a strong genetic interaction between *nrg* and Rac1. These observations are consistent with the possibility that neuroglian acts as an upstream activator of Rac GTPases that in combination with specific guidance cues elicits specific responses in mushroom body axon growth, guidance, and branching.

90. Abstract #90 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

91. **A Proteomic Approach to Identify Developmentally Important Transcriptional Regulators.** Wendy V. Gerber, Frieder Schoek, and Herbert Jaekle. Max Planck Institute for Biophysical Chemistry.

Previous experiments have utilized *Drosophila* genetics to determine the hierarchy and players involved in the terminal/Torso pathway. While genetic experiments have indeed identified a number of important players, it is still not known which transcription factors or transcriptional coregulators are required to mediate huckebein transcription. It is likely that whatever proteins are required are also necessary for other aspects of early development and may not be identified through a genetic screen. Utilizing a different approach, proteomics, combined with subsequent genetics, may lead to further insight into the molecular mechanisms necessary for activation of huckebein (*hkb*). We are using a recently identified enhancer region from the promoter of *hkb*. We have fused binding sites for a TetR bacterial DNA-binding protein to this enhancer region which allows us to isolate specific chromatin fragments from transgenic embryos via immunoaffinity purification with antibodies against TetR. Proteins from "active" and "inactive" chromatin are then compared to identify active specific proteins. We anticipate that we will not only identify factors specific for activation of *hkb* but also factors necessary for general transcriptional activation. Here, we present information regarding the development and progress of this strategy.

92. **Transgenic and Comparative Genomic Analysis Revealed Novel Long-Range Regulation of NKX2-5 in Mouse Development.** X. Chi, F. J. DeMayo, and R. J. Schwartz. Department of Molecular and Cellular Biology, Center for Cardiovascular Development, Baylor College of Medicine.

Nkx2-5 gene encodes a highly conserved homeobox transcription factor with a pivotal role in heart development. None of the transgenic analysis so far can account for its complete expression pattern during development. The goal of this study is to recapitulate the endogenous Nkx2-5 expression pattern in BAC transgenic mice and delineate different regulatory elements for Nkx2-5 expression in the heart and other muscle lineages. We knocked an IRES-hrGFP reporter into the genomic locus of Nkx2-5 in a 120-kb BAC clone and made gap deletions on the 5' flanking sequences of the Nkx2-5 GFP BAC by homologous recombination in *E. coli*. Long-range sequence alignment between mouse and human was done using VISTA program for conserved non-coding sequence (CNS) and novel CNS regions were subjected to transgenic analysis. Nkx2-5 GFP BAC transgenic mouse lines recapitulated the endogenous Nkx2-5 expression in four chambers of the heart, tongue muscle, pylorus sphincter, and the developing spleen. The gap deletion transgenic mouse line, with 26-kb 5' flanking sequences, has similar expression pattern as that of the full-length transgene. Long-range sequence alignment across the species revealed multiple peaks of mouse/human homology in the upstream 26-kb flanking region, some of which corresponded precisely to known enhancers of Nkx2-5. Three novel CNS regions can drive *LacZ* gene expression in the left ventricle, in tongue muscle, and in both atria, respectively. The tongue muscle enhancer is embedded in an adjacent gene from skeletal muscle lineage.

93. **A Search for Targets of the *Drosophila* Neuroblast Temporal Network.** Thomas Brody,* Chad Stivers,* Dan Russ,† Colin Stevenson,† and Ward F. Odenwald*. *Neural Cell-Fate Determinants Section, NINDS, NIH, Bethesda, Maryland; and †Division of Computational Biosciences, CIT, NIH, Bethesda, Maryland.

During *Drosophila* neuroblast lineage development, a characterized temporal network regulates transitions in gene expression, generating a changing repertoire of functionally diverse cells (for review see Brody and Odenwald, Development 129: 3763-3770, 2002). We have undertaken a search for targets of the temporal network constituents PDM-1 and PDM-2, by taking a candidate gene approach, based on analysis of *cis*-regulatory modules (CRMs) of known CNS determinants and other uncharacterized genes. Multiple approaches for in silico analysis of *D. melanogaster* and *D. pseudoobscura* enhancers are described. Clustal alignment reveals that associated with transcribed regions of most genes, one can identify *cis*-regulatory sites based on their evolutionary conservation. Computer-based promoter analysis includes the creation and use of a newly developed software (FlySearch) and use of the program FlyEnhancer (<http://flyenhancer.org/Main>) for identification of CRMs. A motif discovery and search tool, MEME (<http://meme.sdsc.edu/meme/website/intro.html>), was used for finding novel prospective transcription factor binding sites. We have developed a CRM database for inferring potential targets of neural cell-fate determinants. Using FlySearch and FlyEnhancer, we have verified the ability to distinguish known targets of the temporal network by the presence of binding sites for known transcription factors. Expression profiles will be presented of CNS determinants that have been identified using the bioinformatics approach.

94. **A System for Conditional RNA Interference in the Mouse Using the lac Operator-Repressor System.** Tsutomu

Sasaki,*† and Heidi Scrabble†. *Neuroscience Graduate Program, †Department of Neuroscience, University of Virginia. P. O. Box 801392, MR4, Lane Rd., Charlottesville, Virginia, 22908, USA.

RNA interference (RNAi) has become a rapid, efficient, and specific method to silence gene expression in mammalian cells. Expression of short hairpin RNA (shRNA) allows stable silencing of gene expression in cultured cells and as reported recently in the transgenic mouse. Conditional expression of transgenes encoding hairpin RNA might be used to develop model organisms with conditional gene expression. To this end, we have developed a conditional RNAi system using the lac operator-repressor system. We use a modified murine U6 promoter with three lac operators that can be regulated by mammalianized lac repressor, using the lactose analog, IPTG. The promoter activity is repressed to 12% of baseline and de-repressed to approximately 80% of baseline. Using a construct that transcribes a hairpin RNA directed against eGFP gene, we demonstrate that the expression of eGFP protein can be repressed to 13% and de-repressed to approximately 80% of control values. The construct achieved the same level of eGFP repression as siRNA directed against eGFP. Therefore, the lac operator-repressor system is sufficient to control polIII-driven conditional RNAi in vitro. We are currently applying this system to transgenic mouse models to achieve conditional RNAi in mice.

95. Differing Susceptibilities to Systemic RNAi Within the *Caenorhabditis* Clade. Veronica Descotte and Mary K. Montgomery. Macalester College, St. Paul, Minnesota.

Although certain features of RNAi are evolutionarily conserved, the phenomenon of "spreading" exhibited by *C. elegans*, whereby the worm responds systemically to the localized introduction of dsRNA, is more species-specific. Delivery of dsRNA into *C. elegans* by microinjection, soaking, or feeding can lead to the systemic depletion of targeted mRNAs. These observations lead to the hypothesis that a mechanism exists in *C. elegans* to transport the dsRNA or a related RNA product from one cell to another; the protein SID-1 has been identified by Craig Hunter's lab as a critical component of this transport mechanism. However, systemic RNAi is not necessarily a common feature of other animals, such as *Drosophila*. Surprisingly, we have found that even within the *Caenorhabditis* clade, the ability to take up exogenous dsRNA varies. We tested a dozen wild-type strains for susceptibility to RNAi by different delivery methods. Our results indicate that several *C. briggsae* and *C. remanei* strains are capable of a robust systemic RNAi response if dsRNA is delivered via microinjection, but not when delivered by feeding or soaking. Attempts to target maternal mRNAs via soaking or feeding of these strains consistently failed. These results suggest that there are at least two separate mechanisms involved in systemic RNAi via feeding/soaking: one involved in initial uptake across the epithelium, and a second SID-1-dependent mechanism for further transport to other cells/tissues. The strains we tested appear to be deficient in the first mechanism but not the second. Many more species may be capable of "cell autonomous" RNAi, but may lack the transport mechanisms needed for a systemic response.

96. Molecular Characterization of the Chicken *Msx2* AER-Specific Enhancer. Hsu-Chen Cheng,* Chi-Kuang Leo

Wang,† and William Upholt*. *Department of Biostructure and Function, School of Dental Medicine, University of Connecticut Health Center, Farmington, Connecticut 06030-3705; and †Institute of Molecular Biology, Academia Sinica, Taiwan.

Msx2 is a homeodomain containing transcription factor which is expressed during development at sites of epithelial—mesenchymal interactions. In chicken limb buds, *Msx2* is expressed in the apical ectodermal ridge (AER) and in various regions of the limb mesenchyme. To identify DNA sequences responsible for temporal and spatial regulation of *Msx2* expression, we characterized the expression of *LacZ* reporter constructs driven by chicken *Msx2* regulatory sequences in transgenic mice. We have identified a 54-bp enhancer that can direct AER specific reporter gene expression. This 54-bp enhancer contains three 6- to 7-bp regions evolutionary conserved in five vertebrate *Msx2* genomic sequences. Mutations introduced into each of these three regions results in loss of or reduced ability of this enhancer to direct AER expression, indicating that these three regions are important. Moreover, changing the relative orientation by reversing and complementing one of the three regions also results in loss of expression, suggesting that the binding orientation of the transcription factor(s) is also important. We hypothesize that transcription factors bind to these three sites and act cooperatively to direct *Msx2* expression in the AER. To identify the transcription factor(s), we conducted 1-hybrid screening and identified *Dlx5* and *Sox11*. Both *Dlx5* and *Sox11* are present in the AER and each binds to separate conserved elements, supporting their roles in regulating *Msx2* expression. (This work was supported by NIH Grant HD22610 to W.B.U.)

97. Regulation of the Chicken *Msx2* Gene by BMP. Jing Chen, Hsu-Chen Cheng, Maria A. Gionfriddo, and William B. Upholt. Department of BioStructure and Function, School of Dental Medicine, University of Connecticut Health Center, Farmington, Connecticut 06030-3705.

Msx2 is a homeodomain-containing transcription factor. It is expressed during development at sites of ectoderm—mesenchymal interactions. Previous studies have demonstrated that BMPs can lead to the up-regulation of *Msx2* expression in a variety of cells and tissues. In chicken limb buds, *Msx2* is expressed in various regions which overlap with the BMP2 expressing domains. This raises the possibility that BMPs may have a role in regulating *Msx2* expression in limb development. We have shown by RT-PCR that BMP treatment delays the normal decrease in expression of endogenous *Msx2* expression in high-density micromass cultures of undifferentiated mesenchymal cells from the distal subridge region of stage 23/24 wing buds. Chicken *Msx2* genomic clones containing 30 kb including 9 kb of 5' flanking sequence have been isolated. To identify DNA sequences responsible for the BMP-induced up-regulation, we are characterizing the expression of luciferase reporter constructs driven by chicken *Msx2* regulatory sequences in cultured transfected cells. We have compared 9 kb of sequence upstream of the chicken *Msx2* coding sequence with the corresponding region of the mouse *Msx2* gene and have identified a region of 237 bp that is conserved between mouse and chicken and that contains at least one conserved SMAD binding site. (This work is supported by NIH Grant HD22610 to W.B.U.).

98. **Upstream of SoxB Genes: Identifying and Linking Signal Components Required for Neural Induction and Differentiation in *Xenopus laevis*.** Elena Silva Casey,* Derek Strong,† and Richard Harland†. Georgetown University, Washington, DC; and †University of California, Berkeley, California.

There has been much interest in elucidating the signal transduction pathways involved in the formation and patterning of the vertebrate nervous system. A number of molecules have been defined that are involved in neural plate formation, but many steps in the pathways remain unknown. The neural default model states that early neural effector genes are repressed by BMP signaling in nonneural tissues and released from BMP inhibition in neural tissues. While target genes activated directly by BMP signaling have been identified, direct targets repressed by BMP and required for neural plate development have not, leaving a major gap in our understanding of neural induction. Sox2 and Sox3 are strong candidates for primary targets repressed by BMP signaling. They are expressed in early gastrula embryos in a temporally and spatially restricted pattern that corresponds with neural induction and play central roles in neural formation in fish, chick, mouse, and frog. By studying their regulation using genetic, biochemical, molecular, and comparative computer analysis, we are identifying the mechanisms that link BMP inhibition to the expression of neural effectors and therefore, the formation of the neural plate. These proteins have also been implicated in maintaining a neural progenitor population; both genes are expressed in proliferating neural cells and down-regulated when these cells differentiate into specific neural cell types. Therefore, we are identifying factors required for the maintenance and down-regulation of these genes. In this way we can begin to understand proliferation and differentiation as well as induction of neural cells.

99. **Dlx Regulation in the Developing Forebrain: Interactions between *cis*-Acting Regulatory Elements in Transgenic Mice and Zebrafish.** O. Jarinova, Q. Long, and M. Kker. Ottawa Health Research Institute, University of Ottawa, Canada; and Vanderbilt University Medical Center, Nashville, Tennessee USA.

The morphological and functional differences between eukaryotic cells are largely due to differential gene expression. *Dlx* are homeobox genes involved in the development and differentiation of many cell types, including forebrain neurons. *Dlx* genes are organized in pairs that are separated by a short intergenic region and often have overlapping expression patterns. Two enhancer elements, I56i and I56ii, were identified in the *Dlx5/Dlx6* intergenic region of mouse and zebrafish and were shown to target *LacZ* reporter gene expression to the mouse forebrain with patterns highly similar to endogenous *Dlx5/6* expression. To reveal possible differences between *Dlx5/6* regulation in zebrafish and mouse, we designed GFP reporter constructs containing the intergenic enhancers. Analysis of primary transgenic zebrafish suggests differential contributions of I56i and I56ii in forebrain GFP expression. Thus, I56ii may increase the level of transcription specifically in cells where the I56i enhancer is active but does not have intrinsic enhancer activity on its own. We have recently identified a novel conserved sequence, I56vi, that may act as a general enhancer contributing to the overall levels of *Dlx5/6* gene expression. This data suggest that the differential expression of *Dlx5* and *Dlx6* in the forebrain relies on interactions between multiple *cis*-acting regulatory elements. (Sup-

ported by grants from the CIHR and the March of Dimes Birth Defects Foundation.)

100. **Differential *Dlx* Gene Regulation Mechanisms in the Ventral Forebrain of Vertebrates through the Action of Different Enhancers in *Dlx* Paralogs.** Noel Ghanem,*† Gary Hatch,* Olga Jarinova,*† and Marc Ekker*,†. *Ottawa Health Research Institute, and †Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, K1N6N5.

Vertebrate *Dlx* genes are homeobox transcription factors involved in early morphogenesis, notably of the head. They are arranged as three bigene clusters on distinct chromosomes. Four *Dlx* genes, *Dlx1*, *Dlx2*, *Dlx5*, and *Dlx6*, are expressed in developing telencephalon and diencephalon with overlapping patterns and are implicated in differentiation of the basal ganglia and in cortical migration of late born neurons. We have identified, using sequence comparisons, two highly conserved enhancers, I12b and URE2, in five vertebrate species including mouse and human. I12b is located in the intergenic region of *Dlx1/Dlx2*, whereas URE2 is in the 5' flanking region of *Dlx1*. *LacZ* reporter transgenes under the control of I12b or URE2 recapitulated the endogenous expression of both genes in the forebrain of transgenic mice but with spatial and temporal differences. Starting at E12, cells from both the lateral and medial ganglionic eminences showed a strong *LacZ* staining and followed migratory pathways similar to those previously reported for *Dlx*-expressing cells. I12b and URE2 showed no sequence similarities with the two previously characterized *Dlx* forebrain enhancers from the *Dlx5/Dlx6* intergenic region. Therefore, the partially overlapping expression of the two *Dlx* gene pairs in the forebrain may result from two distinct sets of regulatory mechanisms rather than the divergence of an ancestral mechanism of regulation that involved a common enhancer. (Supported by grants from the CIHR and the March of Dimes.)

101. **Cortical Interneuron Migration from the Basal Forebrain Is Mediated by Direct Regulation of the Neuropilin-2 Receptor by DLX Homeobox Genes.** T. N. Le and D. D. Eisenstat. Departments of Pediatrics, Anatomy, Physiology, Biochemistry, and Medical Genetics, University of Manitoba, Winnipeg, Canada.

Understanding homeobox gene specificity has been hampered by the lack of known direct transcriptional targets. *Dlx* genes are expressed in the ganglionic eminences (GE) of the developing forebrain. *Dlx1/Dlx2* double knockout mice die at birth. Phenotypic analysis demonstrates abnormal cortical development, including loss of migration of GABAergic inhibitory interneurons to the neocortex. We have applied ChIP (chromatin immunoprecipitation) to identify transcriptional targets of DLX homeoproteins derived in vivo from embryonic day 13.5 GE. Following cross-linking to enrich for protein–DNA complexes, nucleoproteins were incubated with DLX antibodies and genomic DNA (gDNA) fragment pools, including putative DLX transcriptional targets, were further characterized. PCR for the Neuropilin 2 promoter (NRP2) showed that both DLX1 and DLX2 bind to this regulatory region in situ. Electromobility shift assays confirmed direct binding of DLX1 and 2 to the NRP2 promoter in vitro. Reporter assays demonstrated that both DLX1 and DLX2 repress NRP2 expression, confirming the functional significance of DLX binding to this promoter region and consistent with aberrant

expression of NRP2 in the *Dlx1/2* double knockout mouse. NRP2 is a receptor for Class III semaphorin axonal guidance ligands in the developing forebrain. We have used ChIP to provide direct evidence for NRP2 as a direct *Dlx* homeodomain target from embryonic forebrain tissue in situ. This finding will facilitate our understanding of *Dlx* gene function in cortical development in vivo.

102. **Transgenic Analysis of Genomic Regulation of the *Dll-B* Gene in the Ascidian *Ciona intestinalis*.** Steven Q. Irvine and Frank H. Ruddle. Department of Biological Sciences, University of Rhode Island, Kingston, Rhode Island; and Department of Cellular, Molecular, and Developmental Biology, Yale University, New Haven, Connecticut.

Three members of the *Dlx* homeobox gene family have been found in the ascidian *C. intestinalis*. Two of these, called *Dll-A* and *Dll-B*, are arranged in a cluster. We are examining the genomic regulation of these genes, as a simple case of the common genomic arrangement of developmental genes in clusters conserved over long evolutionary time spans. Preliminary transgenic reporter assays incorporating the promoter and 2 kb upstream of the *Dll-B* coding region suggest that this DNA contains enhancer elements capable of driving expression characteristic of not only *Dll-B*, but aspects of the expression patterns of the other two *Dll* genes as well. This work indicates that other elements repress portions of the activation effects of this fragment to limit endogenous *Dll-B* expression. Further analysis of other regions flanking each gene of the *Dll-A/B* cluster may show whether there are interactions, such as sharing, between enhancer elements and both of the genes.

103. **Molecular Characterization of the bHLH Protein *Mist1*.** Thai Tran, J. Michael Rukstalis, and Stephen F. Konieczny. Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907-1392.

Mist1 is an exocrine-specific basic helix-loop-helix (bHLH) transcription factor that plays a critical role in exocrine pancreas cell development. Mice lacking *Mist1* exhibit disrupted acinar cell organization and defective zymogen granule trafficking. Experiments designed to elucidate the molecular mechanism(s) of *Mist1*'s function suggest that *Mist1* can form both heterodimers as well as homodimers in vitro and in vivo, although the homodimer complex appears to be the preferred DNA binding complex. CASTing experiments have revealed that the *Mist1* homodimer binds preferentially to a specific DNA E-box consensus site known as the TA-E box. Surprisingly, reporter gene assays using artificial promoter gene constructs or pancreas-specific gene constructs suggest that *Mist1* can function either as a transcriptional activator or as a transcriptional repressor, depending on the promoter context. *Mist1* repression activity is mediated by a specific class of corepressors belonging to the mSin3A family, suggesting that *Mist1* represses transcription by indirectly recruiting HDAC1. Interestingly, our studies also demonstrate that *Mist1* is capable of forming complexes with the P/CAF class of coactivators and this interaction may be essential to *Mist1* in activating transcription of target genes. The significance and molecular regulation of how *Mist1* differentially interacts with both corepressors and coactivators are currently unknown. Future studies will focus on confirming the in vivo interactions between *Mist1* and its various protein partners and determining

the effects of these complexes on the overall transcriptional regulation of specific pancreatic genes.

104. **Identification of Transcriptional Targets for Proneural bHLH Factors.** Mary A. Logan,*† Erin Callahan,* Michael Steele,* and Monica L. Vetter*. *Department of Neurobiology and Anatomy, †Interdepartmental Neuroscience Program, University of Utah, Salt Lake City, Utah 84132.

Neurogenesis is orchestrated by multiple extrinsic signals and intrinsic factors. One family of intrinsic regulators is that of the proneural basic helix-loop-helix (bHLH) transcription factors. Genetic and misexpression experiments have established that these factors are critical regulators of neurogenesis across species, although our knowledge of bHLH transcriptional targets which mediate this process is still incomplete. To address this question we performed a screen to identify direct gene targets of two *Xenopus* proneural bHLH factors *Xath5* and *NeuroD*. We expressed hormone-inducible forms of either *Xath5* or *NeuroD* in animal cap ectoderm tissue and activated them with hormone treatment in the presence of a protein synthesis inhibitor to ensure upregulation of direct target genes. To confirm activation of hormone-inducible *Xath5* and *NeuroD*, we performed RT-PCR for known gene targets *XBrn3d* and *Xebf3*. We then performed comparative transcriptional profiling by differential display and isolated 16 bands which appeared to be shared targets of these bHLH factors. Sequence analysis revealed that *Xath5* and *NeuroD* may regulate expression of cell-cycle regulators, transcription factors, neuron-specific structural proteins, and translational regulators. In vivo misexpression experiments and in situ analysis will be performed to confirm that these genes are expressed downstream of proneural bHLHs. Identifying these gene targets will further our understanding of the genetic cascades and cellular processes that drive neuronal differentiation.

105. **Differential Regulation of Gene Expression and Differentiation by Homo- and Heterodimers of the bHLH Protein *Twist*.** Yael Leshem, Viktoria Andreeva, Christian Muentener, Mona Connerney, and Douglas Spicer. Center for Molecular Medicine, Maine Medical Center Research Institute, 81 Research Drive, Scarborough, Maine 04074.

The basic-Helix-Loop-Helix (bHLH) transcription factor *Twist* promotes mesenchymal cell fates but also inhibits both muscle and bone differentiation. *Twist* can form both homodimers (TT) and heterodimers with ubiquitously expressed bHLH E proteins (TE) and these dimers may mediate these different activities of *Twist*. Here we propose that the regulation of *Twist* dimer formation may regulate closure of the cranial sutures of the skull. Haploinsufficiency of the *Twist* gene is associated with premature suture closure, or craniosynostosis. *Twist* is expressed in the suture mesenchyme and osteogenic fronts while the HLH inhibitor *Id* is only expressed in the osteogenic fronts. *Id* preferentially dimerizes with E proteins, and thus we hypothesize that within the osteogenic fronts *Id* dimerizes with the available E proteins forcing *Twist* to form homodimers, while in the intervening suture mesenchyme *Twist* forms heterodimers with free E proteins. We have begun to test this hypothesis by creating "forced" homo- and heterodimers of *Twist* and the E protein E12, where the members of the dimers are attached by a linker. Our data with cell lines expressing the different dimers support this conjecture and indicate that the cellular response to factors such as FGF and

BMP differs profoundly, dependent on which Twist dimer is expressed. We have also identified genes that are differentially regulated by the two dimers and their expression patterns within the sutures are consistent with this regulation and are altered in Twist^{-/-} mice.

106. **Regulators of E(spl) Expression in Imaginal Discs during *Drosophila melanogaster* Larval Development.** Deborah Eastman* and Katy Eby†. *Connecticut College, New London, Connecticut; and †Southwestern University, Georgetown, Texas.

The Notch pathway is critical for determining cell types in developing animals. Upon ligand binding, the Notch receptor is cleaved and translocates into the nucleus where it interacts with Suppressor of Hairless (Su(H)) and activates transcription of downstream target genes. In the absence of activated Notch, Su(H) acts as a transcriptional repressor by recruiting corepressors to the upstream regulatory region of specific genes. In *Drosophila melanogaster* Notch and Su(H) regulate a set of seven genes within the *Enhancer of split E(spl)-Complex (E(spl)-C)*. These genes code for bHLH proteins that are known to downregulate the expression of proneural genes during neurogenesis. All seven *E(spl)* bHLH genes are upregulated by activated Notch, contain binding sites for proneural bHLH proteins, and show similar expression patterns during embryogenesis. However, the expression patterns of these genes vary greatly during larval development. Our results suggest that this variation in expression is not due to the number or affinity of Su(H) binding sites, but may be due to the differential binding of other transcriptional regulators upstream of the *E(spl)* genes. We are examining the possible roles of other transcriptional repressors and activators in the expression of two of the *E(spl)* genes, *mgamma* and *mbeta*.

107. **A BAC-GFP Construct Revealed the Faithful Recapitulation of Endogenous Zebrafish myf5 Expression.** Yau-Hung Chen, * Yun-Hsin Wang, * Monte Westerfield,† and Huai-Jen Tsai*. *Institute of Fisheries Science, National Taiwan University, Taipei, Taiwan; and †Institute of Neuroscience, University of Oregon, Eugene, Oregon USA.

Myf5 functions as a myogenic factor, important in the specification of muscle cells. Although a proximal -82-bp regulatory element had been proposed to drive somite-specific expression in zebrafish myf5, the distal regulatory elements are totally unknown. Here, we designed a system in which the homologous recombination of bacterial artificial chromosome (BAC) clone containing the entire zebrafish myf5 locus was induced. A 100-kb Myf5-containing BAC clone was isolated and fused with green fluorescent protein (GFP) as a reporter. Transgenic analysis showed that GFP signals were first detected at 7.5 hours postfertilization (hpf), increased substantially until 16 hpf, and then declined gradually to an undetectable level. During somitogenesis, GFP signals were distributed mainly in the newly forming somites and segmental plates. Prominent signals occurred transiently in adaxial cells in two-parallel rows, but did not extend beyond the positive-signal somites. These results were exactly the same as what we observed from whole-mount in situ hybridization and immunostaining. Meanwhile, an upstream 10 kb of the zebrafish myf5 was cloned and showed that this regulatory region recapitulated the expression of the endogenous gene except positive signals expressed in notochord cells. Taken together, we suggest that all the regulatory *cis*-elements required for the activation of zebrafish myf-5 were within this BAC

clone, and the elements for repressing notochord expression might exist in the 10- to 100-kb region.

108. **An Intron Segment Represses the Somite-specific Expression of Zebrafish (*Danio rerio*) myf-5 Gene.** Cheng-Yung Lin, Yau-Hung Chen, Hung-Chieh Lee, and Huai-Jen Tsai. Institute of Fisheries Science, National Taiwan University, Taipei, Taiwan.

Myf-5, a basic helix-loop-helix transcription factor that controls muscle differentiation, is expressed in a somite- and stage-specific manner during early embryogenesis. In the mammal, the expressions of mice myf-5 are controlled by discrete and dispersed enhancers specific for particular populations of skeletal muscle precursors. However, the repressive mechanism of myf-5 regulation is poorly understood. We constructed a plasmid which contained the upstream regulatory sequence of zebrafish myf-5 from iV2937 to iV1 and fused with GFP. In the transgenic assay, the somite-specific expression rate was 84% (323/385 survival embryos). But the expression rate was only 2% (5/228 survival embryos) for embryos injected upstream regulatory sequence fused with intron 1, suggesting intron 1 involves the repressive function. Based on deletion analyses of intron 1 (2 kb), we found that (1) segment +502/+835 acted as a repressive element, which was in an orientation- and position-dependent manner; (2) segments +502/+660 and +816/+835 played key elements for repressive mechanism; (3) a 155-nucleotide spacing (+661/+815) was essential for repression, since absence of this 155-nucleotide region dramatically lost its repressive efficacy. Therefore, we identify novel *cis*-acting elements located at the first intron which may play role in the negative regulation of zebrafish myf-5.

109. **Cloning and Characterization of the 5'-Flanking Region for the Ebox Gene.** Woon Kyu Lee, Yong-Man Kim, Nasir Malik, Chang Ma, and Heiner Westphal. LMGD, NICHD, NIH, Bethesda, Maryland.

Ebox is a paired-like homeobox gene. It plays a known role in embryonic stem (ES) cell differentiation into hematopoietic, endothelial, or cardiac cells. To understand the mechanisms responsible for the regulation of Ebox gene expression, the 5'-flanking region of the Ebox gene was isolated from a mouse BAC library. 5'RACE analysis reveals that there is a single transcriptional start site located 130 bases upstream from the translation initiation codon. Transient transfection to express a luciferase reporter gene under the control of serially deleted 5'-flanking sequences reveals that the 84- to 68-bp region contains a positive *cis*-element for efficient expression of the Ebox gene. Through mutation analysis of this region, oligonucleotide competition and antibody supershift experiments in electrophoretic mobility shift assays, we found that the presence of a CCAAT box, a target for the transcription factor NFY (nuclear factor Y), is essential for positive regulation. NFY is known as a factor mediating cell-type-specific and cell-cycle-regulated expression of genes. The Ebox gene is reported to be highly expressed in thymus, skin, and testis. However, we found no difference in expression levels when we probed thymus (2B4), testis (F9), and brain (NS20Y) cell lines with a 1.9-kb 5'-flanking region of the Ebox gene linked to the reporter gene. Thus, while NFY clearly exerts a basic positive control of Ebox expression, our experiments suggest that additional control elements may govern tissue-specificity of NFY-mediated target gene control.

110. **Regulation of Hox Gene *lin-39* during *C. elegans* Vulval Development.** Javier Wagmaister and David Eisenmann. Department of Biological Sciences, UMBC.

We are interested in understanding how extracellular signaling processes regulate Hox gene activity and cell fate specification during the development of the vulva in *C. elegans*. The vulva arises from a group of six hypodermal cells called the Vulval Precursor Cells (VPCs). Vulval specification involves the activation of Wnt and RTK/RAS signaling pathways, which together regulate the expression of the Hox gene *lin-39*, a *Scr* and *Dfd* ortholog expressed in the mid-body region. Our goal is to understand how the Wnt and the Ras pathways converge in the regulation of the Hox gene *lin-39* to specify VPC fates. We are following *lin-39* levels during vulval development using transcriptional and translational *lin-39::GFP* fusions containing the entire *lin-39* genomic sequence. We found that GFP expression from both constructs recapitulate the expression pattern of *lin-39*. The specific expression pattern in the VPCs indicates that *lin-39* is regulated at the transcriptional level. We are now analyzing the expression from both constructs in Wnt and Ras mutants. We are also dissecting the *lin-39* promoter. We found that a 1.3-kb promoter fragment is sufficient for the expression of GFP in the VPCs. We are currently dissecting this fragment to identify specific elements required for Wnt or Ras regulation. Finally, we are analyzing if LIN-39 acts on its own promoter. We found that the first intron of *lin-39* contains two putative LIN-39/CEH-20 binding sites that are conserved in *C. briggsae*, a related nematode. We found that LIN-39/CEH-20 can bind these two sites in vitro and we are currently analyzing if the putative *lin-39* binding sites are important for *lin-39* regulation in vivo.

111. **Prep1 Is Essential in Embryo Development and Its Inactivation Results in Homeotic Defects and Embryonic Lethality in Both Zebrafish and Mouse.** F. Blasi,* F. Argenton,† E. Ferretti,* G. De Florian,† Luis Fernandez,* and M. Bortolussi†. *Department of Molecular Biology and Functional Genomics, Università Vita Salute San Raffaele, and †Department Of Biology, University of Padova, Italy.

Prep1 and Pbx1 dimers regulate the activity of anteriore HOX. We have analyzed the function of Prep1 in development by inactivating the gene in zebrafish and in mouse. In zebrafish, Prep1.1 morpholino antisense oligonucleotides induced embryonic lethality at 6 dpf with morphological similarity with the spacehead class (group II) mutants. Prep-1.1-inactivated embryos show homeotic posteriorization of the hindbrain and inhibition of anterior Hox gene expression. Consequently, abnormalities are observed in cranial nerves development, neural crest cells differentiation with complete disappearance of all neural crest-derived cartilages of the pharyngeal arches. In mouse, the insertion of a retroviral enhancer trap vector in the first intron of the Prep1 gene results in the absence of Prep1 protein. The phenotype of homozygous Prep1 ko mice is strongly influenced by the Sc129 genetic background. When C57BL/6 and Sv129 contribution is about 50:50, homozygous Prep1^{-/-} mice are born normal. Decrease of the Sv129 (80% C57BL/6) results in physical abnormalities while in purer animals (over 90% C57BL/6) embryonic lethality around E15.5 ensues. In both zebrafish and mouse the ko animals display a greatly reduced level of all Pbx proteins, which sometimes are even absent. Thus, most if not all phenotypes of

the Prep1 ko in both zebrafish and mice, probably depend on the absence of Pbx.

112. **A GATA4 Hypomorphic Allele Reveals a Critical Relationship between GATA4 Expression Levels and Cardiac Morphogenesis and Function.** William T. Pu, Qing Ma, and Seigo Izumo. Boston Children's Hospital, Beth Israel Deaconess Medical Center.

The transcription factor GATA4 is an important regulator of cardiac gene expression, and knockout of GATA4 resulted in embryonic lethality at E9.5 due to aberrant ventral morphogenesis. In the process of making a conditional GATA4 allele, we modified the GATA4 locus by introducing a neomycin resistance cassette in the second intron, and a loxP site in the 5' untranslated region. Embryos homozygous for this allele (GATA4^{Tg/Tg}) die around E14.5. In GATA4^{Tg/Tg} hearts, expression of GATA4 mRNA was reduced by 30%, and GATA4 protein was reduced by 87%. With the exception of the modifications to the 5' untranslated region, GATA4 transcript and protein from mutant and wild-type alleles were indistinguishable. In situ hybridization demonstrated a normal pattern of GATA4 gene expression in the heart. All GATA4^{Tg/Tg} hearts had double outlet right ventricle, common atrioventricular canal, and marked hypoplasia of both the trabecular and the compact myocardium. Echocardiographic and intraventricular recordings indicated that GATA4.

113. **Functional Analysis of X-msr in the Developing Vascular and Nervous System.** Kristina K Gleason, Theresa Curtis, Anthoney Lim, Douglas DeSimone, and Margaret S Saha. College of William and Mary, Williamsburg, Virginia.

The development of the vertebrate nervous system and vascular system involves complex spatial and temporal regulation of intricate gene networks, beginning at the earliest stages of embryonic growth. Xenopus mesenchyme associated serpentine receptor (X-msr) is a G-Protein Coupled Receptor that is expressed early and broadly in Xenopus mesoderm and gradually becomes restricted to discrete tissues and cell types in the nervous system and the vasculature, thus suggesting a possible role in their development. The function and regulation of X-msr in the developing vascular system of Xenopus are examined. Morpholino antisense technology reveals a critical role for X-msr in the development of vascular structures as well as in interactions with other vascular genes. Transgenesis (using portions of the upstream regulatory region of X-msr fused to the coding region of GFP) is performed to characterize the promoter activity responsible for both normal and ectopic X-msr expression. Confocal microscopy allows three dimensional visualization of this activity in transgenic animals.

114. Abstract #114 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.
115. Abstract #115 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.
116. **Expression of Rat Alx3 in the Developing Central Nervous System and Pancreatic Islets of Langerhans.** M. Mirasierra and M. Vallejo. Instituto de Investigaciones Biomédicas

Alberto Sols, Spanish Council for Scientific Research (CSIC)/Autonoma University, Madrid, Spain.

Alx3 is a Paired-class homeoprotein that belongs to the Group I aristaless-like transcription factors. In the mouse, Alx3 is expressed in the developing craniofacial mesenchyme and limb buds, but no alterations in structures derived from these tissues have been reported in embryos carrying loss-of-function mutations. Available evidence indicates that redundant functions and complex interactions with other genes exist. Alx3 was originally found in a pancreatic islet-derived cell line, and we found Alx3 expressed in a neural cell line. For these reasons, we investigated expression of rat Alx3 in developing nervous system and pancreas using a specific Alx3 antiserum. Alx3 immunoreactivity was detected in the telencephalon from embryonic day 12 (E12). In E14–E16 embryos, Alx3-positive cells were also found in the diencephalon, spinal cord, and dorsal root ganglia. In the developing cortex and diencephalon, Alx3-expressing cells were generally located in both the ventricular layer and the most peripheral layer, suggesting that Alx3 is present in neural precursors and in differentiated neurons. In pancreatic islets Alx3 was detectable from at least E14, and postnatal studies indicated that most Alx3-positive cells are insulin-producing β -cells. Transient transfection studies indicated that Alx3 participates in the regulation of insulin gene expression by interacting with bHLH transcription factors bound to the E2A3/4 enhancer. These studies indicate that Alx3 may have a wider role in development than previously anticipated.

117. Regulation of Prospero Expression in Longitudinal Glial Cells. Y. Yuasa and Y. Hiromi. National Institute of Genetics, Japan.

In *Drosophila*, the longitudinal glia regulates formation of longitudinal axonal bundles and neuronal survival. A critical factor that enables the function of longitudinal glial cells is Prospero (PROS), a homeodomain transcription factor. PROS is expressed in a subset of longitudinal glial cells. In *pros* mutant embryos longitudinal glial cells form, but fail to differentiate and support axonal growth. We are analyzing the regulation of PROS expression in longitudinal glia. We focused on three transcription factors, homeodomain protein REPO, Ets transcriptional factor PNTP1, and DNA-binding protein Dead Ringer/Retained (DRI/RETN), which is a member of the ARID (AT-rich interaction domain) family. These transcriptional factors are all expressed in longitudinal glial cells. We found that PROS expression was absent or reduced in mutants of any of these three transcription factors. While ectopic expression of each transcriptional factor alone had no effect on PROS expression, when three genes were simultaneously misexpressed, there was a dramatic increase in the number of PROS-positive cells in the epidermis. Within the upstream regulatory region of the *pros* gene, there were consensus binding sites for all three transcriptional factors. Thus PROS could be a direct target gene for these three transcription factors in the longitudinal glia.

118. TEF-1-Dependent Activation of α -Tropomyosin Gene in the Smooth Muscle Cell Lineage. Pierre Thiébaud, Stéphanie Pasquet, Wilfrid Barillot, Michelle Olive, Corinne Fauchoux, and Nadine Thézé. Unité INSERM 441, Avenue du Haut Lévêque, 33600 France.

Tropomyosins (TMs) are actin binding proteins that are encoded by four genes in vertebrates. We have undertaken the analysis of the regulation of the *Xenopus laevis* α -TM gene in the smooth muscle cell lineage. We have established that the U8A4 smooth muscle cell line developed in our laboratory was suitable for the analysis of the transcriptional control of the α -TM gene. This cell line has conserved a smooth muscle cell phenotype and is able to regulate at the transcriptional and posttranscriptional levels an α -TM exogenous gene. Deletions analysis of the *Xenopus* α -TM gene promoter revealed that a 300-bp sequence was sufficient to confer a smooth muscle specific activation of a luciferase reporter gene. We have found by point mutation analysis that a M-CAT sequence was essential for the activity of the promoter. This sequence has been shown to be bound by TEF-1 protein. Immunohistochemistry and Western blot analysis revealed the presence of TEF-1 protein and its nuclear accumulation in serum-free conditions. We demonstrated by gel-shift analysis that the M-CAT sequence could be specifically bound by a TEF-1 protein present in smooth muscle cells. To know whether TEF-1 could activate the α -TM gene according to a smooth muscle pattern in embryonic cells, we have injected TEF-1 mRNA in *Xenopus laevis* embryos and found that smooth muscle α -TM transcripts were present in animal caps explants. This indicates a requirement of this factor for the expression of the gene in the smooth muscle cell lineage.

119. A Pharyngeal Muscle Specific Enhancer from *ceh-22* Is Targeted by PHA-4 and Other Factors. T. Vilimas, A. Abraham, and P.G. Okkema. Department of Biological Sciences, University of Illinois at Chicago, Chicago, Illinois.

C. elegans pharyngeal muscle development involves *ceh-22*, an NK-2 family homeobox gene related to genes controlling heart development in other species. *ceh-22* is the earliest known gene expressed in pharyngeal muscle and is likely regulated directly by factors specifying pharyngeal muscle fate. We have previously implicated the *ceh-22* distal enhancer in initiating *ceh-22* expression. Here we analyze the distal enhancer using functional and comparative assays. The distal enhancer contains three subelements contributing to its activity, and functionally important regulatory sequences are conserved in *C. briggsae*. One subelement, *DE3*, is strongly active in the pharyngeal muscles, and we identified two short oligonucleotides (*de199* and *de209*) contributing to its activity. *de209* enhances transcription specifically in the pharyngeal muscles and is necessary for *DE3* activity, suggesting it is a key site regulating *ceh-22*. *de209* binds the pan-pharyngeal Forkhead factor PHA-4 in vitro and responds to ectopic *pha-4* expression in vivo, suggesting PHA-4 directly initiates *ceh-22* expression through *de209*. Because *de209* enhancer is primarily active in pharyngeal muscle, *de209* must also bind factors functioning with PHA-4 to specifically activate *ceh-22* expression. We have identified several additional factors binding *de199* and *de209*. A *de209*-binding C_2H_2 zinc finger protein, F13H6.1, is expressed in the pharyngeal muscles, as well as epithelial and marginal cells in the pharynx. *F13H6.1(RNAi)* worms arrest as larvae with pharyngeal abnormalities. We hypothesize F13H6.1 and PHA-4 function together to regulate *ceh-22* expression.

120. Identification of N-myc Regulatory Domains Required for Visceral Arches, Somites, and Limb Buds Expression. J. Charron, B. Lachapelle, M. Tremblay, and J.-F. Cadrin-

Girard. Centre de Recherche en Cancérologie de l'Université Laval, L'Hôtel-Dieu de Québec, CHUQ, QC, Canada.

Our knowledge on the regulation of the *N-myc* proto-oncogene expression comes mostly from *in vitro* studies. Very few *in vivo* analyses have been performed to identify the regulatory elements involved in *N-myc* developmental expression. In the present study, we identified DNA domains required for *N-myc* expression in the visceral arches, somites, and limb buds. We showed that the expression of *N-myc* driven by the human *N-myc* sequences previously described to control *N-myc* expression in appropriate cell types *in vitro*, cannot rescue the mouse *N-myc* mutant phenotype, suggesting that regulatory elements necessary for *N-myc* embryonic expression were missing. To identify the regulatory DNA regions involved in *N-myc* expression, transgenic mouse lines carrying *N-myc/lacZ* reporter constructs were generated. β -Gal staining analysis at different stages of gestation revealed that more than 16 kb of mouse *N-myc* genomic sequences are required to recapitulate the entire spatio-temporal expression pattern of the endogenous *N-myc* gene between E8.5 and E11.5. This observation supported the notion that the sequences previously identified by *in vitro* assays were not sufficient to reproduce the *N-myc* embryonic expression pattern. Moreover, regulatory regions involved in directing expression in visceral arches, somites, and limb buds have been identified. Altogether, these findings indicated that the regulation of the spatio-temporal expression pattern of *N-myc* during development necessitates multiple regulatory DNA elements. (Funded by the CRS Inc.)

121. **Identification and Characterization of Indian Hedgehog Target Genes in Limb Development.** Manuela Wuelling, Andreas Ratzka, Markus Wenzel, Sabine Schneider, and Andrea Vortkamp. Max-Planck Institute for Molecular Genetics, Ihnestrasse 73, 12167 Berlin, Germany.

Indian Hedgehog (IHH) is one of the main regulators of chondrocyte proliferation and differentiation, but the genes that are transcriptionally regulated by IHH have been up to now mainly unknown. To identify downstream target genes of IHH signaling we used a PCR based differential screening approach. For this approach, we compared mRNA populations of limb explant cultures that were treated with Sonic Hedgehog (SHH) or with Cyclopamine to activate or suppress hedgehog signaling, respectively. Utilizing the PCR select differential screen we identified 125 genes regulated by IHH. After sequencing, the predicted function of these genes was determined by Blast analysis. Up to 40% of the detected genes have an unknown function, but are homologous to ESTs. 20% are transcriptional regulators and DNA-binding proteins, while 10% of the genes code for membrane proteins or receptors. The other genes represent different classes of proteins including cell-cycle regulators, kinases, transferases, proteases, channels, or ribosomal proteins and range from 8 to 2% of all identified genes. The expression of these genes in developing cartilage and bone was determined by *in situ* hybridization. To confirm regulation by IHH *in vivo*, expression of these genes was analyzed in IHH overexpressing and IHH deficient mice by *in situ* hybridization. In addition, we plan to quantify the differences between the two transgenic mice were quantified by Real-Time PCR to detect changes in gene expression.

122. Abstract #122 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

123. **Somatostatin and Somatostatin Receptor Expression Are Altered in Transgenic Isl-1 Mice.** Hong-Khanh Dinh,* Diane Costanzo,* J. Michael Salbaum¹,† and Claudia Kappen*,†. *Center for Human Molecular Genetics and †Department of Genetics, Cell Biology, and Anatomy, University of Nebraska Medical Center, Omaha, Nebraska 68195-5455.

Somatostatin (SST) is produced in some peripheral organs and throughout the central and peripheral nervous systems. The regulatory peptide acts through five G-protein-coupled receptors (SSTR1-5) encoded by different genes. SST has been shown to modulate the secretion of both exocrine and endocrine cells and to inhibit cell proliferation. We have shown that Islet-1 (Isl-1) transgenic mice exhibit a phenotype characterized by sacral/caudal agenesis, a birth defect associated with maternal diabetes in humans. To elucidate the molecular pathways downstream of Isl-1, real-time PCR was previously utilized to identify genes with altered expression in Isl-1 transgenic mice; somatostatin was among the significantly altered genes. In this study, we evaluated gene expression levels and localization of SST and SSTRs using real-time PCR and *in situ* hybridization. RNA was isolated from the tail bud region of FVB control (+/+ +/+), transgenic control (TA/+ +/+ or TA/TA +/+), and Isl-1 transgenic (TA/+ TR/+ or TA/TA TR/+) mice at embryonic day 10.5, 11.5, and 12.5. *In situ* hybridization was performed on paraffin sections. Activation of particular SSTRs has been identified with various pathways; therefore, identifying which receptors are affected in Isl-1 transgenic mice may further elucidate which pathways may be directly or indirectly perturbed.

124. Withdrawn

125. **Cloning and Functional Characterization of HIF-1 α Upstream Regulatory Regions in *Xenopus laevis*.** Conor W. Sipe, Erika J. Gruber, and Margaret S. Saha. College of William and Mary, Williamsburg, Virginia.

As the final electron acceptor in the electron transport chain, oxygen is essential for the cellular production of ATP in aerobic environments. An important mediator of oxygen-dependent gene expression is the HIF-1 complex. Well characterized in mammals, HIF-1 α is a heterodimer composed of an α and β subunit, although its biological activity is determined by the activity of HIF-1 α . To investigate the role that the regulation of HIF-1 α transcription plays in development of the amphibian *Xenopus laevis*, we have cloned and functionally characterized the upstream regulatory regions of the HIF-1 α gene and described its developmental expression pattern. Our analysis of HIF-1 α 5'-flanking regions suggests that the 200 bp immediately upstream of the transcriptional initiation site is sufficient to drive expression matching the endogenous pattern. This is further supported by the isolation of two HIF-1 α promoter alleles, revealing 100% sequence identity in this proximal region. The expression pattern of HIF-1 α during early embryonic development shows specific upregulation in axial tissues. We also show the presence of two maternally

deposited HIF-1 α mRNAs, whose expression peaks around gastrulation, and is followed by a rapid decline in neurulation when one species drops to an undetectable level.

126. **A Transgenic Analysis of the Regulation of *Xcad3*, a Caudal-Related Gene from *Xenopus laevis*.** S. Reece-Hoyes, Mary E. Pownall, and Harry V. Isaacs. Department of Biology, University of York, UK.

The family of caudal-related (Cdx) proteins have conserved roles in the development of posterior structures in both vertebrates and invertebrates. In mice, *Cdx* gene expression has been shown to be regulated by retinoic acid and Wnt proteins, and evidence exists supporting auto- and cross-regulation by Cdx-family proteins as well as regulation by other homeobox proteins. *Xenopus laevis* has three *Cdx*-family genes: *Xcad1*, 2, and 3. A 3-kb proximal promoter fragment of the *Xenopus laevis* gene *Xcad3* was isolated to investigate the regulatory mechanisms of this gene. A deletion series of this fragment was created in a GFP-expressing vector, and transgenic embryos were generated using these constructs. The entire 3-kb fragment induces expression of GFP in a pattern analogous to endogenous *Xcad3* expression. Surprisingly, no apparent change in the GFP expression pattern occurs with any of the promoter fragment deletions from 3 kb down to the proximal 238 nucleotides. Detailed inspection of the 238 bp promoter fragment reveals a number of putative binding sites for potentially important transcription factors. These include Cdx proteins, homeoproteins, and the effector of the Wnt pathway. The results of mutating these putative binding sites as well as generating further deletions are presented here.

127. **Function of the Novel *C. elegans* Factor PEB-1 Requires Nuclear Localization and DNA Binding Activity.** Laura Beaster-Jones and Peter Okkema. Biological Sciences, University of Illinois-Chicago, Chicago, Illinois.

The *C. elegans* pharynx provides a simple model for studying cell specification and organogenesis. We have described *C. elegans* PEB-1, a novel protein expressed in pharyngeal cells and essential for viability. PEB-1 binds an organ-specific regulatory sequence from the pharyngeal myosin gene *myo-2* and the 158 amino acid DNA binding domain (DBD) is unrelated to other DBDs, although it contains a cys/his motif matching a consensus found in *Drosophila* Mod(mdg4) isoforms. We are using comparative and functional analyses to characterize PEB-1. We identified *peb-1* from the related nematode *C. briggsae* and found Cb-PEB-1 is expressed in a similar pattern to *C. elegans* PEB-1. Ce-PEB-1 and Cb-PEB-1 share highest sequence identity in the N-terminal DBD and a region of the C-terminus including a cys-rich fragment. To test the importance of these conserved domains, we deleted the C-terminal domain and found the DBD alone can rescue *peb-1* mutants albeit very weakly. When residues matching the Mod(mdg4) consensus are mutated, PEB-1 no longer binds DNA in vitro and these proteins cannot rescue *peb-1* mutants. Mutant proteins tagged with GFP are incompletely nuclear localized in vivo. Together these results suggest DNA binding and nuclear localization are essential for *peb-1* function. Further in vitro analyses reveal PEB-1 binds DNA as a monomer and neither the cys/his motif in the DBD nor the cys-rich fragment in the C-terminus bind metal. PCR-assisted binding site selection established a PEB-1 consensus sequence which is bound with equal

affinity and specificity when compared to the PEB-1 binding site in the *myo-2* enhancer.

128. **Transcriptional Response to Hypoxia in *Drosophila* Is Controlled by Fatiga, a Prolyl-4-hydroxylase that Functions as an Oxygen Sensor.** L. Centanin, J. Mondotte, and P. Wappner. Leloir Foundation, Patricias Argentinas 435, (1405) Buenos Aires, Argentina.

HIF-1 is a mammalian heterodimeric transcription factor that controls the transcriptional response to hypoxia. Whereas the α subunit is constitutive, HIF-1 α subunit is regulated at the level of protein stability, being degraded in normoxia and stabilized in hypoxia. Oxygen-dependent destruction of the α subunit requires hydroxylation of two key prolyl residues that is catalyzed by oxygen-dependent prolyl-4-hydroxylases, functioning as oxygen sensors. We have demonstrated the occurrence of a system homologous to HIF-1 in *Drosophila*, being Sima the HIF-1 α homologue. We have also identified the HIF prolyl hydroxylase homologue gene that we have named *fatiga* (*fga*). Here we report *fga* molecular and functional characterization; we found that disruption of *fga* gene results in stabilization of Sima and upregulation of the transcriptional response to hypoxia in vivo. We have identified three different *fga* transcripts (*fgaA*, *B*, *C*) that are expressed at high levels in early and mid embryogenesis. We observed that *fgaB* and *C* but not *fgaA* are upregulated in hypoxia and induced upon Sima ectopic expression. We generated novel *fga* alleles that exhibited stabilization of Sima, upregulation of the transcriptional response to hypoxia and die either at L1, L2 or pupal stages. Analysis of these novel alleles revealed alterations in tracheal morphology. Similar results were observed upon ectopic expression of an hyperactive form of Sima, suggesting that the hypoxia-responsive pathway participates in "hard wired" tracheal development.

129. **Developmental Expression of *Drosophila* RNP-4F, a Fly Homolog of the Human P110/Sart 3 U4/U6 snRNP Recycling Factor.** Jane P. Petschek and Vincent J. Concel. Department of Biology, Case Western Reserve University, Cleveland, Ohio.

In a database search for proteins similar to *Drosophila* RNP-4F we discovered that it is a homolog of the human P110/Sart3 protein. It belongs to a protein family that includes the yeast PRP24 U4/U6 snRNP recycling factor. The human P110/Sart 3 protein, a U6 snRNP protein and U4/U6 snRNP recycling factor, is implicated as a tumor rejection antigen. Similar genes are found in *A. gambiae*, mouse, *C. elegans*, and *Arabidopsis*. Common to this family, and in RNP-4F, is a highly conserved 12 amino acid motif at the extreme C-terminus. *S. cerevisiae* mutants lacking this motif are deficient in U4/U6 snRNP assembly. Whether RNP-4F functions as a splicing factor is unknown. *rnp-4f* is a single-copy nuclear gene that expresses alternatively spliced transcripts. Two cDNAs predict proteins 943 and 639 aa long that exhibit 22% identity and 44% similarity with other members of the family. The 943 aa protein has one RNA recognition motif, 3 HAT domains, and the 12 aa C-terminal motif. The 639 aa protein retains only the HAT domains. The temporal and spatial expression of RNP-4F proteins reveals a complex pattern that appears developmentally regulated. Because the phenotype of a gene-specific mutation is unknown, we are using the RNAi technique to obtain an *rnp-4f* phenocopy.

130. **Promoter Analyses of *SHORTROOT* (*SHR*) and *SCARECROW* (*SCR*).** Noritaka Matsumoto, Juliette Colinas, and Philip Benfey. Development, Cell, and Molecular Biology Group, Department of Biology, Duke University, Durham, North Carolina.

The *Arabidopsis* root displays a clear radial pattern. Starting from the center of the root, four regions or cell layers are observed: stele, endodermis, cortex, and epidermis. Cells of the endodermis and cortex develop from one initial cell (Cortex/Endodermis Initial; CEI) through asymmetric cell division. Analyses of mutants missing one cell layer showed that this cell division and the subsequent cell specification of the endodermis and cortex are regulated by two genes, *SHORTROOT* (*SHR*) and *SCARECROW* (*SCR*), members of the GRAS family of putative transcription factors. *SHR* is transcribed in the stele and the *SHR* protein is localized in the stele and the nucleus of the endodermis. *SCR* is expressed in the endodermis. For finding new genes involved in the radial patterning of the *Arabidopsis* root, it is important to identify upstream genes controlling *SHR* and *SCR* expression. For this purpose, we tried to identify *cis*-regulatory regions in the upstream noncoding sequences of *SHR* and *SCR*. We performed a series of promoter bashing experiments in both genes using ER localized *GFP* as a reporter gene. Moreover we obtained a set of upstream sequences for *SHR* and *SCR* from several species in *Brassicaceae* and compared these to the *Arabidopsis* sequence to find conserved noncoding regions. Combining the information from molecular genetics experiments and comparative genomics, we identified candidate sequences which might regulate the expression pattern of *SHR* and *SCR*.

131. **Transcriptional Regulation in Oocyte-Specific Genes.** Jia L. Song and Gary M. Wessel. Brown University, Providence, Rhode Island.

From a primordial germ cell, oocytes develop into fertilizable eggs in a prolonged process known as oogenesis. The objective of this research is to elucidate the mechanism in which oocyte-specific genes are temporally and spatially expressed in early oogenesis. We hypothesize that oocytes utilize a shared regulatory network that controls the coordinated expression of oocyte-specific genes. We have chosen two oocyte-specific genes, *Yolk Platelet Protein of 30 KDa* (*YP30*) and *Ovoperoxidase* (*OVO*), with distinct functions that are highly expressed in young sea urchin oocytes to examine their transcriptional regulation. Oocytes from the sea urchin *Lytechinus variegatus* are amenable to manipulations, and we can obtain massive quantities of oocytes and culture them after microinjections for a prolonged period of time. We screened a high density arrayed *L. variegatus* genomic library to identify BAC clones containing *YP30* and *OPO*. Approximately 2 kb of the promoter regions of both genes were obtained. 5' nested deletion constructs containing 500, 1000, and 2000 bp upstream of the ATG from both *YP30* and *OPO* have been cloned in front of the green fluorescent protein (*GFP*) construct to identify regulatory regions. Using the FamilyRelations sequence analytical program, we have identified putative conserved regulatory regions within the *YP30* and *OPO* promoters. These *GFP* reporter constructs are being microinjected into sea urchin oocytes for a quantitative analysis of their transcriptional activity. A basic understanding of gene regulation that dictates proper oocyte development will contribute to our overall

knowledge in causes of oocyte pathology and female reproductive biology.

132. **MIZF, an MBD2-Interacting Zinc Finger Protein, Is a Sequence-Specific Transcriptional Repressor.** Masayuki Sekimata and Yoshimi Homma. Department of Biomolecular Sciences, Fukushima Medical University.

MBD2, a methyl-CpG binding protein, is a component of the MeCP1 histone deacetylase (HDAC) complex and plays a critical role in DNA methylation-mediated transcriptional repression. To understand the molecular basis of the methylation-associated repression, we isolated a novel MBD2-interacting zinc finger protein, MIZF, by a yeast two-hybrid system. The subcellular localization of MIZF is distinct from that of MBD2, although both proteins colocalize in some areas of the nuclei; MIZF localizes diffusely in the nucleoplasmic region, while MBD2 preferentially localizes in major satellites. A reporter assay demonstrated that MIZF significantly abrogates transcriptional activities. This repression is attenuated by an HDAC inhibitor, trichostatin A, and is completely dependent on the interaction with MBD2. Moreover, using a method for cyclic amplification and selection of targets (CASTing), we determined the MIZF binding sequence, CGGACB. Electrophoretic mobility shift assays and transient transfection experiments demonstrated that MIZF functions as a sequence-specific transcriptional repressor. Thus, MIZF appears to have both site-specific DNA-binding and transcriptional repression activities, which may be synergistic with MBD2-mediated gene silencing.

133. **Regulation of Neural Crest Apoptosis in *Xenopus laevis* by XSlug and BMP4/Xmsx-1.** Celeste Tribulo,^{*}† Manuel J. Aybar,^{*}† Sara S. Sánchez,[†] and Roberto Mayor^{*}. ^{*}HHMI and MNDB, Faculty of Science, University of Chile; [†]Department of Developmental Biology, INSIBIO (University of Tucumán-CONICET), Argentina.

Apoptosis refers to the naturally occurring cell death that is part of the developmental program of an organism. This loss of cells can be fundamental to some developmental processes and serves many functions, such as sculpting or deleting structures, controlling cell number, and eliminating abnormal cells. Recently, an apoptotic program with high levels of cell death in the region of the neural crest was described in *Xenopus*. Our results confirmed that apoptosis is localized within the neural crest and that during neural crest development BMP4 and Xmsx-1 promotes apoptosis while XSlug acts as an antiapoptotic gene. In addition, we performed rescue experiments in animal caps. We found that in animal caps XSlug and a dominant negative of Xmsx-1 (HDXmsx-1) are able to rescue the apoptosis induced by Xmsx-1. To analyze whether the apoptotic program affected by XSlug/Xmsx was dependent on the Bcl-2/Bax pathway, we coinjected XSlug or HDXmsx-1 plus XBax, and Xmsx-1 plus XR11 (a Bcl-2 homologue). We found that XBax was able to rescue the antiapoptotic effect of XSlug and HDXmsx-1, while XR11 rescued the apoptotic effect of Xmsx-1. Finally, we analyzed by RT-PCR whether Xslug and Xmsx-1 were able to regulate the transcription of some apoptotic and antiapoptotic factors such as caspases and Bcl family members. Our results show that XSlug and HDXmsx-1 are able to control the transcription of caspases 2, 3, 6, 7, 9, and XR11.

134. **The Role of XChk2 Protein Kinase in the Early Embryonic Development of *Xenopus laevis*.** Brian N. Wroble and Jill C. Sible. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

When endogenous DNA is damaged or unreplicated, checkpoint pathways function to arrest the normal, somatic cell cycle prior to M or S phases through the inhibition of cyclin-dependent kinases (Cdks). In *Xenopus laevis*, early embryonic cellular divisions (2-12) consist of rapid cleavage cycles in which gap phases (G1, G2), cell-cycle checkpoint pathways, and an apoptotic program are all absent. Upon the completion of the 12th cellular division, the midblastula transition (MBT) begins and the cell cycle lengthens, acquiring gap phases. In addition, cell-cycle checkpoint pathways and an apoptotic program become functional. In *Xenopus laevis*, cell-cycle checkpoint initiation is largely dependent upon the function of two protein kinases, XChk1 and the less characterized XChk2 (XCds1). To determine the role of the latter, wild-type XChk2 (wt-XChk2) and mutated, catalytically inactive, dominant-negative XChk2 (DN-XChk2) were subcloned into RNA expression vectors and overexpressed in *Xenopus* embryos. Overexpression of wt-XChk2 in *Xenopus* embryos creates a pre-MBT cell-cycle checkpoint in which cellular division slows. Furthermore, premature inhibitory phosphorylation of Cdks (MPF) on tyrosine 15 occurs in embryos expressing exogenous wt-XChk2. In contrast, embryos overexpressing exogenous DN-XChk2 develop normally until they undergo a post-MBT apoptotic death. These data suggest the importance of XChk2 as not only a checkpoint protein kinase, but also as an integral constituent of cell-cycle remodeling events which occur at the MBT.

135. **A Quantitative Analysis of the DNA Replication Checkpoint in *Xenopus laevis*.** Ian Auckland, Amit Dravid, Wei Sha, John J. Tyson, and Jill C. Sible. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

In somatic cells, checkpoint pathways trigger cell-cycle arrest in response to unreplicated or damaged DNA. In the *Xenopus laevis* embryo, checkpoints are not operational until the midblastula transition (MBT). Studies in cell-free egg extracts indicate a threshold concentration of nuclei is required to elicit a checkpoint. We have paired experimental and computation methodologies to develop a quantitative model of the relationship between nuclear concentration and checkpoint engagement. Egg extracts were supplemented with aphidicolin, an inhibitor of DNA replication, and varying concentrations of sperm nuclei and nondegradable cyclin B. We have established that (1) superthreshold concentrations of cyclin B can bypass a DNA replication checkpoint and trigger entry into mitosis, (2) the concentration of cyclin B required to trigger mitosis increases with increasing concentrations of sperm nuclei, and (3) the lag time between addition of cyclin B and entry into mitosis increases with increasing concentration of sperm nuclei. The quantitative data will be fit to our mathematical model and additional studies will be performed to analyze a DNA damage checkpoint.

136. **XCTK1: A *Xenopus* C-terminal Kinesin-like Protein.** Seth Winfree and Sigrid Reinsch. NASA-Ames Research Center, Lockheed Martin Inc., Moffett Field, California.

XCTK1 is 97-kDa kinesin-like protein homologous to human/mouse KIFC3 and is predicted to function as a homodimer.

XCTK1 is present at picomolar levels in eggs, embryos, and cultured cells in a soluble high-molecular-weight complex that is not associated with membranes. XCTK1 localizes to centrosomes in *Xenopus* A6 cells. Overexpression of XCTK1 in A6 *Xenopus* cultured cells disrupts centrosome structure and function. To determine the function of XCTK1 in embryos, we first performed a developmental profile and showed the presence of XCTK1 transcripts at various stages. We used morpholino (MO) antisense treatment to inhibit XCTK1 expression in early embryos. XCTK1 MO treatment has no effect on the development of *X. laevis*. However, overexpression of either full-length XCTK1 (unfused) or the N-terminal cargo and coiled-coil domains fused to GFP (dominant negative) caused early embryonic lethality. Individual blastomeres begin lysis during cleavage stages. Expression of the exogenous XCTK1 was verified by Western blot and immunoprecipitation of early embryos. Microinjection of antibody against XCTK1 was also lethal. Imaging of embryos injected with either antibody or mRNA constructs indicate that cell division is aberrant. This is consistent with our results in A6 cells indicating that overexpression disrupts centrosomal function. The failure of MO treatment to cause a detectable phenotype may indicate that the protein stores present at fertilization are sufficient for early embryogenesis.

137. **Sonic Hedgehog Regulates the Maintenance of Neural Progenitors in the Ventral Spinal Cord.** Saeock Oh and Chin Chiang. Department of Cell and Developmental Biology, Vanderbilt University Medical Center.

Diverse neuronal cell types in the vertebrate central nervous system are generated at specific time and location from multipotential neural progenitors. The secreted signaling molecule Sonic hedgehog (Shh) has been shown to play an essential role in the specification of ventral progenitor domains by regulating the expression of progenitor-specific transcription factors. The effects of Shh signaling are mediated through the Gli family of transcription factors. Analysis of Shh and Gli3 double mutants has provided evidence that the establishment of ventral progenitor domains is largely achieved through downregulation of Gli3 repressor activity. However, the mechanism that regulates the capacity of specific progenitor cells to undergo proliferation or differentiation in the ventral spinal cord is not well understood. Here, we show that in Shh and Gli3 double mutants, a large proportion of precursor cells reenter the cell cycle after mitosis, leading to enhanced cell proliferation and prolonged neurogenesis. Conversely, in mutants where Shh signaling is constitutively active, neuronal differentiation occurs at the expense of progenitor cells, resulting in a significant reduction of proliferating cells. These results reveal a critical role for Shh signaling in regulating the balance between progenitor cell renewal and neuronal differentiation in the neural tube.

138. **FGFR1 Is Essential for Hippocampal Development by Regulation of Radial Glia Proliferation.** Yas Ohkubo,* Ayumi O. Uchida,* Dana Shin,* Juha Partanen,† and Flora M. Vaccarino*. *Child Study Center, Yale University, New Haven, Connecticut, USA; and †Institute of Biotechnology, University of Helsinki, Finland.

FGFs maintain neural stem cells and/or precursor cell pools during neurogenesis. Fgfs and Fgfr genes are distributed in key areas of the CNS throughout development. We found that radial

glia within the developing hippocampus expresses FGFR1 by immunocytochemistry, suggesting that FGFR1 may play a significant role in radial glial proliferation or differentiation. To investigate it, we disrupted the *Fgfr1* gene in radial glia by site-dependent recombination using the Cre-loxP system. The human GFAP-cre transgenic mouse leads somatic gene recombination into radial glia of dorsolateral cortex from E13.5. By crossing this transgenic mouse with *Fgfr1* floxed line, we generated conditional mutant mice lacking the transmembrane and intracellular kinase domains of the *Fgfr1* gene. These *Fgfr1* conditional knockout mice survive and show abnormal hippocampal development. *Fgfr1* mutant have a dramatic decrease in their hippocampal volume (~50%). We also found a decline of radial glia (GLAST/nestin/BLBP) and mitotic cells by BrdU incorporation at various stages of hippocampal development. These data strongly suggest that *Fgfr1* is essential for the proliferative expansion or maintenance of radial glial cells during the hippocampus development. (Supported by NSF0083104.)

139. Abstract #139 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

140. **The Role of E2F1 in Regulating Neural Stem Cells in the Mouse Brain.** Vassiliki Nikolettou, K. L. Ferguson, W. C. McIntosh, J. G. MacLaurin, and R. S. Slack. Ottawa Health Research Institute, University of Ottawa, 451 Smyth Rd., Ottawa, Ontario K1H 8M5, Canada.

Recent studies have indicated the involvement of Rb family proteins in regulating the stem cell population in the brain. Our lab has previously demonstrated that loss of Rb/p107 results in increased stem cell number, as well as higher progenitor proliferation. Moreover, telencephalon-specific Rb^{-/-} embryos exhibit enhanced neurogenesis and cellularity resulting in a 30% increase in brain size by E16.5. These embryos are further characterized by the presence of ectopic mitoses, consistent with the implication that Rb regulates neuronal commitment and terminal mitosis of neural progenitors. The E2F-1, -2, and -3 transcription factors are the key functional downstream targets of Rb. The aim of this study is to determine the molecular mechanisms of Rb-mediated neurogenesis, by investigating the role of E2F1 in neural stem cell regulation. We are currently crossing mice to generate double E2F1:telencephalon-specific Rb mutants, to determine whether loss of E2F1 can rescue the phenotype of the Rb-deficient embryonic telencephalon. Furthermore, we will be characterizing the number and self-renewal capacity of neural stem cells derived from these double knockout animals in vitro using neurosphere assays as well as in vivo by BrdU injections and immunohistochemical analysis. We hypothesize that double knockout animals will exhibit normal stem cell numbers and neurogenesis and will correct the ectopic mitoses observed in the telencephalon-specific Rb^{-/-} brains. (Supported by CIHR.)

141. **Role of Gdf11 in Retinal Neurogenesis.** Joon Kim,* Hsiao-Huei Wu,* Sanja Ivkovic,† Karen Lyons,† and Anne Calof*. *University of California, Irvine, California 92697; and †University of California, Los Angeles, California 90095.

We have shown that growth and differentiation factor 11 (GDF11), which is produced by neuronal progenitor cells and differentiated neurons in the mammalian olfactory epithelium,

mediates feedback inhibition of neurogenesis in this tissue in vitro and in vivo (Wu et al., 2003, Neuron 37, 197-207). To determine if GDF11 plays a similar role in the retina, we examined expression of genes encoding GDF11 and components of its signal transduction pathway, as well as markers for different neural progenitor cell types, in the eyes of wild-type mouse embryos and their littermates lacking functional *Gdf11*. In the developing retina, ganglion cells are among the first neuronal cells to be produced. *Gdf11* expression is evident in the retina at E12.5, and appears to be concentrated in the presumptive ganglion cell layer, where it continues to be expressed at least until birth. BrdU incorporation experiments indicate that there is ectopic overproliferation in the presumptive ganglion cell layer of *Gdf11* null embryos. *Hes1*, a bHLH transcription factor involved in negative regulation of retinal neural cell differentiation, is aberrantly expressed in the region of overproliferation. In contrast, expression of the proneural bHLH factors *Mash1* and *NeuroD* appears to be delayed in the retinas of *Gdf11* knockouts. In addition, the laminar structure of the retina is disrupted in *Gdf11* mutants. These results support the hypothesis that retinal neurogenesis is regulated, at least in part, via a negative feedback mechanism mediated by GDF11. (Supported by grants DC03583 and HD38761 to A.L.C. from the NIH).

142. **Functional Interactions of the Product of the Proto-Oncogene TCL1 during Early Preimplantation Embryo Development in the Mouse.** M. T. Fiorenza,* S. Torcia,* G. Ragona,† M. G. Narducci,† A. Bevilacqua,* G. Russo,† and F. Mangia*. *Department of Psychology, Section of Neuroscience, University La Sapienza of Rome, and †Istituto Dermatologico della Immacolata, Rome, Italy.

The product of the proto-oncogene TCL1 (T-cell leukemia lymphoma 1) plays a key role in T-prolymphocytic leukemia and its overexpression under the control of a VH promoter in transgenic mouse lines results in B cell tumors resembling B-CLL, pinpointing a relevant role of this factor in both T cell and B cell leukemias. Despite its importance in leukemogenesis, however, TCL1 normal/oncogenic function is still unknown. We have recently found that TCL1 is also abundantly expressed in early stages of preimplantation mouse embryo development, where it shuttles between embryo cortex and nuclei according to the cell cycle, and that lack of TCL1 results in an embryo mitotic block at the 4-/8-cell stages (Narducci et al., 2002), making the early mouse embryo the system of choice to understand normal/oncogenic TCL1 function. In light of well-established TCL1 heterodimerization with AKT and consequent enhancement of AKT transphosphorylation activity, we have investigated the intracellular distribution and movement of AKT1, AKT2, and AKT3 isoforms by confocal microscopy and FLIM using TCL1-/AKT-GFP chimeric proteins. We have also analyzed the presence of AKT isoform mRNAs during preimplantation embryo development in either normal or TCL1-KO embryos. Results obtained so far indicate AKT1/AKT2 as putative candidates for functional interaction(s) with TCL1.

143. **Regulation of Teratocarcinoma Stem Cells by the Murine Blastocyst: A Molecular Approach.** J. P. Gaillard, A. Diez, P. Vecino, and J. Arechaga. Department of Cell Biology—University of the Basque Country, 48940 Lejona, Spain.

Proliferation and differentiation of teratocarcinoma stem cells can be controlled by the blastocyst. Oddly, although documented

in the past by the wealth of work that led to the development of gene targeting technology, this regulation has never been approached at the molecular level. To this end, we have embarked in the production of monoclonal antibodies. Owing to its plasticity upon in vitro induced differentiation, CE44 teratocarcinoma cell line was used to immunize rats. Polyclonal antisera were first studied and monoclonal antibodies were further prepared from the spleen of the best responder. Specific clones were selected by means of flow cytometry and kept frozen until screened for their functional properties. We sought those hybridoma supernatants endowed with an antagonist activity in a bioassay in which both the tumor cells and the antibody are microinjected in a blastocyst. Inhibition of the regulatory process was expected to lead to tumor growth and to appearance of a colony after the blastocyst had hatched. Microinjection of cells along with a preimmune serum failed to produce tumor outgrowth. With the same experimental settings, polyclonal antisera reproducibly led to colony formation in short-term cultures. Hybridoma supernatants were first assayed as pooled batches according to this procedure. Antibodies from mixtures able to rescue tumoral growth were further tested individually. Several supernatants have been reproducibly observed for their induction of tumor outgrowth from the injected blastocysts. Molecular characterization of their cognate antigen(s) is currently underway.

144. Withdrawn

145. **Membrane Trafficking and Cytokinesis.** J. Todd Blankenship, Rebecca Farkas, Carmen Robinett, and Margaret Fuller. Stanford University, Stanford, California.

Cytokinesis is the final step in the division of one cell into two. At the beginning of the cytokinetic process, an actin/myosin ring is assembled adjacent to the plasma membrane in the plane of cleavage. When cytokinesis is initiated, the actin/myosin ring constricts. The fold in the surface of the cell created by this constriction is termed the cleavage furrow. We are using spermatogenesis as a system with which to identify factors required for cytokinesis and to study the mechanisms that underlie the ingression of the cleavage furrow. A screen of male steriles has identified five genes required for furrow ingression. We will present the cloning and characterization of the *onion ring* and *funnel cakes* mutants. The genes represented by these mutants are involved in membrane trafficking and thus may implicate a membrane trafficking pathway in the process of cytokinesis. Indeed, our early results indicate that the reason these cells cannot divide is that their ability to traffic membrane to the cell surface is impaired, and thus, these genes may define a multistep membrane trafficking pathway as essential for cell division.

146. **Primordial Germ Cell Development in the Insect, *Rhodnius prolixus*.** Roxane D. Graham and Erwin Huebner. Department of Zoology, University of Manitoba, Canada.

Primordial germ cells (PGCs) are set aside during embryogenesis and are essential to proper germ line and gonad formation during animal development. PGC development has been studied extensively in only a few insects such as *Drosophila*. Our work on the hemipteran, *Rhodnius prolixus*, shows it to be an ideal model, with important differences from *Drosophila*. While the

chronology, some facets of PGC development, and incorporation into the gonads are known, many of the details and mechanisms controlling these processes are largely unknown. This study aims to fully characterize PGC morphology, behavior, and cytoarchitecture for the complete embryonic developmental sequence. The embryonic cytoarchitecture and location of PGCs in *Rhodnius* embryos make it an ideal model system to observe PGC events in living embryos. DIC and video time lapse analysis of embryos provides insight into the behavior and structural morphology of live PGCs at various stages of embryogenesis. Microscopical analysis of fixed, as well as live, preparations (both LM and TEM) show the PGCs have large nuclei and nucleoli, cytoplasmic polar granule-like granules, prominent mitochondrial clusters, and distinct cortical F-actin. Additional cell-surface features, overall PGC morphology, and PGC—PGC and PGC—mesoderm interaction are revealed with SEM techniques. Examination of the contacts between PGCs and the underlying mesoderm (using live preps, TEM, and SEM) we hope will provide insight into the role cell—cell contact plays in directing PGCs to their final destination. This study aims to provide an in-depth and integrated picture of the origin and fate of the PGCs in the semi-long embryo of *Rhodnius prolixus*.

147. **Primordial Germ Cell Development in the Japanese Newt *Cynops pyrrhogaster*.** Yoichiro Tamori and Masami Wakahara. Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo, Japan.

In all sexually reproducing organisms, primordial germ cells (PGCs) differentiate to gametes and are responsible for the continuity of a species. Mechanisms of PGC development are being clarified in some typical model organisms. In anuran amphibians, PGCs are believed to form preformistically by a presence of the germ plasm in oocytes and fertilized eggs, similar to in *Drosophila*. Contrary to this, in urodelan amphibians it is generally accepted that there are no germ plasm in their oocytes and eggs, so we expect that PGCs are epigenetically induced in the lateral plate mesoderm during the embryogenesis as in mammals. To analyze the developmental and molecular mechanisms of the PGC formation in the newt, *Cynops pyrrhogaster*, we isolated at first cDNA clones of *Cynops Dazl* and *Cynops Vasa* genes from adult gonads. *Vasa*, a member of the DEAD protein family of ATP-dependent RNA helicases, is expressed in germ cell lineage in many animals. *DAZ* (Deleted in Azoospermia) and *DAZ*-like (*Dazl*) genes encode RNA-binding proteins that are important for germ cell differentiation in many animals as well. We analyzed the spatiotemporal expression of the *Cyvasa* and *Cydazl* genes during the normal embryogenesis using RT-PCR and in situ hybridization. In addition, we made explants consisted of the animal cap ectoderm and vegetal endoderm pieces from late blastula embryos to confirm whether the *Cynops* PGCs can be induced in animal cap ectoderm in vitro. It is expected that either *Cyvasa* or *Cydazl*, or both are used as specific molecular marker for the PGC formation in vivo as w

148. **Investigating the Molecular Basis of Germ Cell Specification in Planarians.** Ricardo M. Zayas and Phillip A. Newmark. Department of Cell and Structural Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA.

The specification of primordial germ cells is essential for the survival of all sexually reproducing species. Despite the impor-

tance of understanding how the germ cells are formed and how totipotency is established and maintained in germ cells, we are far from understanding these processes in detail. In the best studied invertebrates and many vertebrates, germ cells are specified by maternally supplied, cytoplasmic determinants. However, germ cell determination in many other organisms (e.g., mammals) proceeds epigenetically, requiring inductive interactions. The mechanisms linking these different modes of specification remain unclear. Planarian flatworms—classic models for studying regeneration—make excellent models to investigate epigenetic germ cell specification. Planarians do not segregate their germ cell lineage embryonically; rather, under appropriate conditions, germ cells are formed from stem cells in specific regions of the adult. Two strains of the planarian, *Schmidtea mediterranea*, exist: hermaphroditic, sexual worms; and asexual worms that reproduce only by fission. To date, molecular resources from *S. mediterranea* have been generated from the asexual strain. Here we describe the identification of thousands of expressed sequence tags from the sexual strain of *S. mediterranea*. To identify markers of the planarian germ cells, high-throughput in situ hybridization screens are underway. Combined with functional analysis using RNAi, these studies will shed light upon the mechanisms by which cell—cell interactions can specify germ cell fate and the extent to which these mechanisms have been conserved.

149. **Sexualization by a Putative Sexualizing Substance in the Planarian *Dugesia ryukyuensis*.** Kazuya Kobayashi, Sachiko Arioka, Midori Matsumoto, and Motonori Hoshi. Department of Biosciences and Informatics, Keio University, Japan.

Various metazoans reproduce asexually as well as sexually. Although many of them convert the reproductive mode depending upon the environmental conditions and/or the phase of life cycle, the mechanisms underlying the switch between asexual and sexual reproduction remain hardly known. Some species of planarians have three populations in terms of the reproductive mode; exclusively asexuals, exclusively sexuals and those that switch the reproductive mode seasonally. We have established an experimental system to analyze the mechanism in the planarian, *Dugesia ryukyuensis*. If the worms of an exclusively asexual clone (OH strain) of the species are fed with sexually mature worms of *Bdellocephala brunnea* (an exclusively oviparous species), they gradually develop ovaries, testes, and other sexual organs, then copulate, and eventually lay cocoons filled with fertilized eggs. This suggests that sexually mature worms contain a putative sexualizing substance(s) of poor species-specificity. The fully sexualized OH worms (acquired sexuals) can produce enough of an amount of their own sexualizing substance and keep the sexuality depending upon it. We are trying to identify the sexualizing substance in the acquired sexuals and have so far clarified that it is acidic, heat-stable, smaller than 0.5 kDa, and pronase-resistant. Meanwhile, we are trying to isolate genes that are specifically expressed for the sexualization by using the technique of differential display. Here, we show the sequence of *Durgen 1*, which is specifically expressed in the gonads, most probably oogonia and spermatogonia, of the acquired sexuals.

150. **Reproductive Mode, Ploidy, and Emergence of Eyeless Worms in the F1 Offspring by Random Inbreeding of Acquired Sexuals in the Planarian *Dugesia ryukyuensis*.** Sa-

chiko Arioka, Kazuya Kobayashi, Midori Matsumoto, and Motonori Hoshi. Department of Biosciences and Informatics, Keio University, Japan.

Worms of an exclusively asexual clone, the OH strain, of the planarian *Dugesia ryukyuensis* are triploid ($3n = 21$). However, they are experimentally sexualized if fed with the sexually mature worms of *Bdellocephala brunnea* (an oviparous planaria). We have found that F1 population by random inbreeding of the experimentally sexualized worms (acquired sexuals) is a mixture of asexuals and sexuals (innate sexuals) in a ratio of 1:2 and of diploids and triploids in a ratio of 1:4. However, there is no direct correlation between the ploidy and the reproductive mode. The asexuals in F1 are similarly sexualized by feeding them with *B. brunnea*. Although innate sexuals and acquired sexuals are hardly distinguishable by their appearances, we have found some essential differences between them. Despite triploid biotype of the OH strain, meiotic images observed in both the ovaries and testes of acquired sexuals suggest that eggs and sperm are derived from diploid cells. This result raises an important question on the chromosome behavior during meiosis and fertilization. Finally, we show, for the first time to the best of our knowledge, a spontaneous mutant in planarians. Eyeless worms of both innate sexual and asexual emerged at a rate of 2.5% (95/3797) in the F1 population. The eyeless worms never form normal eyes even if they are allowed to regenerate after surgical ablation, and the inbreeding of eyeless innate sexuals produces only eyeless offspring. Interestingly, all eyeless worms we have observed so far are diploids.

151. **Selective Transport and Packaging of the Major Yolk Protein in the Sea Urchin.** Jacqueline M. Brooks and Gary M. Wessel. Brown University, Providence, Rhode Island.

The major yolk protein of sea urchins is an iron-binding, transferrin-like molecule that is made in the adult gut, but taken up by developing oocytes that are embedded in somatic accessory cells and encompassed by two epithelial layers of the ovary. Here we address the dynamics of yolk transport, endocytosis, and packaging during the vitellogenic phase of oogenesis in the sea urchin by use of fluorescently labeled major yolk protein (MYP). Incorporation of MYP into the accessory cells of the ovary and its packaging into yolk platelets of developing oocytes is visualized in isolated oocytes, ovary explants, and whole animals. When MYP is introduced into the coelom of adult females, it is accumulated by the somatic cells of the ovarian capsule and transported to the oocytes. This phenomenon is specific for MYP and accurately reflects the endogenous MYP profile with correct targeting of this protein to yolk platelets as assessed by colocalization of another yolk protein marker, YP30. We find that oocytes cultured in isolation are endocytically active and capable of selectively packaging MYP into yolk platelets. Furthermore, oocytes that packaged exogenous MYP are capable of in vitro maturation, fertilization, and early development, enabling an in vivo documentation of MYP utilization and yolk platelet dynamics. These results demonstrate that the endocytic uptake of yolk proteins in sea urchins does not require a signal from their surrounding epithelial cells and can occur autonomous of the ovary. In addition, the entire population of yolk platelets is competent to receive new yolk protein input, suggesting that they are all made simultaneously during oogenesis.

152. **Requirement of Localized Maternal Factors for Zebrafish Germ Cell Formation.** Yoshiko Hashimoto, Shingo Maegawa, Kunio Yasuda, and Kunio Inoue. Graduate School of Biological Sciences, Nara Institute of Science and Technology, Japan.

In higher eukaryotes, maternally supplied factors in fertilized eggs play important roles in the determination of primordial germ cells (PGCs). Among those factors, the *vasa* and *nanos* gene have been identified as germ cell determinants and conserved through species. In zebrafish, *vasa* and *nanos1* are expressed in the germ cell lineage, and interestingly, maternal transcripts of these genes are localized at the end of first and second cleavage planes in early embryos. Previous studies have showed that these parts of cytoplasm contain electron-dense granules which include *vasa* mRNA. These observations imply that the cytoplasm at the end of cleavage planes act as germ cell determinants in zebrafish. However, the precise function of the cytoplasm in germ cell formation is not yet known. To investigate whether these parts of cytoplasm are necessary for the germ cell formation, we removed the all four regions of cytoplasm containing *vasa* and *nanos1* mRNAs by glass capillary at four-cell stage. We found embryos lacking *vasa*- and *nanos1*-containing sites neither express PGC markers nor develop normal PGCs. This is the first direct evidence that these parts of cytoplasm play a pivotal role in germ cell formation in zebrafish. Furthermore, we also found that maternal mRNAs of *zDazl* and *brul* are also localized at the cleavage planes in four-cell stage embryos. These novel localization patterns indicate that these genes also participate in germ lineage determination.

153. Abstract #153 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

154. Abstract #154 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

155. **Effect of PHGPx Overexpression on Spermatogenesis in Transgenic Mice.** R. Puglisi, A. Bevilacqua, G. Carlomagno, F. Mangia, and C. Boitani. Department of Histology and Medical Embryology, Department of Psychology, Section of Neuroscience, University "La Sapienza", Rome, Italy.

Selenoproteins, such as phospholipid hydroperoxide glutathione peroxidase (PHGPx), are essential for male fertility in mammals. Evidence has indicated that PHGPx plays not only its canonical role as antioxidant, but also a structural role in the sperm mitochondrial capsule. Moreover a nuclear isoform has been proposed to be involved in sperm chromatin condensation by cross-linking protamine disulfides. In infertile men, defective sperm display an impairment in PHGPx activity. To gain more insight into the function of PHGPx in mouse spermatogenesis, we examined by Northern blotting the expression pattern of this enzyme in highly homogenous populations of germ cells. Our results demonstrate that the level of PHGPx mRNA is markedly low in primary spermatocytes compared to that of round spermatids. On this basis, we have generated a transgenic mouse model, in which expression of mitochondrial PHGPx is driven by the synaptonemal complex protein1 promoter. Histological evaluation of testes from mice overexpressing PHGPx specifically in the

meiotic prophase revealed variable degrees of apoptosis of germ cells, seminiferous tubule degeneration, and delayed differentiation. No obvious defects were observed in somatic cells, whereas haploid germ cells appeared to be reduced, indicating this stage of spermatogenesis as the most compromised by PHGPx overexpression. These results indicate that developmental anticipation of PHGPx expression during germ cell maturation causes an impairment of spermatogenesis, thus supporting the idea that this gene is important for testicular function.

156. **Expression of GDNF/GFR α -1 during the Mouse Spermatogenesis and the Effects of GDNF for Spermatogonia Cell Development.** Yukio Nishina, Hideyuki Kikuchi, Satoru Goto, and Tomoaki Saitoh. Graduate School of Integrated Science, Yokohama City University.

The object of this study was to determine the role of Glial cell-derived neurotrophic growth factor (GDNF) during spermatogenesis. The expression patterns of mRNA for GDNF and the receptor for GDNF (GFR α -1) were evaluated during early spermatogenesis through a quantitative real-time RT-PCR procedure. The expression of mRNA for GDNF declined with progress of postnatal spermatogenesis. In contrast, that for GFR α -1 was relatively low in late embryonic testes, but increased suddenly after birth, highest at 4.5 dpp. Immunohistochemical localization revealed the expression of GDNF in Sertoli cells at from late embryonic stage through adult stage. GFR α -1 was expressed in gonocyte at late embryonic stage, in exclusively spermatogonia in neonatal, adult testes. In the primary culture system for neonatal spermatogonia, we found that the spermatogonia supplied with recombinant human GDNF was maintained more than nonsupplied spermatogonia at 5 days after culture. In BrdU incorporation assay, GDNF in the 1.5 dpp testicular cells culture promoted the BrdU incorporation of spermatogonia, so that GDNF induced DNA synthesis of neonatal spermatogonia. In addition, GDNF had no effect on apoptosis of spermatogonia in the primary culture. In summary, GDNF appears to regulate the proliferation of spermatogonia during neonatal period in mouse testes.

157. **A Ca²⁺-Sensitive Actin-Binding and -Bundling Protein Associated with Sertoli Cell Ectoplasmic Specializations.** Ichiro Tanii, * Hiroshi Iida, † Kazuya Yoshinaga*. *Miyazaki Medical College; †Kyushu University, Japan.

Ectoplasmic specializations are unique actin-related intercellular adhesions in Sertoli—Sertoli cells and Sertoli cells—spermatids junctions. This structure is repeatedly assembled and disassembled at specific periods during the spermatogenic cycle. The turnover of the structure is essential for spermatogenesis, but the regulatory mechanism is yet to be determined. We have identified a protein associated with ectoplasmic specialization using a monoclonal antibody. Immunohistochemistry showed that the antigen was expressed specifically in the testis and brain. In adult testes, the antigen was densely deposited on the sites corresponding to ectoplasmic specializations. Immunoelectron microscopy demonstrated the localization of the antigen on actin bundles. By the immunoaffinity chromatography, the antigen was partially purified from a testicular extract, and the eluate contained three major polypeptides, including the antigenic molecule of 65 kDa. F-actin was bound to immunoaffinity column when the antigen was preloaded on the column and was dissociated from the column with Ca²⁺. The partially purified antigen

facilitated actin-bundling in the absence of Ca^{2+} . The antigen appeared in early postnatal Sertoli cell and was deposited at Sertoli—Sertoli junctions in concert with blood—testis barrier formation. These results suggest that the antigen is possibly a novel Ca^{2+} -sensitive actin-binding and -bundling protein and play a role in actin turnover of ectoplasmic specializations.

158. **SCP/TAPS Gene Expression in *Drosophila*.** G. E. Kovalick and D. L. Griffin. Science and Mathematics, University of Texas of the Permian Basin, Odessa, Texas.

There are 28 members of the SCP/TAPS gene family in *Drosophila*. The members of this family typically encode small secreted proteins that contain an SCP/TAPS domain. The domain averages 154 amino acids in length and contains five relatively well conserved sequence blocks. These blocks are separated by less conserved regions. In *Drosophila*, SCP/TAPS proteins can be divided into two subfamilies. Most proteins belong to subfamily 1. Members of this subfamily display greater sequence divergence and have a larger SCP/TAPS domain, a signal sequence, characteristic sequences called the I and the M sequences, and eight conserved cysteine residues that probably participate in disulfide bonding. Members of subfamily 2 show more sequence conservation, have a smaller SCP/TAPS domain, may not have a signal sequence, and lack both the I and M sequences and the conserved cysteines. Most SCP/TAPS proteins appear to function extracellularly and may have cell signaling functions. However, little is known about SCP/TAPS protein function. As a first step in characterizing the function of these proteins, we have been examining the expression of each of the 28 SCP/TAPS genes in *Drosophila*, using a combination of RT-PCR, Northern, and in situ hybridization. Our results indicate that the majority of these genes display male-specific expression that is localized to the male reproductive system. This suggests that the majority of SCP/TAPS proteins in *Drosophila* have a role in sperm development, sperm maturation, or fertilization.

159. **SLBP Accumulation during Meiotic Maturation Is Required for Normal Histone Synthesis in the Mammalian Egg.** P. Allard,* W. Marzluff,† and H. Clarke*. *McGill University, †University of North Carolina.

The regulation of histone expression in the mammalian egg is thought to be essential for embryonic development. However, little is known of the regulatory mechanisms. In somatic cells, histones are expressed only during S-phase through a posttranscriptional mechanism involving the Stem-Loop Binding Protein that is required for processing, stabilization, and translation of histone mRNAs. We previously showed that SLBP is present in mouse oocytes arrested at the G2/M border. During meiotic maturation, SLBP protein levels dramatically increase. We hypothesized that SLBP accumulation at maturation is required for the synthesis of histone proteins that will be needed during early embryogenesis. RNAi was used to prevent SLBP accumulation by microinjecting SLBP dsRNA into immature oocytes, which were then allowed to mature. Microinjection of SLBP dsRNA in the oocyte efficiently decreased SLBP mRNA and protein levels compared to control injected with *LacZ* dsRNA. Microinjection of dsRNA did not affect the overall rate of oocyte protein synthesis or the frequency of meiotic maturation. To test the effect of reduced SLBP protein levels in the unfertilized egg on histone synthesis, proteins were radioactively labeled and his-

tones were purified by acid extraction. After SDS—PAGE and autoradiography, the rate of histone synthesis in SLBP and *LacZ* dsRNA injected samples were compared. SLBP dsRNA injected cells had a marked decrease by 40-50% in their rate of core histone protein synthesis. Taken together, these results show that SLBP accumulation at maturation is necessary for a proper accumulation of histone proteins in the unfertilized egg.

160. **Structure-Function Analysis of Vg1RNA Binding Protein.** Kinneret Rand, Froma Oberman, and Joel K. Yisraeli. Department of Anatomy and Cell Biology, Hebrew U. Medical School, Jerusalem, Israel.

In *Xenopus* oocytes, morphological and molecular asymmetries between the animal and the vegetal hemispheres help define first an initial polarity and then subsequently the primary axis around which development proceeds. Vg1RNA binding protein (Vg1RBP) binds the vegetal localization element (VLE) in the 3'UTR of Vg1RNA and mediates its association with MT. Vg1RBP also colocalizes with Vg1RNA to the vegetal cortex, and is thought to play a role in its localization during *Xenopus* oogenesis. We found that although most of Vg1RBP is cytoplasmic, there is a small amount present in the nucleus. Vg1RBP is distributed fairly uniformly throughout the oocyte, but gets concentrated only at the vegetal cortex. By using RNA protection analysis we obtain a fine map of the protein recognition sites in the VLE. We mapped the RNA binding domain in Vg1RBP using a mass spectrometry approach. We found a region near the C-terminus that appears to be involved in this specific binding. To identify the motifs in Vg1RBP that mediate vegetal localization and MT association, we fused GFP to different regions of Vg1RBP, and analyzed the ability of these constructs to bind to MT and to undergo localization when injected into oocytes. Our results are consistent with a model in which the minimum unit, sufficient for vegetal localization, is an intact didomain of KH motifs. When we disturbed the didomain, Vg1RBP localization was interrupted. The Vg1RBP-MT association is necessary for this localization, but RNA binding does not. These results suggest specific approaches for inhibiting endogenous Vg1RBP activity, which we are currently testing.

161. **Roles of MPF and MAP Kinase in Morphological Changes that Occur during Oocyte Maturation.** Tomoya Kotani and Masakane Yamashita. Division of Biological Sciences, Graduate School of Science, Hokkaido University, Japan.

Two kinases, maturation-promoting factor (MPF) and mitogen-activated protein kinase (MAPK), are activated during oocyte maturation in all animals studied so far. They are thought to play essential roles in the regulation of morphological changes, including germinal vesicle breakdown (GVBD), chromosome condensation, and spindle formation, but their precise roles are unknown. We investigated the roles of these kinases in oocytes of the frog *Rana japonica*, which enables us to easily distinguish the functions of MPF and MAPK owing to the lack of pre-MPF. Activation of one kinase without activation of the other induced only limited events; GVBD was induced by MPF without MAPK, and reorganization of microtubules at GVBD was induced by MAPK without MPF, but other events were not induced. In contrast, coactivation of MPF and MAPK promoted almost all of the morphological changes that occur during maturation, indicating that these are controlled by cooperation of MPF and MAPK. The

results revealed the functions of MPF and MAPK in each process of sequential morphological changes during oocyte maturation.

162. **The Role of zero population growth in Oogenesis.** Marie Y. Davis, Lilach Gilboa, and Ruth Lehmann. Skirball Institute, NYU, New York, New York.

The gene *zero population growth* (*zpg*) encodes *Drosophila* Innexin4, a germline-specific gap junction channel protein that is expressed from embryogenesis through gametogenesis. Loss of function of *zpg* in both male and female flies results in sterile adults with reduced-size gonads. In newly eclosed females, germ line stem cells (GSCs) are present, but dividing cysts and egg chambers are rare. Previous studies suggest that the loss of early cystoblasts is due to cell death, and *zpg* may be required for the survival and differentiation of cystoblasts.¹ The presence of *zpg* protein in dividing cysts and egg chambers suggests that gap junctions may play additional roles later in oogenesis. However, all previous characterizations of EMS *zpg* mutations have been of strong loss of function alleles, allowing only the study of early defects in oogenesis. To further investigate the role of *zpg* throughout oogenesis, we are now characterizing a weak *zpg* allele. ¹S.I. Tazuke et al., Development 2002, 129, 2529.

163. **The F-Box Protein Slimb Is Required for Egg Chamber Development in *Drosophila Melanogaster*.** Mariana Muzopappa and Pablo Wappner. Fundacion Instituto Leloir, FCEyN-UBA Patricias Argentinas 435, Buenos Aires, Argentina.

E3 ubiquitin ligases select specific substrates for ubiquitination, targeting them for degradation in the ²⁶S proteasome. SCF complexes are a large family of E3 ligases composed by three constant polypeptides -Skp1, Cullin1, and Rbx1-, and a fourth variable adapter, the F-box protein. By utilizing the yeast two-hybrid system and assessing protein-protein interactions in vitro we have defined for the first time the components and overall structure of the basic SCF complex in *Drosophila*. The F-Box protein Slimb was previously shown to participate in wing and limb development in the fruit fly. We generated germ line and somatic mutant clones in the ovary to study the function of *slimb* in oogenesis. We observed that abrogation of *slimb* results in germaria lacking lens-shaped cysts in region 2b and subsequently in encapsulation defects, giving rise to follicles with supernumerary germ cells and more than one oocyte. In addition, *slimb* mutant follicles have ectopic polar cells eventually accompanied by mispositioning of the oocyte in the egg chamber. Finally, we observed that chorion patterning is impaired in mutant clones and that the morphology of the dorsal appendages is variable. All the phenotypes described above were mimicked upon mild overexpression of Dpp/TGF- β or Hedgehog, suggesting that Slimb might negatively regulate these pathways in the ovary. We are performing genetic experiments aimed to define the molecular targets of Slimb during oogenesis.

164. **The Role of sqd in Anterior-Posterior Patterning during *Drosophila melanogaster* oogenesis.** Daniel Finch, Rebecca Ruffle, Lisa Gibson, Mark Hamas, and Amanda Norvell. The College of New Jersey, New Jersey.

In *Drosophila* oogenesis, the anterior-posterior (A-P) and dorsal-ventral (D-V) axes of the egg are established sequentially

by maternal gene products deposited in the developing oocyte. One of the most critical of these is *gurken* (*grk*), which encodes a TGF- α similar to molecule required for patterning both axes. Additionally, the hnRNP protein, Squid (*Sqd*), is required during the later stages of oogenesis when D-V patterning is established. *Sqd* protein functions in the localization of *grk* mRNA to the dorso-anterior corner of the oocyte, which in turn defines the D-V axis of the egg. Interestingly, we have found that oocytes from *sqd* mutant females are also defective in patterning of the A-P axis of the egg chamber. There are several molecular markers that define the A-P pattern of the egg chamber, one of which is the localization of *oskar* (*osk*) mRNA to the posterior of the oocyte. We have observed that *osk* mRNA localization in egg chambers from *sqd* homozygous females is defective. This *osk* mRNA mislocalization defect is most apparent in stage 8-9 egg chambers, and wild-type localization to the posterior appears to be restored by stage 10. These findings suggest that, in addition to its previously described role in regulating *Grk* activity in patterning the D-V axis, *Sqd* protein also functions in patterning the A-P axis. Currently, we are investigating the role of *Sqd* in this process.

165. **A Role for the RNA-Binding Protein LARK in Oogenesis.** R. Galioto, F. Smith, and G. P. McNeil. York College, The City University of New York, New York, New York.

Pattern formation during early *Drosophila* development is governed by maternally inherited genetic factors, which determine the organization and polarity of the two major embryonic axes. The developmental functions of many maternally expressed genes have been studied in detail. In this study, consideration is given to an essential, maternally acting gene called *lark*, which encodes an RNA-binding protein. We have previously shown *lark* is required for early *Drosophila* development. Embryos that lack the maternally-acting *lark*⁺ component arrest in development prior to cellular blastoderm. We believe this developmental defect is due to a defect in oogenesis since the eggs laid by females lacking the *lark* maternal component are very fragile, with many showing no signs of development. LARK protein is expressed during oogenesis in both the developing oocyte and the nurse cells, consistent with a role during this stage of development. In addition, mutations in either of two LARK RNA-binding domains result in sterility, indicating LARK functions as an RNA-binding protein during oogenesis. To examine the potential role of *lark* during oogenesis, we have performed a morphological analysis of ovaries lacking the *lark*⁺ maternal component. In addition, we have looked at when developmental arrest occurs in the LARK RNA-binding domain mutants to see if they show similar defects in oogenesis to those lacking the *lark*⁺ maternal component.

166. **Presence of Cadherin during Oogenesis in the Brown Spider *Loxosceles intermedia*.** A. C. Santiago-Filha, R. Morishita, and C. Faraco. Department of Cell Biology, Uni. Fed. Paraná, Brazil.

Loxosceles intermedia is a poisonous spider, broadly distributed in Southern Brazil, representing a public health problem. The reproductive system of *L. intermedia* females contains a pair of elongated, sac-like ovaries, located in the ventral face of the abdomen. There are oocytes in different stages of development (classified morphologically in stages I to VI) in a common ovary area, covering a large extension of the ventral face of the organ. Phases I and II are characterized by oocytes protruding from the

ovarian epithelium, and the last phases are attached to the ovarian wall by pedicular cells. The oocyte is not surrounded by follicular cells, but has its surface lined by a thick proteic band underlined by the basement membrane of the ovarian epithelium. The only cells in contact with the oocyte are the pedicular cells. Ovaries of young and mature females are examined by light microscopy, and cadherin detection was made by immunocytochemistry. Areas of the ventral epithelium, where oocytes at very early stages of differentiation could be detected, display cadherin expression. Oocytes at phase I show cadherin in their membranes and are surrounded by cadherin-positive cells. Other stages oocytes show cadherin in their surface and at the point of contact with pedicular cells. The presence of cadherin on the oocyte might be involved on cellular adhesion interactions with the adjacent pedicular cells or function in signaling during oogenesis. Cadherin molecules are also seen in the cytoplasm of oocytes at phases I to V. These molecules may be the source of superficial cadherin or they might represent maternal pool for use in the future embryo.

167. **Calcium Release at Sea Urchin Fertilization Depends on Signaling Mediated by the $\beta\gamma$ Subunits of Heterotrimeric G-Proteins.** Ekaterina Voronina and Gary M. Wessel. Brown University, Providence, Rhode Island.

Cytoplasmic calcium release is required for egg activation at fertilization. The pathway leading to calcium release in echinoderms includes activation of a c-Src tyrosine kinase homologue, followed by phospholipase C γ -activation and release of inositol triphosphate. However, the upstream activator of c-Src is not known. The involvement of heterotrimeric G-proteins in the signaling leading to sea urchin egg activation at fertilization appears controversial. We recently identified four $G\alpha$ -subunits of heterotrimeric G-proteins present in the sea urchin egg. We therefore tested whether these proteins were involved in the signaling at egg activation. We found that activation of G_s - and G_q -type G-proteins is required for egg activation and cortical granule exocytosis. The effects of these G-proteins appear to be mediated by the release of $\beta\gamma$ -subunits, which are upstream of the cytoplasmic calcium release. A model integrating proposed input of G-proteins into the framework of established signaling at sea urchin fertilization is presented.

168. **Analysis of Egg Plasma Membrane Proteins at Fertilization.** Sheila A. Haley and Gary M. Wessel. Brown University, Providence, Rhode Island.

Fertilization initiates extensive remodeling of the extracellular matrix of the sea urchin egg, a process that results in the elevation of the fertilization envelope to create the permanent block to polyspermy. Cell surface proteins of the egg are also significantly modified by the enzymes released from the cortical granules at fertilization, in particular, the cortical granule serine protease CGSP1. By traditional biochemical and mass spectrometry analysis, we have begun to identify the egg cell surface proteins involved in fertilization. We have already characterized a transmembrane protein, p160, that is cleaved from the egg surface by CGSP1 at fertilization. The predicted domain structure of p160 includes a transmembrane domain that anchors it in the plasma membrane as well as five CUB domains, which may be involved in protein—protein interactions, either with other p160 molecules or with egg extracellular matrix proteins involved in

the block to polyspermy. p160 appears to interact generally with the extracellular matrix: it is temporarily removed from the zygotes by the cortical granule protease for fertilization envelope formation and then is rapidly replaced as the embryo develops. We are currently examining the egg surface proteins by mass spectrometry. Resulting peptide sequences will be identified via comparison to DNA sequence information from the sea urchin genome database. Furthermore, peptides from fertilized eggs will be analyzed to identify proteins specifically cleaved by the cortical granule protease that may function in the block to polyspermy. These data should result in a clearer understanding of the egg surface and its transformation following fertilization.

169. **Electric Embryos: Endogenous Ion Fluxes and Voltage Gradients in Left—Right Asymmetry.** Michael Levin, Ph.D. The Forsyth Institute/Harvard University, Cambridge, Massachusetts.

The vertebrate bodyplan features striking LR asymmetry of the viscera and brain. What mechanisms are upstream of the cascade of unilaterally expressed factors dictating the situs of the heart and other organs during embryogenesis? We previously showed that in both chick and frog, a system of gap-junctional communication provides a large-scale path for very early information exchange between the L and R sides of the embryo. We now seek to identify mechanisms which determine the directionality of the movement of small molecule carriers of sidedness through gap junctional paths. A broad, multitiered pharmacological screen implicated just four ion flux proteins in LR asymmetry: two K⁺ channels (K_{atp} and K_{vLQT-1}) and two H⁺ pumps (V-ATPase and H⁺/K⁺-ATPase). These proteins produce directly observable K⁺ and H⁺ fluxes that result in consistently asymmetric voltage and pH gradients. The gradients exist by the second cell cleavage in *Xenopus* (far earlier than other characterized endogenous LR mechanisms) and functionally determine the sidedness of the earliest known asymmetric markers in both chicks and frogs. The asymmetry of ion flux is due to asymmetric localization of mRNA and protein encoding these electrogenic gene products. These localizations identify novel subcellular zip codes for both mRNA and protein and provide a link between embryonic polarity, large-scale developmental physiology, and intracellular transport of macromolecules. We propose a model involving gap junctions and ion flux which leverages embryonic asymmetry from motor protein movement along the cytoskeleton.

170. **Elements of Left—Right Patterning: Gap Junctions, pH, and Membrane Voltage.** D. Adams and M. Levin. The Forsyth Institute.

Various experiments have implicated ion flux as important in left—Right patterning mechanisms that act upstream of asymmetric gene expression. We describe investigations into three different aspects of ion flux in *Xenopus* left—right (LR) patterning: the intracellular pH (pH) and membrane voltage (V_m) differences resulting from activity of electrogenic proteins, and gap junctional communication (GJC) as a factor controlling ion flow. **GJC DUCTIN**, originally identified as the transmembrane H⁺ channel of V-ATPase, also forms gap junctions through which H⁺ and possibly small morphogens, pass pharmacological inhibition of DUCTIN disturbs normal LR patterns in *Xenopus*. We are investigating: [1] localization of DUCTIN to determine whether its domain is distinct from that of other V-ATPase proteins, which

would suggest multiple physiologic roles; [2] inhibition of DUCTIN by injection of antibodies or dominant negative constructs to see whether DUCTIN is necessary for normal LR patterning. pH Proton gradients produced by H⁺ pumps affect the pH of cells and alter the function of pH sensitive proteins (such as gap junctions). pH changes may also be a marker of GJC. Thus, we are examining pH using ratiometric dyes to both monitor gap junctions and assess the consequences for other physiologic processes. To build on results with ion probes, we are using a potentiometric dye to map Vm in early embryos. Data suggest that, in *Xenopus* as in chicks, LR asymmetry in Vm is an early indicator of LR distinctions and thus may be part of a conserved cascade that generates vertebrate LR asymmetry.

171. **Difference in the Maternal and Zygotic Contributions of Tumorhead on Embryogenesis.** Chuan Fen Wu, Agnes Pui-Yee Chan, and Laurence D. Etkin. Department of Molecular Genetics, The University of Texas; M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030, USA.

Tumorhead (TH) is a maternally expressed gene in *Xenopus laevis* that when overexpressed increased proliferation of ectodermal derivatives and inhibited neural and epidermal differentiation. However, injection of anti-TH antibodies inhibited cleavage of all blastomeres, not only those contributing to the ectoderm. The injection of TH morpholino antisense oligonucleotide (TH-MO), which inhibits translation of TH mRNA, did not affect early cleavage but inhibited cell division in both the neural field and the epidermis. This was accompanied by the inhibition of neural and epidermal markers. TH-MO did not affect the formation and differentiation of mesoderm and endoderm derivatives. Our overexpression and loss-of-function studies demonstrated that TH plays an important role in differentiation of the ectoderm by regulating cell proliferation. They also supported the conclusion that the maternal component of TH may affect the cell cycle in all cells, while the zygotic component has a germ-layer-specific effect on the ectoderm.

172. **The RNA Binding Protein Vg1 RBP Is Required for Cell Migrations during Early Neural Development.** K. Yaniv,† A. Fainsod,* C. Kalcheim,* and J. K. Yisraeli.* †Department of Anatomy and Cell Biology and *Department of Cellular Biochemistry and Human Genetics, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

Asymmetric distribution of RNA has been observed in a wide variety of embryonic and somatic cells. Localized RNAs can create a heterogeneous distribution of protein through their translation at particular intracellular locations. RNAs are sorted within cells via interactions with specific RNA binding proteins (RBPs) and cytoskeletal elements. Vg1 RBP is one such RNA binding protein that has been implicated in the localization of Vg1 RNA to the vegetal cortex of *Xenopus* oocytes. Vg1 RBP is a member of the conserved VICKZ family of RBPs, expressed only in embryonic and neoplastic cells. We have analyzed the expression of Vg1 RBP during *Xenopus* embryogenesis. Transcripts are detected throughout the prospective neuroectoderm and neural tube, neural crest cells, eyes, and otic vesicles. Using antisense morpholino oligonucleotides (AMO), we find that Vg1 RBP is required for the migration of cells forming the roof plate and, subsequently, for neural crest migration. These cells are properly

determined but remain at their site of origin. Consistent with a role in cell movement, Vg1 RBP asymmetrically localizes to extended processes of neural crest cells that have migrated out from explanted neural tubes. The abnormal phenotypes can be rescued by sense Vg1 RBP mRNA that is not recognized by the AMO. These data suggest an important role for Vg1 RBP in cell migration during development. Furthermore, given its high homology with other members of the VICKZ family, our results emphasize the close relationship between mechanisms controlling cell movements in embryos and in neoplasias.

173. **Regulation of Xnr2 in the Vegetal Marginal Zone.** Carin H. Ezal and William C. Smith. University of California at Santa Barbara, Santa Barbara, California.

The mesoderm of *Xenopus* is patterned along the animal/vegetal axis, with brachyury (Xbra) expressed in the animal marginal zone and *Xenopus* nodal related 2 (Xnr2) expressed in the vegetal marginal zone. The pattern of these two genes appears to mark the future muscle and ventral mesoderm zone derivatives such as blood, respectively. We are investigating the molecular differences between the Xbra and Xnr2-expressing cells. Xnr2 is one of only two genes that is known to be expressed exclusively in the vegetal marginal zone (the other is Xmenf; Kumano, G., Smith, W.C., 2002, Mech. Dev. 118, 1-2). Xnr2 expression is restricted to the ventral marginal zone by endogenous FGF. To study Xnr2 regulation, a Xnr2 promoter—GFP fusion has been constructed containing 4.5 kb of 5' UTR and 2 kb of putative intron 1. A minimal promoter analysis is being carried out with the goal of identifying domains important for expression, and negative regulation by FGF/MAPK signaling. In addition, this GFP fusion construct is being used to trace the fate of cells that expressed Xnr2 in the blastula/gastrula embryo. Our findings should provide further insight into the patterning of the *Xenopus* mesoderm and the role of Xnr2.

174. **Searching for VegT Target Genes and Exploring Their Functions.** Nicola V. Taverner and James C. Smith. Wellcome Trust/Cancer Research UK Institute and Department of Zoology, University of Cambridge, United Kingdom.

We are interested in genes involved in early mesendodermal specification and differentiation during *Xenopus* development. The vegetally localized maternal T-box transcription factor VegT initiates a cascade of gene activation which specifies endoderm and mesoderm. We have overexpressed a hormone-inducible form of VegT and used microarray technology to identify genes which are upregulated by this transcription factor. Genes isolated in this way include known targets, such as *Derrière* and *Mixer*, novel targets showing homology to genes identified in other organisms, and unknown target genes. We are examining the expression patterns of these genes to identify those which are expressed in the mesendoderm at midgastrula stages. We are also testing whether they are activated directly by VegT or by other T-box transcription factors. Finally, we are using morpholino technology to look at the roles of individual VegT target genes and are investigating the effect of "knocking down" members of the *Bix* family of genes. A reduction in Bix protein levels causes a downregulation of genes expressed in anterior mesendoderm.

175. **Ethanol Exposure Affects Spemann's Organizer through Retinoic Acid Signaling.** Ronit Yelin,* Ayala Frumkin,†

Hadas Kot,* Graciela Pillemer,* and Abraham Fainsod.
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Fetal Alcohol syndrome (FAS) is a common set of developmental malformations caused by alcohol consumption during pregnancy. Individuals with FAS exhibit short stature, microcephaly, and facial dysmorphogenesis, including microphthalmia. Using *Xenopus* embryos as a model developmental system, we show that ethanol exposure recapitulates many aspects of FAS including a shortened rostro-caudal axis, microcephaly, and microphthalmia. Temporal analysis revealed that *Xenopus* embryos are most sensitive to ethanol exposure between late blastula and early/mid gastrula stages. This window of sensitivity overlaps with the formation and early function of the embryonic organizer, Spemann's organizer. Molecular analysis revealed that ethanol exposure of embryos induces changes in the domains and levels of organizer-specific gene expression, identifying Spemann's organizer as an early target of ethanol. We show that mechanistically, ethanol is antagonistic to Vitamin A conversion to retinoic acid, and that the organizer is active in retinoic acid signaling. The model suggests an ethanol-dependent reduction in retinoic acid levels that are necessary for the normal function of Spemann's organizer.

176. **The Secreted Frizzled-Related Protein Sizzled Functions as a Negative Feedback Regulator of Extreme Ventral Mesoderm.** Licio Collavin* and Marc W. Kirschner†. *Harvard Medical School, Boston, Massachusetts, USA; and †Trieste University, Trieste, Italy.

The prevailing model of dorsal ventral patterning of the amphibian embryo predicts that the prospective mesoderm is regionalized at gastrulation in response to a gradient of signals. This gradient is established by diffusible BMP and WNT inhibitors secreted dorsally in the Spemann Organizer. An interesting question is whether ventrolateral tissue passively reads graded levels of ventralizing signals, or whether local self-organizing regulatory circuits may exist on the ventral side to control cell behavior and differentiation at a distance from the Organizer. We provide evidence that Sizzled, a secreted Frizzled-related protein expressed ventrally during and after gastrulation, functions in a negative feedback loop that limits allocation of mesodermal cells to the extreme ventral fate, with direct consequences for morphogenesis and formation of the blood islands. Morpholino-mediated knockdown of Sizzled protein results in expansion of ventral posterior mesoderm and the ventral blood islands, indicating that this negative regulation is required for proper patterning of the ventral mesoderm. Sizzled's biochemical activity is apparently very different from that of other secreted Frizzled-related proteins and does not involve inhibition of Wnt8. Our data are consistent with the existence of some limited self-organizing properties of the extreme ventral mesoderm.

177. **BMP-3b and BMP-3 Function as Different Dorsalizing Factors in *Xenopus* embryos.** Shin-ichiro Nishimatsu,* Jun Hino,† Takeharu Nagai,‡ Tsutomu Nohno,* Hisayuki Matsuo, and K. MaKangawa. *Department of Molecular Biology, Kawasaki Medical School, Okayama 701-0192, Japan; †De-

partment of Biochemistry, National Cardiovascular Center Research Institute, Osaka 565-8565, Japan; and ‡Laboratory for Cell Function and Dynamics, RIKEN Brain Science Institute, Saitama 351-0198, Japan.

Bone morphogenetic proteins-3b and -3 (BMP-3b and BMP-3) together represent a unique subgroup of the BMP family. Although structurally similar, we found that BMP-3b and BMP-3 have different functions in *Xenopus* embryos. BMP-3b injected into *Xenopus* embryos triggered autonomous secondary head formation, whereas BMP-3 induced aberrant tail formation. At the molecular level, BMP-3b antagonized nodal-like proteins and ventralizing BMPs, whereas BMP-3 antagonized only the latter. These differences are due to divergent prodomains. Less BMP-3b than BMP-3 precursor is proteolytically processed in embryos. BMP-3b protein associated with a monomeric form of Xnr1, a nodal-like protein, whereas BMP-3 did not. These molecular features are consistent with their expression profiles during *Xenopus* development. *XBMP-3b* is expressed in the prechordal plate, while [*XBMP-3*] is expressed in the notochord. Using antisense morpholino oligonucleotides, we found that the depletion of both xBMP-3b and cerberus, a head inducer, caused headless *Xenopus* embryos, whereas the depletion of both xBMP-3 and cerberus affected the size of the somite. These results revealed that xBMP-3b and cerberus coordinate formation of the *Xenopus* larval head, and that xBMP-3b and perhaps xBMP-3 are involved in the axial patterning of *Xenopus* embryos.

178. **Xnr3 Regulates Convergent Extension Movement via the FGF Receptor.** Chika Yokota,* Matt Kofron,* Mike Zuck,* Doug W. Houston,* Harry Isaacs,† Makoto Asashima,‡ Chris C. Wylie,* and Janet Heasman*. *Division of Developmental Biology, Cincinnati Children's Hospital Research Foundation, Cincinnati, Ohio 45229, USA; †Department of Biology, University of York, York YO10 5YW, United Kingdom; and ‡Department of Life Sciences, The University of Tokyo, Meguro-ku, Tokyo 153-8902, Japan.

Xnr3 is a member of the nodal subfamily of TGF- β molecules and is known as a direct target of the maternal Wnt/ β -catenin pathway. Xnr3 has different activity from the other five nodal-related genes isolated in *Xenopus* in several respects. Nodal-related proteins (Xnr1, 2, 4, 5, 6) induce down stream gene expression via activin receptor-smad2 pathway, but no direct study has been carried out on the Xnr3 signaling pathway. Here we show that Xnr3 is required for convergent extension movement through the induction of Xbra expression. We also show that ectopic overexpression of Xnr3 mRNA in presumptive ectoderm causes convergent extension movement, and expression of Xbra, eFGF, and MyoD. These inductions require the FGF receptor FGFR1, since they are inhibited by the antisense depletion of maternal FGFR1 mRNA in presumptive ectoderms and whole embryos. In contrast, induction of neural markers (NCAM and Nrpl) is not dependent on the FGF receptor. Furthermore, ectopic expression of Xnr3 in animal caps activates MAP kinase, showing that Xnr3 activates the FGF signaling pathway. These findings reveal the essential role of Xnr3 during early inductive events and suggest a novel role for nodal family members, that of regulating cell movements through the FGF receptor.

179. **Role of *Xenopus* Rap2B, a New Player of Wnt/ β -Catenin Signaling Pathway in Dorsalization of Embryonic Axis.**

Sun-Cheol Choi and Jin-Kwan Han. Division of Molecular and Life Sciences, POSTECH, South Korea.

Rap GTPases are members of the Ras G-protein superfamily. No function has yet been attributed to Rap2. We have cloned *Xenopus* Rap2B (XRap2B) and analyzed its functions during early embryogenesis. It is transcribed both maternally and zygotically throughout early development. XRap2B transcripts are first observed in the animal pole of egg and blastula. At the onset of gastrulation, it is also expressed in the dorsal marginal zone and subsequently expands dorsolaterally, being detectable throughout the marginal zone with its stronger signal on the dorsal side. Injection of XRap2B into ventro-vegetal blastomere of four-cell stage embryos induces a secondary axis, which contains ectopic neural tube, somitic muscle, and gut lumen but no notochord. XRap2B-injected VMZs elongate and express dorsal mesodermal, neural, and anterior endodermal markers. In addition, XRap2B-expressing animal caps have the ability to induce the expression of neural and endodermal genes, but not mesodermal tissue. Antisense morpholino oligonucleotide (Mo) knockdown of XRap2B leads to deficiency of anterior development. Depletion of XRap2B inhibits Nodal/Activin signaling and endogenous expression of organizer-specific markers such as chordin, cerberus, and hex. In addition, its depletion blocks the induction of direct target genes such as siamois and Xnr-3 caused by XWnt-8 and β -catenin. However, BMP-4 signaling is not affected by XRap2B knockdown. Taken together, these results suggest that XRap2B lies at the cross-talk of Activin/Nodal and Wnt/ β -catenin signaling pathways in dorsalization of embryonic axis during early development.

180. **Twisted Gastrulation Directly Alters BMP Inhibition by Chordin.** Bruno Reversade, Michael Oelgelschl ger and E. M. De Robertis. Howard Hughes Medical Institute, University of California Los Angeles, Los Angeles, California.

Dorso-ventral patterning of the vertebrate embryos is controlled in the extracellular space by the interaction of BMPs and secreted proteins that include Chordin, Tolloid, and Twisted Gastrulation (Tsg). Chordin binds BMP via its Cysteine-rich (CR) domains and thus antagonizes binding of BMP to its cognate receptor. Cleavage of Chordin by Tolloid at two sites releases BMP, which regains its activity and binds to its receptor. Tsg has a dual activity: in the absence of Tolloid, Tsg makes Chordin a better BMP antagonist by forming a ternary complex, whereas in the presence of Tolloid, Tsg facilitates both the cleavage of Chordin and the dislodgement of BMP from the fragments of Chordin. To address the dual function of Tsg, we generated point mutations in its two conserved cysteine-rich domains. We found that mutations in the N-terminal domain of Tsg which impair BMP binding but not Chordin binding, have hyper-ventralizing activity upon injection in *Xenopus* embryos. Unexpectedly, this ventralization occurs despite the accumulation of endogenous full-length Chordin, suggesting that this phenomenon is independent of Tolloid cleavage. Given that Tsg hyperventralizing mutants are unable to bind BMP, we propose a model in which the carboxy-terminal domain of Tsg regulates Chordin by modulating its avidity for BMP.

181. **RhoA-Mediated Planar Cell Polarity Pathway Is Conserved in Both Invertebrates and Vertebrates.** Gun-Hwa Kim and Jin-Kwan Han. POSTECH, South Korea.

Recent genetic studies in *Drosophila* identified that Planar cell polarity (PCP) pathway is manifested wing, eye, and sensory bristle development. RhoA is known as a key protein that controls cell polarity through Dishevelled, a component of the planar cell polarity (PCP) cascade. Recently, PCP pathway is involved in convergent extension (CE) movements in vertebrate embryos. However, although RhoA activity appears in dorsal marginal zone (DMZ), it is not known whether *Xenopus* RhoA (XRhoA)-mediated PCP pathway is required for normal CE movements during *Xenopus* embryos. Gain and loss of XRhoA function interfere with CE movements in both intact embryos and Keller explants. These effects occur without affecting mesodermal specification. In addition, XRhoA rescues failure to close the neural tube and inhibition of CE movements by DN Xdsh. We find that *Xenopus* Rho kinases-N  (xROK ) are mainly expressed in the dorsal mesoderm and ectoderm above the dorsal lip, also inhibits CE movements and rescues CE inhibition by DN XRhoA. Furthermore, XRhoA mediates Wnt/PCP activation of Jun N-terminal kinase (JNK). Taken together, our results demonstrate that the RhoA/ROK/JNK pathway is conserved in both vertebrate and invertebrate.

182. ***Xenopus* LIM-Homeodomain Protein Xlim5 Regulates Cell Adhesion in Early Ectoderm Development.** Douglas W. Houston and Chris Wylie. Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio.

An initial step in early development is the allocation of cells to one of the primary germ layers, ectoderm, mesoderm, or endoderm. In *Xenopus*, the T-box transcription factor VegT is required for endoderm specification in vegetal cells as well as for the expression of TGF-beta-related growth factors that induce mesoderm in the equatorial region. In the absence of VegT, ectoderm forms in mesodermal and endodermal regions of the embryo, suggesting the existence of early determinants of ectoderm. To identify such factors, we assayed VegT-depleted vegetal explants at stage 11 for the expression of candidate ectoderm regulatory genes by real-time RT-PCR. We found that expression of Xlim5, a LIM-homeodomain protein (Toyama et al., 1995), was increased in VegT-depleted whole embryos and vegetal explants. To test whether Xlim5 has a role in ectoderm specification, we overexpressed Xlim5 mRNA in *Xenopus* embryos. Lineage studies showed that Xlim5 could cause injected cells to enter other germ layers. Expression of Xlim5 in vegetal cells inhibited their ability to sort from animal cells. Xlim5 was not sufficient to induce ectoderm gene expression in prospective endoderm cells. Interference of Xlim5 function using an Engrailed repressor fusion construct (Xlim5-EnR) caused dissociation of animal cap cells. Injection of Xlim5-EnR into animal blastomeres at the 32-cell stage led to a loss of these cells from the ectoderm. Overall, these experiments provide evidence that Xlim5 may not be required for ectoderm specification per se, but is likely to regulate cell adhesion within the ectoderm.

183. **Grainyhead, a Newly Identified Mediator of BMP4 Signaling during Vertebrate Ectodermal Specification.** Jianning Tao,*† Stephen M. Jane,‡ Paul E. Mead,† and John M. Cunningham*. *Department of Hematology/Oncology and †Department of Pathology, St. Jude Children's Research Hospital, Memphis, Tennessee; and ‡Rotary Bone Marrow Laboratory, Melbourne, Australia.

Drosophila Grainyhead (grh) is a developmentally-regulated transcription factor that has been implicated in embryonic patterning. To determine the role of grh in vertebrates, we have identified a *Xenopus* homologue, xGrhl1. This factor shares the DNA binding and dimerization domains characteristic of the *Drosophila* protein and has a pattern of expression that is restricted to nonneuronal ectoderm. Enforced expression of a dominant negative form of xGrhl1 resulted in failure of gastrulation and loss of epidermal specification. Interestingly, gene knock-down by morpholino-mediated depletion of the xGrhl1 protein replicated this defect. Based on these observations and the central role of BMP4 in ectodermal specification, we hypothesized that xGrhl1 is an intrinsic component of this signaling pathway. To test this model, we have utilized animal cap and cell dissociation assays. We have demonstrated that xGrhl1 is modulated transcriptionally by BMP4, but is not an immediate early response gene. Furthermore, enforced expression of xGrhl1 resulted in increased expression of Dlx3, Dlx5, and xAP2 in ectodermal cells, known downstream gene targets necessary for BMP4-mediated epidermal differentiation. In contrast, xGrhl1 overexpression failed to modulate Msx1 or xVent-2 expression. Taken together, we conclude that vertebrate xGrhl1 is a novel downstream molecule in the ectodermal BMP4 regulatory network.

184. **The Novel Gene *Ashwin* Functions in *Xenopus* Neural Development.** Tara B. Alexander and Amy K. Sater. Department of Biology and Biochemistry, University of Houston, Houston, Texas 77204.

The novel gene *Ashwin* is maternally expressed throughout the ectoderm and zygotically in neural tissues. It is a nuclear protein that shares significant sequence similarity to putative proteins identified in multiple vertebrate species. Using a luciferase reporter assay, we show that an *Ashwin* Gal4 DNA-binding domain fusion protein potently represses transcription. Overexpression of *Ashwin* leads to secondary axes, neurulation defects, and anterior truncations. *Ashwin* overexpression does not affect expression of the early organizer markers *Xnot* and *gooseoid*, nor the anterior mesendoderm marker *Hex*, and reductions in the expression of the forebrain marker *Otx2* and the eye marker *Rx* do not become apparent until tailbud stages. Thus, we suggest that *Ashwin* affects secondary rather than primary organizing centers. We are currently analyzing the effects of *Ashwin* on the isthmus organizer and on midline signaling. In naïve ectoderm, *Ashwin* overexpression upregulates the early neural marker NCAM, but not differentiated neural markers, regionally localized neural markers, nor neural crest markers. However, *Ashwin* does potentiate the neural inducing ability of *Noggin*. Interestingly, whole embryos overexpressing *Ashwin* show an increased number of melanophores as well as misregulation of the neural crest marker *Slug*. The differences between our explant and whole embryo studies suggest that the range of phenotypic effects observed in response to *Ashwin* overexpression is the result of context-specific signals. We propose that *Ashwin* acts in the early embryonic ectoderm with additional region-specific factors in neural specification and patterning.

185. **The Cement Gland as a Paradigm for Anterior Ectodermal Position.** Shuhong Li and Hazel Sive. Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology, Cambridge, Massachusetts 02142.

The amphibian cement gland is a mucus-secreting epithelium that forms from extreme anterior ectoderm and that we have used to analyze positional cues in vertebrate ectoderm. We have proposed that the overlap of anterodorsal identity (AD), ventrolateral identity (VL), and ectodermal outer layer identity (EO) results in determination of the cement gland primordium (A + VL + EO = CG). Anterior identity is conferred by the transcription factor *Otx2*. We are constructing the genetic hierarchy that directs cement gland formation by working forward from *Otx2* and backward from cement gland terminal differentiation markers. Using hormone-inducible fusion proteins, we have shown that the homeodomain genes *pitx1* and *pitx2C* lie immediately downstream of *Otx2*. While *pitx* genes can activate terminal differentiation markers in the cement gland, they are unable to do so directly, but require intervening gene expression. To connect terminal differentiation with more upstream genes, we have asked what *cis*-acting elements direct expression of the *Xag1* (Wardle et al., Development 2002, 129, 4387-4397) and *gob4* genes to the cement gland. We find that multiple factors are involved in this process and that members of the ATF/CREB transcription factor family appear to be pivotal.

186. **Arx Expresses During Forebrain Patterning in Early *Xenopus* Development.** M. Wolanski, F. Khosrowshahian, M. Downorowicz, and M. J. Crawford. University of Windsor.

Vertebrate brain development is a multistep process involving a tight regulation of gene expression. The aristaless-related homeobox gene (ARX), which is expressed in the vertebrate central nervous system, specifically in the developing forebrain and floor plate, has been implicated in the differentiation and maintenance of specific neuronal cell types in the human brain. Recently it has been shown that mutations in human ARX are responsible for several classes of X-linked mental retardation syndromes, including Partington syndrome, which is characterized by dystonic movements of the hands and mental retardation. We have cloned the *Xenopus laevis* homolog of ARX and characterized its early spatial expression. xARX shows high similarity at the amino acid level with its homologues in zebrafish, mouse, and human. xARX first expresses in the anterior neural plate and is later localized to the developing forebrain, where it persists in brain regions including the medial and ventral telencephalon, medial diencephalon, and ventral mesencephalon. Overexpression of xARX in *Xenopus* embryos leads to anterior head reduction, brain abnormalities, along with abnormal eye phenotypes, including ectopic eye structures, and retinal extension evident by pigmented optic nerve. These results suggest that xARX may play a role in the developing forebrain and may be involved in normal eye development.

187. **The Regulatory Mechanism of Posterior Neural Specific Gene *FoxB1*.** D. H. Cai H. and Sive. Whitehead Institute.

Neural patterning starts from the onset of gastrulation. Based on the activation/transformation model, dorsal ectoderm would receive a signal from the anterior dorsal mesendoderm that determines both anterior position and neural tissue identity ("activation"). Subsequently, a posteriorizing factor that confers positional information would be released from the more posterior mesoderm which converts part of the tissue previously induced as anterior neural to more posterior neural fates ("transformation"). *FoxB1* is a winged-helix transcription factor, which is

expressed in the midbrain, hindbrain, and spinal cord, but not in the forebrain. Therefore, deciphering the regulatory mechanisms of this gene will provide insights to understand posterior neural development and patterning. A 7.2-kb promoter was isolated by screening a *Xenopus laevis* genomic library. This promoter is capable of driving luciferase expression comparable to endogenous FoxB1. Animal cap assay showed that Combining Noggin and fgf3 strongly upregulates FoxB1 expression with no mesoderm induction. Luciferase activity assay showed that the 7.2-kb promoter could drive luciferase expression with Noggin and fgf3 injection, suggesting this method is usable in analyzing this promoter. Further work will be focused on (1) looking for the Noggin and fgf responsive elements on this promoter, (2) deciphering the transduction pathway of fgf signaling functioning on this promoter, and (3) studying the mechanism of Noggin and fgf synergy.

188. **Induction and Function of *Xenopus* SIX1 in Cranial Placodes.** Samantha A. Brugmann, Petra D. Pandur, and Sally A. Moody. The George Washington University, Washington, D.C.

Cranial placodes are epidermal thickenings that form the major sensory organs of the head. There are two models to explain placode formation. The gradient model postulates that placodes form in a region where there is a specific concentration of neural inducers (e.g., Noggin and Chordin). The boundary model postulates that along with the neural inducer gradient, other epidermal factors are necessary to initiate placode development. *Xenopus* Six1 is expressed in the undifferentiated, superficial layer of neurogenic placodes. We hypothesize that placode formation is induced via the boundary model and that Xsix1 functions to establish/maintain the placodal progenitor cell population. To determine if XSix1 induction is in response to a neural inducer gradient, we created a gradient by overexpressing neural inducers in ventral blastomeres. Ectopic expression of XSix1 was only observed if a secondary axis formed and was restricted to the most anterior pole, suggesting that additional factors are required. Inhibition of Wnt signaling further expanded the Xsix1 domain only in the presence of a neural axis, confirming that a simple neural inducer gradient is insufficient to promote proper placode formation. Because Xsix1 is expressed in undifferentiated placodal cells, and not in the differentiating derivatives, we posited that Xsix1 maintains placodal cells in a stem-like state. In support of this hypothesis, genes that are involved in the early steps of neural differentiation and are expressed in the deeper, more differentiated cells of the placode (e.g., Sox2, Sox3, NeuroD, and Neurogenin) are downregulated in placodes in response to Xsix1 overexpression, (Supported by NIH Grant NS23158.)

189. ***Xenopus* Pitx3 Plays a Role during Eye Development.** F. KhosrowShahian, M. Wolanski, K. Fujiki, and M. J. Crawford. University of Windsor.

Pitx homeodomain proteins are highly conserved regulatory proteins that were first discovered on the basis of their involvement in the transcriptional regulation of pituitary-specific genes. Recently, a third member of the Pitx family, Pitx3, has been identified which expresses in the midbrain and eye. We have isolated a *Xenopus* Pitx3 homolog which plays a role in lens placode development. Pitx3 expression is first detected during early neurulation by in situ hybridization. Subsequently, the gene

undergoes its most intense period of expression through both the lens placode and the lens vesicle development but subsides shortly thereafter when lenses are fully differentiated. Overexpression or interference with Pitx3 function early in development has the effect of expanding the size of the preoptic field as assessed by the early eye marker genes. Interference with endogenous Pitx3 results in abrogation of eye formation and demonstrates that Pitx3 is critical to normal lens formation, and hence, to eye development. Furthermore, lens ectoderm transplantation studies suggest that although Pitx3 does not directly specify lens ectoderm, its early expression is required for the provision of cues which lead to the competence of lens ectoderm to respond to further lens inducing events.

190. **Protein Kinase CK2 Promotes WNT Signaling in *Xenopus* Embryos.** Isabel Dominguez,* Junko Mizuno,* Diane H. Song,* Karen Symes,† and David C. Seldin*. *Department of Medicine and †Department of Biochemistry, Boston University School of Medicine, Boston Massachusetts USA.

Protein kinase CK2 (casein kinase II), a conserved regulatory serine-threonine protein kinase, is highly expressed in cancer, and we have shown that transgenic overexpression of CK2 promotes tumorigenesis in mice. CK2 tumors usually upregulate β -catenin, the critical transcriptional cofactor for the Wnt signaling pathway. Wnts are growth factors involved in embryonic development, but inappropriate Wnt pathway activation occurs in many human cancers through mutation of β -catenin itself or of accessory molecules such as the adenomatous polyposis coli (APC) gene. We have found that CK2 associates with β -catenin and phosphorylates it on specific residues, and in reporter assays, CK2 upregulates β -catenin/LEF-1 transcription (Song et al., 2000). To analyze the regulation of β -catenin by CK2 in vivo, we used *Xenopus laevis* embryos, which exhibit asymmetric expression of β -catenin. Endogenous β -catenin is stable and upregulated in the dorsal side, while in the ventral side β -catenin is continuously targeted for degradation. A tagged β -catenin protein is more stable when injected dorsally than ventrally; coinjection of CK2 α and β along with β -catenin in the ventral side prolongs its half-life. β -catenin in which the primary CK2 phosphorylation site is mutated is degraded more rapidly than wild-type β -catenin. Overexpression of CK2 in the ventral side leads to dorsalization. Thus, CK2, which promotes tumorigenesis and activates the Wnt pathway in human and rodent cancers, appears to be an important regulator of Wnt signaling and axis determination in *Xenopus* embryogenesis.

191. **The Role of *Xenopus* Idax Gene in Noncanonical Wnt Signaling Pathway.** Tatsuo Michiue,* Kenji Sakurai,† Hiroki Kobayashi,† Akira Yukita,† Akimasa Fukui,* Akira Kikuchi,‡ and Makoto Asashima*. *Department of Life Sciences (Biology), Graduate School of Arts and Sciences, †Department of Biology, Graduate School of Science, University of Tokyo, ‡Department of Biochemistry, Graduate School of Medicine, Hiroshima University.

Wnt signaling pathway plays important roles in many developmental events. By now, Wnt pathway is thought to consist of three major subpathways; β -catenin-dependent pathway (Canonical pathway), PCP pathway (Noncanonical pathway), and Ca^{2+} -dependent pathway. Dishevelled (Dvl) is involved with both Canonical pathway and PCP pathway. So, it is suggested that

Dvl-associating factors are also implicated with both pathways. Idax, identified as one of Dvl-binding proteins, is conserved with many species such as human, rat, mouse, and frog. Previous reports showed that Idax interacts with Dvl via PDZ domain and inhibits the canonical Wnt pathway. Here, we report the role of *Xenopus Idax* gene in noncanonical pathway. Dorso-animal injection of xIdax mRNA caused short and shrimp-like axis, which was often seen in the case of PCP pathway-related gene such as dominant-negative form of xWnt11 and Dishevelled. Therefore, it was suggested that xIdax was involved with PCP pathway and affected the gastrulation movement. Animal cap of xIdax injected embryo treated with Activin showed no elongation, which also supports the involvement of Idax with PCP pathway. Interestingly, these defects were seen not only by overexpression of xIdax but by down-regulation with morpholino antisense oligo for xIdax. This result suggests that the adequate level of Wnt signaling is required for the precise gastrulation.

192. **Investigating TGF- β Proteins during the Induction and Patterning of Mesoderm in *Xenopus laevis*.** P. Huw Williams and James C. Smith. Wellcome Trust/Cancer Research UK Institute, University of Cambridge, United Kingdom.

Members of the transforming growth factor type beta (TGF- β) family regulate diverse aspects of growth and development in multicellular organisms. Interference with TGF- β signaling contributes to diseases such as cancer, neurodegenerative disorders, and autoimmune syndromes. Understanding the functions of this family of proteins, and the means by which they exert their effects, is therefore crucial to understanding development, growth, and disease. The structures and functions of the proteins in this family are highly conserved between organisms, and we study the roles of TGF- β proteins during mesoderm formation and patterning in *Xenopus laevis*. We are using two experimental approaches in an effort to understand the roles of TGF- β proteins in early embryonic patterning. Both involve the family members activin, derriere, and nr2, all of which are candidate ligands for a mesoderm-inducing signal that acts in early embryogenesis. In one series of experiments we use real-time RT-PCR to assess whether these three factors, all of which are believed to signal through Smad2, exert different effects on gene expression in animal cap tissue. We discuss both concentration dependent and temporal regulation of target gene expression and present evidence of ligand-specific gene regulation. The second series of experiments investigates the mechanisms used by these molecules to exert their effects over multiple cell diameters. Using conventionally tagged and fluorescent forms of the three TGF- β proteins, we can analyze their subcellular and supracellular distributions during inductive processes.

193. **Inhibition of Mesodermal Fate by *Xenopus* HNF3 β /FoxA2.** Tomomi Haremaiki, Crystal Suri, and Daniel C. Weinstein. Department of Pharmacology and Biological Chemistry, Mount Sinai School of Medicine, New York, New York.

The winged helix transcription factor HNF3 β /FoxA2 is expressed in embryonic organizing centers of the gastrulating mouse, frog, fish, and chick. In the mouse, HNF3 β is required cell-autonomously for the formation of the mammalian node and notochord and can induce ectopic floor plate formation when misexpressed in the developing neural tube; HNF3 β expression in

the extraembryonic endoderm is also necessary for the proper morphogenesis of the mammalian primitive streak. In the frog *Xenopus laevis*, several lines of evidence suggest that the related winged helix factor Pintallavis functions as the ortholog of mammalian HNF3 β in both axial mesoderm and neurectoderm; the role of *Xenopus* HNF3 β itself, however, has not been clearly defined, and is the subject of this study. HNF3 β is widely expressed in the vegetal pole but, as previously suggested, is excluded from the mesodermal component of the anterior endomesoderm. We find that constitutive activation of HNF3 β target genes inhibits mesoderm formation in *Xenopus*, both in explant assays and in vivo. Conversely, endodermal expression of an HNF3 β >—Engrailed repressor fusion protein induces ectopic axes and inhibits head formation. Our studies suggest that HNF3 β target genes function to limit the extent of mesoderm formation in the *Xenopus* gastrula, and point to related roles for *Xenopus* HNF3 β and the extraembryonic component of mammalian HNF3 β during vertebrate gastrulation.

194. **Dkk1 Induces Heart by Stimulating a Diffusible Intermediary Factor.** Ann Foley and Mark Mercola. The Burnham Institute and Department of Pathology, UCSD School of Medicine.

Misexpression of a single factor, the Wnt antagonist Dkk1, in noncardiogenic mesoderm of chicks or frogs induces beating heart tubes. These and other studies suggest that inhibition of the canonical Wnt signaling pathway plays a fairly direct role in determining the heart field. In amphibians the Organizer provides Dkk1 and other Wnt antagonists but embryological studies suggest that an additional signal is required from the endoderm underlying the cardiac mesoderm. Here we asked whether Dkk1 might act on the endoderm to stimulate production of the second factor. To answer this question, we found that two cell-autonomous inhibitors of Wnt/ β -catenin signaling, gsk3 β and dominant negative TCF3, induced heart tissue up to several cell diameters from the source of action. This non-cell-autonomous induction is consistent with a model in which Wnt antagonists stimulate the production of an intermediary heart-inducing factor, perhaps within the endoderm. In support of this model, we found that Cerberus, Cer, and Hex, which are expressed in the dorsal endoderm at relevant stages, upregulate expression of the cardiac markers Tbx5 and Nkx2.5. Epistasis experiments show that Dkk1 and Cer converge on the transcriptional repressor Hex. Since a dominant active Hex blocks both endogenous heart formation and the ability of Dkk1 to induce hearts, we propose that Hex is an effector of Dkk1 signaling and regulates expression of an unknown diffusible heart-inducing protein. As Hex and Cer also mark heart-inducing tissue in the mouse, these results suggest that the signaling "cassette" responsible for heart induction is conserved among vertebrates.

195. **Endogenous Cerberus Activity Is Required for Anterior Head Specification in *Xenopus*.** Ana Cristina Silva,*¹ Mario Filipe¹, Klaus-Michael Kuerner,[‡] Herbert Steinbeisser,[‡] and Jose Antonio Belo^{8,†}. *Instituto Gulbenkian de Ciencia, R. da Quinta Grande, 6, Apartado 14, 2781-901 Oeiras, Portugal; [†]F.E.R.N. Universidade do Algarve, Campus de Gambelas, 8000-010 Faro, Portugal; [‡]Institute for Human Genetics, University of Heidelberg, Im Neuenheimer Feld 328, Germany. (1 Equal contributing authors.)

The function of *Xenopus* Cerberus in the Anterior Dorsal Endoderm (ADE) and its requirement in head specification have not yet been conclusively demonstrated. To gain further insight we designed an antisense morpholino oligonucleotide (CerMO), which efficiently inhibited the induction of secondary head-like structures generated by microinjection of *cerberus* mRNA. Surprisingly, embryos injected dorsally with this morpholino developed no significant defects. Using an ADE-specific promoter, targeted increase of BMP, Nodal, and Wnt activities resulted in synergistic loss of anterior head structures, without affecting the axis. Remarkably, this phenotype was aggravated when the *cerberus* translation was inhibited by the morpholino. Using conjugates of DE/ADE explants, we could demonstrate the requirement of Cerberus in the ADE for the proper induction of anterior neural markers and repression of more posterior ones. Our findings demonstrate the role of Cerberus as a biological inhibitor of BMP, Nodal, and Wnt activities and provide support for an endogenous requirement of Cerberus activity in the leading edge of the ADE for the correct induction and patterning of the neuroectoderm.

196. **Inhibition of Multiple Primitive Streak Formation.** Federica Bertocchini and Claudio Stern. Department of Anatomy, UCL, London, United Kingdom.

In the chick embryo, Vg1, a member of the TGF β family of secreted molecules, can induce an ectopic primitive streak via a cascade of gene activation that includes Chordin, Nodal, and FGF8 (Shah et al., 1997; Skromne and Stern, 2001, 2002; Bertocchini and Stern, 2002). When misexpressed in the anterior marginal zone, Vg1 induces a primitive streak that coexists with the original axis. If Vg1 is placed at 90 degrees from the posterior region, it still induces an axis but the original primitive streak is inhibited. This suggests the presence of an inhibitor, ensuring the formation of only a single axis in the blastoderm. How fast does this putative inhibitor travel, and at which level of the signaling cascade does it act? When Vg1-secreting pellets are placed on opposite lateral sides of the embryo, both induce an axis. However, if the pellets are grafted sequentially (the second 6 hours after the first), only the first one initiates formation of an axis. This experiment suggests that the putative inhibitor takes a maximum of 6 hours to travel the 3 mm distance to the opposite lateral position in the embryo. We investigated at which level of the cascade the inhibition occurs. If we graft Vg1 on one side and Vg1 plus Chordin or Nodal on the other side after 6 hours, an ectopic primitive streak is induced also from the second graft. By contrast, neither Chordin nor Nodal alone can induce an axis 6 hours after a Vg1 graft on the opposite side. This result suggests that the inhibition takes place downstream of Vg1 but upstream of Chordin and Nodal. In addition, since Vg1 induces an ectopic axis together with Chordin or Nodal, but it is not sufficient, the results suggest that a second molecule is induced by Vg1 to bypass the inhibitory step (Bertocchini and Stern, 2002, *Dev. Cell.* 3, 735-744; Shah et al., 1997, *Development* 124, 5127-5138; Skromne and Stern, 2001, *Development* 128, 2915-2927; Skromne and Stern, 2002, *Mech. Dev.* 114, 115-118.)

197. **Inhibition of Differentiation by Chondroitin Sulfate in the Early Chick Embryo.** Satinder Sidhu, Ethan Richard, and David R. Canning. Department of Biological Sciences, Murray State University, Murray, Kentucky.

The central area pellucida epiblast (CAPE) region of the early chick blastoderm gives rise to all subsequent embryonic tissues. At gastrulation it produces axial mesoderm and endoderm under instructive influences from the marginal zone. Later, the anterior CAPE forms the head and anterior CNS due to factors secreted in the prechordal plate. However, the actual cellular mechanisms allocating epiblast cells to specific fates are largely unknown. We have examined the state of specification of CAPE cells from the onset of gastrulation through the definitive head stage. By extirpating CAPE tissue from various stages, we have performed isolation experiments designed to map the time course of the degree of commitment of epiblast to specific cell fates and morphogenetic processes. Our studies have revealed specific roles for molecular components of the extracellular matrix in the allocation of cell fates during gastrulation and head formation. One of these components, the chondroitin sulfate (CS), appears to suppress certain cell fates relative to others. In the pre-gastrula, CS inhibits N-cadherin expression and muscle differentiation, while promoting notochord differentiation. Following gastrulation, CS assists in the formation of head structures from the prechordal plate region. As the head develops, CS delineates nervous tissue areas from mesenchymal tissues. Isolates from the anterior CAPE fail to form head vesicles if CS is experimentally removed during culture, and the delineation of the separate tissues is lost. This mechanism appears to be mediated by suppression of N-cadherin activity during head development.

198. **A Fate Map of the Endoderm of the Early Chick Embryo.** W. Kimura, S. Yasugi, and K. Fukuda. Department of Biological Science, Tokyo Metropolitan University, Tokyo, Japan.

The gut of the vertebrate is divided morphologically and functionally into various organs along the anteroposterior axis during embryogenesis. A previous report shows that the endoderm of 1.5-day-old chicken embryo, which gives rise to the epithelial lining of the digestive tract, already has regional difference, and segregation of stomach/intestinal fates occurs until this stage. The exact timing and the molecular nature involved in the regionalization of the endoderm is still unclear. To approach these questions, the precise fate maps of the endoderm at the early stage of the embryo are essential. In this study, we established a method to label lower layer cells specifically using DiI and traced the fate of labeled cells of the gastrulation-stage (H.H. stage 3-5) chick embryo until embryos reached 1.5 days old (H.H. stage 11-12). At stage 3, the labeled cells which contributed to the embryonic endoderm were found in the lower layer. These cells were in the restricted domain around the primitive streak, and all of them contributed to hindgut endoderm. At stage 4, the area in the lower layer which becomes endoderm expanded around the Hensen's node, and cells which contributed to the dorsal foregut endoderm were found. From stage 4⁺-5, there were the cells which give rise to the ventral foregut endoderm in the lateral side of the head process. These results suggest that the endoderm progenitor cells ingress into the lower layer from stage 3 to stage 4⁺-5, and each cell has different regional fate in the gut depending on the timing of the ingression.

199. **Hypoblast and Anterior Definitive Endoderm Pattern Anterior Identity in Early Chick.** Susan Chapman, Frank Schubert, Gary Schoenwolf, and Andrew Lumsden. MRC Centre for Developmental Neurobiology, King's College, London,

United Kingdom; and Neurobiology and Anatomy, University of Utah, Utah USA.

We demonstrate a role for the chick hypoblast and anterior definitive endoderm (ADE) in patterning anterior neuroectoderm at early developmental stages. Previous studies of head induction in the chick have failed to demonstrate a clear role for these tissues in the overlying ectoderm, whereas data from both mouse and rabbit suggest patterning roles for anterior visceral endoderm (AVE) and ADE. The chick hypoblast has been suggested to be the homologue of the mouse anterior visceral endoderm. In support of this, the rabbit AVE when transplanted to chick embryos induces anterior markers in the chick epiblast. To re-evaluate the role of the hypoblast/ADE (lower layer) in patterning the chick ectoderm, we used rostral blastoderm isolates (RBIs) as an assay, that is, rostral portions of the blastoderm transected at levels anterior to the node. RBIs are therefore free from the influences of Hensen's node and ingressing axial mesoderm tissues that are able to induce *Ganf*, the earliest specific marker of anterior identity in neural plate. We demonstrate, using such RBIs (or RBIs dissected to remove the lower layer with or without tissue replacement), that the hypoblast/ADE is required and sufficient for patterning anterior positional identity in the overlying ectoderm, leading to expression of *Ganf* in neuroectoderm. Our results demonstrate, for the first time, that patterning of anterior positional identity and specification of neural identity are separable events operating to pattern the rostral end of the early chick embryo.

200. Serotonin Is a Novel Very Early Signaling Mechanism in left—right Asymmetry. Takahiro Fukumoto and Michael Levin. The Forsyth Institute.

Our previous work indicated that long-range signaling between the left and right sides of vertebrate embryos is an obligate, early aspect of embryonic left—right (LR) asymmetry. Pursuing the molecular characterization of these signals, we conducted a pharmacological screen which identified serotonin (5HT) as a novel element of the LR pathway. Out of the several dozen gene products which participate in serotonergic signaling, receptor subtypes 3 and 4, the 5HT transporter, and the 5HT degrading enzyme (MAO) are required for correct LR asymmetry in chick and frog embryos. Inhibition of any of these proteins prior to the blastula stage in frog or primitive streak formation in chick specifically induces randomization of the heart and viscera (heterotaxia), as well as of the sidedness of early asymmetrically expressed genes, in the absence of other defects. Immunohistochemical analysis reveals that 5HT and the implicated elements are present in eggs and embryos as maternal products (long prior to the appearance of neurons synthesizing 5HT). 5HT and the relevant proteins exhibit striking subcellular localization patterns indicative of novel intracellular “zip codes” and asymmetries at the earliest stages of development. Moreover, gain of function analysis reveals a previously unknown cytoplasmic site of serotonin activity in contrast to well-described cell membrane 5HT receptors. We are pursuing multiple approaches to characterize fully the dynamics of 5HT signaling and to test specific models by which 5HT-mediated signals carry LR information in the context of known early mechanisms controlling laterality.

201. Characterization of the Role of a Chick Claudin Family Member in Patterning the Left—Right Axis. Annie Simard

and Aimee K. Ryan. Montreal Children's Hospital Research Institute, McGill University, Montreal, Canada.

The classic pattern of organ positioning is highly conserved among vertebrates. Most of the internal organs in the body are unpaired and localized asymmetrically. This permits their efficient packing within the body cavity and is critical for their normal physiological function. In humans, defects in organ situs occur in at least 1/10,000 live births and often have severe consequences. Several components of the left—right patterning cascade are conserved in vertebrates, including the Nodal to *Pitx2* portion of the signaling cassette. *Pitx2* is asymmetrically expressed early in the left lateral plate mesoderm as well as in several organs that are positioned asymmetrically in the body. Molecules downstream of *Pitx2* are expected to be important for the relay of information from the lateral plate mesoderm to the organ primordia and for the morphogenetic events that will lead to the asymmetric development and the positioning of individual organs along the left—right axis. To identify molecules downstream of *Pitx2*, a subtractive screening approach has been used. One of the molecules identified in this study is a member of the claudin family of tight junction proteins. Studies in *Xenopus laevis* have shown that the overexpression of *Xcla* (*Xenopus laevis* claudin) causes laterality defects. We are currently examining the role of claudins in patterning the left—right axis in chick embryos and will present the results of these studies.

202. Antagonism of the SHH Pathway by the Opitz Syndrome Gene MID1: Patterning the Left—Right Axis and Ventral Midline. Alessandra Granata, Dawn Savery, and Nandita A Quaderi. King's College, London, United Kingdom.

Patterning the avian left-right (L/R) body axis involves the establishment of asymmetric molecular signals on the left and right sides of Hensen's node. We have examined the role of the chick homologue of the Opitz syndrome (OS) gene *MID1*, *cMid1*, in generating asymmetric gene expression in the node. *cMid1* is initially expressed bilaterally, but its expression is then confined to the right side of the node. We show that this restriction of *cMid1* expression is a result of repression by *Shh* on the left side of the node. Misexpression of *cMid1* on the left side of the node results in bilateral *Bmp4* expression and a loss of *Shh* expression. Correspondingly, downstream left pathway genes are repressed while right pathway genes are ectopically activated. Conversely, knocking down endogenous right-sided *cMid1* results in a loss of *Bmp4* expression and bilateral *Shh* expression. This results in an absence of right pathway genes and the ectopic activation of the left pathway on the right. Here, we present a revised model for the establishment of asymmetric gene expression in Hensen's node based on the epistatic interactions observed between *Shh*, *cMid1*, and *Bmp4*. We propose that the antagonistic relationship between *cMid1* and *Shh* extends beyond Hensen's node and that *cMid1* is a negative regulator of the SHH pathway. The hypertelorism characteristic of OS patients is reminiscent of that shown by patients with mutations in *PTC* and *GLI3*, contrasting with the hypotelorism displayed by patients with SHH mutations. We are currently overexpressing/knocking down *cMid1* in the axial mesoderm and studying the molecular and morphological consequences of these manipulations. We predict that knocking down *cMid1* will result in hypertelorism, while overexpressing *cMid1* will result in hypotelorism.

203. Abstract #203 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

204. Early Specification of Neural Crest in Gastrulating Chicken Embryos. M. L. Basch, M. Bronner-Fraser, and M. I. Garcia-Castro. California Institute of Technology, Pasadena, California.

In light of recent evidence suggesting that neural specification takes place earlier than previously thought, we decided to investigate the timing in the formation of the neural crest. We identified Pax-7 as the earliest marker for neural crest. In the chick, Pax-7 is expressed earlier and more anterior and dorsal than the previously described Pax-3. Based on its expression pattern, which correlates with the prospective neural folds as early as HH stage 4+, we speculate that Pax-7 might play an important role in the formation of neural crest. To further characterize Pax-7 domain of expression and its relation to the border between neural and nonneural ectoderm, we compared Pax-7 expression pattern to those of other early markers of prospective neural and epidermal fates. We then asked whether Pax-7 is responsible for the specification of the prospective neural crest. Unexpectedly, explant analysis revealed that the regionally restricted potential of the epiblast to generate neural crest precedes the expression of Pax-7. Our fate maps show that the tissue used in the specification assays gives rise to neural crest *in vivo*. Interestingly, this region of the embryo does not coincide with the border of the neural territory established by recent fate maps of the stage 4 epiblast. We are carrying out functional experiments using morpholino-mediated "knock-down" and overexpression studies to elucidate the role of Pax-7 in the formation of neural crest.

205. Neural Tube Signals Are Involved in Otic Vesicle Regionalization in Chick. F. Aragón,* E. Ulloa,* S. Cereghini,† B. Alsina,* F. Giraldez,* and C. Pujades*. *Developmental Biology Group, UPF, Barcelona, Spain; and †CNRS UMR7622 Paris, France.

The inner ear is a complex sensory organ responsible for the senses of hearing and balance in vertebrates. Development of inner ear starts with the formation of the otic placode within the surface ectoderm adjacent to the prospective hindbrain at the 3-5 somites stage. At this stage the hindbrain region of the central nervous system undergoes regionalization along the antero-posterior axis, a segmentation process that leads to the formation of 7-8 morphological bulges called rhombomeres. This process is under the control of specific segmentation genes. Gene inactivation in mice suggested that some of the genes that participate in hindbrain segmentation are also involved in the control of otic development. Recently, it has been shown that the transcription factor *vHNF1* may be one of those genes in zebrafish. The main focus of our research is the analysis of hindbrain molecular signals that may affect inner ear development. Particularly, we want to address which are the roles played by hindbrain segmentation genes—such as the transcription factor *vHNF1*—in the regionalization of the otic vesicle. With that purpose *mVHNF1* was ectopically expressed within the hindbrain by means of *in ovo* electroporation in the chick. *vHNF1* appears to control the expression profiles of *MafB*, *Krox20*, *Hoxb1*, and *Fgf3* in the neural tube. These alterations in the hindbrain segmentation cascade generate a loss of regionalized expression pattern of *Lmx1*

in the otic vesicle. These results support a model where the patterning of the otic vesicle depends on adjacent neural tube cues.

206. Targeted Nonviral Gene Delivery for Studying Neurogenesis in the Chick Embryo. Michael J. deCastro and Gary C. Schoenwolf. Department of Neurobiology and Anatomy and Children's Health Research Center, University of Utah, Salt Lake City, Utah.

Introducing genetic material into the embryo and analyzing the subsequent effects on embryogenesis has greatly aided investigation of the gene expression patterns that drive temporal and spatial development in the chick embryo. Recently, advancements in the field of gene delivery have led to the development of nonviral delivery strategies capable of nontoxic, high-efficiency *in vivo* gene transfer. We are developing a nonviral gene delivery system with cell-specific targeting that will allow highly specific localized gene expression for studying neural development in chick embryos. The targeted gene delivery system is based on a multicomponent peptide/noncationic lipid gene delivery system that has previously been shown to produce high levels of gene expression when injected into chick embryos *in vivo* (Longmuir et al., 2001, *Mol. Ther.* 4, 66-74). The delivery system consists of five components: plasmid DNA, a DNA condensing peptide, a fusogenic peptide that promotes endosomal escape, phosphatidylcholine, and polyethylene glycol conjugated to dioleoylphosphatidylethanolamine. Ligands that selectively bind the FGF receptor 2 are conjugated to the distal ends of the liposome-grafted polyethylene glycol chains, restricting genetic misexpression in the developing embryo to those cells that express FGFR2. Lipoplexes containing a GFP expressing plasmid with a β -actin promoter are injected into EC cultured chick embryos (HH stages 4-6) and transfection efficiency, expression level, and specificity of the system for the target cells is analyzed. (Supported by NIH Grant DC04185.)

207. Interrhombomeric Signaling and the Role of *r4* in Maintaining *EphA-4* Expression in *r5*. F. Cambroner, L. McNaughton, and R. Krumlauf. Stowers Institute for Medical Research, Kansas City, Missouri.

Although rhombomeres are considered autonomous developmental units, it has been demonstrated that interrhombomeric interactions are important in some aspects of their phenotype. We are interested in interactions between neighboring rhombomeric (*r*) segments to know how they affect aspects of segment-restricted gene expression. Using manipulative embryology approaches in avian embryos, we performed *r4* ablations, followed by segmental analysis of gene expression. Our results revealed that interrhombomeric interactions influence the expression pattern in a stage-specific manner. In host embryos of 10 somites or greater there were no changes in the segmental pattern of *Krox-20*, *EphA-4*, and *kreisler* in *r3* and *r5* following *r4* ablation. In embryos of less than 10 somites we found a downregulation of *EphA-4* in *r5*. To further investigate this effect on *EphA-4* and examine the specificity of the signal, we performed heterotopic chick/chick transposition placing *r2*, *r3*, or *r5* in the original position of *r4*. The results with *r3* and *r5* showed a similar time- and stage-dependent effect on *EphA-4* expression as revealed by *r4* ablation, that is, *EphA-4* was specifically downregulated in the host *r5* in embryos of less than 10 somites. In the case of *r2*

transposition the experiments show that the expression of *Epha-4* is maintained in r5. In conclusion, this study uncovered the presence of signals in r4 important for the maintaining of *Epha-4* in r5 that cannot be replaced with another odd rhombomere but may be with an even one. This highlights the importance of interrhombomeric signals in maturation of these segmental units.

208. **Regulating Fgf8 Expression at the Isthmus.** Claire A Canning, Carol Irving, and Ivor Mason. MRC Centre for Developmental Neurobiology, Kings College, London, United Kingdom; and Department of Anatomy, University College, London, United Kingdom.

The midbrain—hindbrain boundary, or isthmus, is a source of signals that are responsible for specification of both the midbrain and the anterior hindbrain. Fibroblast growth factor 8 (*Fgf8*) is expressed specifically at the isthmus and has been shown to be an important component of the isthmus organizer (*IsO*) patterning signal. *FGF8* is induced by a direct interaction between midbrain and anterior hindbrain (rhombomere 1) tissue and has been suggested to involve a diffusible signal. However, the molecular nature of this induction and/or maintenance of *FGF8* remain uncharacterized. Several members of the Wnt family of secreted factors, most notably *Wnt1*, are expressed immediately anterior to the *Fgf8*-positive domain at the isthmus and are therefore potential candidates for this inducing activity. We will present data from experiments using the chick neural system that address the identity of signals that regulate *Fgf8* at the isthmus.

209. **A Role for BMPs in the Formation of the Ventral Hypothalamus.** Liz Manning, Kyoji Ohyama, Sandrine Soubes, and Marysia Placzek. Centre for Developmental Genetics, University of Sheffield, United Kingdom.

The vertebrate hypothalamus develops in the ventral-most regions of the diencephalon, in part arising from cells that share a lineage with more posterior floor plate cells. Previous studies have shown that cells that populate the ventral midline of the chick hypothalamus coexpress the bone morphogenic proteins *BMP2* and *BMP7*. However, their role remains unclear. In this study we analyze the effects of BMPs in the differentiation of this region. Fate mapping and immunolocalization studies demonstrate that the hypothalamic ventral midline cells initially coexpress *BMP2*, *BMP7*, and *Sonic hedgehog* (*Shh*) at HH stage 10, followed by a transient down-regulation of BMPs at HH stage 13, during which *Shh* remains expressed. Subsequently, by HH stage 15, these midline cells reexpress BMPs and down-regulate *Shh* expression. Here we demonstrate using *in vivo* and *in vitro* approaches that both *BMP2* and *BMP7* can down-regulate *Shh* within these cells. Furthermore, our *in vivo* analysis demonstrates a correlation between transient down-regulation of BMPs at HH stage 13 and a synchronised reentry into cell cycle, which appears to result in a dramatic expansion of this ventral progenitor domain. Our analysis suggests a model in which expression of BMPs have two effects on hypothalamic ventral midline cells: first a transient arrest of cell cycle, and second a down-regulation of *Shh*. Our studies therefore suggest that BMPs simultaneously control both the character and the spatial domain of the ventral hypothalamus.

210. **Anterior—Posterior Limb Position Is Dependent on the Relative Timing of Key Embryonic Events.** Tyler C. Prestwich,* Trent D. Stephens,† and Michael R. Stark*. *Brigham Young University, Provo, Utah; and †Idaho State University, Pocatello, Idaho.

To better understand embryonic events leading to the determination of limb position along the anterior—posterior (A-P) axis, we compared pre-limb bud stage developmental morphology of three vertebrate organisms: chicken, goose, and swan. These organisms were selected because of the differences each exhibit with regard to the A-P location of the forelimbs, or more specifically, the number of cervical vertebrae. By observing disparities between the embryonic morphologies of the different species, we were able to identify specifically where morphological distinctions in A-P patterning arise. An overall observation stemming from this analysis was that there were significant differences in the number of somites at specific morphological stages of development when comparing organisms with a different number of cervical segments (i.e., a swan with the three primary brain vesicles clearly visible exhibited 20+ pairs of somites, while a chicken with similar head morphology exhibited 10 pairs of somites). This developmental delay in morphology compared to somite number corresponds very well with differences in the number of cervical vertebrae. We sought to confirm morphological differences and expand our understanding of these deviations with molecular data. Spatial and temporal expression patterns of molecular markers of differentiation—*MyoD*, *Hox* genes, and others—were evaluated. Based on the data obtained, we hypothesize that the relative timing of key embryonic events is critical in determining A-P limb position.

211. **The Mouse Gene Expression Database (GXD): A Resource for Developmental Biologists.** C. M. Smith, D. A. Begley, J. T. Eppig, J. H. Finger, T. F. Hayamizu, D. P. Hill, J. A. Kadin, I. J. McCright, J. E. Richardson, and M. Ringwald. Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, Maine 04609, USA.

The Gene Expression Database (GXD) collects and integrates gene expression information about the developing mouse. By combining diverse types of expression data, GXD provides information about the expression profiles of transcripts and proteins in different mouse strains and mutants, thus enabling insights into the molecular networks underlying developmental and disease processes. Expression patterns are described using an extensive dictionary of standardized anatomical terms, making it possible to record expression results from assays with differing spatial resolution in a consistent manner. GXD is integrated with the Mouse Genome Database, as well as other community databases, to include expression data in a larger biological and analytical context. Our data are primarily acquired from the literature by our curators. However, we have developed the Gene Expression Notebook (GEN) to facilitate the direct submission of data to GXD. GEN functions as a notebook to store and organize expression data and images in the laboratory. It also allows the researcher to select expression data, such as data that due to space constraints in written publications would merely be referred to in text summaries, and submit it relatively effortlessly to GXD. Capturing these data will further enhance GXD's utility as a community resource. GEN is available at <http://www.informatics.jax.org/mgihome/GXD/GEN/>. GXD is accessible through the Mouse Genome Infor-

matics web site at <http://www.informatics.jax.org/>. GXD is supported by NIH Grant HD 33745.

212. Withdrawn

213. **Cdx2 and Eomesodermin Are Essential for the Establishment of the Trophoblast Lineage in the Mouse Embryo.** Dan Strumpf, Chai-An Mao, and Janet Rossant. Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, 600 University Ave., Toronto, ON, Canada M5G 1X5.

The first differentiation event in the mammalian embryo leads to the formation of the ICM and the trophectoderm. The trophectoderm mediates embryo implantation, and later it will contribute to extraembryonic tissues of the trophoblast lineage and eventually to the embryonic portion of the placenta. To dissect the molecular mechanisms that underlie trophoblast lineage establishment, we have studied two trophoblast-specific transcription factors, *Cdx2*, a mouse homologue of *Drosophila* Caudal, and *Eomesodermin* (*mEomes*), a T-box transcription factor. Expression of both is detected in the preimplantation embryo and is specific to the trophoblast from the blastocyst stage. Analysis of homozygous mutants for either of the genes reveals distinct lethal peri-implantation phenotypes: *Cdx2* mutant blastocysts do not expand, do not hatch from the zona pellucida, and fail to implant. Marker analysis indicates that trophectoderm differentiation is faulty in these mutants; *mEomes* mutant embryos implant, but do not express markers indicative of further trophoblast differentiation, and their embryonic development halts. When mutant blastocysts for either of the genes are cultured in vitro, they fail to form an outgrowth, do not express markers of differentiated trophoblast, and trophoblast stem (TS) cell lines could not be derived from these mutants. We conclude that *Cdx2* is a primary factor, essential for specifying the trophoblast lineage, before implantation, whereas *mEomes* is required for further differentiation and the establishment of the trophoblast lineage after implantation.

214. **Cell Movements in the Prestreak Stage Mouse Embryo Epiblast.** Elizabeth M. Morin-Kensicki, Jaime A. Rivera-Perez, and Terry R. Magnuson. Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7264.

We are interested in how cell movements of the mouse embryo contribute to proper development. The emergent model for mouse body axis development relies on events occurring in the proximal region of the egg cylinder at prestreak stages of development. Expression domains for several genes occur as rings in prestreak proximal epiblast that later resolve to the future posterior side of the egg cylinder where the streak forms. In addition, a distal visceral endoderm population shifts toward the future anterior side of the embryo to occupy the anterior visceral endoderm domain. The emergent model thus describes a rotation of a proximodistal axis to give rise to the anteroposterior axis of the embryo. Yet unknown is whether cell movements or cell-cell signaling events alone mediate the restriction of proximal epiblast expression domains to the future posterior side of the egg cylinder. We used vital intracellular labeling techniques in mouse embryos cultured from prestreak (E5.75) to periprimitive streak stages to analyze epiblast cell movements during these stages of

development. We discuss our results relative to development in other vertebrates.

215. **Relationship of Blastocyst Axis to First Cleavage Plane in Mouse Development.** Vernadeth B. Alarcón and Yusuke Marikawa. University of Hawaii, Institute for Biogenesis Research, Hawaii.

Cell division patterns generate asymmetries that are critical for cell fate specification and body axis formation in embryos. Mammals were thought to be exempt from this view, but recent reports suggested that the embryonic-abembryonic (Em-Ab) axis of the mouse blastocyst is orthogonal to the first cleavage plane of the two-cell embryo. To test the universality of this relationship, embryos of two different genotypes, F1 (C57BL/6xDBA/2) and CD-1, were examined. The position of the first cleavage plane was determined by labeling a blastomere with DiI at the two-cell stage, and by monitoring the boundary between fluorescent and nonfluorescent cells in the blastocyst. Blastocysts were sorted into two groups, depending on the angular departure of the first cleavage plane from the plane of the blastocoel cavity floor, which lies perpendicular to the Em-Ab axis. The blastocyst was scored as "orthogonal" when the departure was $\leq 30^\circ$ and "deviant" when $> 30^\circ$. About one-quarter of the blastocysts was categorized as orthogonal, and the remainder was deviant. Thus, the orthogonal orientation of the Em-Ab axis to the first cleavage plane is not prevalent in these genotypes. We then tested whether the angular relationship between the Em-Ab axis and first cleavage plane influence the postimplantation development. We separated the orthogonal from deviant blastocysts and transferred the two groups into surrogates to examine fetuses in late gestation. Both types of blastocysts produced normal fetuses at a similar frequency. Thus, the relationship of the Em-Ab axis to the first cleavage plane does not significantly influence later development.

216. **The *Nodal*/*Smad2* Pathway Patterns Anterior Streak Derivatives in the Developing Mouse Embryo.** S. Vincent and E.J. Robertson. Molecular and Cellular Biology Department, Harvard University, Cambridge, Massachusetts USA.

In mouse, initial anterior identity is imposed on the pregastrulation epiblast by the anterior visceral endoderm (AVE) that overlies the prospective anterior side of the epiblast. As gastrulation proceeds, the AVE is displaced proximally and patterning of the neuroectoderm is assumed by the anterior streak derivatives including the definitive endoderm (DE), prechordal plate (PCP), and notochord. *Nodal*, a TGF β family member is required to specify the initial proximodistal polarity. In the VE, the effector *Smad2* relays *Nodal* signals to promote formation of the AVE. However, the precise function of *Smad2* in the epiblast is poorly defined. To evaluate the specific contributions made by individual components of the *Nodal* pathway in the patterning of the primitive streak (PS), we use two genetic strategies to remove *Smad2* function in the early epiblast and to lower *Nodal* signaling in the posterior epiblast and PS. In both classes of mutant embryos the PS forms and elongates normally; mesoderm formation is unaffected and the AVE is correctly induced and rotates properly. However, the distal population of the streak that includes the gastrula organizer is not patterned properly. As a result, specification of the DE and PCP is affected leading to the loss of identity of the most anterior structures of the brain.

Moreover the depletion of DE progenitors leads to incomplete displacement of VE cells and formation of a primitive gut tube that is a mixture of DE and VE derivatives. Collectively our data suggest that dose-dependent *Nodal* signaling via the Smad2 effector controls the allocation of PCP and DE during gastrulation.

217. Abstract #217 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

218. **Zic3 Is Critical for Early Embryonic Patterning and the Maintenance of Symmetry during Gastrulation.** Stephanie M. Ware, Karine G. Harutyunyan, and John W. Belmont. Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas.

Mutations in the zinc finger transcription factor ZIC3 are associated with human left–right patterning abnormalities (X-linked heterotaxy, HTX1, MIM 306955), and mice null for *Zic3* show a similar phenotype. Analysis of *Zic3* function at early embryonic stages shows that it ensures the fidelity of early embryonic patterning, including the initiation of gastrulation and positioning of the primitive streak. *Zic3*-deficient mice can develop axial duplications, indicating a role for *Zic3* in suppressing ectopic primitive streak formation. In addition, these analyses demonstrate a previously unrecognized requirement for the maintenance of symmetry during the migration and allocation of cells to the primitive streak during gastrulation. These studies identify a novel role for *Zic3* in the proper patterning of the primitive streak in the gastrulation stage embryo.

219. **Screening for Genes Involved in the Establishment of the AP Axis in the Mouse.** Maria-Elena Torres-Padilla,* Stephen Frankenberg,* Roger A. Pedersen,† and Magdalena Zernicka-Goetz*. *The Wellcome Trust/Cancer Research UK Institute and Department of Genetics; and †Department of Surgery, University of Cambridge, Cambridge, United Kingdom.

The anterior–posterior (AP) axis of the mouse embryo is clearly set up by the time of gastrulation, embryonic day 6.5 (E6.5): the primitive streak forms at the future posterior and the anterior aspect of the embryo becomes established about the same time at the opposite site of the embryo. Before this time the embryo exhibits a proximodistal asymmetry defined by the abembryonic and embryonic regions, respectively. “Rotation” of the proximodistal axis will lead to the future AP axis. At E5.5, a subpopulation of the most distal visceral endoderm (VE) cells moves to one side of the proximal region of the embryo. This region corresponds to the future anterior of the embryo and cell migration is essential for its definition. Migrating cells express *Hex* and *Cer-1* such that their expression in the VE shifts from distal to anterior. Concomitantly, the activity of proximally expressed genes, such as *Wnt3* and *Fgf8*, shifts toward the future posterior of the epiblast. Beyond these findings, the molecular basis for the changes in gene expression pattern and initiation of AP polarity in the mouse is by and large poorly understood. To gain a better understanding of the molecular mechanisms underlying the establishment of the AP axis, a gene screening approach was designed to identify genes involved in this process. Preliminary results will be presented.

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221. Abstract #221 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

222. **Mesoderm Induction by the BMP, FGF, and Nodal Pathways.** Diana H. Lee,* David Green,* William S. Talbot,† and Alexander F. Schier*. *Developmental Genetics Program, Skirball Institute of Biomolecular Medicine, Department of Cell Biology, NYU School of Medicine, New York, New York 10016, USA; and †Department of Developmental Biology, Stanford School of Medicine, Stanford, California 94305.

Nodals are essential for the induction of endoderm and head and trunk mesoderm, but not for the formation of tail mesoderm. Accordingly, mesodermal genes such as *ntl/T*, *spt*, and *tbx6* are still expressed at the ventral–lateral margin in the absence of Nodal signaling. It has been unclear how this mesodermal domain is induced. BMPs and their antagonists have been implicated in dorsal–ventral patterning, but mesoderm induction is normal in the absence of BMP signaling. To test for potential overlapping roles of BMP and Nodal signaling in induction of mesodermal genes, we generated embryos that inactivate both signaling pathways. We find that *ntl/T*, *spt*, and *tbx6* are no longer expressed in these embryos, revealing redundant roles of BMP and Nodal signaling in mesoderm induction. FGFs have also been found to be important for mesoderm formation. However, mesoderm induction is normal in the absence of FGF signaling alone. In the absence of both FGF and Nodal signaling, however, there is no mesoderm induction. Further experiments suggest that the BMP, FGF, and Nodal pathways work in parallel in mesoderm induction and that each of these pathways are sufficient, but not required, to induce mesodermal genes.

223. **Characterizing the Cranial Mesoderm in Early Mouse Development.** Kristin Melton, Karin Zueckert-Gaudenz, and Paul A. Trainor. Stowers Institute and Children's Mercy Hospital, Kansas City, Missouri.

Classically, cranial neural crest cells (NCC) were thought to be preprogrammed prior to their emigration. However, recent evidence demonstrates that cranial NCC are plastic and that their migration and differentiation are influenced by the mesodermal, ectodermal, and endodermal tissues they contact. Little is known about the cranial mesoderm that NCC contact. Few cranial mesoderm-specific genes have been identified, and although the mesoderm is described as being subdivided into units called somitomeres, there is little cellular evidence to support their existence. The goal of our study was threefold, as follows: (1) To identify and characterize the spatio-temporal expression of a set of mesoderm-specific genes present in the mouse embryo during early craniofacial development; (2) To analyze the role of candidate genes in anterior–posterior patterning of the cranial mesoderm; and (3) To analyze the role of candidate genes in patterning NCC development. We performed an Affymetrix gene chip microarray screen of cranial mesoderm isolated from 8.5 day mouse embryos. 184 genes, including known mesodermal genes such as *Twist*, *Prx2*, *PDGFR α* , and *FoxC2*, displayed expression > three-

fold stronger compared to a pooled endoderm/ectoderm sample. Thirty-six percent of identified genes were novel, and functional analysis uncovered a diverse group of genes, including transcription factors, ECM components, and endothelial cell markers. Initial in situ analysis identified a number of mesodermal gene candidates that may play a role in NCC development and demonstrated that although the cranial mesoderm is regionalized anterior—posteriorly, there is no genetic evidence for somitomeres.

224. Establishing Endoderm Organ Domains in Mouse and Chick Involves a Posterior FGF Signal. Jennifer Kordich, Robert Opoka, and James M. Wells. Cincinnati Children's Hospital Research Foundation, Cincinnati, Ohio.

Our studies focus on the mechanisms by which endoderm organ domains are initially established along the A-P axis. One day after gastrulation in mouse and chick, cells of the endoderm form a primitive tube and become regionally specified as seen by expression of genes in discrete organ boundaries. Our studies have demonstrated that late gastrulation stage endoderm is patterned along the A-P axis by soluble inductive signals from adjacent mesoderm/ectoderm. One factor, FGF4, is expressed by the primitive streak and can specify posterior endoderm organ domains in a dose-dependent manner in the absence of mesoderm or ectoderm. FGF 5 and 8b are also expressed in the primitive streak at this time, but do not have this activity. Studies of endoderm patterning in vivo further suggest that FGF4 is a posterior determinant of endoderm. Exposing anterior endoderm to exogenous FGF4 repressed foregut morphogenesis and anterior endoderm patterning as measure by HNF3 β /Foxa2 expression. Moreover, FGF4 affected an anterior shift in the pancreatic/duodenal domain and induced ectopic expression of the posterior marker CdxB in the anterior. Our studies additionally implicate FGFR4 in endoderm patterning, and we have identified that FGF binding protein 1 is expressed by posterior endoderm, but not by mesoderm or ectoderm. FGFbp1 is reported to promote FGF diffusion and enhance FGF signaling. We have found that endodermal expression of FGFbp1 is induced by FGF4 in a dose-responsive manner, suggesting that posterior endoderm amplifies an FGF signal. These results implicate FGF signaling in establishing endoderm organ domains along the A-P axis.

225. Lineage Specification and Cell Movement of Extraembryonic Endoderm in the Early Mouse Embryo. Y. Yamanaka, C. Chazaud, and J. Rossant. SLRI, Mt. Sinai Hospital, Toronto, Ontario M5G1X5, Canada.

The primitive endoderm (PE) develops on the surface of ICM in E4.5 blastocysts and gives rise two lineages, visceral(VE) and parietal endoderm after implantation. Recent studies show that VE plays important roles for patterning the embryo proper. It is not clear when and how this lineage is specified, although the cells are clearly different and separated from epiblast at the time of implantation. We have studied the timing of specification of PE in preimplantation embryos by various cell tracing methods and gene expression analysis. We find that the majority of cells in the ICM are specified to be epiblast or PE by the early blastocyst and that this is associated with localized expression of GATA6 in some cells of the ICM. The progeny of the later dividing blastomere at the two- to four-cell transition seem to preferentially contribute to PE. We propose a model for blastocyst lineage

specification in which a combination of cell division order and cellular segregation of key transcription factors can generate all three lineages by the early blastocyst stage. We have also studied the cell movement of VE in pregastrula embryos using cell tracing and embryo culture. The first sign of the anterior—posterior axis in mouse development is observed as cell movement in VE prior to gastrulation and this movement is necessary to establish a correct embryonic axis. We observed active cell movements from the distal to the future anterior from E5.5. Careful microscopic analysis revealed that the anterior visceral endoderm formed a horseshoe shape by E5.8, consisting of at least three cell types. The horseshoe-shaped morphology is consistent with the cell rearrangement model predicted from cell tracing experiments.

226. Smad2 and Smad3 Coordinately Regulate Craniofacial and Endodermal Development. Ye Liu, John C. Thompson, and Michael Weinstein. Department of Molecular Genetics, Ohio State University, Columbus, Ohio.

Smad2 and Smad3 are critical for transmitting TGF beta signals from the receptors at the cell-surface to the target genes in the nucleus. We have recently demonstrated that mice lacking one copy each of *Smad2* and *Smad3* suffered midgestation lethality from liver hypoplasia, while here we show that both the *Smad2* and *Smad3* genes are required at a full dosage for normal embryonic patterning. Animals lacking one allele of each gene exhibit a variably penetrant phenotype in which they display craniofacial and midline defects, in addition to the fully penetrant liver defects. These patterning defects range from mild holoprosencephaly to severe midline defects, and we now show that both the craniofacial and the liver defects are due to problems in the definitive endoderm. We have found reduced expression of genes known to be important for endodermal specification at developmental stages where the *Smad2*^{+/-} *Smad3*^{+/-} embryos fail to exhibit any overt phenotypes in axial or anterior patterning. This endodermal failure, which occurs despite the formation of a relatively normal foregut structure, likely causes the abnormalities seen in craniofacial and midline patterning. We also show that in all the mutants, the definitive endoderm fails to displace visceral endoderm at the anterior ventral foregut. This lack of hepatic endoderm combined with its decreased competency explains the decreased number of hepatoblasts in the later liver primordium and consequently hypoplastic liver in the mutant.

227. Separate Signals from the Neural Plate and the Mesoderm Cooperate to Induce the Preplacodal Domain. Anna Litsiou, Andrew P. Bailey, and Andrea Streit. Department of Craniofacial Development, King's College London, London, United Kingdom.

Precursors for cranial ganglia and for vital parts of the sensory organs arise from a common region of the embryonic ectoderm, the pre-placodal region that is subsequently divided into sensory placodes with specific identities. The preplacodal region is molecularly defined by the overlapping expression of members of the *Dlx*, *Pax*, *Six*, *Eya*, and *Dach* families of nuclear factors. Using these molecular markers, we have investigated the tissue interactions that set up this territory next to the anterior neural plate. We find that anterior, but not posterior, neural plate induces the expression of *Dlx5*, *Pax6*, and *Eya2*, while head, but not trunk, mesoderm is required for *Six4* and *Dach1* expression. These findings suggest that different signals emanating from the neural

plate and the mesoderm cooperate to establish the region where sensory placode precursors are generated. We report on the contribution of FGF, BMP, and Wnt signaling to the inductive interactions that establish the preplacodal domain.

228. **Yes-Associated Protein is Required For Proper Embryogenesis in the Mouse.** Brian Boone,* Elizabeth Morin-Kensicki,† Michael Howell,* Jaclyn Stonebraker,‡ Terry Magnuson,† Wanda O'Neal,‡ and Sharon Milgram1‡. *Department of Cell and Developmental Biology, †Department of Genetics and Curriculum in Genetics and Molecular Biology, ‡Cystic Fibrosis/Pulmonary Research and Treatment Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599.

Yes-Associated Protein (YAP65) was originally identified as a phosphoprotein able to bind the src-family kinase c-Yes in vitro. Recently, several groups have demonstrated that YAP65 can associate with and regulate the activity of transcription factors such as p73, AML1, and TEAD. Although YAP65 may modulate the activity of transcription factors known to have vital roles in embryogenesis, the role of YAP65 in development has never been directly addressed. To examine this, we generated lines of mice carrying a YAP65 targeted allele in which the first exon is replaced with a neo cassette. No YAP65 homozygous mutant progeny was recovered at postnatal stages. Heterozygous crosses show recovery of expected numbers of homozygous mutant embryos at embryonic day 7.5 (E7.5) but failure to recover any homozygous mutant embryos by E12.5. To characterize the requirement for YAP65 in embryogenesis, we have begun an expression analysis of YAP65 in the mouse embryo and have explored disruptions in development of homozygous mutant embryos via marker analysis. Our results indicate that homozygous mutant embryos undergo gastrulation, producing extraembryonic and embryonic mesoderm and patterned neuroepithelium, but show caudal dysgenesis by E8.5. Failure to differentiate viable cell types in the extraembryonic mesoderm including the allantois and the blood cell progenitors of the yolk sac may underlie the embryonic lethality. (Supported by HL63755.)

229. Abstract #229 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

230. **Notch Signaling Is Implicated in Rhombomere Boundary Patterning.** Yi-Chuan Cheng, Qiling Xu, Marc Amoyel, and David Wilkinson. Division of Developmental Neurobiology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom.

The formation of vertebrate rhombomere has been shown to be regulated by several molecules such as Krox-20, *Valentino*, *Hox* genes, ephrins, and Eph receptors. Here we provide the first evidence that Notch signaling is implicated in rhombomere boundary patterning. In mosaic expression experiments, both dominant-active Notch and Suppressor of Hairless (Su(H)) expressing cells contribute to all the rhombomere boundaries, coinciding with the stages when rhombomere boundaries form. Conversely, blocking Notch signaling by dominant-negative Su(H) causes cells to be excluded from the boundaries. In addition, the expression of several rhombomere boundary markers was disrupted at later stages. The effects of modulating Notch

signaling on cell sorting are consistent with the expression pattern of endogenous Notch pathway genes. These results suggest Notch signaling regulates rhombomere cell behavior and may play an important role in rhombomere boundary formation.

231. **Haploinsufficiency of *Smad1* Results in Abnormal Midbrain/Hindbrain Development.** Mark Hester, John C. Thompson, Joseph Mills, and Michael Weinstein. Ohio State University, Department of Molecular Genetics, Columbus Ohio.

Bone morphogenetic proteins (BMPs) are part of the TGF β superfamily of secreted ligands that play important roles during mammalian development. In order for BMPs to relay signals from the cell membrane to the nucleus, they must utilize the BMP-responsive Smads (*Smad1*, 5, or 8) that can bind to certain target genes to modulate their activity. Previous research has shown that *Smad1* is important in the formation of the allantois. However, we have uncovered a partially penetrant lethality in *Smad1* heterozygotes due to previously uncharacterized placental and midbrain/hindbrain defects in the *Smad1*^{+/-} embryos. Of the *Smad1*^{+/-} embryos that display placental defects, there was reduced cellularity and vasculature in the labyrinth. In those mutants displaying midbrain/hindbrain defects, histological analysis revealed hypercellular regions within the dorsal part of the neural tube due to increased cellular proliferation. Since one of the functions of BMPs is to dorsalize the neural tube, genes critical in dorsal/ventral patterning were analyzed such as *Pax3* and *Pax6*. Interestingly, *Pax3* and *Pax6* were upregulated in the mutant, although their expression domains did not differ from the wild-type. The *Pax3* promoter contains sequences known to bind *Smad1* and can be activated by *Smad1* in an in vitro assay. *Sip1*, which has been shown to interact with *Smad1*, is highly expressed in neuronal tissues and can bind to characteristic DNA motifs, one of which is found in the *Pax3* promoter. We therefore hypothesize that *Smad1* forms a complex with *Sip1* at the *Pax3* promoter repressing its gene expression.

232. **The Role of Frizzled 5 Signaling During Early Eye Development.** C. J. Burns,* T. Ishikawa,‡,§ M. M. Taketo,§,¶ S. Fuhrmann,† and M. L. Vetter*. *Department of Neurobiology and Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132; †Department of Ophthalmology and Visual Sciences, University of Utah School of Medicine, Salt Lake City, Utah 84132; ‡Banyu Tsukuba Research Institute (Merck), Tsukuba, 300-0026, Japan; §Department of Pharmacology, Graduate School of Medicine, Kyoto University, Kyoto, 606-8501, Japan; ¶Laboratory of Biomedical Genetic, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033 Japan.

Wnt/Frizzled (Fz) signaling has been shown to regulate cell fate, patterning, and proliferation within the developing central nervous system (CNS). Though some studies have implicated this pathway during eye development, the function of Wnt/Fz signaling during retinal histogenesis is poorly understood. Here, we investigate the role of Fz5 in the developing mouse eye. A detailed analysis of Fz5 expression during retinal development reveals that Fz5 is expressed within progenitors of the neural retina and appears to be reduced in differentiating cells. To determine Fz5 function during eye development, we are analyzing the retina in Fz5^{-/-} mutant embryos. Our data reveal that

mutant and wild-type eyes are morphologically similar at E9.5; however, by E10.5 the *Fz5*^{-/-} embryos fail to form an optic cup. Instead, only a vesicle-like structure and a rudimentary lens vesicle remain, suggesting an early patterning or proliferation defect. Preliminary results show that expression of *Chx10* and *Pax6* are unaltered in the mutant retina at E10.5. Currently, we plan to further characterize the function of *Fz5* during eye development. We will analyze the mutant retina for changes in cell proliferation and cell death, as well as examine additional markers of retinal patterning. Collectively, our initial results clearly demonstrate that *Wnt/Fz* signaling, and *Fz5* in particular, is an important regulator of early eye development.

- 233. *Kahloista* Is Required for Somitogenesis and Formation of a Single Heart Tube in the Mouse Embryo.** A. S. Rakeman,^{*}† and K. V. Anderson^{*}. ^{*}Sloan Kettering Institute, 1275 York Ave., New York, New York 10021; and [†]WGSMS, Cornell University, 445 East 69th Street, New York, New York 10021.

To identify novel genes that direct embryogenesis in the mouse, we have undertaken a systematic ENU mutagenesis screen for recessive mutations. Here, we describe one mutant identified in the screen, *kahloista* (*khlo*). The *khlo* phenotype is first apparent at E8.5. Mutants arrest at this stage with an open and kinky neural plate, cardia bifida, few, poorly formed somites, and a malformed primitive streak. The *khlo* mutation has been mapped to a less than 1 mb region on chromosome 2. In *khlo* embryos, the bilateral heart fields fail to migrate ventrally and instead develop as two symmetric, linear heart tubes. The two heart tubes appear to be patterned correctly, with defined endocardial and myocardial layers and correct regional expression of anterior—posterior markers, and are capable of beating. Paraxial mesoderm is properly specified in *khlo* mutants, but only the first 5–7 most anterior somites condense. These somites remain small and misshapen and fail to express markers of differentiated dermomyotome or sclerotome. At the time when the anterior somites are condensing in *khlo* mutants, patterning in the pre-somitic mesoderm and marker gene expression at somite boundaries appears normal. Since the defects in *khlo* mutants appear in issues undergoing rapid morphogenetic transitions and with little change in patterning, we are currently investigating the role of *khlo* in regulating cell shape changes and cell movements.

- 234. A Caudal—Rostral wave of RALDH2 Conveys Antero—Posterior Information to the Cardiac Field.** T. Hochgreb, V. F. L. Linhares, D. C. Menezes, A. C. Sampaio, N. Rosenthal, and J. Xavier-Neto. Laboratório de Genética e Cardiologia Molecular InCor-HC FMUSP 05403-000 São Paulo-SP, Brazil.

Establishment of antero-posterior (AP) polarity is an early decision in cardiogenesis. Much information has been obtained showing that retinoic acid (RA) is a morphogen that communicates AP polarity to the heart. Utilizing embryo culture, in situ hybridization, morphometry, fate mapping and treatment with the RA pan-antagonist BMS493 we investigated the relationship between cardiac precursors and RA signaling. We defined two phases of AP signaling by RA. The first phase (HH4–7) is characterized by increasing proximity between sino-atrial precursors and the lateral mesoderm expressing RALDH2, while the second phase (HH7–8) is characterized by progressive encircling of car-

diac precursors by a field of RALDH2 which originates from a novel, highly dynamic caudal-rostral wave pattern in the lateral mesoderm. Systemic treatment with BMS493 at stages HH4 to 7, but not at HH8 to 9, abrogate the development of atria and sinus venosa altering the cardiac fate map such that ventricular precursors appear in areas which, in the absence of treatment, contained only sino-atrial precursors. Moreover, local inhibition of RA signaling in the left anterior lateral mesoderm inhibited ipsilateral atrial differentiation. Identification of the caudal-rostral wave of RALDH2 as the endogenous source of RA establishing AP fates in the cardiac field will provide an useful model to approach the intriguing and yet, poorly understood mechanisms that transmit axial information from the embryo to its organs.

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- 237. The Role of *alk8* in Zebrafish Development.** T. L. Payne-Ferreira and P. C. Yelick. Department of Cytokine Biology, The Forsyth Institute and Department of Oral and Developmental Biology, Harvard School of Dental Medicine, Boston, MA 02115.

The zebrafish *alk8* encodes a novel TGFβ family member receptor that acts through Bmp signaling pathways to establish dorsoventral patterning of the early embryo. *alk8* is ubiquitously expressed as a maternal RNA and later is detectable in developing pharyngeal teeth, eye, heart and blood, notochord, muscle, bone, and neural tissue. Microinjection studies of constitutively active (CA) and dominant negative (DN) *alk8* mRNAs reveal that *alk8* is required for neural crest cell (NCC) formation and that Alk8 signaling gradients direct the proper patterning of premigratory NCCs and NCC cell-derived pharyngeal cartilages. Furthermore, an antero—posterior gradient is revealed, where overexpression of Alk8 more severely affects anterior arch cartilages, while decreased Alk8 activity more severely affects posterior arch cartilage formation. To examine *alk8* function in more detail, we are identifying tissue-specific regulatory elements of the *alk8* promoter that can be used to disrupt *alk8* expression in a tissue-specific manner. *alk8* promoter-GFP constructs were generated from an 18-kb genomic *alk8* clone and injected into single cell zebrafish embryos, which were raised and analyzed for tissue-specific GFP expression patterns. Promoter fragments directing GFP expression to blood, heart, muscle, neural tissue, and notochord have been identified. Identified tissue-specific promoter elements are being used to direct the expression of CA and DN *alk8* expression in developing embryos, to reveal tissue specific functions for this novel type I receptor. (Supported by NIDCR grants DE12024 and DE12076.)

- 238. Role of Tolloid in Regulating Chordin Function in the Zebrafish Gastrula.** Shannon Fisher and Jing Xie. Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland.

The BMP inhibitor chordin (*chd*) is required for proper dorsal—ventral patterning during gastrulation. Chd is cleaved by enzymes

related to *Drosophila* tolloid; this is thought to limit the amount of active chd in vivo. There is contradictory data on the role of twisted gastrulation (tsg), suggesting that it can either enhance or suppress chd's ability to inhibit BMPs, and that it promotes cleavage of chd by tolloid at an alternative site. To test the role of chd cleavage, we generated chd in which the two known sites for tolloid are mutated. Injected WT *chd* RNA can rescue *chordino* (*din*) mutant embryos. At similar RNA levels, *chd* with both cleavage sites mutated (*CM_{ab}*) strongly dorsalizes *din* mutants and rescues them at levels 10-fold lower than WT *chd*. To test the relative importance of the two cleavage sites, we mutated only the first (*CM_a*) or second (*CM_b*) site; these RNAs also rescued *din* mutants at levels much lower than WT. Using C-terminal tagged chd, we showed that the WT protein yields the predicted C-terminal fragment. *CM_a* also yields a small C-terminal fragment; in contrast, *CM_b* accumulates only full-length protein. Our data show the importance of chd cleavage to limit its effect as a BMP inhibitor and suggest that cleavage is sequential, with cleavage at the downstream site required first. However, altered cleavage at the first site is also associated with higher BMP inhibitory activity; this contradicts data from in vitro studies, which suggest that only full-length chd binds BMPs with high affinity. We are currently testing the requirement for tsg and tolloid enzymes in the generation of this high-inhibitory activity.

239. **Redundant Tolloid-Related Enzymes Regulate Chordin Activity in the Zebrafish Embryo.** Jing Xie and Shannon Fisher. Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland.

The bone morphogenetic protein (BMP) inhibitor chordin (chd) is required for dorsal—ventral patterning during gastrulation and helps to establish a gradient of BMP activity in the embryo. Chd protein is cleaved in vitro by enzymes related to *Drosophila* tolloid; in vivo, this cleavage is thought to limit the amount of active chd and to release active BMP bound to chd in an extracellular complex. Multiple mouse tolloid-related enzymes are capable of cleaving chordin, although their in vivo roles are unclear. The zebrafish minifin (*mfn*) mutation disrupts a tolloid-related gene. Although *mfn* is expressed during gastrulation, mutants have no early patterning defect. This suggests that tolloid-mediated cleavage does not limit chd during gastrulation. However, we have shown that chd protein resistant to tolloid cleavage is much more effective at dorsalizing the gastrula. These data show that chd cleavage is an important regulatory mechanism and that redundant tolloid-related enzymes may be active during gastrulation. A search of zebrafish ESTs revealed sequences representing two additional tolloid-related genes. Map position and sequence homology suggest that both genes encode enzymes that can cleave chd. One gene is expressed from late gastrulation through somitogenesis in localized areas, while the other is present as a maternal transcript and continues to be expressed widely through early larval stages. Using an assay for chd cleavage in vivo, together with mutant analysis and antisense morpholinos, we are carrying out experiments to determine which enzymes are primarily responsible for chd cleavage in the embryo.

240. **Zebrafish *colgate*-Mediated Inhibition of Wnt Signaling Is Required for Early Dorso—Ventral and Neuroectoderm Patterning.** Roopa M. Nambiar and Paul D. Henion. Neurobiotechnology Center, Molecular, Cellular, and Developmental

Biology Program and Department of Neuroscience, Ohio State University, 105 Rightmire Hall, 1060 Carmack Rd., Columbus, Ohio 43210.

The zebrafish *colgate* mutant displays defects in early dorso—ventral and neuroectodermal patterning. At gastrulation, mutants exhibit a reduction in the expression of shield-specific genes and an expansion of ventrolaterally expressed genes. In addition to these early defects, *col* mutants display a regional posteriorization of the neuroectoderm, manifested by an expansion of posterior fates within the forebrain and mid-hindbrain regions. We are able to correlate these defects to an overactivation of the Wnt signaling pathway. Overexpression of negative regulators of the Wnt pathway such as *dickkopf1*, *gsk3*, and *tcf3* are able to partially rescue the *col* mutant phenotype and antisense morpholinos directed against Wnt8 and Wnt8b are able to rescue distinct aspects of the mutant phenotype. These results suggest that *col* is required for the specification of dorsal and anterior fates by antagonizing Wnt signaling.

241. Withdrawn

242. Abstract #241 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

243. **Are Ephs Important for Adhesion in the Developing Hindbrain?** Julie E Cooke and Cecilia B Moens. HHMI and FHCR, P.O. Box 19024, Seattle, Washington 98102.

Segmentation of the hindbrain into rhombomeres (r) that underlie patterns of neuronal differentiation is a conserved developmental strategy among vertebrates. We are interested in understanding the mechanisms that underlie rhombomere boundary formation. Studies in the zebrafish have shown that Eph receptors and their ligands, the ephrins, are expressed in complementary rhombomere-restricted domains, and that interactions between Eph receptors and ephrins at presumptive rhombomere boundaries result in repulsive responses that play a role in segregation of cells from different rhombomeres. However we have uncovered an adhesive role for Ephs and ephrins within rhombomeres which we hypothesize may be equally important for segmentation. In addition to exhibiting boundary defects, embryos injected with antisense morpholinos (MOs) against EphA4 (expressed in r3 and r5) show neuronal patterning defects at the center of r3 and r5, suggesting an additional role for EphA4 within the segments in which it is expressed. Furthermore, observations of cell behavior in mosaic embryos made by transplanting cells between EphA4 MO-injected embryos and uninjected embryos demonstrate a role for EphA4 in cohesion of cells within a segment, since cells that lack EphA4 function are excluded from EphA4-expressing rhombomeres. We show that this effect is not due to an upregulation of B-ephrins in EphA4 MO-treated cells, and therefore, cannot be explained by Eph-ephrin-mediated repulsion. We present a model in which Eph-dependent adhesion within rhombomeres and Eph-ephrin-dependent repulsion between rhombomeres drive the establishment and maintenance of rhombomere boundaries.

244. Withdrawn

245. Requirements for Retinoic Acid in the Zebrafish Hindbrain.

Angela Nunez and Thomas Schilling. University of California, Irvine, California.

Segmentation of the vertebrate hindbrain into rhombomeres is essential for the anterior–posterior patterning of neurons. Retinoic acid is an essential embryonic signal necessary for patterning these segments, but its roles in neural development within rhombomeres remains unclear. To study how RA controls the locations of newly born neurons and their migrations within the hindbrain, we have analyzed segmental patterns of reticulospinal and branchiomotor neurons in the zebrafish retinaldehyde dehydrogenase 2 (*raldh2*) mutant neckless (*nls*), and in wild-type embryos treated with an antagonist which disrupts retinoid receptors (RAR) signaling. Antagonist treatments cause more severe hindbrain defects than *nls*, suggesting that *raldh2* is not the only source of retinoic acid in the hindbrain. Unlike *nls*, RAR antagonist-treated embryos show an expanded r3 and r4 and do not form rhombomeres 5–7 (*r5-7*). These embryos lack the branchiomotor neurons typical of *r5-7*, and facial neurons form throughout the enlarged r4 domain. Posterior duplications of the Mauthner cell, an identifiable neuron typically restricted to r4, further suggest that disruption of RA leads to transformations in rhombomere identity. To determine which RARs mediate the responses to RA in the hindbrain, we have analyzed their expression by in situ hybridization and have been surprised to find that several are expressed outside the CNS in surrounding mesoderm. We are currently addressing RAR isoform specificity through mosaic analysis and receptor knock-down experiments with morpholinos. Together these methods will further define how the RA signal mediates patterning and RA-dependent gene expression along the vertebrate hindbrain.

246. Genomic Characterization and Cloning of the Zebrafish Neural Crest Mutant, *alyron*.

M. J. Juryneć, * K. Hoshijima, * N. Bahary,† and D. J. Grunwald*. *Department of Human Genetics, University of Utah, SLC, Utah 84112; and †DFCI/Children's Hospital, Hematology Oncology Department, Harvard University, Boston, Massachusetts 02115.

alyron (*aln*) is a recessive, embryonic-lethal zebrafish mutant that has a severe deficit of premigratory trunk neural crest (NC) cells. *aln* was generated by plasmid insertion and is phenotypically similar to the *wnt-1/wnt-3a* double mutant and the *pax3* mutant in mouse. The *aln* mutant has a complete loss of body pigmentation and also exhibits heart defects presumably due to the deficit of premigratory NC. Experiments indicate that the mutation is not linked to any known zebrafish genes. *aln* has been mapped to the distal portion of linkage group (LG) 15. Molecular analysis of the *aln* genomic region indicates that it is a complex mutation consisting of both plasmid insertions and a deletion of telomeric genomic sequences. *aln* fails to complement an overlapping deletion mutant, *c4*, and genetic analyses indicate the phenotype is due to complete loss of function at the *aln* locus. We have used the overlapping region that is deleted in both *aln* and *c4* mutations to define the region encompassing the *aln* gene. The *aln/c4* region is ~250 kb. We have isolated a zebrafish YAC clone that can rescue that *aln* phenotype when injected into the one-cell stage embryo. We have used homologous recombination in yeast to transfer the YAC into a BAC vector. A 25-kb BAC was isolated that also rescues the *aln* phenotype. We are now in the process of using the YAC and BAC clones to identify candidate

genes that may be responsible for the *aln* phenotype. We are also in the process of identifying an ENU induced allele of *aln*.

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249. A Contractile Actomyosin Network Drives Cortical Flows That Establish and Maintain Par Domains and AP Polarities in the Early *C. elegans* Embryo. *Edwin M. Munro, Jeremy Nance,† and Jim Priesst. *Center for Cell Dynamics, Friday Harbor, Washington 98250; and †FHCRC, Seattle, Washington 98109.

In *C. elegans* embryos the anterior/posterior axis set up during pseudocleavage in response to a polarizing cue supplied by the sperm-donated centrosomes and associated microtubules. The response to this cue involves both cortical and cytoplasmic flows, and dynamic changes in the cortical enrichments of the anterior (Par-3, Par-6, and Pkc-3) and posterior (Par-2 and Par-1) Par proteins. However, the mechanisms underlying cortical flows and their relationship to the establishment and maintenance of par polarities remain unclear. Here we show that a contractile meshwork containing F-actin and the nonmuscle myosin NMY-2 is responsible for driving cortical flows during pseudocleavage and that these flows transport anterior Par proteins within the cortex to establish an anterior domain enriched in both the NMY-2 and the anterior Pars. We show that the organization and activity of the actomyosin cortex is regulated by the cell-cycle clock, by Par protein activity and locally by the sperm-donated MTOC. In support of recent results (Cuenca et al., 2003, Development 130(7), 1255-1265), our data point to distinct mechanisms for the establishment and maintenance of Par domains and implicates Par-dependent myosin contractility and cortical flows in both mechanisms. These data suggest a modular mechanism for the establishment of Par domains in response to polarizing cues that could be readily redeployed in other cellular contexts and may help to explain the ubiquitous roles of Par proteins in establishing cellular polarities.

250. Anterior–Posterior Polarity in *C. elegans*: A Role for Spatially and Temporally Regulated Protein Degradation. Geraldine Seydoux, Cynthia Derenzo, and Jason Pellettieri. Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

In *C. elegans*, polarization of the egg begins after fertilization. In response to a cue provided by the sperm, the PAR polarity regulators divide the zygote into distinct anterior and posterior domains, eventually causing segregation of the germ plasm to a single posterior blastomere that will form the germ line. Two independent mechanisms have been implicated in germ plasm asymmetry: posterior enrichment in the zygote before the first cleavage, and degradation of a subset of germ plasm proteins (CCCH proteins) in anterior cells after cleavage (Reese et al., 2000). We have found that degradation of CCCH proteins depends on a novel SOCS-box protein and the E3 ubiquitin ligase subunits

Elongin C and CUL-2. Surprisingly, degradation is activated in anterior cells by MEX-5 and MEX-6, two CCH proteins that are themselves targeted for degradation, suggesting an autoregulatory mechanism for restricting degradation to anterior cells. Degradation also appears to be temporally regulated. We have identified a new gene minibrain-kinase-2 (*mbk-2*) that is required to link fertilization to the onset of maternal protein turnover. *mbk-2* is essential not only for CCH protein degradation, but also for timely turnover of meiotic spindle proteins, and for germ plasm asymmetry in the zygote. We propose that spatially and temporally regulated degradation of maternal proteins is one of the mechanisms that drives the transition from symmetric egg to patterned embryo.

251. **The *scu-1* Gene Is Required for Anterior—Posterior Axis Specification and Meiotic Exit in the Early *Caenorhabditis elegans* Embryo.** Rebecca Lyczak, Sarah Kirkegaard, and Bruce Bowerman. Department of Biology, Ursinus College, Collegeville, Pennsylvania and University of Oregon, Eugene, Oregon.

In the nematode *C. elegans*, sperm entry into the oocyte triggers resumption of meiosis and establishment of the embryonic anterior—posterior (A-P) axis. Axis formation is thought to be driven by the sperm-donated centrosome, which associates with the cortex at the posterior pole and initiates polarized changes in both the cytoplasm and the cortex of the embryo. However, little is known about the exact mechanisms by which a cue from the sperm elicits these events, the proteins involved, and the relationship between the cell cycle and axis specification. We have identified a new mutant in *C. elegans* in a gene we call *scu-1* for (sperm cue abnormal), which may provide a link between cell-cycle regulation and embryonic polarity. The *scu-1* gene product is paternally supplied and is required to ensure a close association between the sperm pronuclear/centrosome complex with the posterior cortex. As a result, *scu-1* embryos lack the earliest signs of anterior—posterior polarization and fail to properly localize determinants in the embryo. In addition to a role in axis polarization, the *scu-1* gene product is required for timely exit from meiosis. Meiosis I and II both complete in timing similar to wild-type; but decondensation of the DNA and nuclear envelope formation is delayed in these mutants. The coupling of the meiotic exit and polarity defects in *scu-1* mutant embryos reveals that axis formation and cell-cycle regulation are intimately linked in the early embryo.

252. **Left—Right Asymmetry, Handedness, and *spn-1* Function in Early *C. elegans* Embryos.** W. B. Wood, R. Rivera, and D. Bergmann. University of Colorado, Boulder, Colorado; and MPI-CBG, Dresden, Germany.

L-R asymmetry is first apparent at the four- to six-cell stage, when the spindles in blastomeres ABa and ABp orient along the L-R axis and then skew 20°, always in the same direction, to give an embryo with dextral handedness. The *spn-1(it143ts)* mutation at 25° causes 70% maternal-effect embryonic lethality, with 40% sinistral animals among the survivors, all of which are fertile. Sinistrality results from randomization of the above spindle orientations, to produce sinistral, dextral, and inviable embryos. *spn-1* encodes a Gα protein involved in control of centrosomal movements that define the ABa and ABp spindle orientations. *spn-1(RNAi)* in adult hermaphrodites (soaking) mimics the

it143ts phenotype. *spn-1(RNAi)* (feeding) throughout larval development causes sterility, suggesting a second role for *spn-1* in gonadogenesis or gametogenesis. In genetic tests for functionally related genes, *spn-1(it143)* interacts with mutations in *par-3*, *par-4*, and *par-6*, all encoding components of the AB-cell cortex. We have also identified and are characterizing suppressors of *spn-1(it143)*. Our results show for the first time that heterotrimeric G proteins can play a role in establishment of L-R asymmetry and handedness choice; however, we do not know whether the initial symmetry-breaking event occurs before, during, or after the SPN-1-mediated process of AB-cell spindle orientation. In collaboration with A. Hyman and co-workers at the MPI-CBG in Dresden, we are investigating causes of the skewing described above and testing for prior cellular and molecular L-R asymmetries at the four-cell stage using GFP reporters for cellular markers and candidate proteins.

253. **Mutagenesis Screening in the Ascidian *Ciona savignyi*.** Jason Tresser, Di Jiang, and William Smith. Department of Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, California.

Ascidians are sessile marine invertebrates that have a rich history as models for developmental studies. The larvae (tadpole) stage represents the most basic chordate body plan, making it an attractive model for understanding developmental mechanisms of chordates in general. The recent sequencing of the *Ciona* genome has indicated ascidians have relatively few genes (~16,000) compared with vertebrates, presumably due to less genetic redundancy. This fact, along with their short generation time, transparent embryo, and invariant cell lineage makes the ascidian ideal for genetic studies of development. We have used the chemical mutagen *N*-ethyl-*N*-nitrosourea (ENU) to induce mutations in the ascidian *Ciona savignyi*. Sperm from ENU-treated adults was crossed to wild-type eggs to produce an F1 generation. These F1 were self-fertilized and screened for recessive zygotic mutations during early development. Various mutations such as abnormalities in the tail, central nervous system, and head morphology have been identified. Outcrossing and screening of the F2 generation has confirmed the mutations are heritable. We are in the process of characterizing several of these mutants.

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255. **Roles for *ik2*, a *Drosophila* IκB Kinase, in Anterior—Posterior and Dorsal—Ventral Embryonic Patterning.** R. S. Shapiro and K. V. Anderson. Sloan-Kettering Institute and WGSMS at Cornell University, New York, NY 10021.

The Rel/NF-κB transcription factors are essential for *Drosophila* dorsal—ventral (D/V) embryonic patterning and the immune responses in *Drosophila* and mammals. Rel family proteins are regulated by the degradation of their inhibitory proteins, IκBs. In mammals, IκB is targeted for degradation by phosphorylation by an IκB kinase (IKK). The *Drosophila* genome encodes two IκB-like kinases. *DmIKKβ* is essential for antibacterial immunity, but is not required for embryonic patterning. We identified five recessive lethal mutant alleles of the second IKK, *ik2*. All of the rare escaper homozygous

mutant adults have abnormal bristle morphology. To investigate whether *ik2* encodes the putative IKK that acts in embryonic D/V patterning, we examined embryos produced by females carrying homozygous *ik2* mutant germ line clones. These embryos are bicaudal, with a duplicated abdomen in place of the head. Many of these embryos have expanded Twist expression domains, indicating a D/V patterning defect. The eggs produced by *ik2* mutant germ line clone females also have D/V polarity defects. Since the loss of function of *ik2* affects both the anterior-posterior and the D/V embryonic axes, we are analyzing the asymmetric distribution of maternal determinants in the oocyte. We will present evidence that the actin cytoskeleton is impaired in mutant oocytes. Because of the aberrant ovarian cytoskeleton, and the fact that bristles are actin-based structures, we are investigating a role for *ik2* in regulating the actin cytoskeleton and therefore proper localization of maternal determinants in the oocyte.

256. **Genetic Interaction between Dispatched and Hh Signaling Components.** Hua Tian and Andrew P McMahon. Harvard University, Cambridge, Massachusetts.

Genetic analyses in *Drosophila* have demonstrated that the 12 pass transmembrane protein Dispatched is essential for releasing Hedgehog protein from secreting cell. We reported here are cloning of two Dispatched homologues, mDisp1 and mDisp2. mDisp1 was enriched in plasma membrane when overexpressed in COS7 cell. Its expression domains during early embryogenesis overlap with most of Hh signaling centers. We show that mice with impaired Disp1 function (exon 2 deletion) exhibit defects indicative of impaired long-range Hh signaling. Through genetic combination with null alleles of Ptc, Shh, and functional null allele of Disp1, we established a system in which we can systematically reduce the available Hh protein released from its source and study the concentration-dependent Hh signaling events in vivo. We examined three Hh signaling centers: ventral forebrain, ventral CNS, and ZPA of the limb, and demonstrated that cell types that generally depend on high levels of Shh activity for their specification are most sensitive to attenuation of Shh signaling.

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258. **Calcium and CaM Kinase II Are Involved in Polarization and Germination of *Silvetia compressa* Zygotes.** Rongsun Pu* and Kenneth R. Robinson†. *Department of Biological Sciences, Kean University, Union, New Jersey 07083; †Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.

Using microinjected fluorescent calcium indicators, we demonstrate here the formation of cytosolic Ca^{2+} gradients in dark-grown zygotes of the marine brown alga, *Silvetia compressa*, prior to germination. Germination occurs from the site of elevated Ca^{2+} , as in zygotes polarized by unilateral blue light. Using the self-referencing Ca^{2+} -selective probe, we measured highly localized influx of Ca^{2+} during photopolarization, indicating that extracellular stores supply at least some of the Ca^{2+} needed to construct a gradient. Bath-applied KN-93, an inhibitor of calcium/calmodulin-dependent kinase II (CaM kinase II), inhibits

germination, but does not interfere with photopolarization of the zygotes, consistent with the view that calmodulin is not involved in the initial response to light. Germination is also inhibited by an injected inhibitory peptide for CaM kinase II. The requirement for active CaM kinase II for germination ends about 2 hours before overt germination. We conclude that Ca^{2+} gradients, generated in part by localized calcium entry from the seawater, are an essential part of polarity development and expression in these cells, regardless of the nature of the external polarizing cue. Calmodulin and CaM kinase II are involved in interpreting (but not in establishing) the calcium gradient, allowing germination to occur at the site of elevated calcium, but CaM kinase II appears not to be involved in the initial process of germination. We are currently using Western blots to identify CaM kinase II in *S. compressa* cells.

259. **Par1 and par6 Homologs in the Leech *Helobdella robusta*.** Xiaoyun Ren and David A. Weisblat. Department of Molecular and Cell Biology, 385 LSA, University of California, Berkeley, California 94720-3200, USA.

On the basis of current molecular phylogenies, bilaterally symmetrical animals fall into three major groups, Deuterostomia, Ecdysozoa, and Lophotrochozoa. The par genes have been found to play a fundamental role in establishing epithelial cell polarity in deuterostomes and ecdysozoans. In the ecdysozoans (*Drosophila* and *C. elegans*), the pars seem to have an additional function in establishing embryonic anterior-posterior polarity. Whether the par genes play a similar role in establishing cell or embryonic polarity in lophotrochozoans is not known. To address this question and to gain additional information on the evolutionary history of the functions of par genes in general, we are studying par homologs in embryos of the glossiphoniid leech *Helobdella robusta* (Lophotrochozoa; phylum Annelida). We have isolated one par1-like and two par6-like genes from *Helobdella* and have generated a polyclonal antibody against HRO-PAR1. Our preliminary results show that HRO-PAR1 is expressed in the zygote and is localized to cellular junctions in later embryonic stages.

260. ***toast*^{b460} Is Required for Neural Crest Development and Hematopoiesis in Zebrafish Embryos.** Min An and Paul D. Henion. Department of Neuroscience and Neurobiotechnology Center, Ohio State University, Columbus, Ohio 43210

The zebrafish *toast*^{b460} mutant was isolated in an early pressure screen for mutations that affect neural crest development. *toast*^{b460} was identified based on a complete lack of neural crest-derived melanocytes. It is a recessive embryonic lethal mutation that has been mapped to LG9. Through phenotype analysis, we have attempted to determine the function of the *toast*^{b460} locus in the early development of neural crest cells in zebrafish. We found a dramatic deficiency in the number of trunk neural crest cells at early stages. This deficiency appears to be due to programmed cell death within the neural crest domain of the ectoderm. Much later in development, neural crest cells did appear, but failed to undergo migration and differentiation. In addition to the defect in neural crest development, blood development is also affected in *toast*^{b460} mutant embryos. In mutant embryos, the number of erythroid precursor cells is decreased and migration of these cells to the dorsal mesenteric region fails to occur. In addition, the number of myeloid precursor cells was also

decreased and the remaining cells failed to differentiate into granulocytes and macrophages. Transplantation of cells between mutant and wild-type embryos at blastula stages revealed that *toast*^{b460} is required cell-autonomously during development of the neural crest and is required non-cell-autonomously for hematopoiesis. These results indicate that *toast* locus is normally required for neural crest development and hematopoiesis in zebrafish.

261. Abstract #261 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

262. **Sox10 Participates in the Early Development of Neural Crest by Controlling Its Specification and Survival.** S. M. Honoré, M. Aybar, and R. Mayor. HHMI and MNDB, Fac. Science, University of Chile, Chile.

The Sox family of transcription factors has been implicated in the development of different tissues during embryogenesis. Several mutations in humans and mice and zebrafish have shown that depletion of Sox10 activity produces defects in the development of neural crest derivatives. We have isolated the *Xenopus* homologue of the Sox10 gene and shown that it is expressed in prospective neural crest and otic placode regions from the earliest stages of neural crest specification. Loss of function experiments using morpholino antisense oligos against Sox10 produce a loss of neural crest precursors as well as an increase in apoptosis and a decrease in cell proliferation in the neural folds, suggesting that Sox10 could work as a survival factor in neural crest precursors in premigratory stages. An additional late role of Sox10 on neural crest derivatives was studied in experiments where the development of melanocytes and ganglia induced in vivo and in vitro were blocked by inhibiting Sox10 activity. We also show that Sox10 expression is dependent on FGF and Wnt activity. Finally, we used inducible forms of the wild-type and dominant negatives for the Snail and Slug genes to establish the position of Sox10 in the hierarchical cascade of gene activation required for neural crest specification. Our results indicate that Sox10 may lie between Snail and Slug in the genetic cascade of transcription factors that controls neural crest development.

263. **Early Events in Neural Crest Cell Induction: Specification, Markers, and Signals.** M. I. Garcia-Castro, M. Basch, and M. Bronner-Fraser. California Institute of Technology, Pasadena, California.

Neural crest cells, which generate peripheral nervous system and facial skeleton, arise at the neural plate/ectodermal border via an inductive interaction between these tissues. Wnts and bone morphogenetic proteins (BMPs) play roles in neural crest induction in amphibians and zebrafish. Here, we show that, in avians, Wnt6 is localized in the ectoderm and in vivo inhibition of Wnt signaling perturbs neural crest formation. Furthermore, Wnts induce neural crest from naive neural plates in vitro in a defined medium without added factors, whereas BMPs require additives. Our data suggest that Wnt molecules are necessary and sufficient to induce neural crest cells in avian embryos. Additionally, we have characterized the potential of epiblast cells from the early gastrulating chick embryo to generate neural crest cells and analyzed the expression of candidate markers for neural crest precursors. Our results suggest an earlier neural crest cell specifi-

cation than previously thought and highlight differences between the assumed neural plate/ectodermal border and the expected origin of neural crest cells.

264. **Chordin and Noggin Function as BMP Antagonists In Vivo to Regulate Mammalian Neural Crest Development.** Ryan M. Anderson, Rolf W. Stottmann, and John Klingensmith. Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710.

Bone Morphogenetic Proteins (BMPs) appear to play many roles in early neural crest development. Here, we explore the functions of BMP antagonism in mammalian neural crest development, using mouse embryos lacking the BMP antagonists Chordin and/or Noggin. We find that Noggin has a significant role in neural crest development, in which Chordin is partially redundant. During neural crest formation, *Noggin* shows robust expression in the dorsal neural folds, while *Chordin* may be found at a low level throughout neural tissue. BMP signal transduction is increased in the early dorsal neural tube with the elimination of *Noggin* and is further increased when *Chordin* is also absent. The neural/surface ectoderm boundary is expanded with reduced BMP antagonism, with corresponding expansion of the neural crest. In neural tube explants, recombinant BMP increases expression of neural crest competence markers, but not of migratory neural crest. Changes in *Wnt* gene expression with decreased BMP antagonism suggests that as in chick embryos, Wnts may synergize with BMPs to regulate neural crest development in mouse. In mutant embryos, most neural crest cells migrate normally; however, some neural crest subpopulations undergo ectopic apoptosis. Altogether, our results demonstrate that Chordin and Noggin function as BMP antagonists in vivo to ensure normal specification and development of the mammalian neural crest.

265. **Graded Potential of Neural Crest to Form Cornea, Sensory Neurons, and Cartilage along the Rostrocaudal Axis.** Peter Lwigale, Gary Conrad, and Marianne Bronner-Fraser. California Institute of Technology, Pasadena, California; and Kansas State University, Kansas.

Neural crest cells arising from different rostrocaudal axial levels form different sets of derivatives: cranial neural crest forms cartilage, cornea, and cranial ganglia; cardiac neural crest forms the outflow tracts of the heart; trunk crest forms sympathetic and dorsal root ganglia. Here, we test whether these differences in derivatives are due to differences in developmental potential by challenging cardiac and trunk neural crest cells in the midbrain environment and examining their long-term differentiation into cornea, trigeminal ganglion, and branchial arch cartilage. Although both cardiac and trunk neural crest migrate to the periocular region, they do not contribute appropriately to the cornea. Cardiac neural crest cells make only a small contribution to keratocytes or endothelium and often form ectopic masses on the dorsal corneal surface. Trunk neural crest cells only differentiate into melanocytes regardless of their location in the cornea. After grafting, the trigeminal ganglion is reduced in size, apparently due to a significant decrease in somatosensory neurons most pronounced for truncal grafts. Those few neurons that differentiate do, however, make appropriate connections to their peripheral targets in the cornea. In the first branchial arch, cardiac neural crest contributes to the quadrate but only nominally to Meckle's cartilage. Trunk neural crest cells form no cartilage

after transplantation and only sparse individual trunk melanocytes are found in the mandibular process. These results suggest a graded rostrocaudal loss in neural crest populations with respect to their ability to form somatosensory neurons and cartilage even after transplantation to a permissive environment.

- 266. Genomic and Functional Analyses of Neural Crest Induction.** Laura S. Gammill, Constanza Gonzalez, and Marianne Bronner-Fraser. California Institute of Technology, Division of Biology 139-74, Pasadena, California 91125.

The vertebrate neural crest is a migratory stem cell population that arises within the central nervous system to generate diverse structures throughout vertebrate embryos, including the peripheral nervous system and the bones and cartilage of the head. The events that cause certain neural cells to become different from their neighbors and migrate are not clear. As a first step toward understanding this process, we combined embryological techniques with array technology to describe 83 genes that provide the first gene expression profile of a newly induced neural crest cell. Our results reveal a sequential "migration activation" process that reflects stages in the transition to a migratory neural crest cell and suggests that migratory potential is established in a pool of cells from which a subset is activated to migrate. We are testing this hypothesis by exploring the functions of various products from our screen, including the receptor neuropilin-2, a novel chemokine, and gene with no known homology. We report our results using chick electroporation and mouse knockouts, combining *in vivo* and *in vitro* assays to dissect the pathways that regulate the formation of a migratory neural crest cell.

- 267. Transcription Factor AP-2 Knockdown in Zebrafish Embryos Disrupts Head Skeleton, Autonomic Neurons, and Melanocytes.** Erin K. O'Brien,* Claudia d'Alençon,† Jeff Schoenebeck,‡ Jeffrey C. Murray,§ Miguel L. Allende,† Bruce D. Gelb,¶ Deborah Yelon,‡ Judith S. Eisen,|| and Robert A. Cornell**.*Department of Otolaryngology, University of Iowa College of Medicine, Iowa City, Iowa; †Depto de Biol, Fac de Ciencias, Unive de Chile, Santiago, Chile; ‡Developmental Genetics Program, Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, New York; §Department of Pediatrics, University of Iowa College of Medicine, Iowa City, Iowa; ¶Departments of Pediatrics and Human Genetics, Mount Sinai School of Medicine, New York, New York; ||Institute of Neuroscience, University of Oregon, Eugene, Oregon; and **Department of Anatomy and Cell Biology, University of Iowa College of Medicine, Iowa City, Iowa.

Orthologues of transcription factor AP-2 are expressed in the neural crest of many vertebrates. Mouse AP-2 α KO embryos show defects in some neural crest derivatives; however, they die at birth because of a failure of ventral body wall closure, limiting their use for analysis of AP-2 in neural crest patterning. We show that in contrast, zebrafish embryos injected with AP-2 morpholinos (MO) complete early development normally, although similar to mouse AP-2 α mutants, they have reduced pharyngeal skeleton and cranial ganglia neurons. In addition, enteric neurons and sympathetic neurons are substantially reduced in zebrafish MO embryos, while glial cells of cranial ganglia appear normal in number. Finally we document defects in melanophore number, differentiation, and migration. We are testing the hypothesis that

effects on melanophores largely result from decreased expression of growth factor receptor *c-kit*.

- 268. Notch Signaling in Differentiation of Neuronal Precursor Cells.** Katja Piltti,* Satu Kuure,* Seppo Vainio,† Hannu Sariola,* and Kirmo Wartiovaara*. *Developmental Biology, Institute of Biomedicine, Biomedicum Helsinki, P.O. Box 63, FIN-00014 University of Helsinki, Helsinki, Finland; †Department of Biochemistry, University of Oulu, P.O. Box 3000, FIN-90014 University of Oulu, Oulu, Finland.

Notch signaling is essential for the maintenance of neuronal progenitor pools in developing and adult central nervous system (CNS). Notch pathway also inhibits neurogenesis and oligodendrogenesis by directing differentiation into astroglial direction. However, the mechanisms by which Notch signaling activates astrocyte-specific gene expression are not yet known. We have studied *in vitro* neuronal progenitor cells derived from brains of Jagged1 transgenic mice embryos. In our culture system we have found differences in cell proliferation, renewal (ability to form colonies), and differentiation between transgenic and wild-type littermates. Initially transgenic neuronal progenitors proliferate faster than controls but in long-term culture the proliferation of Jagged1 progenitors decreases when compared to controls. Besides this, single transgenic progenitor cells diminish their ability to form colonies during prolonged culture. Our results show that overexpression of Jagged1 promotes astroglial and inhibits neuronal differentiation of neuronal precursors *in vitro*.

- 269. Notch Activation Induces Apoptosis in Neural Progenitor Cells through a p53-Dependent Pathway.** Xudong Yang, Rüdiger Klein, and Jie Shen. Center for Neurologic Diseases, Brigham and Women's Hospital, Program in Neuroscience, Harvard Medical School, Cambridge, Massachusetts.

Notch signaling is involved in a variety of cell-fate decisions during development. Here we investigate the effects of Notch activation in neural progenitor cells using a conditional transgenic strategy. Expression of a constitutively active form of Notch1 in early neural progenitor cells but not postmitotic neurons selectively induces extensive apoptosis, resulting in a markedly reduced progenitor population. Notch activation in neural progenitor cells also leads to increased proliferation and elevated levels of nuclear p53 and transcriptional up-regulation of the target genes Bax and Noxa. The promotion of apoptotic cell death by Notch activation can be completely suppressed by p53 deficiency. These results show that during early neural development Notch activation in neural progenitor cells can induce apoptosis through a p53-dependent pathway.

- 270.** Abstract #270 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

- 271. Neurogenesis and Proliferation Control by Her5 at the Midbrain—Hindbrain Boundary.** A. Geling, P. Chapouton, A. Tallafuss, and L. Bally Cuif. TUM and GSF-IDG, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany.

Neurogenesis in the vertebrate midbrain—hindbrain (MH) is organized around a nondifferentiating stripe at the MH boundary (MHB). The zebrafish bHLH factor Her5 is a crucial (i) inhibitor of

neurogenesis and (ii) activator of proliferation at the MHB (Geling et al., 2003, Development 130, 1591-1604). We now aim at providing molecular bases for these activities. By combining gain- and loss-of-function, we show that Her5 and Notch activities are independent, but that blocking Her5 function reveals at the MHB a proneural-like domain undergoing lateral inhibition. We identify a cross-regulatory loop where *zco2* is necessary for but subsequently downregulated by *her5* expression at the MHB. While Her5 inhibits *ngn1* expression, we show that it does not affect other candidate targets such as *zash1a* and *zash1b* expression. We propose that a proneural domain is initiated by Zco2 across the MH and later split by Her5 to prevent neurogenesis progression at the MHB, upstream of Notch and via the inhibition of *ngn1* expression. To approach the role of Her5 on cell proliferation, we trace *her5* progeny using *her5:egfp* transgenics. We show that GFP-positive cells are maintained at the MHB until adulthood, in a location that also incorporates BrdU. We demonstrate that adult MHB GFP-positive cells transplanted into embryos can integrate, divide, and show morphological signs of differentiation. Our results suggest crucial functions for Her5 in attributing specific differentiation properties to zebrafish MHB cells throughout life.

- 272. Absence of Motoneurons in the Caudal Part of the Avian Spinal Cord Is Induced by the Local Environment.** Nuno Afonso and Martin Catala. CNRS UMR 7000/University of Paris 6, Paris, France.

At the caudal-most level of the avian embryo (somites 47 to 53), the spinal cord is devoid of ventral roots. In this study we show that the absence of motor nerves is due to a default in motoneuron (MN) progenitor differentiation and not to their selective elimination by programmed cell death. The caudal-most neural tube (CNT) has normal ventral patterning as demonstrated by the expression of FoxA2 right up to the tip of the tail. Furthermore, the secreted molecule Shh and its membrane receptor Ptc, as well as the downstream target genes responsible for MN differentiation (i.e., Pax6, Nkx6.1, and Olig2), are all expressed at this level, suggesting a normal dorso-ventral patterning of the spinal cord. However, MNR2, which is normally expressed by committed MN progenitors and whose expression is upregulated by Olig2, is not expressed at this level. These results suggest that there is a blockade in the MN fate induction cascade between the two stages characterized by the expression of these two transcription factors. Nevertheless, the neuroepithelial cells of the CNT express Delta1, a marker of cell-cycle exit, and are therefore able to withdraw from the cell cycle. Moreover, we have performed heterotopic and heterochronic transplantations in which a quail CNT is placed at the level of the brachial region of a chick host. We observe that some of the transplanted cells are able to differentiate into MN as confirmed by the expression of MNR2 and Isl1 (a postmitotic MN marker). Despite this potential these cells are normally unable to differentiate due to the presence of an environmental extracellular factor which we are currently trying to identify.

- 273. Analysis of the Wnt Signaling Pathway in Cell Fate Decisions in the Developing Spinal Cord of Zebrafish.** Jennifer Bonner, Matthew Grierson, and Richard I Dorsky. University of Utah, Salt Lake City, Utah.

The Wnt signaling pathway has been implicated in the regulation of many developmental processes. However, the role of Wnts in spinal cord development has remained somewhat elusive. The main objective of this research is to understand how the Wnt pathway is governing spinal cord development in the zebrafish, *Danio rerio*. To do this, we are misexpressing a repressor form of the downstream effector of Wnt signaling, tcf3 (delta-tcf) under control of the heat shock promoter during the time that cell fate decisions are made in the spinal cord. With global heat shock, we have found that expression of delta-TCF results in a loss of islet gene expression both in developing primary and secondary motoneurons, while Rohon-Beard neurons are unaffected. The loss of islet gene expression occurs in newly formed motoneurons as well as preexisting motoneurons. This suggests that cell fate decisions are altered in these embryos. To determine this, markers specific for other cell fates in the spinal cord, such as interneurons, will be investigated in heat-shocked embryos. In a similar approach, we will address the role of tcf3 in single cells, by using a laser to activate delta-tcf in single spinal cord cells. So far, we have been able to reproducibly activate delta-tcf in spinal cord cells with minimal damage to the tissue. This line of research will provide insight into the roles of Wnt signaling in the developing spinal cord.

- 274. Specification and Patterning of Zebrafish Interneurons.** J. Bates, J. S. Eisen, and K. E. Lewis. Institute of Neuroscience, University of Oregon, Eugene, Oregon, USA.

Interneurons (INs) constitute most of the neurons in the vertebrate brain and spinal cord and they function in almost all neural circuits and behaviors. We are using zebrafish as a model system to investigate how INs develop and function. Recent studies in embryonic chick and mouse have implicated a number of transcription factors (TFs) in the specification of different INs. Different combinations of TFs are expressed in distinct dorsal-ventral progenitor domains in the spinal cord, where they specify the expression of additional TFs in the postmitotic INs generated by these domains. In at least some cases, expression of particular postmitotic TFs determines later IN characteristics such as the direction of axon projections, enabling us to link IN development and function. Many of the TFs implicated in IN specification in chick and mouse are also expressed in the embryonic zebrafish spinal cord, suggesting that, as for motoneurons, mechanisms of IN specification are conserved across vertebrate species. Investigating how INs develop and function in chick and mouse is not trivial, as these vertebrates have large numbers of INs with a diversity of morphologies, synaptic connections, and functions. In contrast, the zebrafish spinal cord has a small number of distinct classes of morphologically identifiable INs, several of which are already correlated with specific neural circuits and behaviors. We will describe studies in which we have begun to address two related questions: (1) What combinations of TFs are expressed by each distinct class of zebrafish spinal cord IN? and (2) What signaling pathways are involved in specifying different IN fates? (Supported by NIH Grant NS23915.)

- 275. The Role of Paraxial Mesoderm in Patterning Zebrafish Primary Motoneurons.** K. E. Lewis and J. S. Eisen. Institute of Neuroscience, University of Oregon, Eugene, Oregon, USA.

The vertebrate nervous system consists of many specialized cell types that form at distinct, characteristic positions. How this precise pattern of different cell types is produced is unclear. We are using zebrafish as a model to investigate how neurons acquire appropriate, position-specific fates. Zebrafish primary motoneurons (pmns) are an early-developing population of ventral neurons. There are three distinct pmn subtypes, each identifiable by its characteristic gene expression, axonal trajectory and soma position. Several lines of evidence suggest that different pmn fates may be specified by signals from paraxial mesoderm (either presomitic mesoderm and/or somites). We have analyzed a number of mutations that have different effects on paraxial mesoderm and/or somite development to investigate this hypothesis. We find that mutants with severely reduced paraxial mesoderm form pmns with hybrid subtype identities, consistent with our hypothesis that paraxial mesoderm-derived signals are required to specify different pmn subtypes. In contrast, specification of different pmn subtypes occurs normally in mutants that lack morphological somite boundaries and at least some aspects of antero-posterior somite patterning. However, the normal regular spacing of different pmn subtypes is disrupted in these mutants, suggesting that the correct positioning of different pmn subtypes may require somite boundary formation and/or antero-posterior somite patterning. (Supported by NIH Grant NS23915.)

276. Abstract #276 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

277. The Segmental Columnar Identity of Spinal Motor Neurons Is Dependent Upon the Status of Retinoid Receptor Activation. Shanthini Sockanathan*,† and Thomas M. Jessell†. *Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, Maryland; and †HHMI, Center for Neurobiology and Behaviour, Columbia University, New York, New York.

The establishment of functional motor circuits is dependent upon the coordinated generation of specialized groups of motor neurons (MNs) which have distinct settling positions, molecular identities, and specific peripheral axonal projection patterns. Although much is known of the early events involved in the establishment of a generic MN identity, very little is understood of the molecular signals involved in the subsequent specialization of spinal MNs. One potentially relevant signaling molecule is retinoic acid (RA), which is synthesized by RALDH2 in the paraxial mesoderm directly abutting the developing spinal cord. Expression of RALDH2 in non-limb-level mesoderm is significantly delayed with respect to that at limb levels, suggesting a role for RA signaling in the development of segmentally restricted motor columns. By manipulating retinoid receptor activation within newly generated spinal MNs using dominant negative and constitutively active receptor isoforms, we have shown that the formation of limb-level specific MNs is dependent upon the activation of retinoid receptors, whereas the absence of retinoid signaling is critical for non-limb-level MN generation. Moreover, our results suggest that the source of RA for this phenomenon is derived from RALDH2 expression in the paraxial mesoderm. Taken together, these results suggest that the establishment of segmentally restricted motor columns is a consequence of the generation of differential rostrocaudal zones of retinoid receptor activation within postmitotic MNs.

278. Oligodendrocyte Precursor Specification in the Chick Spinal Cord: Implication of a High Level of Shh Activity and Identification of C-Sulf1 as a New Marker of the Oligodendrocyte Lineage. C. Danesin, N. Escalas, X. Ai, C. Emerson, and C. Soula. UMR5547, CNRS/UPS.

In the embryonic chick spinal cord, oligodendrocytes (OLs) originate from the ventral-most domain of the neuroepithelium (NE) characterized by the expression of Nkx2.2. The specification of oligodendrocyte progenitors (OLP) is a late event in neurogenesis and depends on the activity of the morphogen Shh. We have shown that optimal doses of Shh required to induce OLP from ventral progenitors are higher than those required to induce ventral neurons, leading to the attractive hypothesis that the Nkx2.2+ progenitors switch to an OL fate in response to an increased activity of Shh in the ventral NE. In accordance with this hypothesis, we have observed a strong accumulation of Shh at the apical surface of Nkx2.2 progenitors just before OLP specification. Moreover, we have recently shown that a premature overexpression of Shh in the neural tube leads to a marked anticipation of OLP specification, supporting the hypothesis that the induction of the OL lineage from Nkx2.2+ progenitors can be driven by a local increase of the Shh concentration. We have recently isolated the chick orthologue of the Sulfatase1 (C-Sulf1) and have shown that this gene is a novel marker of OL lineage in the chick embryonic spinal cord. Its expression in the NE is restricted to the Nkx2.2 domain and starts just before OLP specification. The putative function of Sulf1 in modulating the sulfation state of HSPG, involved in the regulation of several signaling pathways, including Shh, suggests that the modification of the Shh gradient observed at the time of OLP specification may be mediated by Sulf1.

279. Developmental Defects in Cerebral Cortex and Olfactory Bulb Demonstrate a Crucial Role of Vax1 during the Development of the Telencephalon. Paola Tagliatela*,†, Jose Miguel Soria*, and Stefano Bertuzzi*. Dulbecco Telethon Institute at CNR-ITB, Segrate (MI), Italy; and †Newron Pharmaceuticals, Gerenzano (MI), Italy.

Two different types of cellular migration can be distinguished in the developing telencephalon: (1) a radial migration pathway, followed by projecting neurons in the neocortex, which are generated from precursor cells located in the underlying ventricular zone; and (2) a tangential migration pathway, followed by interneurons that populate the cortex and the olfactory bulb, migrating across rather long distances. During development, tangentially migrating cells originate in the ventricular zone of the basal telencephalon. This area contains two distinct progenitor domains, the lateral and the medial ganglionic eminences (LGE and MGE), generating the striatum and the globus pallidus, respectively. We present data indicating that mice lacking the homeobox gene Vax1 show a developmental defect affecting the basal ganglia, which seem strikingly expanded and poorly differentiated. As a consequence, Vax1^{-/-} mice show a reduced stream of neural stem cells that reach the olfactory bulb, following the tangential trajectory known as Rostral Migratory Stream. We also observe a strong reduction in the number of GABAergic interneurons present in the cerebral cortex. These findings indicate that, in the absence of Vax1, the progenitors of tangentially migrating neurons do not develop properly, highlighting an im-

portant role of Vax1 in the determination processes of tangentially migrating interneurons.

280. **Rhombomere-Specific Regulation of First-Order Relay Visceral Sensory Interneurons.** Gary O. Gaufo, Sen Wu, and Mario R. Capecchi. Department of Human Genetics, HHMI, University of Utah, Salt Lake City, Utah 84112-5331.

Hox genes are required for the generation of cellular diversity along the anteroposterior axis of the developing organism. In the PNS and CNS, the specification of multipotential neural crest cells and distinct classes of motor neurons are established functions of Hox genes. However, the role of Hox genes in the development of first-order sensory relay interneurons in the CNS is unknown. By loss-of-function analysis we show that the segmental appearance of Mash1-dependent D2 visceral noradrenergic interneurons of the solitary tract nucleus requires distinct combinations of Hox genes. Furthermore, we show that Hox genes are epistatic to Phox2b, a major postmitotic determinant of visceral noradrenergic interneurons. Finally, we discuss a mechanism by which Hox genes impinge upon the differentiation of visceral noradrenergic interneurons and to the overall neuronal diversity of the developing hindbrain.

281. **Axon Segmental Border Crossing in the *Drosophila* CNS Is Regulated by *nerfin-1*.** Alexander Kuzin, Chad Stivers, Thomas Brody, and Ward Odenwald. Neural Cell-Fate Determinants Section, NINDS, NIH, Bethesda, Maryland.

Our efforts are directed at understanding the molecular events that control cell-identity decisions during *Drosophila* CNS development. We have identified a Zn-finger gene, *nerfin-1*, from a cDNA library screen for genes expressed during embryonic CNS development (Stivers et al., *Mech Dev* 2000, 97, 205-210; Brody et al., 2002, *Mech Dev* 2002, 113, 41-59). *nerfin-1* belongs to a conserved Zn-finger transcription factor subfamily, with human, mouse, and nematode cognates. Immunolocalization studies reveal that the Nerfin-1 protein is nuclear and is detected in most, if not all, ganglion mother cells (neural precursors that divide once to produce neurons). Its expression in the nascent neurons is transient. We have generated both recessive lethal null mutations and homozygous viable truncated protein mutations in *nerfin-1*. Thus far, our phenotypic analysis of *nerfin-1* null mutants has shown that neural precursor lineage development is normal, however, axonal outgrowth and pathfinding are severely disrupted. Longitudinal axon connectives that interconnect ganglia fail to develop. Immunostaining with axonal markers reveals that neurons generate axons but they fail to extend across segmental boundaries. Although the intraganglion axon commissures form, they too show morphological defects. Recent gain-of-function experiments reveal that overexpression of Nerfin-1 in neurons results in fewer commissural axons and greater axon densities crossing segmental boundaries. We are currently testing known axon guidance genes to determine if they are targets of Nerfin-1 transcriptional regulation.

282. **Tumor Suppressor Protein Discs-Large (Dlg) and its Interactor Partner of Inscuteable (Pins) Play Important Role in *Drosophila* Brain Development.** Anna Radovic, Peter J Bryant. UC Irvine, Developmental Biology Center.

Brain development requires close coordination between the production of cells and their differentiation into functioning neurons. In *Drosophila*, the brain develops in a series of steps starting from the embryo when the neuronal stem cells (neuroblasts) are formed. These undergo asymmetric cell division (ACD), producing an apical neuroblast (NB) that continues dividing, and a basal ganglion mother cell (GMC) that differentiates into neurons. After a period of growth during the first and second larval instars, in the third instar a new set of ACD's forms the adult brain within the structural framework of the larval brain. Our research is focused on two ACD participants, that function in both central and peripheral nervous systems: the tumor suppressor protein Discs-large (Dlg) and a protein that interacts directly with the SH3 domain of Dlg called Partner of Inscuteable (Pins) encoded by pins. Several different mutants of dlg cause neoplastic overgrowth of imaginal discs and brains in the larva. One of the pins partial deletions also causes brain overgrowth in the larva, but complete loss of Pins results in a smaller brain. We investigated the function of Dlg, Pins and Dlg-Pins complex in ACD process and brain development by following distribution of different participants of ACD's, cell cycle markers, and brain growth pattern in dlg and pins mutants. We were able to further understand mechanisms by which these two factors affect localization of other cell fate determinants, which in turn affects the balance between proliferation and differentiation necessary for normal brain development.

283. **Fate of Ventricular Zone Derived Aggrecan-Expressing Cells in Culture.** M. S. Domowicz, J. G. Henry, M. M. Mueller, and N. B. Schwartz. Department of Pediatrics and Biochemistry and Department of Molecular Biology, University of Chicago, Chicago, Illinois 60637.

The developmentally regulated expression of aggrecan in chick brain begins at embryonic day 7, increases up to day 13, then markedly decreases after day 16, and is not expressed posthatching. Whole-mount in situ hybridization detects aggrecan by E6 in the ventricular zone (VZ) of the ventral midbrain, by E8 expression progresses caudally through the optic tectum VZ, and is observed in the telencephalon VZ and hindbrain in a characteristic pattern. The time period and pattern of expression of aggrecan suggest that it may be expressed by glia precursors. To analyze this possibility, a culture system enriched in aggrecan-expressing cells was established from E12 tectal VZ in DMEM-containing EGF/bFGF and assessed for differentiation ability. The aggrecan-expressing cells do not express markers of astrocytes (GFAP), oligodendrocyte precursors (PLP), radial glia cells (GLAST), and neurons (Class III- β -tubulin) as determined by in situ hybridization. The level of aggrecan produced by the cultures was dependent on the presence of EGF and bFGF when the cultures were changed to defined F12/DMEM (1:1) media supplemented with G5 or Neurobasal media supplemented with B27; VZ-derived cells expressed GFAP, PLP, and GLAST but not Class III- β -tubulin, indicating that aggrecan-expressing cells have the potential to differentiate in culture to different cell types of the glial lineage. Taken together, these results suggest that aggrecan-expressing cells represent a subset of glia precursors that may give rise to differentiated glial cells in vivo.

284. **Regulation of In Vitro Fate Specification of Gonadotropin-Releasing Hormone Neurons by FGF Signaling.** John C. Gill and Pei-San Tsai. Department of Environmental, Popula-

tion, and Organismic Biology and the Center for Neuroscience, University of Colorado, Boulder, CO 80309-0334.

Neurons that synthesize and release gonadotropin-releasing hormone (GnRH) are the most upstream neuroendocrine regulators of vertebrate reproduction. Unlike most neurons in the brain, the fate of GnRH neurons is specified outside the central nervous system (CNS) in the nasal placodes of the embryonic nose. GnRH neurons, after fate specification, migrate into the brain and extend axons to target the median eminence for hormone release. We hypothesized that members of the fibroblast growth factor (FGF) family, known for their potent neurotrophic actions in the developing CNS, regulate GnRH neuron fate specification. In this study, we examined if SU5402, a FGF receptor (FGFR) antagonist, alters GnRH neuronal fate specification in cultured embryonic nasal placodes isolated from E10.5 mouse embryos, an age when GnRH neurons were first observed. Nasal placodes maintained in serum-free medium for 3 days *in vitro* (3DIV) have significantly higher GnRH neurons than controls at Day 0 (0 DIV), demonstrating our culture system supports the fate specification of GnRH neurons. Addition of SU5402 significantly blocked the emergence of GnRH neurons in cultured nasal placodes, suggesting a role of FGF signaling in GnRH neuronal fate specification. The newly emerged GnRH neurons did not incorporate bromodeoxyuridine (BrDU), a mitotic marker, when a pulse of BrDU was given during the first 24 hours of culture, demonstrating GnRH neurons were specified from a population of postmitotic precursors. These results demonstrate the activation of FGFR is critical in the final stages of GnRH neuronal fate specification, particularly the progression from postmitotic precursors to the GnRH expressing phenotype.

285. Withdrawn

286. Pax6 and Hes1 Transcription Factors Direct Retinal Neuron Identity. Carrie N. Stair, Gary T. Philips, Hae Young Lee, Emily Wroblewski, Nadean L. Brown, and Grant S. Mastick. Biology Department, University of Nevada, Reno, Nevada; Divisions of Developmental Biology and Ophthalmology, Children's Hospital Research Foundation, Cincinnati, Ohio.

The retina produces a stereotyped sequence of differentiated cell types, beginning soon after optic cup formation. The first neurons are specified as retinal ganglion cells (RGCs), activating a diagnostic set of genes, including both general neuronal differentiation and RGC-specific genes. We have characterized null mutations in two genes that participate in early eye development: the paired-box homeodomain factor Pax6 and the bHLH gene Hes1. Both mutants have precocious neurogenesis within the retinal epithelium, indicating repressive roles in neurogenesis. To determine the identity of these precocious neurons, immunofluorescence and *in situ* hybridization were used to identify the presence of neuron-specific markers. The first wild-type neurons express RGC markers such as β -III-tubulin, doublecortin, Islet1, Math 5, and Brn3b. In Hes1 mutants, the precocious neurons develop as RGCs, expressing markers as in wild-type. In contrast, precocious retinal neurons of Pax6 mutants express β -III-tubulin, Doublecortin, and Islet1, but fail to express two critical RGC markers, Math5 and Brn3b. Amacrine neurons, as predicted by a previous study (Marquardt et al., Cell 2001, 105, 43), were not found, implying a novel early function for Pax6 in RGC identity. Taken together, our results suggest that RGC formation is

regulated by separable genetic steps, which set the timing of neurogenesis and specification of neural identity.

287. Abstract #287 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

288. Precocious Retinal Neurons: Pax6 and Hes1 Regulate the Timing of Retinal Neurogenesis in Mice. Grant S. Mastick, Gary T. Philips, Carrie N. Stair, Hae Young Lee, Emily Wroblewski, and Nadean L. Brown. Biology Department, University of Nevada, Reno, Nevada; and Divisions of Developmental Biology and Ophthalmology, Children's Hospital Research Foundation, Cincinnati, Ohio.

The vertebrate optic vesicle first undergoes morphogenesis to form the optic cup, followed soon after by differentiation of the initial neurons, retinal ganglion cells (RGCs). We have found that these two processes are coordinated by two transcription factors, Pax6 and the bHLH gene Hes1. Pax6 has a critical role in eye development, with eye morphogenesis apparently arrested at the optic vesicle stage in Pax6 mutant embryos. Surprisingly, we found that neurogenesis in Pax6 mutant optic vesicles was not arrested, but accelerated as numerous neurons differentiated precociously, more than a day early than in wild-type. Hes1 mutants showed similar defects in eye morphogenesis and likewise accelerated neurogenesis. However, comparison of the two mutants revealed considerable differences in proneural and differentiation markers. Hes1 mutants precociously activated several proneural bHLH genes, and an appropriate set of RGC markers, including Math5. In contrast, Pax6 mutants activated only one of the examined proneural factors, Mash1, and the precocious neurons expressed generic neuronal markers (and no amacrine markers). These results identify both Pax6 and Hes1 as critical for setting the timing of retinal neurogenesis. Subsequently, Pax6, but not Hes1, is required for coupling neuron differentiation to specification. The differing molecular phenotypes in these mutants identify two distinct but overlapping genetic pathways in the control of the timing of retinal neurogenesis.

289. Retinal Cell-Fate Determination: Importance of Intracellular Developmental Programs. Michel Cayouette, Ben A. Barres, and Martin Raff. MRC Laboratory for Molecular Cell Biology and Cell Biology Unit, University College London, London, United Kingdom, WC1E 6BT; and Department of Neurobiology, Stanford University, Stanford, California 94305-5125.

Cell diversification in the developing nervous system is thought to involve both cell-intrinsic programs and extracellular signals, but their relative contributions remain uncertain. In the mammalian retina, different cell types develop on a predictable schedule from multipotent retinal progenitor cells. A current view is that progenitor cells pass through a series of competence states, progressively changing their responsiveness to instructive extracellular cues, which also change over time. Here, we use a novel clonal-density culture system to test to what extent intrinsic programs in embryonic retinal progenitor cells control cell fate choices in the developing rat retina. We find that embryonic day 16-17 (E16-17) rat retinal progenitor cells develop similarly in serum-free clonal-density cultures and in serum-containing reti-

nal explants develop similarly in the number of times they divide, the cell types they generate, and in the order in which they generate the different cell types. These unexpected results indicate that, from at least E16-17 onward, rat retinal progenitor cells are already heterogeneous and step through their own developmental program independently of instructive extracellular signals, challenging the current views about how progenitor cells diversify to produce the various retinal cell types. This is the strongest evidence so far that intrinsic developmental programs in retinal progenitor cells help control cell-fate choices in the retina.

290. **Zac1 Function in Cell Fate Specification in the Retina.** Lin Ma, Natasha Klenin, Laurent Journot, Sarah McFarlane, and Carol Schuurmans. Department of Biochemistry and Molecular Biology, 2153 Health Sciences Center, University of Calgary, Canada.

The retina serves as an ideal model system to study cell fate specification as it consists of only six neuronal subtypes and a single glial cell. Cell fate specification in the retina is a complex process influenced by both intrinsic factors and extrinsic signals. Many transcription factors, including homeodomain and basic-helix-loop-helix family members, are known to act in a combinatorial manner to specify the identity of distinct neuronal and glial cell types. We have found that the zinc finger transcription factor mZac1 is expressed at high levels in multipotent retinal progenitors in the developing mouse retina. In the postnatal retina, mZac1 expression is initiated in subsets of amacrine, horizontal, retinal ganglion cells, and Müller glia. Overexpression of mZac1 in *Xenopus* embryos strongly biases retinal progenitors toward a Müller glia cell fate. In addition, retinal ganglion cells derived from mZac1 overexpressing progenitors are found in clusters and have abnormal morphologies. Currently we are examining whether mZac1 influences cell fate specification directly, as opposed to regulating apoptosis and/or cell-cycle kinetics. Taken together our studies suggest that the zinc finger protein mZac1 may participate with other transcriptional regulators to specify cell fates in the vertebrate retina.

291. **Use of Transgenic *Xenopus* embryos to Identify Critical Regulatory Regions in the Xath5 Promoter.** David A. Hutcheson,* Nadean Brown,† and Monica L. Vetter†. *Department of Neurobiology and Anatomy, University of Utah School of Medicine, Salt Lake City, Utah 84132; and †Divisions of Developmental Biology and Ophthalmology, Children's Hospital Research Foundation, Cincinnati, Ohio 45229.

In the neural retina, expression of the basic helix-loop-helix factor Xath5 commences at st. 24 when retinal neuron differentiation begins, but is quickly downregulated in postmitotic differentiated cells. Prior work has indicated that Xath5 functions as a regulator of retinal neuron differentiation and has been linked specifically to the differentiation of retinal ganglion cells. To investigate the mechanisms that regulate the expression of *ath5* in the vertebrate retina, we are using the *Xenopus laevis* REMI transgenic technique pioneered by K. Kroll and E. Amaya. Using a GFP reporter and 3.3 kb of genomic sequence 5' to the Xath5 gene, we have created transgenic *Xenopus* tadpoles. The spatial and temporal pattern of transgene expression resembles the Xath5 in situ hybridization pattern. This

indicates that this promoter sequence is sufficient to direct the expression of Xath5 to the appropriate tissues. Using a series of deletions and point mutations, we have isolated a 201-basepair region of the Xath5 promoter which is able to drive expression of the GFP transgene in the developing retina. Sequence analysis of this region and comparison with mammalian *ath5* homologues identified several highly conserved binding sites. Among these are two E-boxes, or bHLH factor binding sites. In the context of this 201-bp promoter, we find that these E-boxes are essential for retinal expression, suggesting that Xath5 expression is regulated in vivo by bHLH factors.

292. **Role of Murine *Irx2* and *Irx5* Homeobox Genes during the Development of CNS and Neural Retina.** Mélanie Lebel,*† Chi Wa Cheng,‡ Vijitha Thanabalasingham,* Xiaoyun Zhang,* Shuk Han Cheng,‡ and Chi-chung Hui*,†. *Hospital for Sick Children, †University of Toronto, ‡City University of Hong Kong.

Irx genes are mouse homologues of the *Drosophila Iroquois* homeobox genes and several studies have suggested that these genes play a role in organ patterning. In higher vertebrates, *Irx3* is involved in the specification of neural progenitors along the dorso-ventral axis of the nervous system. *Irx4* plays a role in the specification of the ventricular heart chamber and in the neural retina development. The six *Irx* genes found in mouse exhibit specific expression in several organs, including the central nervous system, the neural retina, and the heart. To study the role of *Irx* genes during mouse development, we generated *Irx2* and *Irx5* mutant mice. Both *Irx2* and *Irx5* null mice are viable. *Irx2* null mutants do not exhibit any apparent defects. *Irx5* null mutants are smaller and have defects in differentiation of retinal neurons. *Irx2* and *Irx5* null embryos showed no significant defect in the patterning of their nervous system. Functional redundancy between *Irx* genes has been previously shown in several species and may be responsible for the absence of strong developmental defects in all the *Irx* mutants reported so far. To evaluate the functional redundancy between *Irx2* and *Irx5*, we have generated *Irx2;Irx5* null mutants. *Irx2;Irx5* mutant mice have reduced viability and show a more severe phenotype than either single mutant, supporting the notion that *Irx2* and *Irx5* possess overlapping functions during mouse development. Results of these studies will be presented.

293. **Role of XBH1 in Ganglion Cell Fate Commitment within the *Xenopus* Retina.** Lucia Poggi,* Teresa Vottari,* Joachim Wittbrodt,† Giuseppina Barsacchi,* and Robert Vignali*. Dipartimento di Fisiologia e Biochimica, Università di Pisa, Italy; †Developmental Biology Programme, EMBL, Heidelberg, Germany.

A complex interplay between extrinsic and intrinsic factors acts on precursor cells and leads to specify different cell types in the developing vertebrate retina. To date, a limited number of genes involved in this process have been identified. Vertebrate homologues of the *Drosophila* BarH genes are expressed in retinal ganglion cells during development. We addressed the role of the *Xenopus* Bar gene XBH1 in retinal ganglion cell development and its possible interaction with the proneural genes Xath5 and Xath3, also involved in ganglion cell determination. We show that XBH1 has a unique role in promoting ganglion cell fate within the retina. Lipofections of retinal precursors with XBH1

DNA constructs lead to an increase of ganglion cell frequency in derived clones. In addition, chimeric XBH1 fusions to engrailed repressor (XBH1EnR) or VP16 activation (XBH1VP16) domains, respectively, increase or decrease the number of ganglion cells. Moreover, while XBH1 is able to increase Xath5 and Xath3 ability to promote ganglion cell fate in lipofection experiments, XBH1VP16 suppresses this effect of atonal-related genes. Injection of XBH1 mRNA into animal caps turns on expression of the XBrn3 ganglion cell markers. This effect is reproduced by XBH1EnR, and suppressed by XBH1VP16, mRNA. XBH1VP16 similarly suppresses XBrn3 expression in Xath5 injected caps. Our results strongly suggest that XBH1 acts as a late repressor downstream of Xath3 and Xath5 in the genetic pathway leading to ganglion cell fate commitment in the *Xenopus* retina.

294. **VegT, a Vegetal Maternal Molecule, Represses Retinal Development in *Xenopus*.** Bo Yan and Sally A. Moody. Department of Anatomy and Cell Biology, The George Washington University, Washington, DC 20037.

Elucidating retinal cell fate determination is essential to understanding normal retinal development, congenital, and disease-related retinal defects. Previous studies demonstrate that a vegetally localized maternal factor represses the ability of embryonic cells to contribute to retina. Here, we investigated the regulating effects on retina formation of a candidate maternal transcription factor-VegT, which is essential for endoderm formation. VegT misexpression is carried out by injecting VegT mRNA (5-200 pg) plus GFP mRNA into one animal blastomere of 16-cell stage embryos that contribute significantly to the eye. The eye formation was inhibited significantly (62-87%) in a dose-dependent manner. Sectioning of the embryos revealed that an ectopic, GFP-labeled tissue mass was formed, suggesting that retinal precursors were directed to another embryonic fate. Whole-mount in situ hybridization showed that misexpressed VegT repressed expression of three neural genes (Sox3, Rx, and Notch-1) and induced the expression of endoderm, an endoderm marker. To deplete endogenous VegT, morpholino antisense oligonucleotides corresponding to VegT were injected into animal blastomeres at two-cell stage. VegT-depleted embryos had enlarged eyes (40%) and expanded domains of Sox3, Rx, and Notch-1 expression. Depletion of VegT in vegetal blastomeres lead to reduced gut development. Taken together, our data demonstrated that VegT changes retina cell fate from ectoderm to endoderm. Therefore, VegT, a maternal molecule, plays an essential role in repressing retinal development in the vegetal region of the embryo. (Supported by NIH Grant EY10096.)

295. Withdrawn

296. Abstract #296 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

297. **Zebrafish *lmo4* Coorthologs in Embryonic Development.** Catherine McCollum and Mary Ellen Lane. Rice University, Houston, Texas.

We have identified a zebrafish gene for the LIM domain protein, *lmo4*. We show that this gene *lmo4b* plays a role in fin, craniofacial, and otic development, as well as eye patterning and pigmentation. We have also identified a closely related gene (74%

amino acid identity) and we demonstrate through sequence comparison and phylogenetic analyses that this gene is a second *lmo4* ortholog, which we designate *lmo4a*. A partial genome duplication in teleost fish occurred approximately 100 Mya, and about 30% of all mammalian genes have two zebrafish orthologs. Determining the spatial distribution of *lmo4a* and further misexpression and knockdown studies will provide information for functional overlap.

298. **FGF Signaling Is Required for Neuroblast Determination during Otic Development.** Berta Alsina, Montse Coll, Encarna Ulloa, Cristina Pujades, and Fernando Giraldez. Biologia del Desenvolupament, DCEXS-Universitat Pompeu Fabra, Barcelona, Spain.

The generation of otic neurons is initiated by the early specification of neural precursors in the otic placode epithelium. We have analyzed the expression and function of FGF10 and FGF-signaling during the early stages of the development of otic neurons. FGF10 is expressed in a highly restricted domain overlapping the presumptive neurogenic (proneural-sensory) domain of the otic placode. A study of the expression pattern of FGF10, proneural, and neurogenic genes revealed the following temporal sequence in the onset of gene expression: FGF10-LFng > Ngn1-Dl1-Hes5 > NeuroD-NeuroM. Neural differentiation genes NeuroD/M persist in ganglionic neuroblasts along Islet1/2 and Tuj1, but not FGF10, or neural determination genes Ngn1 and Delta1. Overexpression of FGF10 or FGF10 delivery in vivo promotes an increase in NeuroD or NeuroM, but not Delta1. These effects occur only within the proneural-sensory domain of the otic vesicle. FGF receptor inhibition in otic placode explants causes a severe reduction in NeuroD and Delta1 expression with no change in nonneural genes similar to Lmx1. FGF receptor inhibition does not interfere with neuroblast delamination or proliferation. FGF10 and the inhibition of FGF receptor signaling cause mirrored images on cell determination and cell proliferation, FGF10 inducing the former and reducing the latter. We suggest that local activity of FGF10 is required for the transit of precursors toward a state of cell determination and that it does so by silencing cell division.

299. **An Intricate Choreography of Cell Movements Is Involved in the Formation of the Chick Olfactory Placode.** Sujata Bhattacharyya, Marianne Bronner-Fraser, and Andrea Streit. California Institute of Technology, Pasadena, California 91125; and King's College, London, United Kingdom.

Neurogenic placodes are focal ectodermal thickenings present in stereotypic positions in the vertebrate embryonic head. Their derivatives are responsible for much of our sensory perceptions in the craniofacial region. The olfactory placode which gives rise to the olfactory epithelium of the nose has a dual origin within the embryo. It initially arises within the anterior neural folds and the adjacent ectoderm. Little else is known about the early stages of its induction and formation. To trace definitively the origin of these cells, we generated a fate-map and compared it with patterns of gene expression in the chick olfactory region. Small populations of cells were labeled with DiI and DiO from HH stage 6 to stage 10 and their derivatives were analyzed 2 days later. At head-fold stages, olfactory placode precursor cells are spread out over a broad domain and in certain areas share a common origin with lens, epidermal, and/or neural precursors. As the neural

folds close, the olfactory precursors appear to converge anteriorly within the ectoderm. The lens and nasal precursors seem to sort out from each other around HH stage 8, at which time, *Pax-6* is differentially upregulated in the region fated to form the lens and *Dlx-5* expression is enhanced in the anterior area where nasal precursors accumulate. To further study the cell movements that lead to the eventual formation of the olfactory placode, we performed confocal time-lapse analysis. We conclude that considerable cell mixing is involved in the formation of this placode. Molecular cues that could serve as possible chemoattractants for the nasal precursor cells are being investigated.

300. **The *C. elegans* *Otx*-related Genes Specify Distinct Sensory Neuron Identities.** Anne Lanjuin,* Miri K. VanHoven,† Cornelia I. Bargmann,† Julia K. Thompson,* and Piali Sengupta*. *Department of Biology, Brandeis University, Waltham, Massachusetts 02454; and †HHMI, Departments of Anatomy and Physiology, University of California-San Francisco, San Francisco, California 94143.

OTX transcription factors have a conserved role in the patterning of brain and sensory structures. We demonstrated that *ttx-1*, one of the three *C. elegans* *Otx*-related genes, is crucial for the specification of the AFD thermosensory neurons (Satterlee et al, 2001). We now show the remaining two *Otx*-related genes, *ceh-36* and *ceh-37*, are also required for the specification of distinct sensory neuron types. CEH-37 acts to promote AWB olfactory neuron development by regulating the expression of the LIM homeobox gene *lim-4*. CEH-36 is expressed in the ASE and AWC chemosensory neurons and is required at distinct steps in their development. It is required broadly for AWC olfactory neuron differentiation, yet plays a more limited role in the development of the ASE gustatory neurons. We investigated whether the distinct phenotypes of the *Otx* mutants are a consequence of divergence in gene functions and/or in spatiotemporal expression patterns. We find that these *Otx* genes, as well as the rat *Otx1*, gene are functionally equivalent for AWB and AWC but not AFD neuron specification. Interestingly, each *Otx* gene is sufficient to impart either AWC or AFD neuron characteristics to other sensory neuron types, suggesting that cellular context plays a critical role in dictating the specific identity adopted. We suggest that the *C. elegans* *Otx*-like genes have evolved to specify different sensory neuron types through changes in their regulatory sequences, as well as through changes in their individual activities.

301. **Cell Fate Specification Along the A-P Axis of the Intermediate Mesoderm.** Hila Barak,* Lea Rosenfelder,* Thomas Schultheiss,† and Ram Reshef*. *The Faculty of Biology, Technion, Haifa, Israel; and †Molecular Medicine Unit, Beth-Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts.

Many embryonic tissues show clear anterior—posterior (A-P) patterning, which can be observed morphologically and molecularly. For example, in the chick embryo kidney tissue is only generated from intermediate mesoderm (IM) located posterior to the sixth somite axial level. To gain insight into the mechanisms of A-P patterning of the kidney and of A-P patterning in general, the current study focuses on the problem of how the border at the sixth somite level between kidney-generating and non-kidney-generating IM is formed. A comprehensive set of tissue manipu-

lation experiments using chick-quail chimeras were performed to address when and where the anterior border of kidney gene expression is specified. Results obtained from these studies have shown that the prospective IM region in the primitive streak (PS) of early embryonic stages (HH 3-6) is already competent to respond to inductive signals. Interestingly, we have found that these signals are present along the whole axis even at early stages as ectopic expression of kidney markers, such as *Pax2* and *Lim1*, was obtained in anterior non-kidney-generating IM. However, only at early stages (HH 3-4) the prospective IM region in the PS is competent to respond to inhibitory signals that are located along the migratory pathway and therefore, anterior to the border; normally IM regions do not express kidney genes. In this study we propose a model to explain the shift of the anterior border of IM gene expression posteriorly during the evolution of kidney development.

302. **BMP Signaling Regulates Mesoderm Patterning in Mouse Embryogenesis.** Shigeto Miura,* Shannon Davis,† John Klingensmith,† and Yuji Mishina*. *Molecular Developmental Biology Group, LRDT, NIEHS/NIH, Res. Triangle Park, North Carolina 27709; and †Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710.

Bmpr1a encodes the BMP type IA receptor for bone morphogenetic proteins (BMPs) 2 and 4. Bmpr1a mutant embryos fail to gastrulate. To assess the later functions of BMP signaling, we established a Bmpr1a conditional allele. MORE mice that express Cre in the epiblast generate an epiblast-specific lack of Bmpr1a. Mutant embryos gastrulated successfully; thus, BMP signal transduction is critical in extraembryonic tissues for normal gastrulation. Histological and marker analyses of mutant embryos at E7.5-8.5 revealed that (1) lateral plate mesoderm was underdeveloped; (2) somites were expanded laterally; (3) node and notochord seemed to develop normally; (4) primordial germ cells were decreased; and (5) the heart did not form, although heart mesoderm was specified at E7.5. Tunnel assays of mutant embryos detected increased apoptosis in heart mesoderm beginning from the head fold stage. As a possible mechanism to explain these observations, we hypothesized that BMP signaling from the proximal end of the embryo might be important for establishing regional domains of mesoderm emigrating from the primitive streak, such that lateral mesoderm is promoted by BMP while more medial domains do not require BMP. Accordingly, presumptive paraxial (somitic) mesoderm should be expanded toward the proximal end of the primitive streak in the mutant embryo. To test this hypothesis, we are undertaking marker analyses, lineage analyses, and experiments using explant culture. Our results will be reported.

303. **Regulation of Cardiac Differentiation by Cell Adhesion Molecules.** Ryan Ausborn, Samuel Zwenger, and Duane A. Hinton. Department of Biological Sciences, Fort Hays State University, Hays, Kansas.

Abnormal differentiation of the cardiac mesenchyme has been implicated as a primary cause of heart malformations which account for the majority of clinically significant birth defects (Clark, 1987). Many are undoubtedly the result of errors in inductive signaling or cell/extracellular matrix interactions. Using a mouse embryonic stem (ES) cell model, this study will characterize the

ECM receptor interactions that induce cardiac myocyte differentiation. ES cells are permanent cell lines which can be propagated and experimentally manipulated in vitro. For example, ES cells that are allowed to differentiate do so by first forming cellular aggregates, termed embryoid bodies, that after 9-10 days differentiate into beating cardiac myocytes in vitro. Our early results demonstrate that an early cardiac cell differentiation gene, *Nkx-2.5*, is expressed as early as day 4 of differentiation and continued to increase its expression through day 12 (the last time point taken). Immunoblot analysis of cardiac myosin expression was first observed on day 8, 1 day before visualization of beating cardiac myocytes on day 9. We are using RT-PCR and immunoassays to examine the expression of β -1,4-galactosyltransferase (GalTase), as well as α 6A, α 4, B1 integrins. Integrin and GalTase antibodies, integrin-specific cell binding domain peptides, and GalTase-specific perturbation reagents will be used to block ECM/receptor interactions to determine the effects on cardiomyocyte differentiation with expression of cardiac myosin and dHAND. Our early results suggest that GalTase is expressed in these differentiating cells and that perturbation of GalTase in early stages of differentiation leads to dissociation of the embryoid bodies after 2 days of differentiation.

304. **Notch Promotes Epithelial-Mesenchymal Transitions during Heart Development and Transformation.** J. L. de la Pompa,* L. A. Timmerman,† J. Grego,* J. M. Pérez-Pomares,‡ A. Raya,§ F. McCormick,† and J. C. Izpisua-Belmonte§. *Departamento de Oncología Molecular, Institut de Recerca Oncològica (IRO), Barcelona, Spain; †UCSF Cancer Center, San Francisco, California USA; ‡Departamento de Biología Animal, Universidad de Málaga, 29071 Málaga, Spain; and §Gene Expression Laboratories, The Salk Institute, La Jolla, California USA.

The Notch pathway regulates cell-fate choices in embryonic and adult tissues by lateral inhibition and lateral induction mechanisms. Disruption of Notch has severe developmental consequences and overexpression of activating alleles is oncogenic in mammals. Here we show that Notch functions in a field of endocardial cells during heart valve development in a manner consistent with lateral induction. Notch-expressing endocardial cells undergo a TGF β -mediated epithelial-mesenchymal transition (EMT), to cellularize developing cardiac valves. Loss of Notch activity attenuates expression of Notch pathway elements, TGF β 2 and its receptors, prevents local expression of the snail repressor, and stabilizes expression of the cellular adhesion molecule VE-cadherin, resulting in loss of endocardial EMT. Conversely, transient ectopic expression of activated Notch1 (Notch1IC) in zebrafish embryos results in abnormally enlarged and hypercellular cardiac cushions. Overexpression of Notch1IC in endothelial cells in vitro also induces attenuation of VE-cadherin expression, loss of contact inhibition, and apparent EMT. We conclude that Notch plays an unexpected, novel role in the promotion of EMT in both development and cancer progression, in part via regulation of the cellular adhesion system.

305. Abstract #305 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

306. **The Role of Prox1 in the Specification of Lymphatic Endothelial Cell Fate.** Natasha Harvey and Guillermo Oliver. Department of Genetics, St Jude Children's Research Hospital, Memphis, Tennessee USA.

The homeobox gene *Prox1* is the first gene identified that is absolutely essential for development of the mammalian lymphatic vasculature. *Prox1*-null embryos die at approximately E14.5 and display a multitude of phenotypic abnormalities in addition to the failure to form lymphatic vessels, including disruptions in development of the lens, liver, and CNS. Detailed analysis of *Prox1* expression during embryogenesis revealed that *Prox1* is initially expressed in a subpopulation of endothelial cells in the cardinal veins; these cells ultimately bud, proliferate, and migrate to create the entire lymphatic vasculature. In the absence of *Prox1* expression, the endothelial cells which initially bud from the cardinal vein never acquire a lymphatic phenotype, instead they maintain a blood vascular phenotype. This indicates that *Prox1* is a master gene required to specify the fate of lymphatic endothelial cells. In an effort to understand the mechanism of *Prox1* activity during lymphangiogenesis, we have adopted an approach to analyze the molecular profile of lymphatic endothelial cells at various stages of vascular development. Here we present some of the molecular events regulated by *Prox1* that are important for the generation of the lymphatic vasculature.

307. **PPARgamma Ligands Promote Human Leukemia NB4 Cells to Myeloid Differentiation.** Etsuko Yasugi,* Akiko Horiuchi,† Emiko Okuma,* Masami Nakatsu,* Kumiko Saeki,* Isao Uemura,‡ and Akira You*. *International Medical Center of Japan, Tokyo, Japan; †International Christian University, Tokyo, Japan; and ‡Tokyo Metropolitan University, Tokyo, Japan.

Peroxisome proliferator-activated receptor- γ (PPAR γ), a member of the nuclear receptor superfamily, is known to promote adipocyte or macrophage differentiation and also to regulate lipid metabolism. Pioglitazone (Actos, AD), an insulin-sensitive drug, has recently been identified as a synthetic ligand and 15-deoxy- δ -12,14-prostaglandin J₂ (PG) as natural or endogenous ligand for PPAR γ , respectively. In this study, we examined the effects of PPAR γ ligands on cell differentiation and lipid metabolism in human leukemia NB4 cells, derived from a patient with acute promyelocytic leukemia. It is known that these cells undergo morphological granulocytic maturation when exposed to 1 μ M all *trans*-retinoic acid (ATRA). Treatment of the cells with each PG (4 μ M) or AD (50 μ M) markedly suppressed proliferation but failed to induce differentiation. Combined treatment of the cells with PPAR γ ligand and ATRA (1 nM) resulted in morphological granulocytic differentiation and an increase in the number of cells positive for nitroblue tetrazolium staining, whereas treatment with ATRA (1 nM) alone showed no effect. During the process of differentiation in NB4 cells, we observed marked accumulation of lipid droplets in cytoplasm stained with Nile red. These findings demonstrated that the treatment with PPAR γ ligands stimulated significantly differentiation of NB4 cells together with lipogenesis.

308. **What Prevents *Xenopus* Primordial Germ Cells from Entering an Endodermal Fate?** Mary Lou King and Thiagarajan Venkataraman. Department of Cell Biology, University of Miami School of Medicine, Coral Gables, Florida.

Primordial germ cells (PGC) in *Xenopus* are specified through the inheritance of germ plasm. During gastrulation, PGCs remain totipotent while surrounding cells in the vegetal mass become

committed to endoderm. We asked if PGCs were transcriptionally repressed as they are in *C. elegans* and *Drosophila*, and therefore unable to respond to maternally inherited signals at the midblastula transition (MBT). PGCs were isolated from dissociated embryos at pre- and post-MBT stages. We used mAbs to determine the phosphorylated state of ser2 and ser5 in the CTD of RNA Pol II. We found that at pre-MBT, both PGCs and somatic cells contain P-ser5, suggesting initiation events have occurred. However, PGCs do not acquire P-ser2 until neurula stages, suggesting PGCs are transcriptionally repressed at the elongation step. Ser2 is phosphorylated by Cdk-9/cyclin T and these proteins are currently being analyzed. Consistent with these observations, results from RT-PCR analyses show that while somatic cells express Bix4 and Xnr1 at MBT, PGCs do not. To identify what new genes are transcribed at neurula by PGCs, we used a PCR-based subtractive cloning strategy. As expected, only neurula stages yielded new amplified products. Among the new zygotic PGC transcripts identified are a putative transcription factor bearing a BTB/POZ domain and cell signaling molecules. Current studies are focused on the function of these new PGC transcripts. Our results suggest that transcriptional repression may be an ancient mechanism for preserving PGC totipotency. (This work was supported by NIH Grant GM33932 to M.L.K.)

309. **Regulation of tra-1 by Sex-Specific Phosphorylation, Processing, and Nuclear Localization.** Mara Schvarzstein and Andrew M. Spence. Department of Molecular and Medical Genetics, University of Toronto, Toronto, Canada M5S 1A8.

The *tra-1* gene is the terminal global regulator of sex determination in *C. elegans*. Its activity is required for all female somatic cell fates. Its major product, TRA-1A, is a zinc-finger transcription factor of the Gli family. Western blot analysis of nematode lysates with an antibody against TRA-1 protein reveals striking differences between hermaphrodites and males. Lysates of both sexes contain a band of apparent molecular weight 140 kDa, approximately the predicted size of TRA-1A (125 kDa), but hermaphrodites accumulate several bands of apparent molecular weights 90-100 kDa that are absent from males. We suggest that these bands derive from TRA-1A by sex-specific proteolysis. The more slowly migrating bands in the 90-100 kDa size range are phosphorylated forms of the smaller bands. Males and hermaphrodites also differ greatly with respect to the subcellular localization of TRA-1 as assayed by immunofluorescence. Whereas adult males exhibit diffuse TRA-1 immunoreactivity throughout the cell in most tissues, adult hermaphrodites show predominantly nuclear TRA-1 immunofluorescence in somatic nuclei and distal germ line nuclei. We suggest that TRA-1A activity is regulated by sex-specific phosphorylation and proteolysis as well as by differential subcellular localization. Truncated, hermaphrodite-specific isoforms of TRA-1 may be responsible for specifying female cell fate by repressing known target genes. The regulation of TRA-1A may be analogous to that of *Drosophila* Ci and mammalian Gli2, both of which undergo ligand-independent proteolysis to yield transcriptional repressors.

310. **Homeoproteins in Prostate Development.** K. Economides,* Y. Hu,* M. Reynon,* S. Price,* N. Desai,* M. Capecchi,† M. Shen,* and C. Abate-Shen*. *Center for Advanced Biotechnology and Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey; and †HHMI, Depart-

ment of Human Genetics, University of Utah, Salt Lake City, Utah.

A process of differentiation and branching morphogenesis involving epithelial—mesenchymal interactions underlies the formation of many vertebrate organs, such as the lungs, kidneys, mammary glands, and prostate. However, the mammary gland and prostate are somewhat unique because their formation is sexually dimorphic and they display the ability to regress, regenerate, and rebranch depending on the serum concentrations of sex hormones. For example, the prostate undergoes significant atrophy in the absence of testosterone, but regrows in the presence of testosterone. The branching morphology and differentiation of various cell types within the prostate is coordinated by the expression of various molecules that inhibit or activate branching, including growth factors, signaling molecules, and homeodomain transcription factors such as Nkx3.1 and the Hoxb13. We are currently studying the roles of Hoxb13 and Nkx3.1 in the assignment of epithelial identity and branching morphology within the prostate since both exhibit roles in branching morphogenesis as well as proper differentiation of the luminal epithelium. Mice that are homozygous mutants for Hoxb13 display a complete loss of ventral prostate identity, while mice that are mutant for Nkx3.1 display defects in prostate branching morphology and prostatic secretions. We have been studying the potential collaborative roles of Hoxb13 and Nkx3.1 in prostate morphogenesis by utilizing knockout mice for these homeodomain transcription factors and will present our latest findings.

311. **Endothelial Cell Signaling.** Ondine Cleaver and Doug Melton. Harvard University, Cambridge, Massachusetts.

Endothelial cell signals have been shown to be important during both pancreas and liver development. Embryos lacking a dorsal aorta are deficient in pancreatic gene expression, while embryos with a hypervascularized pancreas show an increase in endocrine differentiation. In vitro cultures of prepancreatic endodermal explants demonstrate that aortic endothelial tissue is required for initiation/maintenance of pancreatic differentiation. In this report, we show that signals from endothelial cell lines can substitute for aorta endothelium in these explant cocultures, by promoting the expression of pancreatic markers. We also demonstrate that endoderm not fated to become pancreas can be redirected to take on a pancreatic fate in these explant cultures. To identify candidate molecules for the inductive endothelial signal, we characterize endothelial gene expression using RT-PCR and microarray analysis. We also demonstrate that endothelial cells can signal to differentiating embryonic stem cells and can alter their differentiation potential.

312. **Withdrawn**

313. **Expansion of Nestin-Positive Precursors in Embryonic Pancreas by TGF- α .** F. Esni and S. D. Leach. Department of Surgery. Johns Hopkins University, Baltimore, Maryland.

Nestin is an intermediate filament protein, expressed in neuronal precursor cells in which their differentiation is regulated by EGF signaling. We have rigorously mapped the expression pattern of nestin and its correlation with different pancreatic markers from E10.5 to adult pancreas. Furthermore, to study the effect of EGF signaling on nestin-positive precursors differentiation, intact

E10.5 dorsal pancreatic rudiments were cultured in the presence or absence of TGF- α . Our confocal studies revealed that nestin was indeed expressed in a subset of undifferentiated epithelial cells on E10.5. Interestingly, while a population of p48-positive cells expressed nestin until E15.5, no coexpression of nestin and *ngn3* could be detected. After E15.5, epithelial nestin expression was extinguished upon differentiation of different cell types. Our in vitro studies showed that nestin-positive epithelial cells could not be detected in control explants following a 7-day culture period. However, in explants treated with TGF- α a population of nestin-positive epithelial cells persisted and exocrine differentiation was prevented, even while α - and β -cell differentiation were unchanged. These data suggest that nestin is expressed in pancreatic exocrine precursor cells. However, as these cells become differentiated they also lose nestin expression. Furthermore, TGF- α seems to have an inhibitory effect on pancreatic exocrine differentiation in vitro, resulting in maintenance of a nestin-positive precursor pool. These data suggest that TGF α regulates an exocrine stem cell pool in developing pancreas.

314. **Response of Single Cells to Growth Factors.** F. C. Wardle and J. C. Smith. Wellcome Trust/Cancer Research UK Institute, Tennis Court Road, Cambridge, CB2 1QR. United Kingdom.

The embryos of triploblastic animals have three germ layers: mesoderm, endoderm, and ectoderm. In *Xenopus* embryos, soon after the midblastula transition, cells in the marginal zone are induced to form mesoderm by a secreted signal, likely to be a member of the TGF- β family. TGF- β signaling is also required for endodermal fate in the *Xenopus* embryo. Analysis of single cells in culture, or by heterotopic and heterochronic transplantation, suggests that cells of the early *Xenopus* embryo are not fully committed to ectodermal, endodermal, or mesodermal fate until the end of gastrulation. RT-PCR analysis of single cells at the start of gastrulation confirms that cells can be isolated that are "mesendodermal" or "mesectodermal". These cells, which are found in the marginal zone, express a combination of markers that are traditionally thought to be endodermal (*sox17*, *mix1*), mesodermal (*xbra*, *gsc*, *myf5*), or ectodermal (*keratin*). Addition of the TGF- β family member, Activin, to ectodermal explants (animal caps) can mimic the induction of mesoderm and endoderm. To understand how cells respond to an inducing factor at the single-cell level, ectodermal cells were exposed to activin, and the change in gene expression over time in single cells was followed. The results of these experiments will be discussed.

315. **Myosin Heavy Chain Expression in Embryonic, Adult, and Regenerating Muscle in Zebrafish.** Y. Frank Chan, Daniel A. Fernández and S. H. Devoto. Wesleyan University, Middletown, Connecticut, USA.

Unlike in mammals, muscle fiber types are spatially segregated in the axial musculature of fish. Fast fibers form the bulk of the myotome, while slow, intermediate, and tonic fibers are located more superficially and are far less numerous. In zebrafish, the most popular fish model for studying muscle development and growth, only two fast MyHC isoforms have been cloned and these have only been characterized in embryos. We have cloned and characterized those two and three novel myosin heavy chain isoforms in embryonic, adult, and regenerating axial muscle. Four are fast isoforms (*myhc1*, *myhc2*, *myhc3*, and *myhc4*) and the

other one is a slow isoform (*myhc5*). In larvae *myhc1*, *myhc2*, and *myhc3* are expressed in the bulk of fast fibers; *myhc4* is expressed in both fast and slow fibers, and *myhc5* is expressed only in slow muscle fibers. In adults, the expression pattern is more complex. Of the four fast isoforms, only *myhc2* is exclusively expressed in fast fibers. The slow isoform, *myhc5*, is predominantly expressed in slow fibers but other fibers also show some expression. The expression of these myosin genes is mosaic within the fast muscle, suggesting that fast fibers may not all be identical. The mosaic pattern resembles what has been observed in many other teleost species using histochemical techniques such as PAS, SDHase, and ATPase staining. Following muscle injury, regenerating muscle fibers transiently express all isoforms, independently of their position in the myotome. Our results suggest that muscle fiber type identity in zebrafish may be more complex than previously believed.

316. **Dlx5 Regulates Chondrocyte Differentiation at Multiple Stages.** Giovanni Levi,* and Andrew J. Bendall†. *CNRS UMR8572, Laboratoire de Physiologie, Museum National d'Histoire Naturelle, 7 rue Cuvier, 75005 Paris, France; and †Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario, N1G 2W1, Canada.

Endochondral ossification, in which cartilaginous templates are progressively replaced by marrow and bone, represents the dominant mode of development of the axial and appendicular skeleton of vertebrates. Chondrocyte differentiation within the cartilaginous core of these skeletal elements is tightly regulated, both spatially and temporally. Here, we describe the expression of *Dlx5* in the cartilaginous core of limb skeletal elements in chicken and mouse embryos. We find that *Dlx5* is one of the earliest genes expressed in condensing limb mesenchyme that will give rise to the limb skeleton. Later, when proliferating and differentiating chondrocytes are found in spatially distinct regions of the cartilaginous model, *Dlx5* is expressed in the zone of hypertrophy and in mitotically active chondrocytes in the flattened zone that are poised to differentiate, but not in the rapidly proliferating chondrocyte pool. Consistent with this pattern of expression, we show that forced expression of *Dlx5* potentiates early and late chondrocyte differentiation and inhibits proliferation in cultured cells. Examination of the limbs of mutant *Dlx5* mouse embryos revealed a delay in chondrocyte maturation compared with wild-type littermates. Together, these data reveal a positive role for *Dlx5* during multiple stages of chondrocyte differentiation and, with previous studies of *Dlx5* function during osteogenesis, identify *Dlx5* as a general regulator of differentiation in the mouse skeleton.

317. Abstract #317 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

318. **Mouse Intraflagellar Transport Proteins Regulate Hedgehog Signaling.** Danwei Huangfu, Andrew Rakean, and Kathryn Anderson. Sloan-Kettering Institute, Cornell University, Ithaca, New York.

We discovered a novel role for an evolutionarily conserved transport mechanism in the Hedgehog (Hh) pathway. First observed in *Chlamydomonas reinhardtii*, intraflagellar transport (IFT) is a system that transports molecules essential for the

generation and maintenance of flagellar and cilia. Defects in IFT proteins can lead to cilia-related diseases such as situs inversus, polycystic kidney disease, and retinal degeneration. No connection, however, has been made between IFT and the Hh pathway. *wimble* (*wim*) was identified as an ENU-induced mutation causing exencephaly and frequent reversal of heart looping. *wim* embryos failed to specify ventral cell types in the neural tube and had randomized left–right asymmetry. Expression of *Patched1* (*Ptch1*), a transcriptional target of Hh signaling, was greatly reduced in *wim*, suggesting Hh signaling was blocked. Epistasis analyses between *wim* and known components in the Hh pathway showed that *wim* acted downstream of *Ptch1*, *Smoothed*, and *Rab23*, and upstream of or parallel to *Gli3*, a transcription factor at the end of the pathway. *wim* encoded the mouse homolog of IFT172, an IFT protein in *Chlamydomonas*. Nodal cilia were absent in *wim*, confirming that *wim* was a true functional homolog of IFT172. This led us to test whether kinesin-II, the IFT motor, was also required in the Hh pathway. Ventral neural fates failed to be specified in mutant embryos lacking a kinesin-II subunit Kif3a as in *wim*. Similar to *wim*, Kif3a acted in the Hh pathway downstream of *Ptch1*. Thus, a novel role of IFT proteins was identified, raising the possibility that IFT proteins are required for trafficking of essential signaling molecules in the Hh pathway.

319. **The Disintegrin-like AmpA Protein Acts to Regulate Cell Fate Specification in *Dictyostelium discoideum*.** Hoa Ho, Timothy Varney, and Daphne Blumberg. University of Maryland Baltimore County, Catonsville, Maryland.

The *ampA* gene encodes a novel protein that modulates cell–cell and cell–substrate adhesions, as well as cell fate specification, in *Dictyostelium*. It is expressed by a population of cells called the anterior-like cells (ALCs). A large proportion of AmpA is secreted while some of the protein remains cell-associated. Deletion of the *ampA* gene results in an alteration of the tightly regulated prespore-prestalk ratio. Analysis of reporter gene expression of *ampA* null strains and chimeras indicates that secreted AmpA is necessary to prevent premature expression of a prespore gene marker. In the *ampA* null cells, this marker is expressed even in cells that are destined to adopt the prestalk fate. A supernatant source of AmpA added extracellularly can prevent this premature misexpression of the prespore marker. A 9 amino acid “active site” sequence in the ornatin domain is identified as necessary and sufficient for preventing cells from premature prespore gene expression. Furthermore, preliminary data suggests that a factor, called “prespore-inducing factor” or “PIF,” accumulates extracellularly in the absence of AmpA and is capable of inducing cells to activate prespore reporter expression. It is postulated that the ALCs secrete AmpA, which antagonizes PIF to prevent neighboring cells from assuming the prespore fate. AmpA expressed in the ALCs is also required for their rapid migration which plays an essential role in the shaping of the fruiting bodies. These results demonstrate the distinct roles for the AmpA protein during the processes of cell fate specification and cell migration during development of *Dictyostelium*.

320. **Notch and Wnt Signaling in Early Leech Development.** Foster C. Gonsalves and David A. Weisblat. Department of Molecular and Cell Biology, 385 LSA, University of California, Berkeley, California 94720-3200, USA.

Previous studies have shown that fates in the two-cell stage of the leech, *Helobdella robusta* are determined by the differential inheritance of intracellular determinants (teloplasm; Astrow et al., 1987; Nelson and Weisblat, 1992). Despite this determinacy, it has also been shown that the protein encoded by a wnt-class gene (HRO-WNTA) is expressed stochastically at an early point in the two-cell stage of *Helobdella*, seemingly as part of a homeostatic regulation of cell adhesion (Huang et al., 2001). Here we present further evidence for stochastic interactions in the two-cell stage. Specifically, we observe transcripts of a notch-class gene (*Hro-notch*) in a stochastic pattern that precedes that of HRO-WNTA protein. Further, we observe a similar pattern of expression for activated MAPK, which has been implicated in D quadrant specification in molluscan embryos (Lambert and Nagy, 2001). Thus the leech embryo, traditionally regarded as a prime example of mosaicism, is nonetheless activating at least three major cell-signaling pathways at the earliest possible point of embryonic multicellularity.

321. **Analyzing the Role of *Drosophila* Neuralized in Notch-Mediated Lateral Inhibition.** Cosimo Commisso and Gabrielle Boulianne. Program in Developmental Biology, Hospital for Sick Children, and Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada.

The Notch signaling pathway is important for the development of a vast number of tissues in many multicellular organisms. One of the well-characterized functions of Notch signaling is in the process of lateral inhibition whereby a single cell within an equivalence group can inhibit surrounding cells from differentiating into specific cell fates. Lateral inhibition occurs repeatedly during *Drosophila* development and is dependent upon the neurogenic genes, which encode components of the Notch signaling pathway. We have been studying the function of one of these neurogenic genes, *neuralized*, to determine its role in lateral inhibition mediated by the Notch signaling pathway. In previous studies, we have shown that Neuralized acts cell autonomously to inhibit neural cell fates within proneural regions. Conserved regions within Neuralized include two Neuralized Homology Repeats (NHR1 and NHR2) and the C-terminal RING finger domain. The NHR regions are required for Neuralized localization to the plasma membrane, whereas the RING finger has E3 ubiquitin ligase activity in vitro. Mutations in the RING finger abolish in vitro ubiquitin ligase activity and fail to rescue neurogenic embryos. To further characterize the function of Neuralized, we have generated transgenic flies expressing a mutant form of Neuralized and examined its effects on *Drosophila* development. We will show that the mutant protein acts as a dominant negative and that the resulting phenotypes can be useful for identifying factors that genetically interact with *neuralized*.

322. **The Role of *extramacrochaetae* in the Development of Stalk and Polar Cells in the *Drosophila* Ovary.** J. C. Adam and D. J. Montell. Department of Biological Chemistry, Johns Hopkins Medical School, Baltimore, Maryland 21205.

The polar cells are pairs of specialized cells found at the anterior and posterior poles of developing *Drosophila* egg chambers and are involved in several important signaling processes during oogenesis. The polar cells are related by lineage to the

stalk cells, a group of 4-7 cells that separate adjacent egg chambers, and similar signaling pathways, such as the Notch and JAK-STAT pathways, are involved in the specification of both cell types. We undertook an over-/misexpression screen to identify genes involved in specification of polar cell fate. This screen identified *extramacrochaetae* (*emc*), which encodes an HLH protein known to act by sequestering bHLH proteins downstream of Notch. EMC is expressed in proliferating cells in the gerarium, as well as in the follicle cells surrounding most of the egg chamber, but is reduced in the differentiating polar and stalk cells. Consistent with this expression pattern, ectopic expression of *emc* affected both polar and stalk cells. Unexpectedly, while the effect of *emc* overexpression in the polar cells resembled that of activated Notch, its effect in stalk cells differed from that of activated Notch. This suggests that the *Drosophila* ovary presents a useful system in which to study the relationship between Notch and EMC, which may be even more complex than previously appreciated.

323. **The Role of *serpent* and *Abdominal B* in Fat Cell and Muscle Specification in *Drosophila melanogaster*.** Deborah K. Hoshizaki and Jennell M. Miller. Department of Biological Sciences, University of Nevada, Las Vegas, Nevada.

The precursors to the *Drosophila* fat body are arrayed in a complex but stereotypic pattern within the mesoderm of the developing embryo. The specific positions in which different groups of precursor fat cells arise reflect intrinsic differences among mesodermal cells. These differences are determined, in part, by various patterning cues provided along both the dorsal/ventral and the anterior/posterior axis. The differentiation and maintenance of precursor fat cells are dependent upon the expression of *serpent*, a GATA transcription factor gene that is sufficient to induce fat cell formation. *serpent*'s initial expression in the mesoderm is controlled by a variety of dorsal/ventral and anterior/posterior cues. Within the lateral region of the posterior mesoderm, the homeotic selector gene *Abdominal B* represses *serpent*. Here, we present evidence that within the dorsal region of the posterior mesoderm, *AbdB* is critical for activation of *serpent* and the subsequent specification of the precursor dorsal fat cells. Furthermore, within the dorsal mesoderm *Abdominal B* and *serpent* can independently repress circular visceral muscle formation. We suggest that, in the appropriate context *Abdominal B* and *serpent* might participate in a molecular switch between visceral muscle and fat-cell specification.

324. **Molecular and Functional Characterization of a Novel RING-Protein in *Drosophila* Eye Development.** Chonnetta Jones,* Rita Reifegerste,† and Kevin Moses*. *Department of Cell Biology, Emory University, Atlanta, Georgia; and †Zentrum fuer Molekulare Neurobiologie, Universitaetsklinikum Eppendorf, Hamburg, Germany.

hedgehog (*hh*) encodes a short-range diffusible signaling molecule which regulates the progression of the morphogenetic furrow in *Drosophila* eye development. Hypomorphic mutations in the *hh* gene lead to an arrest in furrow progression and photoreceptor cell differentiation. Our laboratory has conducted a dominant modifier screen to identify novel genes that interact with *hedgehog* in eye development by using a viable heteroallelic genotype, *hh[1]/hh[13C]*. A mutation isolated from the genetic screen affects early embryonic development, including segmen-

tation and nervous system development. Initial studies suggest that the affected gene acts genetically upstream of *hedgehog* to promote Hedgehog function. We have molecularly characterized the genomic locus that is affected and determined that a novel ORF has been disrupted in this mutant. Furthermore, this ORF encodes a RING-protein that is highly conserved and has putative homologs in yeast, worms, mice, and humans. We will discuss the current molecular and genetic approaches that we are performing to more clearly determine its relationship to Hedgehog and its role in *Drosophila* development.

325. **Dpp, Notch, and Ey: Control of Tissue Specification and Proliferation in the *Drosophila* Eye-Antennal Disc.** K. L. Kenyon, S. S. Ranade, and F. Pignoni. Harvard Medical School/MEEI, Cambridge, Massachusetts.

In *Drosophila*, the adult eye, antenna, and head cuticle structures originate from a single progenitor epithelium known as the eye-antennal disc. The specification of regional identity within this disc involves the coordinated efforts of signaling pathways and selector genes during larval development. Numerous transcription factors such as Eyeless, Eyes absent, and Distal-less as well as the signaling molecules Dpp and Notch have been cast as likely mediators of this process, though much remains unknown as to their direct contribution. In this study, we present a new perspective on the roles of Ey, Dpp, and Notch in the specification of regional identity. We show that induction of eye and antenna primordia depends upon specific domains of *dpp* expression that appear during the second larval stage. We also find that the Notch pathway does not control the Ey/Pax6 pathway as currently proposed. Although Notch is not required for the establishment of eye identity, Notch does influence the emergence of an eye primordium through its control of cell proliferation within the disc. Finally, we present evidence indicating that Ey plays a broad role as a selector factor in eye-antennal disc development.

326. **A Screen for Suppressors of *mom-2* TS Embryonic Lethality.** Tuba Bas,* Takao Ishidate,† and Craig C. Mello*,†. *University of Massachusetts Medical School, Worcester, Massachusetts; and †Howard Hughes Medical Institute.

In this study, we used the soil nematode *C. elegans* as our model organism to study animal development. In four-cell stage *C. elegans* embryos, signaling between a posterior blastomere, P2, and a ventral blastomere, EMS, is required to specify endoderm. Components of conserved Wnt signaling pathway take part in the process, namely secreted protein MOM-2/Wnt, the receptor MOM-5/Frizzled, and downstream components such as SGG-1/GSK3 β , MIG-5/Dishevelled, and WRM-1/ β -catenin. To look for new components of this pathway, we have initiated suppressor genetic screens beginning with MOM-2/Wnt. For these screens we have utilized two temperature-sensitive alleles of *mom-2* (*ne834* and *ne874*). We used ENU as mutagen and screened for suppression of embryonic lethality at the nonpermissive temperature. Three million worms were mutagenized for *ne874* and six dominant suppressors were identified. Five of these have second site mutations in the *mom-2* gene, indicating potential intragenic suppression. Three of these have been outcrossed and two have been confirmed to exhibit linked suppression, while the third exhibits partial suppression linked to the original allele as well as a second site suppressor that has yet to be mapped.

Finally, the sixth suppressor appears to be unlinked. One million worms were mutagenized for *ne834* and three suppressors were identified including at least one unlinked suppressor. The other two are subject to ongoing experiments. The next step in this study is to clone the intergenic suppressors. Through analysis of these new mutants, we hope to gain further understanding of the role of Wnt signaling in embryonic development.

327. ***C. elegans pvl-5* Maintains Epithelial Cell Identity by Preventing *ced-4*-Independent Apoptosis.** Pradeep M. Joshi and David M. Eisenmann. Biological Sciences, UMBC, Baltimore, Maryland 21250.

The apoptotic or PCD machinery is actively kept in check by multiple evolutionarily conserved mechanisms to ensure the survival of the cell. We describe here a gene involved in regulating PCD during *C. elegans* vulval development. The *C. elegans* vulva is derived from six vulval precursor cells (VPCs) born in the first larval stage (L1). During L1, 12 neuroectoblast cells (P cells) migrate into the ventral midline and divide to give a neuron and a Pn.p epithelial cell. Six of the 12 Pn.p cells (P3.p–P8.p) become VPCs, while the rest fuse with the surrounding syncytium. We show that *pvl-5* is required for the generation of the correct number of Pn.p cells by preventing PCD of the Pn.p cells. *pvl-5(ga87)* was isolated in a screen for mutants with a Protruding vulva (Pvl) phenotype. In *pvl-5* mutants there are on average 7.0 Pn.p cells in the ventral midline compared to 11 in wild-type animals resulting in fewer cells to form the adult vulva. Lineage analyses in *pvl-5* mutants reveal that the 12 Pn.p cells are born correctly but later die. Pn.p cell death is suppressed by *lof* mutations in *ced-3* caspase and *gof* mutations in Bcl-2 homolog *ced-9*, suggesting the cells are dying by PCD in *pvl-5* mutants. Also, both *ced-9* and *pvl-5* function to prevent Pn.p cell death as the double mutant has fewer cells than either mutant alone. However, the Pn.p cell death is not suppressed by *lof* mutations in proapoptotic *egl-1* and APAF-1 homolog *ced-4*. This is surprising as loss of *ced-4* suppresses all PCD in the worm. This suggests loss of *pvl-5* function leads to the activation of PCD by a mechanism that is distinct from that seen in other somatic cells.

328. **Function of a FMRFamide-Related Neuropeptide Gene Family in *C. elegans*.** Kyuhyung Kim, Nartono Tjoe, and Chris Li. Department of Biology, Boston University, Boston Massachusetts.

FMRFamide (Phe-Met-Arg-Phe-NH₂)-related peptides (FaRPs) have been found throughout the animal kingdom where they are involved in many behaviors. With the completion of the genome of *C. elegans*, 23 FaRP-encoding genes, *flp-1* through *flp-23*, were identified. The cell-specific expression pattern of 19 *flp* genes was determined using *green fluorescent protein (GFP)* reporter constructs under the control of different *flp* promoters. Each *flp* gene is expressed in distinct, and sometimes overlapping, sets of cells. To determine the function of the *flp* genes in *C. elegans*, deletion mutants of eight *flp* genes were isolated by screening libraries of chemically mutagenized *C. elegans* and are being characterized. To identify genes that may regulate expression of the *flp* genes, genetic screens were used. Transgenic animals expressing a *GFP* reporter construct under the control of a *flp-1* or *flp-12* promoter were mutagenized and mutants showing altered expression of these reporters were isolated. Thus far, 10 *mof* (modifier of *flp*) genes have been identified. These genes include the paired-like

homeodomain protein UNC-42, the nuclear hormone receptor FAX-1, and the LIM homeodomain protein LIM-4. *mof-3* mutants show increased *flp-1* expression in the AVK neurons and appear to function downstream of *unc-42* and *fax-1*. *mof-6* mutants show decreased *flp-12* expression in the SMB neurons only at the adult stage, suggesting that the function of MOF-6 is to maintain expression of *flp-12* in the specific cell type. *mof-6* encodes a novel protein and acts downstream of *lim-4*. These results suggest that different *flp* genes are regulated by distinct pathways.

329. **Roles of a *C. elegans* Fer-type Nonreceptor Tyrosine Kinase in Morphogenesis and Wnt Signaling.** Aaron P. Putzke and Joel H. Rothman. MCD Biology, University of California-Santa Barbara, Santa Barbara, California 93106.

FRK-1 (Fer related kinase-1), the *C. elegans* homologue of the Fer NRTK, is most similar to the truncated isoform of the Fer kinase, FerT. In early embryos, FRK-1 localizes to cell boundaries and nuclei; however it becomes nonnuclear later in embryogenesis, when most cells are postmitotic (prior to epidermal enclosure). Expression is seen in most epithelial tissue types. Consistent with a role in epithelial morphogenesis, RNAi of *frk-1*, and a chromosomal deficiency that deletes it, lead to embryonic lethality with severe defects in morphogenesis resulting from a failure in enclosure and elongation, and a block to epidermal differentiation. These defects are rescued by *frk-1(+)*, and by mammalian FerT. These functions are apparently independent of the FRK-1 kinase activity, as a kinase-dead form is also able to rescue. In mammalian cells, Fer kinase interacts with β -catenin in the membrane cadherin complex. We found that FRK-1 becomes primarily nuclear-localized in mutants of *hmp-2*, a *C. elegans* nonnuclear β -catenin. While HMP-2 has not been implicated in Wnt signaling, we found that loss of *frk-1* rescues the gutless phenotype caused by the absence of WRM-1 (the nuclear, Wnt-involved β -catenin) in a HMP-2-dependent manner, suggesting that HMP-2 can function in the Wnt signaling pathway in the absence of FRK-1. We have also observed genetic interactions between *frk-1* and other components of the Wnt pathway, implicating it as a negative control of the pathway. We will present additional evidence for FRK-1 in membrane adhesion and Wnt signaling in *C. elegans*.

330. **Molecular Pathways Controlling the Assembly of the Spinal Monosynaptic Reflex Circuit.** S. Hippenmeyer,*,† E. Vrieseling,*,† D. R. Ladle,*,† T. Portmann, *,† T. M. Jessell,‡ and S. Arber*,†. *Biozentrum, University of Basel, Department of Cell Biology, Basel, Switzerland; †Friedrich Miescher Institute, Basel, Switzerland; and ‡HHMI, Columbia University, Department of Biochemistry and Molecular Biophysics, New York, New York, USA.

Dorsal root ganglia (DRG) neurons transmit sensory information from the periphery to the central nervous system. Recent evidence showed that the expression of ER81 and Pea3—members of the Pea3 subfamily of ETS transcription factors—is regulated by target-derived neurotrophic factors. ER81 expression by proprioceptive DRG neurons is essential for the establishment of proprioceptive afferent projections into the ventral spinal cord. To study how ETS transcription factors control proprioceptive neuron differentiation and to determine whether ETS transcription factors have the capacity to overcome the dependence of DRG neurons on peripheral signals, we generated mouse mutants

where ETS transcription factors of the Pea3 subfamily are expressed ectopically in DRG sensory neurons at different developmental stages. We found that DRG neurons from mice expressing a potent transcriptional activating variant of Pea3 early during development become independent of neurotrophic support for neuronal survival and neurite outgrowth but that the development of projections in the target regions of mutant DRG neurons was affected. In contrast, expression of the same protein in proprioceptive neurons at late developmental stages can rescue the projection phenotype of proprioceptive afferents in *Er81* mutants arguing for the importance of a sequential activation of transcription factors during DRG neuron development.

331. **Early Induction of the Neural Crest in *Xenopus*.** R. Mayor, S. Villanueva, M. Aybar, A. Glavic, G. Acuña, C. Tríbulo, S. M. Honoré, F. Bastidas, and E. Rodríguez. Universidad de Chile, Chile.

The neural crest segregates from the dorsal portion of the neural tube and migrates through the embryo to generate a highly pluripotent cell population, able to generate a variety of cell types. The cellular and molecular mechanisms that control neural crest induction and differentiation are only now beginning to be unraveled. We will present data that support a model of neural crest induction in three steps. We have characterized the first step of neural crest induction as the specification of the entire border of the neural plate as anterior neural fold. This step depends on a gradient concentration of BMP. In the second step additional signals such as Wnts, FGF, and retinoic acid transform the most posterior region of the anterior neural fold into prospective neural crest cells. This transformation corresponds to the posteriorizing signals that works on the neural plate. In this second step the Notch/Delta signaling plays an important role on the specification of the borders of the neural crest territory. The third step, that takes place once the neural tube is closed, requires higher values of BMP, as an inhibition on BMP activity at this step leads to a decreasing in the expression of the neural crest markers. The combination of all these signals at different steps leads to the activation of specific genes in the neural crest cells. We have shown that some of these genes work in the specification of the neural crest (such as *Snail*), while others work as survival factors (such as *Slug*, *Sox10*). A final model where all these extracellular signals and transcription factors are considered will be discussed.

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335. **Retinoic Acid Signaling Patterns Anterior Lateral Plate Mesoderm.** B. R. Keegan,* J. L. Feldman,* G. Begemann,† P. W. Ingham,† D. and Yelon*. *Developmental Genetics Program, Skirball Institute, New York University School of

Medicine, New York, New York; and †Centre for Developmental Genetics, University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN, United Kingdom.

Regionalization of each embryonic germ layer requires the integration of multiple signaling pathways. Our data show that retinoic acid signaling plays a critical role in the regionalization of the anterior lateral plate mesoderm into myocardial, myeloid, and endothelial progenitors. We find that blocking retinoic acid signaling, either via mutation or with a pharmacological inhibitor, results in excess myocardial tissue. Conversely, application of exogenous retinoic acid represses the formation of myocardial tissue. Other mesoderm derivatives respond differently to retinoic acid signaling; for example, blocking retinoic acid signaling results in a loss of endothelial and myeloid precursors in the anterior lateral plate mesoderm. These results suggest that retinoic acid signaling contributes to a balance between myocardial and nonmyocardial fates. Analysis of myocardial phenotypes in double mutants suggests that retinoic acid signaling functions upstream of *Fgf8*, *Hand2*, and *Gata5*, three factors that have been shown to promote myocardial differentiation. In addition, using a pharmacological inhibitor of retinoic acid receptors, we have shown that retinoic acid activity is required between 80% epiboly and tailbud to restrict myocardial differentiation. Together, our results identify a novel and early role for retinoic acid signaling during cell fate assignment in the anterior lateral plate mesoderm.

336. **FoxC1 and FoxC2 Regulate Somitic versus Intermediate Mesoderm Fate Determination.** Thomas Schultheiss,* Richard James,* Brigid Hogan,† and Bettina Wilm†. *Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts; and †Vanderbilt University School of Medicine, Nashville, Tennessee.

The forkhead family genes *FoxC1* and *FoxC2* are expressed in the paraxial mesoderm and, at lower levels, in the intermediate mesoderm of mouse and chicken embryos. Compound homozygous mouse mutants for *FoxC1* and *FoxC2* exhibit loss of somite gene expression and expansion of intermediate mesoderm (IM) markers into the paraxial mesoderm domain. Ectopic expression of *FoxC1* or *FoxC2* in chick embryos via in vivo electroporation results in repression of the IM transcription factor *Pax-2*, interference with formation of the nephric duct and tubules, and ectopic expression of the somite transcription factor *Pax-7* in the IM. Interestingly, manipulation of *FoxC1/2* levels does not appear to affect expression of lateral plate genes: In the compound mouse mutants, the lateral plate marker *FoxF1* is not expanded medially, and ectopic expression of *FoxC1/2* in the lateral plate does not lead to ectopic *Pax-7* expression. Based on these findings, we propose a model in which *FoxC1/2* act to promote somite formation within a group of embryonic cells that is competent to give rise to either somitic or intermediate mesoderm.

337. **A Forward Genetic Approach to Understanding Endodermal Organ Formation in Zebrafish.** Elke A. Ober, Heather Verkade, Holly A. Field, Duc Dong, Pia Aanstad, and Didier Y. R. Stainier. University of California-San Francisco, Department of Biochemistry and Biophysics, 513 Parnassus Ave., San Francisco, California 94143.

Despite the importance of endodermal organs, we have a rather limited understanding of their development. Screening for muta-

tions that affect the formation of the gut tube and its derivatives has been difficult, due to problems in visualizing internal organs using conventional microscopy. To circumvent this problem, we have used the transgenic gut GFP line expressing GFP throughout the developing digestive system, to carry out a forward genetic screen. We have screened 1035 genomes and identified more than 100 mutants that affect various steps of endodermal organogenesis—formation of the liver, the exocrine pancreas, endocrine pancreas, the gut tube uniquely, or in various combinations. From this wealth of mutant lines, data on selected examples of different classes will be presented. In particular, the identification of the mutant *prometheus* (*prm*) has proven that using a GFP-based approach for mutant identification is very powerful. *prm* mutants display a complete absence of a liver at 48 hpf, when in wild-type the liver is clearly recognizable. Intriguingly, this mutant displays no other morphological phenotypes, suggesting that *prm* is specifically involved in early hepatocyte formation. Surprisingly, the hepatic tissue in *prm* mutants recovers, resulting in viable and fertile adult. Therefore, *prm* will be informative to study not only early steps of liver development, but also aspects of later steps such as regeneration and organ size regulation. This mutant phenotype is unique as no similar phenotype has been reported in other species.

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340. **Regulation of Synaptogenesis by the Cadherin/Catenin Adhesion Machinery.** Masatoshi Takeichi, Hideru Togashi, and Kentaro Abe. RIKEN Center for Developmental Biology, Kobe 650-0047, Japan; and Graduate School of Biostudies, Kyoto University, Kyoto 606-8502, Japan.

How neurons recognize other neurons and establish their stable contacts is poorly understood. Cell—cell adhesion is regulated by many surface molecules; among them, the cadherin/catenin complex plays a major role. This adhesion machinery is also localized in synaptic cell—cell contacts, and so we have been investigating its role in synaptogenesis. Our recent studies revealed that, when cadherin function in hippocampal neurons is blocked by use of a dominant-negative cadherin, their synaptic organization and spine morphogenesis are severely perturbed not only structurally but also functionally. Genetic loss or overexpression of α N-catenin, a cytoplasmic molecule mediating cadherin-actin interactions, causes aberrant spine dynamics. Mutation of DN-cadherin, a cadherin specifically expressed in the *Drosophila* nervous system, induces disorganized synapse formation. Furthermore, mice in which cadherin genes are mutated show various behavioral abnormalities. These findings suggest that the cadherin/catenin adhesion system plays a critical role in synaptic contact formation, as well as spine dynamics that is thought to be important for synaptic plasticity and remodeling.

341. **Differential Use of an Upstream Cleavage Site in proBMP-4 Provides a Mechanism for Tissue-Specific Regulation of BMP-4 Activity.** R. Hackenmiller,* T. Nakayama,* D. C.

Goldman,* H. Kulesa,† B. L. M. Hogan,† and J. L. Christian*.*Oregon Health and Science University, Oregon; and †Vanderbilt University Medical School.

Previous studies in our lab have shown that proBMP-4 is sequentially cleaved at two sites within the inactive prodomain. Cleavage occurs initially at an optimal furin consensus motif adjacent to the mature ligand domain (S1 site) and subsequently at an upstream minimal furin consensus motif within the inactive prodomain (S2 site). In *Xenopus* embryos, BMP-4 synthesized from ectopic precursor in which the upstream site is noncleavable is less active, signals over a shorter range, and is rapidly degraded relative to BMP-4 cleaved from native precursor. To test the hypothesis that ordered cleavage of endogenous BMP-4 is essential for normal BMP activity, we generated mice carrying a targeted mutation at the BMP-4 allele that renders the upstream site (S2) uncleavable. Homozygous mutants show a dramatic reduction in the number of primordial germ cells, consistent with reduced BMP-4 activity in the extraembryonic ectoderm, while patterning of other tissues known to be sensitive to BMP dosage, such as the limbs and kidneys, appears normal. Furthermore, levels of mature BMP-4 protein are severely reduced in some, but not all, tissues of mutant mice relative to wild-type siblings, consistent with cleavage at the S2 site being regulated in a tissue-specific fashion. Our data suggest that tissue-specific cleavage of the S2 site of proBMP-4 provides a mechanism for tissue-specific regulation of BMP activity and signaling range during vertebrate development.

342. **The Morphogen Sonic Hedgehog Is an Axonal Chemoattractant That Collaborates with Netrin-1 in Midline Axon Guidance.** Frédéric Charron,* Elke Stein,* Juhee Jeong,† Andrew P. McMahon,† and Marc Tessier-Lavigne*.*Department of Biological Sciences, Howard Hughes Medical Institute, Stanford University, Stanford, California; and †Department of Molecular and Cellular Biology, The Biolabs, Harvard University, Cambridge, Massachusetts.

During nervous system development, axons are guided to their targets by attractive and repulsive guidance cues. In the embryonic spinal cord, commissural neurons send axons to the midline in response to the floor plate chemoattractant Netrin-1. We previously provided genetic evidence that floor plate cells also produce an additional chemoattractant(s) for commissural axons. We show that the morphogen Sonic hedgehog (Shh) can mimic the Netrin-1-independent chemoattractant activity of the floor plate in vitro and that it can act directly as a chemoattractant on isolated axons. Loss-of-function experiments using cyclopamine-mediated inhibition of the Shh signaling mediator Smoothened (Smo) or conditional inactivation of *Smo* in commissural neurons indicate that Smo activity is required for most or all of the Netrin-1-independent chemoattractant activity of the floor plate in vitro and for the normal projection of commissural axons to the floor plate in vivo. These results provide evidence that Shh, acting via Smo, is an additional midline-derived chemoattractant for commissural axons and show that a morphogen can act as an axonal chemoattractant.

343. **Transduction of Frizzled Signaling by a Heterotrimeric GTP-Binding Protein in *Drosophila*.** Vladimir L. Katanaev,* Romina Ponzelli,† Michel Semeriva,† and Andrew Tomlinson*.*Department of Genetics and Development, Colum-

bia University, 701 West 168 Street, r. 1120, New York, New York 10032, USA; and †Laboratoire de Genetique et Physiologie du Developpement, IBDM-CNRS-INSERM-Universite de la Mediterranee, 13288 Marseille, France.

Frizzled proteins are 7TM-type receptors transducing the Wnt/Wingless signals crucial for many aspects of development and cancerogenesis, as well as signals controlling convergent extension/planar cell polarity (PCP). In PCP epithelial cells are uniformly polarized within the plane of the epithelium. This phenomenon is similar to the gradient-decoding behavior that occurs in yeast chemotropism and leukocyte chemotaxis. Heterotrimeric $G\alpha\beta\gamma$ proteins usually relay the signal from 7TM receptors, yet trimeric G proteins have not been implicated in PCP signal transduction. Here we show that a trimeric G protein G_o is a transducer of the PCP Frizzled signal in *Drosophila*. Removal or overexpression of G_{ao} disrupts PCP; G_{ao} physically interacts with Frizzled in vitro and colocalizes with it in vivo. G_{ao} plays different roles in the PCP depending on the guanine nucleotide bound to it. We propose a model describing G_o function in PCP, which may represent a general scheme eukaryotic cells utilize to detect extracellular gradients through 7TM receptor.

344. Antagonists of Hedgehog Signaling and Dorsal—Ventral Neural Patterning. Jonathan Eggenschwiler, Oleg Bulgakov, Tiansen Li, and Kathryn V. Anderson. Princeton University, Princeton, New Jersey; MEEI/Harvard Medical School, Cambridge, Massachusetts; and Sloan Kettering Institute.

Discrete cells fates are specified along the dorsal—ventral axis of the vertebrate neural tube in response to regional signals emanating from surrounding tissues. The nature of the signals and the mechanisms by which they act are only partially understood. Using mouse genetics, we have studied the roles of genes required for neural patterning to proceed normally. Two genes, one encoding a member of the Rab family of vesicle transport proteins and the other encoding a member of the FK506-binding protein family, show similar requirements in neural patterning. In both mutants, ventral fates are ectopically expanded into lateral territories; lateral fates are shifted dorsally, and dorsal fates are not specified. Such changes in dorsal—ventral patterning are reminiscent of the effects of Sonic Hedgehog (SHH). SHH is a ligand produced by the notochord, lying at the ventral pole of the neural tube. SHH signaling has been shown to be both necessary and sufficient for the specification of ventral neural fates. A series of genetic experiments demonstrated that both of the genes we are studying encode intracellular antagonists of the hedgehog signaling pathway; their loss leads to ligand-independent pathway activation. Moreover, these experiments have uncovered the existence of SHH-independent mechanisms for specifying positional information all along the dorsal—ventral neural axis. Ongoing experiments are aimed at understanding the roles of these proteins at the molecular level within the hedgehog signaling pathway.

345. Morphogenetic Signaling and the Endocytic Pathway. Marcos A. González Gaitán. MPI für Molekulare Zellbiologie und Genetik, Dresden, Pfotenhauerstrasse 108, 01307 Dresden, Germany.

Morphogenetic signals, such as the *Drosophila* TGF β -homolog Dpp, are secreted from a source and traffic throughout the target tissues. This way, they form concentration gradients which endow the receiving cells with positional information. We show

that DPP stays only transiently at the extracellular matrix of the target tissue, implying that only short-range Dpp propagation can be explained by unhindered diffusion of Dpp in between cells. Instead, our results using mutants in dynamin and endocytic Rab proteins show that Dpp long-range propagation involves intracellular traffic of the ligand through the endocytic pathway. In particular, we showed that Rab5 and Rab7 determine the range of Dpp signaling throughout the tissue. Our data suggest Dpp propagation throughout the tissue occurs intracellularly via the endocytic pathway. We propose that the slope of the Dpp gradient, and therefore pattern and size of the wing, is determined at the receiving cells by the ratio between the Rab7-mediated degradation of Dpp and its Rab5-dependent recycling to be released and eventually signal at the next cells. We also explored the possibility that signal transduction is initiated from an endosomal compartment. In particular, SARA is essential to initiate Activin-like signal transduction from an apical endosomal compartment.

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347. Neural Induction in *Xenopus*. E. M. De Robertis, H. Kuroda, B. Reversade, M. Oelgeschläger, E. Pera, A. Ikeda, O. Wessely, L. Zakin, and C. Coffinier. Howard Hughes Medical Institute and Department of Biological Chemistry, University of California, Los Angeles, California, USA.

Morpholino knockdown of Chordin in *Xenopus* caused mild ventralized phenotypes similar to those of the zebrafish chordin mutant. A strong requirement for Chordin in dorsal development was revealed by experimental embryology assays. First, dorsalization by lithium chloride treatment was completely blocked by Chd-MO. Second, Chd-MO inhibited elongation and muscle differentiation in Activin-treated animal caps. Third, Chd-MO completely blocked the induction of central nervous system (CNS), somites, and notochord by organizer tissue transplanted to the ventral side of host embryos. Unexpectedly, transplantations into the dorsal side revealed a cell-autonomous requirement of Chordin for neural plate differentiation. Expression of Chordin at the blastula stage in a novel signaling center, called the preorganizer, is required for CNS formation. This region is distinct from the Nieuwkoop center, which is located more vegetally in dorsal endoderm and expresses Nodal-related factors. The preorganizer expresses Chordin, Noggin, and Follistatin under the control of β -Catenin and does not require Nodal signaling. The preorganizer region gives rise to brain anteriorly and floor plate in more posterior regions. At the blastula stage, preorganizer tissue is already specified and required to form brain tissue. The discovery of the blastula preorganizer center helps explain the old problem of planar versus vertical signals in neural induction.

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349. Genetic Dissection of Pattern and Chamber Formation in the Developing Mammalian Heart. R. P. Harvey, D. Lai, D. Elliott, M. Solloway, C. Biben, O. Prall, and F. Stennard.

Victor Chang Cardiac Research Institute, 384 Victoria Street, Darlinghurst 2010 Australia.

Important questions in developmental biology, evolution, and cardiology are how the lineages of the heart are specified and how cardiac chambers are formed. Anatomical, electrophysiological, and gene expression data suggest that chamber muscle is formed in discreet domains at the outer curvature of the looping heart as a specialization of a more primitive and ancient muscle type. The integration of anterior/posterior and dorsal/ventral patterning information is required for chamber specification, and in mice lacking the Nkx2-5 homeodomain transcription factor gene, chamber differentiation is blocked at an early stage. The neuregulin/ErbB signaling system has previously been shown to be necessary for formation of trabeculae, the spongiform layer of myocytes that forms only in chamber myocardium. Using knock-out mice, we have now shown that this signaling pathway is critical for specification and/or maintenance of chamber myocardium acting through Nkx2-5 and a host of other transcriptional regulators. Our studies suggest a key role for endocardium in heart patterning and chamber formation in the vertebrate heart and highlight an important evolutionary step in progression from a simple pulsatory linear vessel-like heart, as seen in *Drosophila*, to a multichambered organ with complex form and a specialized muscle type.

350. **The Hypoxic Response in *Drosophila* Depends on the bHLH-PAS Protein Similar and the Prolyl-4-hydroxylase, Fatiga, That Operates as an Oxygen Sensor.** Pablo Wappner, Maximiliano Irisarri, Sofia Lavista-Llanos, Juan A. Montotte, and Lázaro Centanin. Fundación Instituto Leloir, Patricias Argentinas 435, Buenos Aires (1405), Argentina.

HIF-1 is a mammalian heterodimeric bHLH-PAS transcription factor that controls the transcriptional response to hypoxia. We have identified a homologous system in *Drosophila* and characterized its activity in vivo during development. We generated transgenic lines carrying hypoxia-inducible reporters and showed that transcriptional activation is most pronounced in tracheal cells. We found that the bHLH-PAS proteins Similar (Sima) and Tango function as HIF- α and β homologues respectively and demonstrated a conserved mode of regulation for Sima by oxygen. Sima protein was upregulated in hypoxia and accumulated in the nucleus. Experiments performed in transgenic lines demonstrated that Sima degradation and nuclear localization rely on the Pro850 residue, presumably modified by an oxygen-dependent prolyl-4-hydroxylase. Consistent with this, abrogation of the *Drosophila* Egl-9 prolyl-hydroxylase homologue, Fatiga, caused both stabilization and nuclear localization of Sima, indicating a central involvement of the hydroxylase/ubiquitin system in both processes. We generated novel *fatiga* loss of function alleles that revealed an involvement of the prolyl-hydroxylase system in normal patterning of major tracheal branches. These observations establish an interesting link between the cellular oxygen sensing machinery and genetically controlled developmental programs.

351. **The Role of reaper and sickle in Developmental Apoptosis.** Christian Peterson, Reena Patel, Ginger Carney, Barbara Taylor, and Kristin White. CBRC, Massachusetts General Hospital/Harvard Medical School, Cambridge, Massachusetts; and Department of Zoology, Oregon State University, Corvallis, Oregon.

In *Drosophila*, three genes, *hid*, *grim*, and *reaper* (*rpr*), act as global initiators of apoptosis. Deletion of all three genes completely blocks developmental apoptosis in the embryo. A fourth gene *sickle* (*skl*) has recently been identified in the same region of the genome. Although *Sk* resembles *Rpr*, *Grim*, and *Hid* in function, its role in developmental apoptosis has yet to be demonstrated. The distinct expression patterns of these genes during development, and in response to known proapoptotic signals, suggests that there might be specific requirements for these genes in developmental cell death. To test this, we have generated deletions that remove various combinations of these genes. Using a deletion that removes *rpr*, we found that this gene is not required for viability and that *rpr* mutant embryos shows no detectable defects in overall levels of apoptosis. However, *rpr* mutant adults have an enlarged central nervous system. This is due to the survival of neuroblasts that normally die early in development. In the absence of *rpr*, these cells survive and continue to function as neural stem cells. Removal of both *skl* and *rpr* results in an even larger nervous system. The nervous system defect in these double mutants is under investigation. Both *rpr* and *skl* have also been implicated in DNA damage-induced apoptosis. The *Drosophila* p53 homolog, *Dmp53*, is required for DNA damage induced apoptosis and directly induces *rpr* expression in response to irradiation. Deletion of *rpr* alone or both *rpr* and *skl* results in a reduction in DNA damage induced apoptosis, but does not completely block this death. This indicates that there must be other proapoptotic targets of *Dmp53* activity.

352. **Regulation of Cell Death by Pax-3 During Neural Tube Development.** Shoba Thirumangalathu and Mary R. Loeken. Joslin Diabetes Center and Harvard Medical School, Cambridge, Massachusetts.

Pax-3 is required for development of neuroepithelium, neural crest, and *mosmitc* mesoderm. The mutant Pax-3 allele, *splotch* (*Sp*), causes neural tube defects (NTD) and defective cardiac neural crest development in homozygous embryos. Previously, we showed that Pax-3 deficiency is associated with neuroepithelial apoptosis and that p53 deficiency prevents apoptosis and NTD in *Sp/Sp* embryos. This suggests that the function of Pax-3 is to prevent p53-dependent apoptosis. Here we show that p53 deficiency also protects *sp/Sp* embryos from at least some cardiac neural crest defects, as *Sp/sp* embryos that carry germ line p53 mutations survive past day 16.5 and are viable at delivery on day 18.5. To determine the stage of neuroepithelial development that depends upon Pax-3, we have obtained embryos from *Sp/+* \times *Sp/+* matings and frequent times after the onset of Pax-3 expression on day 8.5 and stained them with acridine orange to detect apoptotic cells. Acridine orange-positive neuroepithelial cells were detected at the future location of an exencephalic defect of 6-8 somite *Sp/Sp* embryos. No acridine orange-positive cells were detected in control (*Sp/+* or *w.t.*) embryos. These results indicate that neuroepithelial apoptosis caused by Pax-3 deficiency ensues within hours of normal onset of Pax-3 expression. Since some studies have found that Pax-3 expression correlates with progression throughout the cell cycle, Pax-3 may prevent withdrawal from cell cycle and subsequent p53-mediated apoptosis.

353. **PTEN Tumor Suppressor: Development and Disease.** Hong Wu, Matthias Groszer, Gang Li, and Xin Liu. Howard Hughes Medical Institute, Department of Molecular and

Medical Pharmacology, and Department of Pathology and Laboratory Medicine, UCLA School of Medicine.

PTEN tumor suppressor is frequently mutated in human cancers, including glioblastomas, as well as endometrial, prostate, and breast cancers. Besides carcinogenesis, PTEN may play important roles in development, suggested by its ubiquitous expression pattern in embryos as well as by neurological disorders and virginal hypertrophy of the breast associated with PTEN germline mutations in humans. However, the early embryonic lethality of conventional *Pten*^{-/-} mice precluded further studies of PTEN functions during animal development. We have generated mice with either brain- or mammary-specific deletion of the *Pten* gene and our results suggest that PTEN plays an essential and cell autonomous role in regulating neuronal stem cells and in controlling the proliferation, differentiation, and apoptosis of mammary epithelial cells.

354. **Ancient Pathways Programmed by Small RNAs.** Phillip D. Zamore. University of Massachusetts Medical School, Massachusetts.

The discovery 5 years ago that double-stranded RNA can trigger sequence-specific, posttranscriptional gene silencing transformed our understanding of how eukaryotes protect and regulate their genes. Central to this "RNA silencing" mechanism is the use of ~21 nt RNA guides that provide sequence specificity. Insights from plants, animals, and fungi have together revealed an elegant and conserved cellular machinery that defends the genome against parasitic nucleic acids and regulates the expression of key developmental transcription factors. Remarkably, regulation of gene expression by the RNA silencing machinery can occur not only at the level of mRNA stability, but can also reflect mRNA-specific repression of protein synthesis or alterations in the structure of the chromatin or DNA of specific genetic loci. In particular, endogenous small RNA regulators called microRNAs (miRNAs) are emerging as an exciting class of developmental regulators in both animals and plants. Both miRNAs and siRNAs are generated by the same enzyme, Dicer, and function in remarkably similar, if not identical, complexes. The function of miRNAs and siRNAs can be interchanged in vitro and in vivo simply by altering the degree of complementarity between the small RNA and its mRNA target. However, we do not yet know if this common set of RNA silencing proteins corresponds to a common complex with multiple activities or a series of distinct but related protein-RNA machines that perform disparate effector functions. Experiments that probe the links between the RNAi pathway and other manifestations of RNA silencing promise to resolve this as yet unsettled debate.

355. **The *Drosophila* SDE3 Homologue *armitage* Is Essential for Axial Polarization of the Oocyte Microtubule Cytoskeleton and Translational Repression of *oskar* mRNA.** Heather A. Cook, Birgit S. Koppetsch, and William E. Theurkauf. University of Massachusetts Medical School, Massachusetts.

Embryonic axis specification in the fruit fly *Drosophila melanogaster* begins during early oogenesis, when the microtubule cytoskeleton is polarized along the anterior-posterior axis and microtubule-dependent transport leads to asymmetric localization of several mRNAs. Anterior-posterior axis specification also depends on the correct spatial and temporal regulation of mRNA translation. For example, translation of *oskar* mRNA,

which is critical for posterior patterning and germline specification, is normally repressed until it is properly localized to the posterior pole of the oocyte during midoogenesis. Local protein production then triggers assembly of poleplasm, which contains determinants for specifying the abdomen and germline. We find that mutations in the *armitage* (*armi*) gene disrupt polarization of the oocyte microtubule network, block *oskar* mRNA localization, and lead to premature *oskar* translation. Armi protein associates with the microtubule network and accumulates in the oocyte during early oogenesis. The *armi* gene encodes a homologue of SDE3, a presumptive RNA helicase required for homology-dependent posttranscriptional gene silencing (PTGS) in *Arabidopsis*. Consistent with a conserved function for these proteins, we find that Armi is required for silencing of the testis-expressed *Stellate* gene, repression of which is mediated by small homologous RNAs. Based on our findings, we propose that *oskar* mRNA translation, polarization of the oocyte microtubule network, and embryonic axis specification are regulated by a germline PTGS system.

356. **Targets and Functions of Plant Micro-RNAs.** Brenda J. Reinhart,* Matthew W. Rhoades,*† Earl G. Weinstein,* Lee P. Lim,*† Christopher B. Burge,† Bonnie Bartel,† and David P. Bartel*,†. *Whitehead Institute of Biomedical Research, Cambridge, Massachusetts; †Department of Biology, MIT, Cambridge, Massachusetts; and †Department of Biochemistry and Cell Biology, Rice University, Houston, Texas.

MicroRNAs are an extensive class of 22-nt noncoding RNAs thought to regulate gene expression in metazoans. We identified 16 *Arabidopsis* micro-RNAs, many of which have differential expression patterns. Eight are absolutely conserved in the rice genome. Plant microRNA loci potentially encode stem-loop precursors similar to those processed by Dicer (a ribonuclease III) in animals. Mutation of an *Arabidopsis* Dicer homolog, CARPEL FACTORY, prevents microRNA accumulation, demonstrating that similar mechanisms direct micro-RNA processing in plants and animals. The previously described roles of CARPEL FACTORY in the development of *Arabidopsis* embryos, leaves, and floral meristems suggest that the micro-RNAs may play regulatory roles in plant development. We predicted regulatory targets for the *Arabidopsis* microRNAs by identifying mRNAs with near complementarity. Many of the predicted targets are members of transcription factor gene families involved in developmental patterning. Moreover, many of these target sites are conserved in rice. The near-perfect complementarity between plant micro-RNAs and their potential targets suggests that many plant micro-RNAs act similarly to small interfering RNAs and direct mRNA cleavage. The potential targeting of developmental transcription factors suggests that some plant micro-RNAs may function to clear maternal regulatory transcripts from daughter cell lineages.

357. **The *Arabidopsis* heterochronic Gene ZIPPY Is an ARGONAUTE Family Member.** Christine Hunter, Gang Wu, Hui Sun, and R. Scott Poethig. University of Pennsylvania, Philadelphia, Pennsylvania.

In the course of development, plants pass through a juvenile, adult, and reproductive phase, each with characteristic morphological traits. We have isolated an *Arabidopsis* heterochronic mutation, *zippy*, that causes a premature juvenile to adult tran-

sition, but does not affect the timing of reproductive development. Cloning of *ZIPPY* revealed that it is a member of the *ARGONAUTE* gene family. *ARGONAUTE*-like genes are found in a wide range of organisms, where they have known functions in RNAi, stem-cell maintenance, and the establishment of organ polarity. This family also includes the *C. elegans* heterochronic genes *ALG-1* and *ALG-2*, raising the possibility that plants and animals use a common mechanism to regulate developmental timing. We will discuss the contribution of *ZIPPY* to developmental timing and transgene silencing and present preliminary evidence on the expression of microRNA-regulated target genes in *zippy* mutant plants.

358. **Systemic RNAi and the Intercellular Transport of RNA.** Craig P. Hunter, William Winston, Evan Feinberg, Marie Sutherlin, Christina Molodowitch, Frances Chu, and Caitlin Ferguson. Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts.

RNAi in *C. elegans*, whether induced by ingestion or injection of double-stranded RNA (dsRNA), spreads throughout the organism and is even transmitted to the progeny (Fire et al., Nature 391, 806). We are investigating how gene-specific RNAi silencing information, most likely dsRNA, is transmitted between cells. We use forward genetic screens to identify cellular components required for systemic silencing and then use these gene products to study the process in *C. elegans* and in heterologous systems. In our initial genetic screen we isolated over 200 mutants in at least five SID genes (Systemic RNAi Defective; Winston et al., Science 295, 2456). We have identified two of these, SID-1 and SID-2, whose structure, subcellular localization, and expression pattern have been informative for how dsRNA can be transported into and between cells.

359. **Quaking-6 Regulates GLI1 Translation through the 3'UTR.** O. Lakiza,* E. Villavicencio,* D. Walterhouse,* E. B. Goodwin,† and P. Iannaccone*. *Children's Memorial Institute for Education and Research, Northwestern University, Feinberg School of Medicine, Chicago, Illinois; and †Department of Genetics, University of Wisconsin, Madison, Wisconsin.

Sexual cell fate in the nematode *C. elegans* is, in part, controlled by the translational repression of the sex determining gene, *tra-2*, by two elements, called TGEs (*tra-2* and *GLI* elements) located in the 3'UTR. Binding of the STAR protein GLD-1 to the TGEs inhibits *tra-2* translation allowing for hermaphrodite spermatogenesis. Previously, we showed that the TGE control is a conserved process. Both the *C. briggsae tra-2* and the human *GLI1* mRNAs have 3'UTR elements that are functionally identical to TGEs. Moreover, one isoform of the mouse homologue of GLD-1, called Quaking-6 (QKI-6), can repress translation of TGE containing RNAs both in worms and in translation extracts. Our data suggest that QKI-6 regulates *GLI1* in mammals. Reporter transgenes with the WT *GLI1* 3'UTR are translationally inhibited in mouse embryos and NIH3T3 cells (which express QKI-6), while transgenes with mutant 3'UTRs lacking TGEs are not repressed. Moreover, QKI-6 specifically binds the WT *GLI1* 3'UTR but not a 3'UTR that lacks the TGE both in vivo and in vitro. Reduction of QKI-6 activity by siRNA in NIH3T3 cells disrupts translational control of reporter transgenes that carry the WT *GLI1* 3'UTR. In conclusion, TGE control regulates *GLI1* translation in vivo and is conserved between worms and mammals. (Supported in part by P01ES10549.)

360. Abstract #360 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

361. **Slalom Encodes a PAPS Transporter Essential for Segment Polarity and Dorsal—Ventral Axis Determination in *Drosophila*.** F. Lüders,* H. Segawa,† D. Stein,‡ E. M. Selva,§ N. Perrimon,§ S. J. Turco,†S.J. and U. Häcker*. *Lund University; †University of Kentucky; ‡University of Texas; and §Harvard University, HHMI.

slalom (*sll*) was identified in a genetic screen for zygotic lethal mutations associated with maternal effect phenotypes. Embryos maternally and zygotically mutant for *sll* show larval cuticle phenotypes indistinguishable from mutants in the *wg* or *hh* genes. Molecular characterization of *sll* mutants suggests that the *sll* gene encodes a putative transmembrane transporter. Using a yeast-based vesicle transport assay and an anti-Sll antibody, we show that *sll* encodes a PAPS transporter located in the Golgi. PAPS is a sulfate donor necessary in the Golgi for the sulfation of heparan sulfate (HS) chains, which has been shown to be essential for Wg and Hh signaling. Our data suggest that lack of sulfation of HS is responsible for the defects in growth factor signaling observed in *sll* mutants. In situ hybridizations to whole-mount embryos show that *sll* mRNA is supplied maternally and, at later stages of embryogenesis, expressed specifically in the developing salivary glands. A very similar expression pattern has been reported for the putative 2-O-sulfotransferase pipe. pipe is expressed in the ventral follicle cell epithelium where it regulates the maternal signaling cascade establishing the dorsal—ventral axis of the egg. We show that *sll* mutant follicle cell clones are associated with dorsalized phenotypes very similar to those seen in pipe mutants. Our data suggest that *sll* is necessary in female follicle cells for the establishment of the dorsal ventral embryonic axis and provide evidence that pipe is a sulfotransferase.

362. **Nodal Signaling Specificity Revealed by DNA Microarray and Phylogenetic Footprinting Analyses.** Ken W. Y. Cho,* Tadayoshi Hayata,* Souichi Ogata,* Yongchol Shin,* Dennis Kibler,† Steve Hampson,† Arnold Liao,‡ Qiangdong Zeng,‡ Peter Jablonski,‡ and Matthew Trunnell‡. *Developmental and Cell Biology, †Information Computer Science, University of California, Irvine, California 92697 USA; and ‡GeneData (USA), Inc., Waltham, Massachusetts 02451 USA.

To better comprehend how mutations in activin/nodal signaling affect various developmental processes at the level of transcription, we used DNA microarrays. We identified over 50 activin/nodal growth factor targets, most of which have never been implicated in activin/nodal signaling. Interestingly, we find that only 50% of these genes are regulated by a FoxH1 (Fast1) transcription factor and the remaining genes are regulated by a FoxH1-independent signaling pathway, implicating the involvement of a transcription factor mediated by nodal signaling other than FoxH1 in early mesoderm formation. When all of the activin/nodal target genes are characterized by whole-mount in situ and by large-scale microarray clustering analyses, we find that FoxH1-dependent target genes are mostly expressed in the Spemann's organizer, while FoxH1-independent target genes are expressed in the entire marginal zone, suggesting that the two groups of genes are induced by two different mechanisms. As part of our attempt to uncover the identity of another critical tran-

scription factor(s) mediating early nodal signaling, we examined the promoter regions of other vertebrate orthologs (human, mouse, fugu, and *Xenopus*). We report on our comparative promoter (phylogenetic footprinting) analysis to detect common sequence motifs shared among genes that are regulated similarly.

363. Abstract #363 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

364. **Radial Patterning in Arabidopsis: A Moving Target.** P. N. Benfey, K. Gallagher, A. Paquette, K. Nakajima, G. Sena. Duke University, Durham, North Carolina.

Plant embryos consist primarily of two stem-cell populations known as meristems, one that will make the root and the other that makes the shoot. Determining how the cells in these meristems are able to control their own division and the differentiation program of their progeny to form organs is one of the major questions of plant development. We have uncovered evidence for a signaling center located in the internal tissues of the *Arabidopsis* root that provides pattern information through cell—cell movement of a transcription factor to the surrounding cell layer. In the root of *Arabidopsis*, we have characterized mutations in which specific meristem cells fail to divide, or their progeny acquire the wrong identity. Analysis of mutations in the SCARECROW (SCR) and SHORT-ROOT (SHR) genes indicates that they are key regulators of radial patterning in the root. Both SHR and SCR are members of the GRAS family of putative transcription factors. SHR acts in a non-cell-autonomous fashion to regulate the amount of RNA that is made by the SCR gene. Analysis of SHR localization revealed protein both in the stele and in the cells immediately adjacent to it, indicating that SHR is able to move from the stele to the adjacent layer. Ectopic expression of SHR results in supernumerary cell layers and altered cell specification, indicating that SHR is both necessary and sufficient for cell division and cell specification in the root meristem. Efforts to identify the mechanism of this highly regulated protein movement will be discussed.

365. **Positional Information and Cell Behavior during Zebrafish Somite Segmentation: Roles of Fgf Signal and T-box Genes.** Hiroyuki Takeda. Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo, Japan.

The temporal and spatial regulation of somitogenesis is governed by an oscillator mechanism that is composed of genetic circuit involving the Notch signaling pathway. The oscillation in cells is finally coordinated and translated into a cyclic wave of expression of Notch-related genes such as hairy and L-fringe. The wave sweeps caudorostrally through the presomitic mesoderm (PSM) and finally arrests at the future segmentation point in the rostral PSM. By experimental manipulation and analyses in zebrafish segmentation mutants, we found that the level of Fgf/MAPK activation (highest in the caudal PSM) serves as a positional cue within the PSM that regulates progression of the cyclic wave. Once the level of Fgf signaling declines in the rostral PSM, the oscillation wave becomes stabilized, leading to the segmental expression of a number of genes which then establish segment polarity and initiate morphological somite formation. One of the zebrafish segmentation mutants, fused somites (*fss*), is defective in this final step of segmentation. We recently cloned

the responsive gene for *fss* mutant: the gene encodes a novel T-box transcription factor (*fss/tbx24*) that is expressed in the intermediate to rostral PSM. Phenotypic analyses of *fss* mutants suggested that *Fss/Tbx24* is an essential factor in the maturation process of the PSM, leading to a segmentation-competent state. Interestingly, *fss*^{−/−} cells are preferentially allocated to the posterior compartment of the somite when transplanted into wild-type embryos. Our recent data on time-lapse analyses of wild-type and mosaic embryos will also be presented.

366. **Asymmetric Divisions and Cell Signaling during Epidermal Cell Specification in Arabidopsis.** Dominique Bergmann, Wolfgang Lukowitz, and Chris Somerville. Carnegie Institution, DPB, Stanford, California.

The epidermis of *Arabidopsis* leaves and stems is simple, consisting of mainly three cell types: trichomes, stomatal guard cells, and pavement cells. Pairs of guard cells flank a pore through which water and CO₂ are exchanged, and can modulate the width of this pore. The complex of guard cells, pore, and subtending airspace is termed a stomate. The distribution of stomata in the epidermis is nonrandom, and guard cells normally obey several patterning rules: (1) they are formed through a stereotyped lineage of asymmetric divisions; (2) they are patterned locally so that two stomatal complexes are never in contact; and (3) the overall numbers of stomatal complexes are controlled globally in response to environment cues. Local pattern is likely to involve classic cell-surface-mediated signaling based on the identification of a receptor-like molecule, TMM, and a subtilisin protease, SDD1, that may process the signal. In an extensive screen for mutants that violated any of the patterning rules, we identified five new loci that affect local pattern. One of these mutationally defined loci, *YODA*, encodes a component of a conserved MAP kinase signaling pathway and acts as a switch to control cell fate in the epidermis. In the absence of *YODA*, the majority of epidermal cells become stomata. Conversely, when an overactive or unregulated form of *YODA* is expressed in plants, no stomata are formed. The potential targets of this MAPK signaling pathway and *YODA*'s relationship to the other known stomatal patterning genes will be presented. (Nadeau and Sack, *Science* 296, 1697-1700; Berger and Altmann *Genes Dev.* 17, 1119-1131).

367. **Establishment of Left/Right Asymmetry in the Nervous System of *C. elegans*.** Robert J. Johnston, Jr., Sarah Chang, and Oliver Hobert. Columbia University, New York, New York.

The molecular mechanisms of differential pattern formation along the left/right axis in the nervous system are not well understood. The nervous system of the nematode *C. elegans* displays several examples of L/R asymmetry, including the directional (biased) asymmetry displayed by the two ASE taste receptor neurons, ASE left (ASEL) and ASE right (ASER). While bilaterally symmetric in regard to several criteria, these two neurons display distinct chemosensory capacities which correlate with the L/R asymmetric expression of three guanylate cyclase genes, *gcy-5*, expressed only in ASER, and *gcy-6* and *gcy-7*, expressed only in ASEL. To elucidate the molecular mechanisms involved in establishing left/right asymmetry, we have performed genetic screens in which we identified 29 mutants that fall into three phenotypic classes. These classes include mutants that lose ASE cell fate, mutants in which the right cell takes on left cell

transcriptional output character, and mutants in which the left cell takes on right cell character. Positional cloning of several of these mutants reveals that several symmetrically and asymmetrically expressed transcription factors and cofactors, including three homeobox genes (Nkx, Otx, LIM class), a Zn finger transcription factor, a bromodomain containing gene, and a *Groucho* homolog, are required to restrict *gcy* gene expression to either the left or the right ASE cell. Our results suggest that ASE cell fate starts from a symmetric ground state and that a complex series of transcriptional regulatory interactions then leads to a diversification of L/R-specific features that ultimately serves to increase its functional capacities.

368. **one-eyed pinhead and Nodal Signaling in Left—Right Patterning.** Rebecca D. Burdine,* Steven R. Zimmerman,† and Alexander F. Schier†. *Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544; and †Skirball Institute, NYU Medical Center, New York, New York 10016.

one-eyed pinhead (*oep*) is an EGF-CFC family member that is absolutely required for Nodal signaling. Using mutations in *oep*, we have explored the role of Nodal signaling in left—right patterning. MZ*oep* embryos injected with *oep* RNA appear wild-type, but have left—right defects in the viscera and brain. We postulate these defects are due to a loss of Nodal signaling at a later time in development and are not rescued by our earlier RNA injection. Thus, we refer to these injected *oep* embryos as late zygotic *oep* (LZ*oep*). To determine when Nodal signaling is required for left-right patterning, we have created transgenic *oep/oep* fish where *oep* expression is controlled by the *hsp-70* promoter. Our recent results on this work will be presented. In LZ*oep* embryos, expression of left-side specific genes in the lateral plate mesoderm (LPM) is absent. Using transplantation techniques, we have demonstrated a previously unrecognized role for *oep* in the midline to allow gene expression to occur outside the midline in the LPM. Further manipulations reveal that the presence of the midline in LZ*oep* embryos represses gene expression in the LPM. This is in contrast to the current models postulating the midline acts solely as a barrier to restrict asymmetric gene expression. Our hypothesis is that an *oep*-dependent event in the midline overcomes the midline repressor (potentially *lefty-1*) to allow for expression in the LPM. This mechanism may be necessary to prevent gene expression in the LPM from occurring until the correct time in development.

369. **Mechanisms of Asymmetric Division in *C. elegans* Embryos.** Kelly Colombo and Pierre Gonczy. Swiss Institute for Experimental Cancer Research (ISREC), CH-1066 Epalinges/Lausanne, Switzerland.

Asymmetric divisions are crucial for generating cell diversity and rely on proper coupling between polarity cues and spindle positioning. In one-cell stage *C. elegans* embryos, polarity cues set by the PAR proteins govern an imbalance of net pulling forces acting on spindle poles that mediate spindle positioning. As a larger net force pulls on the posterior spindle pole, the spindle elongates asymmetrically and the first division is unequal, generating a larger anterior blastomere and a smaller posterior one. During an RNAi-based functional genomic screen, we uncovered two genes, *gpr-1* and *gpr-2*, that play a key role in spindle positioning in one-cell stage *C. elegans* embryos. *gpr-1* and *gpr-2*

encode essentially identical GoLoco-containing proteins distantly related to *Drosophila* PINS and mammalian AGS3. Despite having normal polarity cues, *gpr-1/gpr-2*(RNAi) embryos exhibit no posterior spindle displacement and have a symmetric first division. A similar phenotype has been previously observed in embryos lacking the $G\alpha$ subunits *goa-1* and *gpa-16*. By performing in vivo laser-mediated spindle severing experiments, we established that forces pulling on spindle poles are weak and equal in *gpr-1/gpr-2*(RNAi) and *goa-1/gpa-16*(RNAi) embryos, thus explaining the lack of posterior displacement. Strikingly, we found that GPR-1/GPR-2 are enriched on the posterior cortex during mitosis in a *par-3*- and *par-2*-dependent manner. These findings suggest a working model in which the extent of net pulling force depends on the level of cortical $G\alpha$ activity, which is regulated by AP polarity cues through GPR-1/GPR-2.

370. **Mouse Gastrulation: Tissue Patterning and the Emergence of Embryonic Axes.** Patrick P. L. Tam. Embryology Unit, Children's Medical Research Institute, University of Sydney, Locked Bag 23, Wentworthville, NSW 2145, Australia.

Investigations of cell fates and the expression domain of molecular markers in the early postimplantation mouse embryo show that progenitors of the extraembryonic and embryonic tissues are regionalized along the proximal to distal axis of the pregastrulation embryo. By modeling the mouse embryo in a Disk-Ring-Sector configuration, it becomes evident that the proximo-distal distribution of tissue progenitors corresponds to a radially symmetrical tissue pattern in the epiblast. Emergence of the embryonic axes therefore requires the breaking up of this radial symmetry. This is accompanied by the directional movement of the cells in the visceral endoderm and the epiblast, the installation of signaling activities that specify anterior—posterior polarity of the embryo and the orderly allocation of the tissue progenitors to the germ layers during gastrulation. The establishment of embryonic body plan is influenced by the activity of a multitude of transcription factors and signaling pathways associated with the organizer in the gastrulating embryo. The gastrula organizer is composed of a dynamic population of progenitor cells of the axial mesoderm and other germ layer tissues, as cells are recruited into and allocated from this population constantly during gastrulation. Embryological studies reveal that the activity of the gastrula organizer is a function of the morphogenetic and lineage potency of its derivatives. Analysis of mutant embryos that are deficient of gut endoderm reveals that the definitive endoderm may also play a crucial role in embryonic patterning and axis morphogenesis.

371. **Cell Polarity in *Arabidopsis* trichomes.** Martin Huelskamp. Botanical Institute III, University of Cologne, Cologne, Germany.

Arabidopsis trichomes are single polyploid epidermal cells with a stereotypic branching pattern. During development, the cell establishes new polarity axis several times, which are ultimately expressed in the spatial arrangement of the branches. Several lines of evidence suggest that the unicellular *Arabidopsis* trichomes are evolutionary derived from multicellular ones where branch induction is caused by a certain cell division pattern. It is therefore assumed that the mechanism responsible for polarity establishment is derived from the cell division machinery. We are studying genes involved in branch formation

and cell-shape control. Recent progress on their role in cell polarity and growth directionality will be presented.

- 372. Functional Studies of Regeneration in the Planarian *Schmidtea mediterranea*.** Alejandro S  nchez Alvarado, Peter W. Reddien, Adam Bermange, Nestor Oviedo, and Sofia M. C. Robb. Department of Neurobiology and Anatomy, University of Utah School of Medicine, 20N 1900E, Salt Lake City, Utah 84132.

Planarians (flatworms) belong to the phylum Platyhelminthes, an abundant and diverse group of animals considered to mark significant advances in the evolution of the Metazoa. The free-living, freshwater planarians are among the simplest triploblasts displaying bilateral symmetry and complex organ systems. Planarians are well known for their ability to regenerate and regulate their scale and proportion depending on environmental cues. However, little is known about the molecular events guiding the sophisticated and often plastic biological properties displayed by these organisms. Our laboratory has established clonal lines of sexual and asexual biotypes of the planarian *Schmidtea mediterranea* to facilitate molecular studies of this phylum. Using large-scale, temporospatial gene expression determination and loss-of-function assays (RNAi), we have begun a systematic molecular dissection of the biological attributes displayed by planarians. Here we report on the production and characterization of RNAi loss-of-function phenotypes in *S. mediterranea* and discuss these results in the larger context of regeneration and the regulation of stem cell population dynamics. (Supported by NIH NIGMS RO-1 GM57260.)

- 373. Isolation and Characterization of Planarian Stem Cells using FACS.** Kiyokazu Agata, Maki Asami, Norito Shibata, Testutaro Hayashi, and Ryoichi Okumura. Laboratory for Evolutionary Regeneration Biology, RIKEN CDB.

Planarian may have pluripotent stem cells which support highly regenerative ability and asexual reproduction. However, we have not identified them at cellular and molecular levels. In previous studies we have tried to identify these cells through the use of molecular markers and investigated the cellular events during regeneration using cell-type-specific markers. We found that planarian pluripotent stem cells may be present throughout the mesenchymal space, from head to tail, and that they are specifically eliminated by X-ray irradiation. Interestingly, the stem cells appear committed in a position-dependent manner prior to migrating to the organ rudiments or blastema. Transplantation experiments suggested that pluripotent stem cells may be regulated by intercalation. Recently, we have succeeded in identification of X-ray-sensitive cell fractions (X1 and X2) by fluorescence activating cell sorter (FACS) and then by characterizing these cells by single-cell PCR analysis and a laser scanning microscope. These analyses suggest that X1 and X2 may contain proliferating committed cells and resting stem cells, respectively. Here we would like to introduce molecular characterization of these cells and the genes involved in stem cell regulation.

- 374. Heart and Fin Regeneration in Zebrafish.** Kenneth D. Poss, Alex Nechiporuk, Stephen L. Johnson, and Mark T. Keating. Children's Hospital, Boston, Massachusetts; and Washington University School of Medicine, St. Louis, Missouri.

Zebrafish possess a remarkable regenerative capacity, allowing them to regenerate spinal cord, retina, fins, and even heart muscle. Very little is known about regeneration at the molecular level. We are interested in applying molecular and genetic tools available for the zebrafish model system to understand the factors necessary for organ regeneration. First, we have shown that mutations that block fin regeneration in a temperature-sensitive manner can be positionally cloned. *mps1* and *sly1* are two genes that are induced following fin amputation and are essential for the proliferation that drives fin regeneration. This forward genetic approach to regeneration (1) allows a new, molecular dissection of regeneration, and (2) generates conditional mutants useful for studying vertebrate biological processes in a new manner. Also, we have found that adult zebrafish can regenerate heart muscle following partial ventricular resection. Thus, a robust example of cardiac regeneration can now be analyzed and manipulated in zebrafish. Continued forward genetic and candidate gene approaches in zebrafish promise to reveal molecular mechanisms of organ regeneration in vertebrates.

- 375. Runx2 Is Required for FGF and Shh Signaling during Tooth Development.** Xiuping Wang, Thomas Aberg, Takashi Yamashiro, and Irma Thesleff. Institute of Biotechnology, University of Helsinki, Helsinki, Finland.

The developing tooth germs provide an easily manipulated and powerful system for studying sequential and reciprocal interactions between epithelial and mesenchymal tissues. *Runx2* (*Cbfa1*) is a runt domain transcription factor that is essential for osteoblast differentiation and tooth morphogenesis. Mutations of one allele of *Runx2* gene in humans are responsible for cleidocranial dysplasia, a syndrome characterized by hypoplasia of intramembranous bones and supernumerary teeth. *Runx2* knockout mice completely lack bone formation. Their teeth arrest at late bud stage. Our previous studies have shown that *Runx2* is expressed in dental mesenchyme and regulated by epithelial signals, such as Fgfs. To clarify the role of *Runx2* during tooth development, we searched for the downstream targets of *Runx2* gene. We found that the expression of *Fgf3* and *Shh* was downregulated in *Runx2* mutant teeth. Bead induction experiments showed that *Runx2* is required in the dental mesenchyme for mediation of epithelial signals and involved in both FGFs and Shh signaling pathways regulating expression of *Fgf3* and *Patched* (*Ptc1*), respectively. Most enamel knot marker genes were expressed normally in mutant upper molars, while downregulated in lower ones. In contrast, *Runx3* was upregulated in upper molars, which may compensate for *Runx2* in the mediation of Shh signals. We conclude that *Runx2* functions in the dental mesenchyme and mediates both FGF and Shh signals during the transition of tooth bud to cap stage.

- 376. Cell Growth, Lineage Switching, and Patterning during Tail Regeneration in *Ambystoma mexicanum*.** Elly M. Tanaka. Max-Planck Institute of Molecular Cell Biology and Genetics, Berlin, Germany.

In the Axolotl, tail amputation results in perfect regeneration of all structures including the spinal cord. This amazing process occurs by forming a zone of progenitor cells called the blastema while the regenerating spinal cord forms a tube of GFAP-positive neuroepithelial cells that extends into the blastema. How are cells recruited from the mature tissue to form the regenerating

blastema cells? Does the blastema represent a homogeneous population of pluripotent cells, or a subdivided population of restricted progenitors? By following individual GFP-labeled cells in live animals during tail regeneration, we have found that neural progenitors from the spinal cord exit the spinal cord and form mesodermal derivatives such as muscle and cartilage. This indicates that during the process of regeneration, cells acquire broad plasticity. We are investigating the mechanisms that control this lineage switching. Second, to facilitate molecular studies in the Axolotl, we have initiated an Axolotl EST project. We have so far sequenced 18,000 inserts from regenerating tail and embryonic cDNA libraries resulting in 9677 unique sequence contigs. We are using this sequence information both to generate cell-type specific markers for studying cell identity during regeneration and to identify transcripts important for regeneration.

377. Neural Crest Cells: Patterning and Regeneration via Stem Cells during Craniofacial Development. Paul Trainor, Morphula Remboutsika, and Robin Lovell-Badge. Stowers Institute for Medical Research, Kansas City, USA, and National Institute for Medical Research, London, United Kingdom.

Cranial neural crest cells are a pluripotent migratory population that gives rise to the majority of the nervous, skeletal, and connective tissue in the vertebrate head. Combined with their capacity for self renewal, neural crest cells are often considered to be stem-cell-like. Classic models for craniofacial development have argued that neural crest cells are autonomous in that their identity and fate is established in the neural tube prior to the commencement of their migration. Through a combination of interembryo cell transplantation and transgenic analyses in mice, we demonstrate at the genetic and cellular level that neural crest cells are very plastic in mammals. This has important evolutionary implications as neural crest cells are synonymous with the evolution of the vertebrate head. Given that the majority of congenital craniofacial abnormalities arise through defects in neural crest cell development, we have begun transplanting neurospheres/stem cells into the neural plate and observed that they can regenerate migrating neural crest cells which follow appropriate pathways and express typical neural crest cell markers. Sox2 appears to be an integral signal for maintaining neurospheres in a proliferative and undifferentiated fate and Sox2 is downregulated as neurosphere stem cells generate migrating neural crest cells. Currently we are screening for genes that pattern the identity and/or migration pathways of cranial neural crest cells which should our understanding of the genetic basis underlying congenital craniofacial abnormalities.

378. Abstract #378 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

379. Control of Monoallelically Expressed Genes. Elena Allen,*† Silvia Diaz-Perez,* Gyorgyi Csankovszki,§¶ Veronica Gallegos,* Michelle Blanco,* Frances Tong,* Peter Kraft,‡ Rudolf Jaenisch,§¶ Steve Horvath*,‡ and York Marahrens*. *Department of Human Genetics, †Department of Mathematics, and ‡Department of Biostatistics, University of California-Los Angeles, Los Angeles, California 90095, USA; §Department of Human Genetics, University of California-Los Angeles, California 90095; and

¶Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142.

Autosomal random monoallelic genes: There is a growing body of evidence that homologous pairing underlies monoallelic gene expression. Since homologous pairing preferentially occurs at heterochromatin, we asked whether autosomal genes subject to random monoallelic expression are flanked by repeats. A statistical analysis of repeat content in the regions surrounding monoallelically and biallelically expressed genes revealed that random monoallelic genes were flanked by significantly more LINE-1 sequence, longer and younger LINE-1s, fewer CpG islands, and less SINE sequence than biallelically expressed genes. The sequence characteristics that distinguished the high-LINE-1 category were used to identify 1300 additional genes from the human and mouse genomes as candidate genes for monoallelic expression. These genes are being examined for monoallelic gene expression. **X-inactivation:** The transcribed Xist allele on the inactive X chromosome (Xi) has been shown to help maintain X-inactivation. It has been assumed that the nontranscribed Xist allele on the active X chromosome (Xa) is inert. Here we show that (1) the Xist allele on the active X chromosome functions to maintain late replication timing and histone H4 deacetylation on the Xi; (2) the functions of the two Xist alleles are distinct, yet partially redundant; and (3) some regions of the X chromosome are more easily controlled by Xist than other regions.

380. Abstract #380 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

381. EMAGE-Edinburgh Mouse Atlas of Gene Expression. J. Christiansen, S. Venkataraman, A. Waterhouse, D. Houghton, N. Burton, Y. Yang, B. Hill, P. Stevenson, J. Bard, M. Kaufman, R. Baldock and D. Davidson. MRC Human Genetics Unit and Division of Biomedical Sciences, University of Edinburgh, Edinburgh, United Kingdom.

EMAGE is a database of spatially mapped gene expression patterns in the developing mouse embryo. All EMAGE data is housed in a standard framework: the EMAP Digital Atlas. This Atlas consists of a standardized nomenclature for anatomical structures present at all Theiler stages of development and at least one 3D digital embryo model at most Theiler stages. As the embryo models are 3D objects, virtual sections can be taken through these in any plane to reveal internal anatomical detail. EMAGE expression data is mapped into this framework both by using text (to the anatomical nomenclature) and by spatially (to corresponding regions within the embryo models). Whole-mount data are mapped in 2D as a domain that is projected onto the "surface" of an EMAP embryo model. Section and OPT data are mapped into the 3D space of the digital EMAP embryo models. Searching EMAGE data are performed by spatial-based queries whereby 2D or 3D query domains are defined by the user or by more conventional text-based methods. Free software to search EMAGE can be downloaded from the EMAP web site. The same software can also be used to prepare private databases for in-lab data management or to prepare electronic submissions to EMAGE. Submissions to the central database can alternatively be made by sending specimens directly to the EMAGE Editorial Office for data entry. Editorial staff are available for help and advice with the

submission process and all other aspects of the database. EMAGE is publicly accessible from <http://genex.hgu.mrc.ac.uk/>.

382. **Identification of Downstream Targets of the Nodal Signaling Pathway.** James Bennett,* Kathy Joubin,* Simon Cheng,* Pia Aanstad,† Hans Lehrach,† and Alexander F. Schier*. *Developmental Genetics Program, Skirball Institute, New York University School of Medicine, New York, New York; and †Max-Planck-Institut für Molekulare Genetik, Berlin, Germany.

Nodal signaling is critical for a variety of processes during vertebrate development, including gastrulation and left–right patterning. Although many of the molecules that play a role in the transduction of Nodal signals are known, the current list of genes known to be regulated by Nodal signals is incomplete. We identified putative Nodal regulated genes by differential RNA hybridization against a zebrafish cDNA library of about 14,000 cDNAs. Putative Nodal-regulated genes were also identified by screening zebrafish gene expression databases for genes expressed in the margin or shield of early gastrula-stage embryos. Putative Nodal-regulated genes were then screened by *in situ* hybridization against wild-type and maternal-zygotic *one eyed pinhead* embryos, which lack all Nodal signaling. This led to the identification of 57 unique Nodal-regulated genes. We are currently analyzing the function and regulation of these genes.

383. **Efficient Enhancer Trapping Using Sleeping Beauty in Zebrafish.** A. E. Davidson, D. Balciunas, S. Hermanson, Z. Welle, S. Sivasubbu, M. P. Cliff, and S. C. Ekker. Arnold and Mabel Beckman Center for Transposon Research, University of Minnesota, Department of Genetics, Cell Biology and Development. Minneapolis, Minnesota 55455.

We are developing the *Sleeping Beauty* (SB) transposon as a tool for gene discovery in zebrafish, *Danio rerio*. Enhancer trap vectors were generated within an improved SB transposon (pT2) using truncated versions of EF1 α to vary the threshold of activation required from *cis*-regulatory cassettes. Pilot screens resulted in efficient transgenesis and expression rates over 30%. The S2 construct resulted in a line that expresses GFP specifically in the hatching gland (HG) and has bred Mendelian to the F4 generation. Molecular characterization of the locus determined that the HG insertion was transposase-mediated. SB10 mRNA was injected into homozygous HG embryos and a consensus excision footprint was detected. These results led to a large-scale screen using the S2EF1 α promoter-based transposon. To date, we have screened 312 fish by single-pair intercrosses, resulting in an estimated transgenesis and expression rate of 29%. A series of transgenic fish have been isolated with tissue-restricted GFP expression patterns in a variety of cell types. Class I patterns represent regionally localized expression profiles, whereas class II lines represent cell-type-specific expression patterns. A new fluorescent reporter system was used to detect postembryonic expression of trapping events in the zebrafish larvae. These SB-based enhancer traps are effective at identifying a large array of novel regulatory elements and pathways in zebrafish.

384. **Design of an 8K *Xenopus* Microarray for Investigating Neural Development.** Vijay R. Dondeti, Melissa A. Wright, and Margaret S. Saha. College of William and Mary, Williamsburg, Virginia.

Microarray technology is powerful and commonly used to study gene expression. Readily available commercially for human and mouse, there are many model organisms, such as *Xenopus*, for which commercial arrays do not exist, despite the fact that there is considerable sequence data. The growing availability of reasonable options for custom array services now makes it feasible to develop microarrays for many different organisms. While probe design has received considerable attention in the literature, selection of the candidate genes of interest must precede this. Here we propose a method (here employed for *X. laevis*) to aid in the selection of candidate genes for such an endeavor, starting with existing microarrays (general purpose as well as more specialized arrays, for example, neural development) from other organisms. The accession numbers of these genes are retrieved and the sequences downloaded using Batch Entrez and blasted against the Unique database of the organism of interest, since it provides a nonredundant view of the transcriptome. Blast results are parsed using Perl, and sequences homologous to input sequences are selected based on user defined criteria ($E < 1e-15$). With results stored in an Access database, this system not only lets us effectively select candidate genes for the microarray, but also annotate them, for use in data interpretation of microarray experiments on neural development in *Xenopus*.

385. **mRNA-Tagging and Microarray Experiments to Identify *C. elegans* UNC-4 Targets.** S. E. Von Stetina,* P. J. Roy,† S. K. Kim,‡ D. M. Miller, III*. *Department of Cell and Developmental Biology, Vanderbilt University Medical Center, Nashville, Tennessee 37232-8240; †Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada; and ‡Department of Developmental Biology and Genetics, Stanford University Medical School, Stanford, California 94305.

The mechanism whereby neurons recognize and synapse with their appropriate targets is poorly understood. We are using *C. elegans* as a model to address this question. Mutations in the UNC-4 homeodomain transcription factor disrupt backward locomotion. In *unc-4* mutants, the usual inputs to VA motor neurons are replaced with synapses from interneurons normally reserved for their lineal sisters, the VBs. UNC-4 and UNC-37/Groucho function together in VAs to repress VB-specific genes. While *unc-4* target genes have been discovered, our genetic experiments have revealed that they are not involved in synaptogenesis. As the *unc-4* target genes that *do* mediate synaptic choice have remained elusive, we have opted to use a whole genome approach to identify all of them. We will isolate VA-specific mRNAs by using the *unc-4* promoter to drive expression of a FLAG-tagged poly(A) binding protein (PAB-1). Cell-specific mRNAs will be immunoprecipitated from wild-type, *unc-4*, and *unc-37* animals carrying an *unc-4::FLAG::PAB-1* transgene. UNC-4 target genes should be expressed at higher levels in *unc-4* and *unc-37* derived samples compared to wild-type. To optimize this approach for neuron-specific mRNA, we have used the immunoprecipitation protocol to isolate mRNA from animals expressing FLAG::PAB-1 driven by a pan-neural promoter. The results of microarray experiments using the *C. elegans* Affymetrix genome array will be presented.

386. **Characterization of the *C. elegans* Embryonic Regulatory Network Specified by PAL-1.** L. Ryan Baugh,* Kate Hill-Harfe,*† Andrew A. Hill,† Joanne C. Wen,* Donna K.

Slonim,[†] Eugene L. Brown,[†] and Craig P. Hunter*. *Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts; and [†]Department of Genomics, Wyeth Research, Cambridge, Massachusetts.

Maternal PAL-1 specifies the C lineage of the *C. elegans* embryo by activating a lineage-specific transcriptional program (Hunter and Kenyon, 1996). Zygotic PAL-1 maintains this program while the C lineage is patterned to give rise to multiple cell fates and lineage-specific morphogenetic behaviors (Edgar et al., 2001). To understand how master regulators control and integrate diverse developmental processes, we have used microarrays to identify PAL-1-regulated genes specifically expressed in the C lineage. We first generated transcript profiles of high temporal resolution from the 4- to 190-cell stage in wild-type (Baugh et al., 2003). To identify genes regulated by PAL-1 activity, we then profiled mutants that either lack a C lineage or that have multiple somatic lineages specified as C. Hundreds of candidate genes have been identified and ordered by their time of activation. Candidates encoding putative transcription factors and signaling molecules have been confirmed as PAL-1 target genes by scoring reporters following RNAi of *pal-1* and its repressor *mex-3*. We are now using RNAi to functionally characterize the validated set, and we are mining the genome sequence for motifs that may correspond to transcription factor binding sites. It is our goal to determine the structure of the regulatory network specified by PAL-1 by determining the full set of regulatory relationships among these genes.

387. Withdrawn

388. **Piggybac-based Insertional Mutagenesis in the Presence of Stably Integrated P Elements in *Drosophila*.** S. Nystedt,* M. Padash Barmchi,* C. Horn,[†] E. A. Wimmer,[†] and U. Häcker*. *Lund University; [†]University of Bayreuth.

P element-mediated mutagenesis has been used to disrupt an estimated 25% of genes essential for *Drosophila* adult viability. Mutation of all genes in the fly genome, however, poses a problem since P elements show significant hotspots of integration. In addition, advanced screening scenarios often require the use of P element-based tools similar to the generation of germ-line mosaics using FLP recombinase-mediated recombination or gene misexpression using the UAS/Gal4 system. These techniques are P element-based and can therefore not be combined with the use of P elements as mutagenic agents. To circumvent these limitations we have developed an insertional mutagenesis system using non-P-element transposons. An EYFP-marked piggyBac-based mutator element was mobilized by a piggyBac specific transposase source expressed from a Hermes-based jumpstarter transposon marked with ECFP. In a pilot screen we have generated 798 piggyBac insertions on FRT bearing 3rd chromosomes of which 9% have sustained a putatively piggyBac-related lethal hit. The FRTs present on the target chromosome remained stably integrated during the screen and could subsequently be used to generate germ-line clones associated with maternal and zygotic phenotypes. PCR-based analysis of insertion loci shows that 57% of the insertions are in genes for which no P element insertions have been reported. Our data demonstrate the potential of this technique to facilitate the quest for saturation mutagenesis of the *Drosophila* genome. The system is *Drosophila*

nonspecific and potentially applicable in a broad spectrum of nonmodel organisms.

389. **Microarray Analysis of Sexual Dimorphism in *Drosophila*.** Michael J. Parisi,* Rachel Nuttall,[†] Jining Lu,* Cathleen Chan,[†] Marina Vainer,[†] James Minor,[§] Alex Lash,[‡] Scott Eastman,[†] James Malley,* Daniel Naiman,[¶] and Brian Oliver*. *National Institutes of Health, Bethesda, Maryland; [†]Incyte Genomics, Palo Alto, California; [‡]National Library of Medicine, Bethesda, Maryland; [§]Novation Bioscience, Palo Alto, California; and [¶]Johns Hopkins University, Baltimore, Maryland.

Sexual dimorphism in *Drosophila* is notably evident in the adult body plan and is particularly conspicuous in gonads where the germ line differentiates into the highly dissimilar cell types; eggs and sperm. To better understand the mechanisms governing sexual dimorphism in adult *Drosophila*, we have examined sex-selective gene expression profiles using a microarray platform that represents 93% of the genes annotated in version 1.0 of the *Drosophila* genome. We have focused on global patterns of sexually dimorphic gene expression attributable to sex (whole adult males vs. females), soma (gonadectomized males vs. females), and gonads (testes vs. ovaries). Our studies reveal that approximately 15% of the genetically encoded information in the *Drosophila* genome is sex-specifically deployed. Germ-line development shows an especially high degree of differential gene expression with the greatest complexity seen in testes. In addition, we are finding that the chromosomal location of sex-biased genes exhibits a nonrandom distribution pattern throughout the genome with a lower number of male-biased genes found on the X chromosome. In addition to chromosomal distribution patterns, preliminary evidence suggests that sex-differentially expressed genes are localized within small, discrete chromosomal regions.

390. **Increased Local Rates of Cerebral Protein Synthesis in Fragile X Knockout Mice.** Mei Qin, Julia Kang, and Carolyn Beebe Smith. Unit on Neurobiology, NIMH, Bethesda, Maryland 20892.

Fragile X syndrome, the most common form of inherited mental retardation, is caused by the lack of expression of a single protein, Fragile X mental retardation protein (FMRP), encoded by the gene *Fmr1*. FMRP is an RNA binding protein that may play a role in transport and localization of mRNAs and activity-dependent translation. In vitro preparations FMRP has been shown to suppress translation of certain mRNAs. The aim of our study was to ascertain whether FMRP functions as a suppressor of translation in the CNS in vivo. We measured local rates of cerebral protein synthesis (ICPS_{leu}) with the quantitative autoradiographic [¹⁴C]leucine method (Smith et al., PNAS 1988, 85, 9341-9345) in seven male *Fmr1* knockout mice (KO) and seven wild-type male littermates (wt) at 4 months of age. Rates were higher (by 5-20%) throughout the brain in the KOs compared with Wt, and in 15 of the 54 regions examined differences were statistically significant (Student's *t*-test). These regions include frontal, cingulate, and parietal cortex, presubiculum, pyramidal cell layer of hippocampus, subregions of thalamus, hypothalamus, and amygdala. Our results show that in the absence of FMRP overall rates of cerebral protein synthesis are higher in the brain, particularly in regions involved in learning and memory.

These findings are of interest in light of the deficiencies exhibited by *Fmr1* KOs on behavioral tests and are consistent with the hypothesized role of FMRP as a suppressor of translation.

391. **Requirement for *Zic2* during Mouse Neurulation.** Carles Gaston-Massuet,* Paul Elms,† Deborah Henderson,* Ruth Arkell,† and Andrew Copp*. *Neural Development Unit, Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, United Kingdom; and †Laboratory of Early Development, Mammalian Genetics Unit, MRC Harwell, Oxfordshire, OX11 0RD, United Kingdom.

Neurulation is a fundamental embryonic event by which the neural tube is formed. Impairment of the process of neurulation leads to a range of anomalies known as neural tube defects (NTD). The mouse *Zic* gene family is composed of four members, *Zic1-4*, encoding 2C2H-like zinc finger transcription factors. To understand the role of *Zic* genes in the generation of NTD, we have studied their expression patterns in the posterior neuropore region at the time of closure of the neural tube, E8.5-9.5, in CD1 mouse embryos and homozygous mutants *curly tail* (*ct*), *loop tail* (*Lp*), and *splotch* (*Sp^{2H}*) that exhibit NTD. *Zic2* expression in homozygous mutant embryos is normal, indicating that these mutant genes are not upstream of *Zic2* in the generation of NTD. Additional evidence for the requirement of *Zic2* during neurulation is provided by the study of the phenotype of a new *Zic2* allele, Kumba (*Zic2^{Ku}*). The *Zic2^{Ku}* allele has been recovered from an ENU-mutagenesis screen. Analysis of *Zic2^{Ku/Ku}* embryos reveals failure of the neural tube to close with absence of bending of the neural epithelium at the dorsolateral hinge points (DLHPs). We conclude that *Zic2* is required for normal neural plate morphogenesis and, when disrupted, absence of DLHPs formation leads to spina bifida as seen in *Zic2^{Ku}* homozygous embryos.

392. **The Effect of Methylation Cycle Intermediates on Neural Tube Closure.** L. P. E. Dunlevy, A. J. Copp, and N. D. E. Greene. Neural Development Unit, Institute of Child Health, UCL, 30 Guilford Street, London, WC1N 1EH, United Kingdom.

Periconceptional supplementation with folic acid has been shown in clinical trials to reduce the recurrence of neural tube defects (NTD) in humans by up to 70%. The folic acid cycle directly interacts with the methylation cycle via the methionine synthase mediated remethylation of homocysteine to methionine. In humans, elevated homocysteine levels have been detected in maternal serum in NTD-affected pregnancies, leading to the suggestion that excess homocysteine may be directly causal. In contrast, methionine has been suggested to be essential for normal neural tube closure to occur. To test the hypothesis that abnormalities in the methylation cycle are responsible for some cases of folic acid responsive NTD, mouse embryos were cultured in the presence of homocysteine thiolactone (Hcy) or methionine throughout the period of cranial neural tube closure. Embryos cultured in the presence of Hcy at concentrations of 500 μ M or greater were growth retarded and developed blisters and abnormal somites. Despite this general toxic effect of Hcy exposure, there was no increase in the incidence of NTD, suggesting that elevated maternal Hcy does not directly cause NTD in humans. Embryos cultured in the presence of methionine at concentrations of 5 mM and above specifically developed NTD. The growth and develop-

ment of the embryos were otherwise unaffected by methionine treatment. To investigate the underlying mechanism behind methionine-induced NTD, apoptosis levels in the forebrain and DNA methylation levels both globally and in CpG islands have been measured in methionine-treated and control embryos.

393. Abstract #393 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

394. **Gene Expression Profiling of Apoptosis-Sensitive and Resistant Brain Regions in a Mouse Model for Fetal Alcohol Syndrome.** W. C. Dunty, Jr., B. Duong, S. O'Buckley, K. K. Sulik, and M. F. Miles. Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599; and Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, Virginia 23298.

Mouse models of alcohol-related birth defects illustrate abnormal hindbrain patterning and apoptosis. The purpose of this investigation was to identify differences in gene expression that may be responsible, conferring susceptibility or resistance to ethanol-induced apoptosis in the embryonic mouse CNS. Comparisons were made between microdissected sensitive (hindbrain) and resistant (midbrain) brain regions from control C57BL/6J embryos and those collected 2 hours after maternal ethanol administration (2.9 g/kg) on E9.5. Expression profiling analysis was performed on pooled mid- or hindbrains using Affymetrix oligonucleotide arrays. Bioinformatic analysis revealed that ethanol exposure elicited dynamic changes in genes involved in glycolysis as well as Wnt, IGF, and retinoic acid signaling pathways. Furthermore, expression profiling between control regions revealed basal differences in genes that may serve a neuroprotective role prior to treatment. Relative RT-PCR was used to confirm trends that were identified by microarray analysis. The identification of changes in region-specific gene expression have increased our understanding of the signaling cascades that mediate ethanol-induced apoptosis and have provided insight into the selective vulnerability of certain brain regions affected in human alcohol-related neurodevelopmental disorders. (Supported by NIH AA 11605, 13200, 13205, and 13678.)

395. **Ese2 Is Crucial for the Extraembryonic Ectoderm.** Peter L. Pfeffer, Helen Davey, Angela Beaton, and Phil L'Huillier. AgResearch, Ruakura, Private Bag 3123, East St., Hamilton, New Zealand.

Ese2/elf5 is a member of the large family of Ets transcription factors. We have independently isolated this gene from the mouse and now found it to be expressed from embryonic day 5.5 to 8 specifically in the extraembryonic ectoderm (ExE) and its derivative, the chorion. To address the function of *ese2* in the ExE, the gene is insertionally inactivated by homologous recombination. Two lines of knock-out mice were generated from different targeting events. Homozygous *ese2*^{-/-} embryos died before day 10, already showing growth retardation as early as day 7.5 using either 129/Sv or BALB/c strains. Some embryos surviving to day 9.5 showed evidence of anterior development though *otx2* expression was severely reduced and mosaic. At day 8.5 mutant embryos resembling a hollow ball of epithelium with occasional folding of the epiblast and/or centrally located masses of cells.

Whole-mount in situ hybridization with brachyury revealed mislocalized and reduced expression suggesting problems in mesoderm formation. *Otx2* was expressed in a patchy pattern in distal regions, indicating perturbations to the anterior–posterior axis. At day 7.5 no embryonic-extraembryonic constriction or amniotic bulge was visible, nor were BMP4 transcripts detected in the extraembryonic regions. This points to a lack of correctly specified ExE in *ese2*^{−/−} embryos, resulting in a lack of signaling to the subjacent embryonic ectoderm. Both the expression and the loss of function phenotype of *ese2* argue that this gene is crucial to the correct specification of the ExE and thus, indirectly, for the correct patterning of the embryo proper.

396. A Conditional Knockout Mouse for the OFD Type I Syndrome: A Possible Tool for the Study of Limb Development.

M. I. Ferrante, A. Barra, and B. Franco. TIGEM, Telethon Institute of Genetics and Medicine, Naples, Italy.

The Oral-Facial-Digital type I syndrome (OFDI) is an X-linked dominant, male lethal developmental disorder characterized by malformations of the face, oral cavity, and digits, often associated with polycystic kidneys and CNS defects. The digital abnormalities include syndactyly, brachydactyly, clinodactyly, and polydactyly. We have identified the gene responsible for this disorder, named OFD1. To date, OFD1, ubiquitous in human, has an unknown function. *Ofd1* expression, revealed by immunohistochemistry and in situ hybridization experiments, is ubiquitous during mouse development. To investigate the pathological mechanism and the normal functioning of OFD1 in development, we decided to generate a conditional knockout mouse by using the cre-loxP system. An unexpected lethality was observed for the newborn chimeras deriving from two independent ES clones: these chimeras displayed skeletal malformations, excess of cartilage, and severe limb abnormalities (polydactyly and syndactyly). We demonstrated that the neomycin gene interferes with the splicing of the *Ofd1* mRNA, impairing *Ofd1* protein production in our male ES cells and mimicking a KO phenotype. We removed the neo gene from the locus using the Flp-FRT system, the viable and normal chimeras obtained are currently bred to wild-type mice. Our conditional KO mouse will be a powerful tool to study the role of *Ofd1* in different tissues, given the availability of several cre transgenic lines. Particularly, the limb being an excellent model for studying basal mechanisms of development, we intend to focus our study on limb development, using limb-specific cre transgenic lines.

397. Cross-Species RNAi: dsRNAs from Two *Ascaris* Genes Sterilize *Caenorhabditis*.

Ge Gao, Sheela Raikar, Lejla Mutapic, Ruth Montgomery, and Karen Bennett. Molecular Microbiology and Immunology Department, University of Missouri, Columbia, Missouri.

The parasitic nematode *Ascaris lumbricoides* is the most ubiquitous human parasite in the world, infecting an estimated 1/4th to 1/3rd of the world's population. *A. lumbricoides* females lay ~250,000 eggs per day! A closely related species *A. suum* parasitizes swine and is also extremely prevalent. Many species of parasitic nematodes are rapidly developing resistance to the current drugs of choice, including ivermectin and albendazole. It has been reported that some nematodes are resistant to most, if not all, anthelmintics. We reasoned that RNA interference (RNAi) might be applicable as a novel antiparasitic agent. Because

ascaris worms, similar to most species of parasitic worms, cannot be maintained in the laboratory, we decided to begin our studies by testing *A. suum* genes in *C. elegans*, the free-living nematode in which RNAi has been best characterized. With the help of GenBank and WormBase, we identified *Ascaris* ESTs that had stretches of identity with *Caenorhabditis* for > 21 nucleotides, with an overall match of > 80% for more than 250 basepairs, and amplified some of the cDNAs from *Ascaris* ovarian RNA. Thus far, after injection into *C. elegans*, two *A. suum* genes have resulted in the desired cross-species RNAi effect of eliminating the next generation. One causes F1 embryonic lethality and the other results in sterile F1 adult worms. Although both genes are highly conserved, neither has a mammalian counterpart with an exact 21 nucleotide match. We plan to test other candidates and to expand our studies to the more-applicable feeding method of dsRNA delivery.

398. Zebrafish (*Danio Rerio*) Caveolin-1a and Caveolin-1b: Indispensable Roles in Embryo Development.

Ping-Ke Fang, Keith Solomon, Liyan Zhuang, Michael R. Freeman, and Pamela C. Yelick. Department of Urology, Children's Hospital, Harvard Medical School, Boston, Massachusetts 02115.

Caveolins are multifunctional signaling proteins associated with cholesterol-rich (caveolar) membrane domains. Caveolin-1 (−/−) mice can develop normally, with relatively subtle functional deficits. To further investigate the possible roles of caveolins in development, zebrafish (*Danio rerio*) caveolin-1 cDNAs were cloned using degenerate oligonucleotide/polymerase chain reaction (PCR) and rapid amplification of cDNA ends. Two zebrafish isoforms, *cav1a* and *cav1b*, were identified, encoding distinct proteins of 181 and 148 amino acids, respectively. Although *cav1a* and *cav1b* cDNA 3' termini are identical, their 5' termini are distinct and nonoverlapping, possibly due to alternative splicing. Whole-mount in situ hybridization (WISH) revealed a perinuclear expression pattern for *cav1* mRNA in 24 h postfertilization embryos, consistent with the expectation that *Cav1a* and *Cav1b* proteins are membrane targeted. *cav1* mRNA expression levels were highest in adult zebrafish, in comparison to earlier developmental stages. Potential functions for zebrafish *Cav1a* and *Cav1b* were explored by targeted depletion using an antisense morpholino oligomer approach. Injection of embryos with either *cav1a* or *cav1b* antisense morpholinos at the one-cell postfertilization stage resulted in pronounced systemic defects including, but not restricted to, the eye, brain, heart, and tail. *cav1a* morpholino effects were more pronounced than those observed with *cav1b* morpholino. Targeted depletion of either *Cav1* isoform results in lethal phenotypes at approximately 2 days postfertilization, while embryos injected with 4-mismatch control morpholinos developed normally. These results demonstrate that, unlike the mouse, caveolin-1 is likely to play important roles in organogenesis in the early stages of zebrafish development.

399. The Zebrafish G12 Gene Is Required for Nuclear Positioning and Cell Migrations during Early Development.

S. S. Reinsch and G. C. Conway. Life Sciences MS239-11, NASA-Ames Research Center, Moffett Field, California.

After fertilization Zebrafish embryos undergo synchronous cleavage to form a blastula of cells sitting upon a single

multinucleate yolk cell. At the beginning of gastrulation these cells undergo extensive cell migrations to form the major body axes. We have discovered a gene, G12, which is required for cell migrations and positioning of nuclei in the large syncytial yolk cell. Overexpression of a G12—GFP fusion protein is not toxic and shows that the protein localizes inside the yolk cell to the yolk nuclei, microtubules, and to the margin between the blastomeres and the large yolk cell. Morpholino (MO) injection into the one-cell embryo or into just the yolk syncytium completely inhibits cell migrations, doming of the yolk cell, and positioning of nuclei around the margin. This effect can be partially rescued by injection of G12—GFP encoding RNA. Given the known role of microtubules in nuclear positioning of yolk nuclei in Zebrafish, we investigated the microtubules in morpholino injected and rescued embryos. We find that microtubules are sparse and disorganized in MO-injected embryos and are restored to normal organization upon G12—GFP rescue. G12 plays a pivotal role in organization of microtubules during early development. G12 is highly conserved in vertebrates and two homologues exist in the human genome. One of the human homologues is amplified in aggressive breast tumors.

400. ES-Derived Neural Progenitor Differentiation In Vitro and In Vivo. J. Carpentino, S. Becker, J. Thorne, J. Naegele, and L. Grabel. Wesleyan University, Middletown, Connecticut.

Embryonic Stem (ES) cells are currently being examined as a potential therapy for patients suffering from various neurodegenerative diseases. Consequently, it is imperative that researchers develop protocols for generating neural progenitors in vitro as well as testing their potential in vivo. We currently generate ES-derived neural progenitors using serum-free defined media and subsequent culture in the presence of FGF. To test their in vivo potential, progenitors derived from a ROSA ES cell line that constitutively express β -galactosidase are labeled with the fluorescent membrane dye PKH-26 and transplanted into the mouse hippocampus, an identified area of adult neurogenesis. Our preliminary studies indicate that the ES-derived neural progenitors survive, and based on expression of neuronal and glial markers, differentiate. We are currently investigating the ability of transplanted progenitors to populate a lesioned hippocampus induced by kainic acid. In addition, we are testing the hypothesis that Hedgehog signaling plays a role in promoting the proliferation and differentiation of neural progenitors.

401. The Differentiation of ES Cells into Neurectoderm and Neurons: a Role for Hedgehog Signalling. Sandy Becker, Peter Maye, Jeffrey Thorne, Henrike Siemen, Chungyu Cai, Joseph Carpentino, and Laura Grabel. Wesleyan University, Middletown, Connecticut 06459.

The prospect of using human embryonic stem (ES) cells for transplantation therapy has prompted great interest in defining the conditions that promote their differentiation into various progenitor cell types. In the mouse system, ES cells can differentiate into neural progenitors following formation of an embryoid body intermediate. Embryoid bodies differentiate from ES cells in suspension culture and initially contain a core of stem cells surrounded by an outer layer of visceral endoderm. The core subsequently cavitates and forms an epithelial embryonic ectoderm layer, which may then differentiate, based upon marker expression, into neurectoderm. Treatment with retinoic acid

promotes the differentiation of nestin-positive apparent neural stem cells within the ectoderm core. We previously demonstrated that the outer embryoid body layer is a source of Indian hedgehog (Ihh) while the core responds by upregulating expression of the *Ptch1* gene. To test the role of Hedgehog signaling in production of neural progenitors and their derivatives, we have used ES cell lines carrying loss-of-function mutations for either *Ihh* or *Smo*, which encodes a cell surface receptor required for Hedgehog signaling, or a mutation in the *Ptch1* gene, which relieves *Ptch1* inhibition of Smo action. Our data suggest that Hedgehog signaling promotes the differentiation of an ectoderm core capable of producing neural progenitors upon retinoic acid treatment. In addition, the *Ihh* and *Smo* mutant cell lines appear to be defective at generating neural stem cells as well as neurons and glia in outgrowth culture, whereas the *Ptch1* mutant cell line can generate substantial levels of these cell types even in the absence of retinoic acid treatment.

402. Vascular Development Research Using Human Embryonic Stem Cells. S. Gerecht Nir, S. Cohen, and J. Itskovitz-Eldor. Biotechnology and Medicine Faculty, Technion-Israel Institute of Technology, Department of OB/GYN, Rambam Medical Center, Haifa, Israel. Department of Biotechnology, BGU, Be'er-Sheva, Israel.

Vasculogenesis mainly refers to the process of embryonic vessels formation. Since the means to study human vasculogenesis are limited, the mechanisms involved in its regulation remain unclear. Human embryonic stem cells (hESCs) provide a unique cell population for exploring human vasculogenesis. We aimed to analyze the vasculogenic potential of hESCs and to search for a human vascular progenitor. hESCs were allowed to initiate mesodermal differentiation, and an endothelial-enriched cell population was isolated. FACS, RT-PCR and immunofluorescence analyses revealed expression of specific endothelial markers in the differentiated hESCs, and further up-regulated early lineages markers of both endothelial and hematopoietic cells. Further differentiation studies were performed with suitable cytokines, and 3D assays for vessel formation and inhibition. The progenitor population was able to differentiate into endothelial cells, and up-regulate vascular smooth muscle cells. Sprouting and tube-like network structures were observed once cultured in matrigel and collagen gel, and inhibited by adding anti-vascular endothelial cadherin to the culture. Electron microscopy revealed typical endothelial cell arrangement within the matrigel containing lumen, lipoprotein capsules, and Weibel-Palade bodies. This work further strengthens previous works claiming that different markers seem to play an important role in human but not in mouse vasculogenesis. Therefore, we conclude that hESCs may serve as research model for exploring human vascular development. please identify which author is associated with which facility

403. Making of Spontaneously Beating Cardiac Muscles on Biodegradable Matrices From Embryonic Stem Cells. Jihwan Song,* Hyung Min Chung,* Tae-Sun Hwang,* Kwang Yul Cha,* Jae Kyun Park,* Won Suk Kang,* Sung Pil Yoo,† Ilkwon Kim,† and Byung-Soo Kim†. *Cell Gene Therapy Research Institute, Pochon CHA University College of Medicine, Seoul, Korea, and †Department of Chemical Engineering, Hanyang University, Seoul, Korea.

With the development of tissue engineering and stem cell technologies in recent years, the hope for regenerative medicine is continuously growing. Among various kinds of stem cells, embryonic stem (ES) cells can serve as the ideal source for repairing damaged tissues or organs as they have the capacity to differentiate into all the cell types that constitute the body. Here we show that mouse ES cells can form spontaneously beating cardiomyocyte-like structures on three different types of biodegradable polymers: poly glycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA) and type I collagen. We first used cystic embryoid bodies (EBs) containing cardiomyocytes with a portion of 10–20%, which were dissociated into single cells before seeding onto PGA matrix. In the second experiment, dissociated ES cells that were directly obtained from culture were seeded onto three different types of biodegradable polymers, anticipating that these ES cells may recapitulate the spontaneous differentiation in the suspension culture. Interestingly, morphological studies using scanning electron microscopy (SEM) and conventional histology showed that the seeded cells were firmly attached on the matrices within a few days, which were later shown to differentiate into various cell types in a couple of weeks. Immunohistochemical staining using Troponin T antibody demonstrated the formation of functional cardiomyocytes. Taken together, these results strongly suggest the possibility of making three-dimensional cardiac muscle tissues from ES cells. This work was supported by a grant from Korea Food Drug Administration (Bio 032-1).

404. **Development of *in vivo* Imaging Techniques for Trafficking of Subventricular Zone Neural Stem Cells (SVZSC).** Mary Owen,*† Jeff Bulte,† Francesca Cicchetti,** Iris Chen,§ Christopher Owen,¶ Xukui Wang,§ Meixiang Yu,§ Kimmo Jokivarsi,§ and Anna-Liisa Brownell§. *Department of Biology, Simmons College, Boston, Massachusetts, †Radiology Department, Johns Hopkins School of Medicine, Baltimore, Maryland, **Unit #233, de Neuroscience, CHUL, Ste-Foy, Quebec, Canada, ‡Departments of Pediatrics, §Radiology and ¶Neurosurgery, Massachusetts General Hospital, Boston, Massachusetts.

Adult progenitor cells hold promise for therapeutic treatment of disabling diseases that are due to the loss of specific cell types. However, stem cell therapies will require the ability to assess the fate of transplanted cells and to ensure that they are viable and traffic to the appropriate location, restoring function. We have developed protocols for labeling neural progenitor cells and for monitoring cell trafficking by high resolution magnetic resonance imaging (MRI) and positron emission tomography (PET). We have labeled rat subventricular zone stem cells (SVZSC) and a control cell line (PC 12) utilizing mion (dendrimer-encapsulated super paramagnetic particles, MD-100) for MRI and ¹⁸F-fluorodeoxyglucose for PET. These cells have been implanted in the striatum or rostral migratory stream of normal (non-lesioned) Sprague-Dawley rats and imaged. Post-mortem histological analysis of rat brains has been conducted to assess viability of transplanted cells and distribution of mion-labeled cells. As clinical use of such essential tracking protocols will require long-term study of slow moving cells, future work includes transfection of progenitor cells with PET reporter genes for which safe and effective radionuclide probes will be developed.

405. **The Role of E2F-4 Transcription Factor in Regulation of Mammalian Neural Stem Cells.** V. A. Ruzhynsky, J. L. Vanderluit, and R. S. Slack. Neuroscience Research Institute, University of Ottawa, Ottawa, Canada.

Rb family of proteins (pRb, p107, p130) and its regulatory targets are important for cell cycle regulation in neural stem cells. *In vitro* neurosphere assays and *in vivo* ablation studies indicate that p107 but not Rb regulates the neural stem cell population in the mouse brain (Dr J. Vanderluit, manuscript in preparation). One of the downstream targets of the pocket proteins is the E2F family of transcription factors. This family includes six E2F members and their heterodimerizing partners D1 and D2 proteins. E2F4 is important for terminal mitosis and differentiation in a number of developing systems. Considering the evidence that p107 regulates stem cells in the brain, our objective is to establish the role of its potential target, E2F4 transcription factor, in the regulation of the number and differentiation of neural stem cells pool in the mammalian brain. We used E2F4 knockout mice to investigate the function of E2F4. Neuroepithelial cells derived from the telencephalon of E2F4 knockout embryos at E13.5 produced a lower number of primary neurospheres. At the same time, late passaged differentiating neurospheres from E2F4 knockout mice have a higher number of BrdU-positive cells. These results indicate that E2F4 transcription factor is important for regulation of the number of stem cells in the developing nervous system. More experiments are in progress to delineate the role of the E2F4 transcription factor in proliferation and differentiation of neural precursors.

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408. **Effects of Wnt1 on Neural Precursor Cells.** Shigemi Hayashi and Andrew P. McMahon. Department of Molecular and Cellular Biology Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138.

Wnt genes, a large gene family that encodes secreted ligands, elicit diverse signaling cascades to regulate pattern formation in various organisms. In mice, two of nineteen Wnt genes, Wnt1 and Wnt3a have been shown to be required for patterning of the central nervous system (CNS). A series of mouse genetics experiments have suggested that activation of Wnt1 signaling can expand the number of neural precursor cells (NPCs) during embryogenesis. To further investigate the effect of Wnt1 on proliferation and differentiation of embryonic and adult NPCs *in vivo* and *in vitro*, we have established a mouse transgenic model system in which Wnt1 can be expressed in a temporally regulated fashion by a drug inducible Cre-loxP system. Two transgenic lines have been generated. One transgenic line is CAGGCre-ER line that broadly expresses tamoxifen inducible form of Cre. This transgenic line elicited efficient recombination in the developing CNS, as well as in the adult brain of the subventricular zone and the subgranular zone of dentate gyrus of the hippocampus, two areas that contain adult neural stem/precursor cells. The other transgenic line expresses a fusion protein of Wnt1 and green

fluorescence protein (GFP) from ubiquitous expression locus Rosa26 upon the Cre-mediated excision of the stop cassette DNA sequences. When neural specific Nestin-Cre transgenic mouse was used to express Wnt1-GFP in the developing CNS, embryos displayed enlarged size of the brain, verifying the activity of Wnt1. We are currently investigating roles of Wnt1 in proliferation and differentiation of embryonic and adult NPCs both *in vivo* and *in vitro*.

409. **Characterization of Neural Stem Cells Derived From Human Pediatric Brain Tumors.** Houman D. Hemmati, Ichiro Nakano, Michael Masterman-Smith, Alexandra Lowry, Simon Bababeygy, Benjamin Rafii, Miguel Minera, Jorge Lazareff, Harley I. Kornblum, and Marianne Bronner-Fraser. Division of Biology, California Institute of Technology, and Departments of Pediatrics and Pharmacology and Division of Neurosurgery, UCLA School of Medicine, , California.

Pediatric brain tumors (PBT) might arise by transformation of proliferating neural stem or progenitor cells. This theory is supported by observations that many PBT arise from the ventricular zone, express nestin, and give rise to multiple types of cells including neurons and glia. In this study, we explore the properties of PBT by isolating and characterizing multipotent, self-renewing progenitors from PBT samples. Propagation of five dissociated PBT using the same methods as for human neural stem cells results in the production of proliferating spheres. These spheres could be passaged at clonal density, with resultant secondary spheres giving rise to cellular elements with the characteristics of neurons and glia. Gene expression analysis reveals that tumor-derived spheres express many genes characteristic of neural stem cell-derived spheres, including nestin, CD133 and musashi-1. Multi-passaged tumor derived spheres migrate and produce neurons and glia when transplanted into neonatal rat brain. Tumor-derived spheres can be passaged for over four months in culture and give rise to single cells expressing markers of both neurons and astrocytes. These data suggest that PBTs derive from cells with many of the characteristics of neural stem cells. Further studies may exploit this culture system for the identification of diagnostic markers and therapeutic targets for PBT.

410. **Neural Stem Cells Induce Reprogramming of Neural Cell Types in Co-Culture Accompanied by Connexin 43 Protein (Cx43) Expression in Interacting Cells.** Arshak R. Alexanian and Shekar N. Kurpad. Neuroscience Research Labs, Department of Neurosurgery, VAMC, 5000 W. National Ave 151, Milwaukee, Wisconsin 53295.

Recently, neural stem cells were demonstrated to differentiate into virtually every cell type when they were injected into blastocysts *in vivo* or cultured *in vitro* with differentiating embryonic stem cells. These studies indicate that extracellular factors or cell-cell interaction might be sufficient for reprogramming cells into pluripotent status. In this study we investigated the plasticity of differentiated neural cell types *in vitro* by nurturing them with neural stem cells. We also studied the Cx43 expression level in these interacting cells. To this end, we plated the neural cells derived from the cortex of ROSA26 transgenic mice in the Neurobasal A /B27 medium in the presence of bFGF. After 1 day, the medium was changed to Neurobasal A/B27 without bFGF. Under this condition, little neural viability was

maintained for weeks, and there was no incorporation of BrdU. Two weeks later bFGF was added to these cultures, however, no BrdU incorporation and cell proliferation was detected during the next 2 weeks. Then we plated adult neural stem cells isolated from the hippocampus of CD-1 mice on top of these cells. After 1.5 weeks, cultures were fixed and triple stained for b-gal activity and immunocytochemistry for BrdU and Cx43. Results showed that ROSA26 derived mature neural cell types incorporated BrdU that coincided with Cx43 expression. We conclude that some neurons and/or glia can change their differentiated state in co-cultures with multipotent neural stem cells, and Cx43 can play a role in such a phenomenon.

411. **Growth and Differentiation of Mammalian Auditory Hair Cell Progenitors.** Angelika Doetzlhofer,* Patricia M. White,* Jane E. Johnson,** Neil Segil,*† and Andrew K. Groves*†. *Gonda Department of Cell and Molecular Biology, House Ear Institute, Los Angeles, California 90057; †Department of Cell and Neurobiology, University of Southern California, Los Angeles, California 90033; **Center for Basic Neuroscience, University of Texas Southwestern Medical Center, Dallas, Texas 75390.

Sensorineural deafness affects a significant portion of the population, and it is largely due to the loss of sensory hair cells in the cochlea of the inner ear. In mammals, these sensory hair cells are only formed during embryogenesis, and if lost regeneration of cochlear hair cells does not occur. In contrast birds and other non-mammalian vertebrates are able to regenerate hair cells by mitotic stimulation of quiescent progenitor cells, probably the supporting cells themselves, which persist into adult life. Whether the lack of hair cell regeneration in mammals is caused by a depletion of hair cell progenitors during embryogenesis or by a lack of appropriate stimuli in the adult is unknown. To better understand the molecular control of progenitor cell fates within the developing inner ear, we established a culture system in which both hair cells and supporting cells can be generated from mitotic progenitors. These hair cells express both molecular and ultrastructural features of cochlear hair cells. The culture conditions necessary for the production of sensory hair cells and the capacity of postnatal cochlear epithelial cells to generate hair cells *in vitro* will be discussed.

412. **Neural Stem Cell Properties and *Xenopus* Spinal Cord Regeneration.** K. Sato, A. Corn, R. Karcavich, R. C. Smith,* and E. A. G. Chernoff. IUPUI Center for Regen. Biol. and Med., Eli Lilly and Co.*

Gap replacement regeneration occurs in *Xenopus laevis* tadpole spinal cord lesioned at stage 50, but fails at stage 62. The ependymal cells (ventricular zone glia) are highly mitotic, contributing to the regeneration outgrowth. Subtractive screens show differential expression of Notch-1 pathway members in stage 50 cord. These include Enhancer of Split-Related protein (ESR-7) and the Notch ligand Delta-2. *In situ* hybridization shows that ESR-7 and Delta-2 are expressed in ependymal cells. Other members of the Notch pathway are examined including the neural progenitor and stem cell marker Nrp-1, the *Xenopus* homolog of Musashi-1. Musashi-1 maintains Notch signaling by inhibition of Numb. Nrp-1 is strongly expressed in regeneration competent *Xenopus* cord. It is localized in ependymal cells and dorsal subependymal cells in cord and forebrain. An inhibitor of neuronal differentia-

tion outside of the Notch pathway, Id3 (Inhibitor of DNA-binding) is also expressed in ependymal cells. Id3 has been associated with stem cell properties in other tissues. A *Xenopus* tadpole ependymal cell culture system was devised to permit experimental manipulation. Stage 50-derived ependymal cells express Nrp-1/Musashi-1 in culture. The ependymal cells respond to EGF and FGF-2 by producing neurospheres like late embryonic or adult mammalian neural stem cells. In vivo and in vitro expression of genes associated with neural stem cell properties and neurosphere formation suggest *Xenopus* spinal cord regeneration competence is correlated with neural stem cell behavior of ependymal cells. Supported by NSF, Indiana 21st Century Res and Tech Fund, Eli Lilly and Co.

413. **Expression of the *Xenopus* DECD-box RNA Helicase, XDDX39, During Development and Limb Regeneration.** M. W. King,** A. L. Mescher,*† M. W. Harty,† M. C. Muzinich†, R. C. Smith***, A. W. Neff,†**. *IU School of Medicine, †Eli Lilly and Co., **IU Center for Regenerative Biology and Medicine, Indiana.

We have cloned genes from regeneration-competent (st 53) versus -incompetent (st 59) *Xenopus* hindlimbs. One gene from blastema-enriched cDNA is the *Xenopus* homolog of the human DECD-box RNA helicase, DDX39, which has recently been shown to be expressed in various stem cells and is identified with the property of “stemness”. As an initial approach to XDDX39’s developmental significance and possible role in tissue repair and regeneration, we have investigated its expression pattern in normal development and hindlimb regeneration. XDDX39 is localized, at st 17, only to regions around the developing nervous system including the neural tube, neural crest, and to a lesser extent the paraxial mesoderm. Later (st 30-39) XDDX39 is expressed in the developing abdominal muscle cells as these migrate ventrally. Differentiated myotomes did not express XDDX39. During hindlimb development XDDX39 localization is mesenchymal only, with distinct patches of labeling at st 53, expression in the digit tips at st 55, and no expression by st 57. XDDX39 expression during hindlimb regeneration is dynamic. In st 53 and 55 regenerates faint labeling is seen at 3-days with definite expression seen at 5 and 7 days. In st 57, XDDX39 is re-expressed and up-regulated distal to the amputation plane and proximal to the wound epithelium at 1 day and present in 3-, 5-, and 7-day pseudoblastemas. Expression of XDDX39 in early development and limb regeneration is highest in undifferentiated or early differentiating cells, suggesting a gene involved in aspects of differentiation.

414. **reg6 Is Required for Branching Morphogenesis During Blood Vessel Regeneration in Zebrafish Caudal Fins.** Cheng-chen Huang,* Nathan Lawson,† Brant Weinstein,† and Stephen Johnson*. *Department of Genetics, Washington University Medical School, St. Louis, Missouri 63110; †Unit on Vertebrate Organogenesis, NIH, Bethesda, Maryland 20892.

Zebrafish reliably regenerate their fins following amputation. Here, we use transgenic fish that express EGFP in blood vessel endothelial cells to study blood vessel regeneration in wild-type and mutant regenerating caudal fins. During the first eight days of fin regeneration, blood vessels follow stereotyped patterns of growth and morphogenesis, including wound healing, vessel reconnection, plexus formation and remodeling, and vessel prun-

ing. This is followed by a transition to simple angiogenic outgrowth at day 8 that persists until regeneration terminates (at ~day 30). Amputated blood vessels heal their ends by 24 hours post amputation (hpa) and then reconnect arteries and veins to resume blood flow at wound sites by 48 hpa. Regenerative vessel growth begins soon after vessel reconnection. Initially, the vessels regenerate by growing excess vessels to form unstructured plexuses. Remodeling of the plexus generates arteries and veins beginning two days after plexus formation. The mode of vessel growth switches by 8 days post amputation (dpa) to growth without a plexus intermediate. Temperature-sensitive mutants for *reg6* have profound defects in blood vessel regeneration. At the restrictive temperature, *reg6* regenerating blood vessels first fail to make reconnections between severed arteries and veins, and then form enlarged vascular sinuses rather than branched vascular plexuses. Our results suggest that the *reg6* mutation causes defects in branch formation and/or angiogenic sprouting.

415. **Zebrafish Fin Regeneration as a Model System for Adult Angiogenesis and Anti-Angiogenic Therapy.** Joanne Chan,* Peter E. Bayliss,* Geoffrey Whitehead,† Mark Keating,† Jeanette M. Wood,** and Thomas M. Roberts*. *Department of Cancer Biology, Dana-Farber Cancer Institute and the Department of Pathology, Harvard Medical School, Boston, Massachusetts; †Department of Cell Biology, Department of Cardiology, Howard Hughes Medical Institute, Harvard Medical School, Children’s Hospital, Boston, Massachusetts; **Novartis, Pharma AG, Basel, Switzerland.

Targeting blood vessels in anti-angiogenic therapy is a promising strategy in the treatment of cancer as well as other angiogenesis-dependent human diseases. We have developed a novel approach to study angiogenesis in the adult zebrafish as a living vertebrate model organism. During caudal fin regeneration in the adult, blood vessels are also regenerated in a precise pattern. Using live angiography, the progression of this neovascularization can be easily monitored over time. This process is robustly regulated by the specific VEGF receptor inhibitor, PTK787/ZK222584; thus providing a chemical genetic dissection of this complex process in an adult animal. In the presence of PTK787/ZK222584, tissue regrowth can be separated from regenerative angiogenesis as skin, pigment, nerve and bone can regenerate up to 1 mm in the absence of new blood vessels. This adult angiogenesis model has also provided a reliable readout for diverse chemical compounds that exhibit high affinities towards the PDGF receptor, the MEK kinase as well as the target of rapamycin (TOR). This study demonstrates the potential use of the zebrafish adult as a novel angiogenesis model, both for the assessment of potential anti-angiogenic compounds and for dissecting the molecular mechanisms of neovascularization.

416. **Bone Patterning is Altered in the Regenerating Zebrafish Caudal Fin Following Ectopic Expression of *shh* and *bmp* Signaling.** A. Smith,*† E. Quint, and M. A. Akimenko*†. *Ottawa Health Research Institute; †Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada.

Zebrafish fins regenerate following amputation or injury. We have shown that genes involved in the sonic hedgehog (*shh*) signaling pathway are expressed during regeneration in patterns suggesting their role in the secretion and patterning of regenerat-

ing dermal bone. We have established an *in vivo* method of gene transfection to ectopically express genes in specific locations of the regenerating fins. We found ectopic expression of both N-shh and bmp2b can induce ectopic bone deposition and alter the patterning of the regenerating fin. Also, treatment of zebrafish with cyclopamine, an alkaloid known to inhibit shh signaling, causes a dose-dependent inhibition of fin outgrowth. This implicates N-shh and bmp2b in stimulating the secretion of bone matrix, possibly by influencing the differentiation of bone-secreting scleroblasts. Also, ectopic expression of N-shh within the blastema, but not bmp2b, results in a downregulation of endogenous shh and significant reductions in length of the regenerating fin rays. These results suggest that shh expression domains may be defined by an autoregulatory feedback loop. Injection of chordin, an inhibitor of bmp signaling, also results in a reduction in fin ray length and alters bone regeneration. We are now investigating the cellular and molecular mechanisms behind the reduction in length of the regenerate following ectopic expression of both N-shh and chordin and examining the regulatory interactions between shh signaling and other signaling pathways (FGF, Wnt) in the regulation of fin regeneration.

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418. **Essential Role for *puma* in Development of Post-Embryonic Neural Crest-Derived Lineages in Zebrafish.** N. B. Parker, J. M. Turner, D. M. Parichy. Institute for Cellular and Molecular Biology, University of Texas, Austin, Texas 78712.

Little is known about the developmental mechanisms underlying adult form. A useful system for identifying these mechanisms is the metamorphosis of fishes and amphibians, in which an embryonic or larval phenotype is transformed into that of the adult. In the zebrafish, *Danio rerio*, metamorphosis entails changes in a variety of organ systems including fins, skin, pigment pattern, peripheral nervous system, as well as sensory and digestive systems. To better understand the genetic and cellular bases for post-embryonic development, we are conducting screens for zebrafish mutants that fail to undergo the normal larval-to-adult transformation. Here, we show that *puma* mutant zebrafish exhibit a nearly complete failure of pigment pattern metamorphosis. We show that *puma* is required during metamorphic stages and acts autonomously to the pigment cell lineage. Moreover, we show that *puma* mutants exhibit defects in the peripheral nervous system and lateral line sensory system that arise during the larval-to-adult transition. Finally, molecular marker analyses of *puma* mutants reveal severe deficits in pigment cell and glial lineages. Since these cell types are derived originally from embryonic neural crest cells, our results suggest a model in which *puma* is required to recruit post-embryonic neural crest-derived stem cells during zebrafish post-embryonic development.

419. **Labeling of Multipotent Progenitor Cells in the Planarian *Schmidtea mediterranea*.** George Eisenhoffer and Alejandro Sánchez Alvarado. Department of Neurobiology and Anatomy, University of Utah School of Medicine, 20N 1900E Salt Lake City, Utah 84132.

Research that involves the activation and migration of multipotent progenitor cells will have implications that are critical to understanding the regeneration of organs and tissues in multicellular organisms. The flatworm *Schmidtea mediterranea* possesses a population of totipotent stem cells called neoblasts. These cells are responsible for the ability of planarians to regenerate. Previous studies have shown that the neoblasts are the only mitotically active cells in *S. mediterranea*. The presence of reporter genes in the host genome can be used to track the behavior of neoblast progenitors and their subsequent progeny. This study reports on a number of strategies to transiently and permanently label the neoblast population. Efficient labeling of the neoblasts will allow for the *in vivo* evaluation of their migratory and differentiation properties. More importantly, robust and reproducible labeling of neoblasts will provide a valuable tool for studying *in vivo* and in real time, the population dynamics of planarian stem cells. Supported by NIH NIGMS RO-1 GM57260.

420. **Stem Cells, Regeneration and Allometry in the Planarian *Schmidtea mediterranea*.** Nestor J. Oviedo^{1,†} and Alejandro Sánchez Alvarado*. *Department of Neurobiology and Anatomy, University of Utah school of Medicine, 20N 1900E Salt Lake City, Utah 84132; †Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela.

The developmental plasticity of planarians is manifested by the ability of small body fragments to regenerate complete worms and the capacity of these animals to grow and degrow depending on the abundance of food. The source of such plasticity lies in a remarkable population of multipotential stem cells known as neoblasts. In order to further characterize these biological attributes and the attendant stem cells, we have begun to study genes in a clonal line (CIW-4) of the asexual biotype of the planarian *Schmidtea mediterranea* that: 1) may serve as markers to study the cell biology of neoblasts; 2) are expressed during various stages of regeneration; and 3) display discrete, and quantifiable expression patterns that are useful for the study of scale and proportion regulation in planarians. Supported by NIH NIGMS RO-1 GM57260.

421. **Studies of *wnt* Cascade Genes in Planarians.** Chiyoko Kobayashi*, Katsuhiko Mineta[†], Masumi Nakazawa[†], Kazuho Ikeo[†], Takashi Gojobori[†], Kiyokazu Agata*. *Lab. E. Regeneration. Biol., RIKEN CDB; †Cent. Info. Biol. and DDBJ, National Institute of Genetics.

wnt cascade genes act for axis formation and morphogenesis. Recently, several *wnt* cascade genes have been identified in *Hydra*, and their expression patterns suggested that WNT signaling molecules may act in their axis formation as in other animals. Here, we focused *wnt* cascade in planarians to investigate whether WNT signals are involved in body axis formation also in planarians. We found a clone which has similarity to *wnt* gene from planarian EST database, and sequenced this clone completely. This clone has 1431 bp length and codes 402 aa including initial methionine. As results of homology search and alignment analyses, this gene was confirmed as a homology of *wnt* genes, named *DjwntA*. *DjwntA* is expressed in the brain and ventral nerve cords. Interestingly, it is gradually expressed in the posterior region of the brain. During regeneration, expression of

DjwntA is observed in their blastema region at mid stage of regeneration after 3 days of cutting]. In order to investigate the function of *DjwntA*, we performed RNAi experiments. Ds *DjwntA*-injected animals formed ectopic eyes similar to the phenotype of *Djndk*-knockdown planarians (Cebrià et al., 2002). However, ectopic eyes were formed in only the brain region but not in throughout the body. And the brain slightly expanded posteriorly in ds *DjwntA*-injected animals. These results suggest that *DjwntA* may be involved in antero- posterior patterning of the brain rather than the brain formation. We also reported results of other wnt cascade genes identified by the planarian EST project.

422. **An RNAi Screen for Regeneration Genes in the Planarian *S. mediterranea*.** Peter W. Reddien, Adam Bermange, and Alejandro Sánchez Alvarado. Department of Neurobiology and Anatomy, University of Utah, 20N 1900E, Salt Lake City, Utah, 84132.

Planarians possess a remarkable ability to regenerate missing body regions. The development of molecular methods for the study of planarian biology in recent years should allow the molecular dissection of regeneration in this classic and understudied model. RNA-mediated gene interference (RNAi) is an effective method of gene inactivation in planarians. We are utilizing RNAi to initiate a systematic and unbiased screen for regeneration defects in the planarian *S. mediterranea*. Double-stranded RNA for RNAi can be expressed from a cDNA in bacteria, fed to planarians, and cause specific gene inactivation. We are generating a library of planarian cDNAs in a vector that generates double stranded RNA in bacteria and using this library to screen for genes that control regeneration. A pilot RNAi screen has been performed using genes with predicted roles in cell cycle, metabolism, patterning, cell fate, and stem cell regulation. The results from this pilot screen have served to identify regeneration phenotypic classes and optimize screening methodologies. We are currently developing methods, utilizing *in situ* and antibody markers for specific cell types, for the phenotypic analysis of regeneration defects.

423. **An RNAi Screen for Regeneration Genes in the Planarian, *Schmidtea mediterranea*.** Adam L. Bermange, Peter W. Reddien, and Alejandro Sánchez Alvarado. Department of Neurobiology and Anatomy, University of Utah School of Medicine, Salt Lake City, Utah 84132.

The remarkable process of regeneration has intrigued scientists for over 200 years. Despite this fascination, very little investigation into the molecular events underpinning regeneration has been possible, largely due to the limited regenerative capacities of the commonly studied model systems. Planarians, by contrast, possess tremendous regenerative powers and many of the tools necessary to study these organisms at the molecular level are now in place. Our group is undertaking the first screen to identify regeneration genes in the planarian model. Using the species *Schmidtea mediterranea*, for which 4,500 independent cDNAs have been isolated, we are systematically eliminating gene function through double-stranded RNA (dsRNA)-mediated gene interference (RNAi) and screening for regeneration-defective phenotypes. dsRNA can be introduced either by injection or ingestion, the latter being the favorable method for large-scale screening. A vector has been engineered, compatible with Gateway cloning technology, allowing for efficient ectopic dsRNA production in *E.*

coli transformants, which can then be fed to planarians. An initial screen of 35 genes has enabled us to optimize our screening protocols. Here, we report on the characterization of a number of phenotypic classes generated by the initial screen.

424. **Role of Meis Genes in Proximodistal Specification During Limb Regeneration in the *Ambystoma mexicanum*.** Karen Echeverri,* Nadia Mercader,† Elly M. Tanaka,* and Miguel Torrest†. *Max Planck Institute for Molecular Cell Biology and Genetics, Pflotenhauerstrasse 108, Dresden, Germany; and †Departamento de Inmunología y Oncología, Centro Nacional de Biotecnología, Universidad Autónoma, Madrid, Spain.

Axolotls have the amazing ability to perfectly regenerate a limb from any level in response to amputation. One of the main unanswered questions is how the regeneration blastema interprets the information necessary to regenerate the missing portion of the limb. During chick and mouse limb development restriction of Meis gene expression to a proximal zone has been shown to play an important role in patterning the proximodistal axis. To address the issue of how a limb is patterned during regeneration we have begun studying the expression of Meis genes during regeneration of the axolotl forelimb. Cloning of the axolotl Meis genes indicates a high conservation of these genes between species. Initial data suggests that Meis genes are downstream targets of retinoic acid. Overexpression of Meis in the regeneration blastema results in an arrest of limb regeneration at the blastema stage or defects in patterning of the limb regenerate, suggesting an important role of Meis in patterning of the regenerate. By combining overexpression studies with fluorescent lineage tracing we can look at the fate of cell populations overexpressing the Meis genes to determine if overexpression can confer a proximal fate to a distal blastema cell. We will also determine if the expression of Hox genes is affected by overexpressing Meis genes.

425. **Accessory Limb Formation From Skin Wounds and Nerve Deviation.** Tetsuya Endo, Susan V. Bryant, and David M. Garediner. Department of Developmental and Cell Biology, University of California, Irvine, California.

We are investigating the mechanisms of limb regeneration in the axolotl (*Ambystoma mexicanum*), with an emphasis of the genesis of blastema cells (dedifferentiation). Although an essential early process in limb regeneration, dedifferentiation is difficult to study because the early phase of limb regeneration consists of both specific and non-specific events induced by trauma to the amputated limb. To analyze dedifferentiation as a process distinct from non-regenerative responses, we have studied accessory limbs induced by skin wounds and nerve deviation. This experimental model has been described previously, however, the frequency of limb induction was relatively low. We have optimized this system to obtain a high frequency of induced outgrowth with minimal trauma to the limb. If a nerve is severed at the elbow and deviated to a superficial skin wound in the upper arm, a blastema-like outgrowth is induced. Although these outgrowths form a cartilaginous element, they eventually regress without forming a limb. If however, a piece of skin from the opposite side of the limb is grafted to the nerve-deviated wound site, the outgrowth continues to develop and forms a limb. This model allows for mechanistic studies of the critical components required for limb regeneration; a wound epithelium, a nerve

supply and connective tissue cells (fibroblasts) with diverse positional information. We hypothesize that the outgrowths arise by dedifferentiation of dermal and connective tissues in response to signals from the wound epidermis and nerves that stimulate fibroblasts to form a blastema.

426. Development of Transgenic *Ambystoma mexicanum* to Study Cell Fate during Tail Regeneration. Lidia Okoniewska and Elly M. Tanaka. MPI-CBG, Dresden, Germany.

Adult urodele amphibians can fully regenerate lost anatomical structures by forming a mound of undifferentiated cells called blastema, formed at the amputation plane. We would like to define all the cell types that contribute to the blastema, and whether dedifferentiation or stem cell activation is the predominant mechanism. Moreover, it is not known if the blastema consists of pluripotent cells or of multiple populations of cells whose fate is already determined. In order to follow long-term cell fate during regeneration, we are developing transgenic axolotls that stably express fluorescent proteins in specific cell types. We are currently evaluating several methods of transgenesis successfully used in other systems. Dedifferentiation is a complex process involving downregulation of known markers of differentiation. If the cells change fate and turn off cell-type specific plasmids, we cannot follow their ultimate fate. Therefore we will use the cre-lox recombination system to irreversibly activate fluorescent protein expression in specific cell types. This system allows us to ubiquitously express a double reporter gene together with cre recombinase gene driven by cell-specific promoter such as muscle. Activation of cre recombinase cuts out the GFP and activates constitutive DsRed expression only in muscles. Therefore if a muscle fiber dedifferentiates and changes its lineage, the descendants will remain DsRed+, and we will be able to follow it as it forms other cell types.

427. An Axolotl EST Database: Gene Information for Studying Regeneration. B. Habermann,* A.-G. Bebin,* M. Volkmer,* S. Herklotz*, K. Eckelt,* K. Pehlke,† H. H. Epperlein,† H. K. Schackert,** G. Wiebe,* and E. Tanaka*. *Max Planck Institute CBG, Pfotenhauerstrasse 108, 01307, Dresden, Germany; †Institute of Anatomy, Medical Faculty of the Technical University of Dresden, Fiedlerstrasse 42 01307 Dresden, Germany; and **Department of Surgical Research, Medical Faculty of the Technical University of Dresden, 01307 Dresden Germany.

Ambystoma mexicanum (Axolotl) is a well-established model organism for comparative embryology, evolution and regeneration studies. A comprehensive set of gene sequences from the organism would greatly aid research in these fields. In order to produce EST sequence information from the Axolotl, we prepared two cDNA libraries from six day tail blastema and from 16 to 22 stage neural tubes. Approximately 18,000 clones were sequenced, resulting in 9677 unique sequence contigs. To date, the libraries were still yielding 42% (blastema) and 50% (neural tube) unique clones. By sequence comparison to the proteomes of selected model organisms a putative identity for 6150 contigs (53%) could be assigned. Among the most abundant cellular functions attributed to the contigs were RNA metabolism and cell cycle control, which is a characteristic for proliferating cells such as those found in the blastema and the neural tube. Regeneration involves the re-expression of developmental genes, and as expected, many

transcription factors involved in embryonic development were found in the 6 day regenerating tail blastema library. The cDNA and contig sequences as well as their annotations in term of homologs, domains and assigned function were collected in an axolotl database, available via <http://www.mpi-cbg.de>.

428. Patterning the Regenerating Axolotl Spinal Cord: The Role of Pax7. Levan Mchedlishvili,* Hans. H. Epperlein,* Elly M. Tanaka†. *TUD, Medical Faculty Carl-Gustav-Carus, Institut of Anatomy Fetscherstr 74, 01307, Dresden, Germany; and †Max Planck Institute for Molecular Cell Biology and Genetics Pfotenhauerstrasse 108, 01307, Dresden, Germany.

The Axolotl can regenerate a fully functional spinal cord in response to tail injury. It is not known how the diversity of different neuronal cell types is regenerated during this process. We are using Pax7 as a marker to understand the process of spinal cord patterning during regeneration. Pax7 is a member of the paired box-containing gene family that is expressed in neural progenitor cells throughout the neural fold during early development. Later, it is restricted to the dorsal progenitor cells of the neural tube. During regeneration we find Pax7 in the dorsal neuro-epithelial cells of the regenerating spinal cord. We see no evidence for Pax7 expression in both dorsal and ventral cells during regeneration. Surprisingly, we also found Pax7-positive progenitors in the adult Axolotl spinal cord. This suggests that the mature spinal cord already harbors spatially-restricted progenitor cells and we hypothesize that the Pax7+ cells of the mature spinal cord will give rise to the dorsal Pax7+ cells of the regenerating structure. Are Pax7-expressing adult Axolotl spinal cord cells able, in response to injury, to give rise to definitive dorsal neuronal and glial cell populations in order to regenerate a functional spinal cord structures? Is Pax7 expression in the cells caused by environmental factors, or are Pax7-expressing cells able to maintain their identity even when placed in a different environment? To answer this and another questions we are using methods such as single cell electroporation and transplantation, as well as histological observations of regenerated and adult spinal cord in the Axolotl.

429. Purification of a Serum Factor That Drives Newt Myotubes Into S-Phase. Werner L. Straube,* David N. Drechsel,* Jeremy P. Brockes,† and Elly M. Tanaka*. *Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; and †Department of Biochemistry, University College London, London, United Kingdom.

Newts belong to a class of tailed amphibians that can perfectly replace complete body parts after loss. This includes the regeneration of different tissue types such as skin, muscle, connective tissue, bone and spinal chord. A requirement for this regeneration is the de-differentiation of cells at the wound site into a mass of proliferating cells that replace the missing tissue. In order to identify factors that initiate de-differentiation, we are purifying a thrombin-activated serum activity termed S-Phase Re-entry Factor (SPRF). SPRF activity provides a link between wounding and regeneration and is found in sera from different sources including humans. After activation by thrombin SPRF drives differentiated cultured newt A1 myotubes but not those from mouse (C2C12) into S-phase. We are using these cultured newt A1 cells to identify this serum activity where the percentage of myotubes stimulated to undergo DNA replication serves as a

quantitative assay for the purification. After three column chromatography steps-cation exchange, anion exchange and Heparin affinity-we have achieved a 200-fold purification starting from a commercially available crude thrombin preparation. We are currently scaling-up this procedure and investigating additional modes of separation such as hydrophobic interaction and lectin-affinity chromatography in order to attain further purification and identify this key factor.

440. **A Morphant Screen Identifies an Essential Role for Syndecan-2 in Vascular Development.** Eleanor Chen, Aubrey Nielsen, Spencer Hermanson and Stephen C. Ekker. University of Minnesota Medical School Department of Genetics, Cell Biology and Development.

Vascular formation is a highly conserved vertebrate process, and yet many key molecular players involved are unknown. We have developed a morpholino-based gene targeting screen to identify novel gene essential for vascular development, using zebrafish, *Danio rerio*, as the model system. From our vascular screen, we have identified Syndecan-2, a cell-surface heparan sulfate proteoglycan, as an essential factor for angiogenic sprouting and remodeling during development. The cytoplasmic tail is important for the vascular function of Syndecan-2 as forced expression of a cytoplasmically-truncated form of syndecan-2 results in embryos with angiogenic defects. The vascular function of Syndecan-2 is conserved as zebrafish and mouse syndecan-2 are similarly expressed in the vascular mesenchyme, and human syndecan-2 can restore angiogenic sprouting in syndecan-2 morphants. Finally, we show that co-targeting of syndecan-2 and vegf (vascular endothelial growth factor) results in synergistic inhibition of angiogenesis, and exogenous VEGF-121 or VEGF-165 protein restores sprouting in syndecan-2 morphants, suggesting that Syndecan-2 and VEGF function in the same pathway during angiogenesis.

441. **Mutation of *Vmhc* Disrupts Contractility of the Embryonic Zebrafish Ventricle.** Hope Coleman, Eli Berdugo, Trisha Bruno, Felix Olale, and Deborah Yelon. Developmental Genetics Program, Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, New York.

Proper circulation depends upon coordinated contractions of each chamber of the two-chambered zebrafish embryonic heart. This organ is composed of an atrium and a ventricle: two morphologically, physiologically, and molecularly distinct chambers. We have identified a zebrafish mutation, *half-hearted*, which causes ventricular developmental defects that impair circulation. *half-hearted* mutants have a non-contractile ventricle and a grossly normal atrium. Additionally, ventricular sarcomeres fail to assemble normally in *half-hearted* mutants. Through candidate gene analysis we demonstrate that the *half-hearted* locus encodes the ventricular myosin heavy chain, *Vmhc*. Immunohistochemical analysis indicates a lack of myosin heavy chain in the *half-hearted* ventricle. Furthermore, in one *half-hearted* allele, we find a point mutation that creates an early stop codon in *vmhc*. In complimentary studies, we have identified a zebrafish locus that is essential for atrial contractility and encodes the atrial myosin heavy chain, *Amhc*. In conjunction with the *amhc* mutation, *half-hearted* is a powerful tool to examine the molecular, morphological, and physiological

changes and compensations in the myocardium that result from disruption of chamber-specific cardiac myosin heavy chains.

442. **Development of Adenosinergic Heart Rate Regulation in Wildtype and Slow mo Mutant Zebrafish Embryos.** Kerri S. Warren and Leigh E. Smith. Department of Biology and Marine Biology, Roger Williams University, Bristol, Rhode Island.

The slow mo (*smo*) gene is required for normal pacemaking in the zebrafish heart. Homozygote mutants lacking *smo* gene function display a reduced heart rate and a reduced cardiomyocyte pacemaker current (*I_h*). As the rate-setting defect is obvious from the start of rhythmic cardiac activity, we are using *smo* mutants as a tool to examine the relative role of the pacemaker current in cardiac pacing and the development of heart rate regulation. Adenosine is an early humoral regulator of heart rate, acting in mouse embryos before the adrenergic or muscarinic systems function. Adult zebrafish have been shown to express adenosine receptors (*A2a* subtype) and embryonic fish heart rate is affected by chronic blockade of adenosine *A1* receptors. We therefore sought to determine the developmental timing of adenosinergic control of wildtype heart rate and to investigate whether regulation by adenosine is present and active in *smo* pacemaking. We find that acute treatment of *A1* adenosine receptor antagonist DPCPX (1,3-dipropyl-8-cyclopentylxanthine) raises heart rate in 35 hour, 40 hour and 72 hour wildtype and *smo* embryos, but not in 27 hour embryos, suggesting that adenosinergic *A1* receptors are present and functioning in *smo* mutants and that the onset of endogenous adenosine rate depression appears after cardiac pacing becomes dependent on *smo* function.

443. **The Role of MEF2C in Cardiac Morphogenesis.** Linh Vong, Weizhen Bi, Katharine O'Connor, Changyou Li, and John J. Schwarz. Albany Medical College, 47 New Scotland Avenue MC8, Albany, New York 12208.

Ablation of the transcription factor, MEF2C, results in an extremely hypoplastic ventricular region. Histological analyses of embryos at various stages of cardiac development indicate that null embryos do not form a normal linear tube heart. Instead of an expansion of the crescent to form the bulbous ventricular region of the tube stage heart, only the region near the midline appears to expand as the inflow and outflow regions of the heart grow apart. These observations suggest that the transition from a crescent to a linear tube stage heart is defective. Current studies are focused on determining if there are differences in either cellular proliferation or death in crescent and linear tube stage hearts that may account for these observations. Cardiomyocytes from MEF2C null embryos were observed to be more rounded and disorganized compared to their wildtype counterparts, which are elongating at this time. Additionally, the later expansion of the ventral part of the heart to form the ventricular chambers does not occur in the null embryos. Thus, MEF2C may be required for both the early expansion of the crescent to form the bulbous tube heart and the later expansion to form the ventricular chambers. Supporting this notion, the expression of *HAND1*, which is normally found in the ventral, posterior region of the heart, corresponding to the future left ventricle, is delayed and drastically reduced in the null embryos. Moreover, gene expression analysis of isolated wildtype and null embryonic hearts by

quantitative RT-PCR (QPCR) show a 8-fold decrease in the expression of MITR, a known repressor of MEF2 activity.

434. **Circulation Is Established in a Step-Wise Pattern in the Mammalian Embryo.** K. E. McGrath, A. D. Koniski, J. Malik, and J. Palis. Pediatric Dept, University of Rochester.

The establishment of circulation in mouse embryos was investigated by analyzing the redistribution of yolk sac-derived primitive erythroblasts and definitive hematopoietic progenitors within the developing vascular system. We found a proximal/distal asymmetry of hemangiogenic differentiation within the yolk sac at embryonic day 8.0 (E8.0). Small numbers of erythroblasts first enter the embryo proper at 4 to 8 somite pairs (sp) (E8.25), concomitant with the proposed onset of cardiac function. However hours later (E8.5), the vast majority of erythroblasts still remain in the yolk sac. A steady state of approximately 40% erythroblasts in the embryo proper is not reached until 26-30 sp (E10). Additionally, erythroblasts are unevenly distributed within the embryo's vasculature before 35 sp. These data imply that fully functional circulation is not established before E10. This timing correlates with vascular remodeling, suggesting that vessel arborization and/or smooth muscle recruitment is required. Furthermore, initial vascular remodeling appears to occur under conditions of uneven circulation. We also examined the distribution of committed definitive hematopoietic progenitors during early embryogenesis. Prior to E8.0, all progenitors were found in the yolk sac. When normalized to circulating erythroblasts, there was a significant enrichment (20 to 5-fold) of progenitors in the yolk sac compared to the embryo proper from E9.5 to E10.5. These results indicate that the yolk sac remains a site of hematopoietic progenitor production and/or preferential adhesion even as the fetal liver becomes the predominant hematopoietic organ.

435. **Quaking, an RNA Binding Protein Required for Proper Myelination, Is Also Essential for Cardiovascular Development.** Jennifer L. Northrop, Lihua Lai, Lanette Pool, Karen K. Hirschi, and Monica J. Justice. Departments of Molecular and Human Genetics, Molecular and Cellular Biology, and Pediatrics, Baylor College of Medicine, Houston, Texas 77030.

One out of every 150 infants in the United States is born with a significant congenital heart defect. Identifying the role of genes essential to cardiovascular development in vertebrate model organisms provides a window of insight into mechanisms of congenital cardiac defects in humans. We have recently identified that quaking, an RNA binding protein, has a previously unsuspected and essential role in cardiovascular development in the mouse embryo. The quaking locus was initially identified in 1964 from a spontaneously occurring mouse mutation that resulted in seizure activity and shaking due to a disruption in myelination. An allelic series of engineered point mutations in the quaking gene produced embryonic lethal defects, demonstrating that quaking has a critical role in development prior to the onset of myelination. Our studies show that quaking is expressed in the yolk sac endoderm, adjacent to the mesodermal-derived developing blood islands where differentiation of blood and endothelial cells first occurs. In addition, vessels within the embryo proper exhibit similar defects in endothelial cell maturation and smooth muscle cell recruitment. Quaking is also highly expressed in the developing mouse heart and our current studies delineate its role

in early cardiac development. While previous studies of quaking have shown that its expression is essential for postnatal myelination, these results demonstrate a novel and critical role for quaking in cardiovascular development.

436. **The Mechanisms of Action of Ethanol on Extraembryonic Vascular Development: Involvement of Oxidative Stress, Retinoic Acid Signaling and VEGF Expression.** A. C. Tufan,* N. L. Satioglu-Tufan†. Department of *Anatomy, †Pathology, Pamukkale University School of Medicine, Denizli, Turkey.

Previously we have examined the effect of EtOH exposure on extraembryonic vascular development using the chick embryo area vasculosa in shell-less culture and demonstrated that, in terms of extraembryonic vascular development, an early (at HH stage 11), single, low-dose (10%) EtOH exposure may have an acute, short-term positive effect, whereas moderate- (30%) or high- (50%) dose EtOH exposure may severely perturb this process, disabling the necessary absorption of the nutrients for the embryo to develop properly. In this study, we elucidated the mechanisms of action of EtOH on extraembryonic vascular development by testing the hypotheses that: 1) generation of reactive oxygen intermediates through the metabolism of EtOH may trigger the apoptotic process in individual cell groups of the embryo and its surrounding membranes; 2) EtOH alters the action of retinoic acid signaling; 3) EtOH alters the expression of growth regulatory factors in the embryo and its surrounding membranes. α -Tocopherol (0.05 M) or all-trans-retinoic acid (10^{-8} M) co-treatment of 50% EtOH treated cultures significantly decreased the mortality rate and recovered the embryonic growth and extraembryonic vascular development. Vitelline membrane specimens from EtOH exposed groups immuno-stained for VEGF showed altered expression in a dose dependent manner. Thus, the mechanisms of action of EtOH on extraembryonic vascular development may involve establishment of oxidative stress, perturbation of retinoic acid signaling, and alteration of the VEGF expression.

437. **The Role of the bHLH Transcription Factor *HAND1* in Extraembryonic Vasculature Development.** Yuka Morikawa and Peter Cserjesi. Department Cell Biology and Anatomy, LSU Health Sciences Center, New Orleans, Louisiana 70112.

The basic helix-loop-helix (bHLH) transcription factor *HAND1* is expressed in numerous tissues during development including the heart, neural crest derivatives, and extraembryonic membranes. To investigate the role of *HAND1* during development, we generated a *HAND1* null mouse by replacing a portion of the gene with b-galactosidase. *HAND1* null mice survive to the nine somite stage at which time they succumb due to numerous developmental defects including delayed development and abnormal heart formation. However, little is known about the specific functions of *HAND1* during development. One striking defect in *HAND1* null embryos is in development of the yolk sac vasculature. Vascular formation occurs in two stages: vasculogenesis when endothelial cells accumulate and aggregate to form the vascular primordium, and angiogenesis during which the primitive vascular network is extended by budding and branching. This is followed by formation of a smooth muscle wall around the endothelial tubes. We examined the distribution of endothelial cells in *HAND1* null yolk sacs using the endothelial-specific

marker PECAM and show that vasculogenesis occurs but vascular refinement is arrested. We examined the expression of a number of genes regulating angiogenesis and show that *VEGF* and *ang1*, and the bHLH factor *HAND2*, a factor with a high degree of homology with *HAND1*, are up-regulated in *HAND1* mutant membranes. Interestingly, *HAND1* mutant yolk sacs also show abnormal distribution of smooth muscle cells. These results suggested that *HAND1* is required for yolk sac angiogenesis and vascular smooth muscle organization.

438. **An ENU Mutagenesis Screen to Isolate Cardiovascular and Hematopoietic Lethal Mutations Using a Mouse Balancer Chromosome.** Kathryn Hentges,* Hishashi Nakamura,* Maritess Alviento,* Bradley Hasson,* Allan Bradley,† and Monica J. Justice*. *Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, Texas, 77030; and †Sanger Centre, Cambridge, United Kingdom.

Balancer chromosomes are a useful tool for mutation isolation, however, they have not previously been used in mice. We used a Cre/*loxP*-engineered Chromosome (Chr) 11 balancer in a genetic screen designed to isolate recessive mouse mutations induced by *N*-ethyl-*N*-nitrosourea (ENU). We have isolated 55 lines of mice on Chr 11 that are lethal before weaning. The time of death has been determined for many mutant lines, and ranges from pre-implantation to two weeks after birth. An examination of mutant phenotypes reveals that many of the mutants are defective in the hematopoietic or cardiovascular system. The phenotypes in these mutants include: heart formation outside the amnion, internal hemorrhages late in gestation, and abnormal heart tube looping. Markers of cardiac mesoderm, vascular endothelium, and hematopoiesis are being analyzed in mutants with phenotypes that suggest cardiovascular or hematopoietic defects. Pair-wise complementation testing has been performed to determine if any of the mutants are allelic. Continuing mutation isolation on this and other mouse chromosomes will identify novel members of pathways required for hematopoiesis and cardiovascular development.

439. **Transgenesis and Insertional Mutagenesis in *Xenopus*.** Emin Kuliyeve, Michelle J. Hamlet, Joanne R. Doherty, Haiqing Zhu, Donald A. Yergeau, and Paul E. Mead. Department Pathology, St. Jude Children's Research Hospital, Memphis, Tennessee.

Our laboratory studies the development of blood and blood vessels in the vertebrate embryo. To identify genes involved in these processes we have developed a rapid transposon-based insertional mutagenesis technique in frogs. We have used the Sleeping Beauty transposase system to stably integrate a variety of transposon targets into the *Xenopus* genome. The transposon transgenes integrate with very low copy number early during development and are passed through the germ line at the expected Mendelian frequencies. Using transposition, we have generated both standard transgenics with GFP reporters and have also initiated insertional mutagenesis screens to identify genes involved in hematopoiesis. Data will be presented on our success with pilot screens using both gene trap and enhancer trap transposons. We have now initiated large-scale enhancer and gene trap screens in the diploid frog *Xenopus tropicalis*. The high-

throughput strategy that we have developed for these insertional mutagenesis screens will also be presented.

440. **The Role of Tbx4 and Hox Genes in Lung Bud Formation.** Jun-ichi Sakiyama and Atsushi Kuroiwa. Division of Biological Science, Graduate School of Science, Nagoya University, Nagoya, Japan.

We are studying on the transcriptional mechanisms determining the positional specification and controlling the morphogenesis of the lung during embryonic development. We found that Tbx4 was specifically expressed in the lung primordium mesoderm overlapping with the expression domain of Fgf10, encoding a lung bud-inducing factor, in chick. Tbx4 misexpression and inhibition of endogenous Tbx4 function indicated that Tbx4 is necessary for the expression of Fgf10. Ectopic Tbx4 or Fgf10 in the esophagus mesoderm induced ectopic bud formation and ectopic expression of Nkx2.1, a respiratory endoderm marker, in the underlying esophagus endoderm. Thus, Tbx4 specifies the lung primordium mesoderm and controls lung bud formation and respiratory endoderm differentiation through Fgf10 activation. On the other hand, to understand the mechanism of the lung bud positioning, we focused on Hox genes that are expressed in the visceral mesoderm in a region-specific manner along the A-P axis. The anterior border of the Hoxb-6 expression domain was just located at the position where the primary bronchus would arise. Misexpression of Hoxb-6 in the visceral mesoderm located anteriorly to the intrinsic expression domain caused the anteriorly and/or medially shift of the primary bronchus position correlating with the shift of the anterior border position of the artificial Hoxb-6 expression domain, implying the relationship between the anterior border of the Hoxb-6 expression domain and position of the primary bronchus. Hoxb-6 misexpression also influenced on the elongation process of the primary bronchus.

441. **Retinoid Regulation of Lung Bud Initiation.** Tushar Desai, Sarah Malpel, and Wellington Cardoso. Boston University School of Medicine, Boston, Massachusetts.

Several studies have shown that retinoic acid (RA) is required for lung development. To investigate the molecular basis of this requirement, we disrupted RA signaling in embryonic day (E) 8.5 foregut explants in vitro with the pan retinoid antagonist BMS493, and we followed development of lung and other foregut derivatives. When explants are treated prior to the 15 somite stage, lung buds fail to form while other derivatives are relatively unaffected. Fgf10 expression is undetectable in the foregut at the prospective lung region, however signals are preserved in thyroid primordia. Lack of RA signaling does not prevent specification of lung cell fate in the foregut endoderm, since the prepulmonary endoderm continues to express Ttf1. Site-specific regulation of Ttf1 by RA is demonstrated by preserved Ttf1 levels in thyroid but reduced levels in the prospective lung area. Treatment with RA receptor isotype selective agonists and antagonists suggests that RAR α may be involved in maintaining Ttf1 levels in the foregut endoderm, while RAR β may be responsible for Fgf10 gene regulation in the mesoderm.

442. **A Forward Genetic Screen in Mice for Genes that Regulate Lung Development.** Charlotte H. Dean,† Roberta Rivi,* Elizabeth Lacy,* Kathryn Anderson,* and Lee A. Niswander†. *Memorial Sloan Kettering Cancer Center and

†Howard Hughes Medical Institute, 1275 York Avenue, New York, New York 10021.

Embryonic lung development requires a coordinated series of cellular and molecular events that results in the elaborate branching pattern observed in the adult lung. To date, relatively little is known about the genes that are crucial for these processes. Using the chemical mutagen ethylnitrosourea (ENU) we have undertaken a forward genetic screen in mice to identify genes critically required for lung development. We have identified several lines of mice with aberrant lung development and they exhibit a range of phenotypes from small underdeveloped lungs (line 48B) to fusion of one (line 46) or more (line 20) lobes. We are currently analyzing the nature of these defects using histology and molecular markers for different aspects of lung development. In addition we are carrying out chromosomal mapping studies to uncover the genetic mutation underlying each phenotype. These studies will lead to a better understanding of lung development and disease.

443. **Distinct Time Windows for FGF Signaling during Lung Morphogenesis.** A. K. Perl,* B. Spencer-Dene,† C. Dickson,† J. Whitsett*. *CCHMC, Ohio; and †ICRF, London, United Kingdom.

Murine lung formation initiates on E9.5 by ventral evagination from the foregut endoderm. Until E12.5 respiratory tubules elongate and branch dichotomously to form the bronchial tree. Lateral budding after E12.5 forms the tubular structures of the bronchioles, which elongate and dichotomously branch until E16.5. Around E16.5 a second wave of lateral buds initiates and forms the terminal buds, the alveolar structures of the mature lung. To dissect temporal requirements for FGF signaling during lung morphogenesis we conditionally expressed FGFR antagonist Sprouty4 (Spry4) or FGFR inhibitor FGFR-Hfc. Transgene expression was directed to respiratory epithelial cells of the developing lung using the surfactant protein C promoter, driving the reverse tetracycline transactivator protein (SPC/rtTA). FGFR2IIIb signaling was investigated by conditional inactivation of the floxed FGFR2IIIb gene using a SPC/rtTA regulated CRE recombinase. Expression of Spry4 or FGFR-Hfc until E12.5 resulted in reduced lobe formation and inhibition of dichotomous branching. The effect of Spry4 expression was reversible when Dox treatment was terminated before E13.5 but not thereafter. Expression of Spry4 or FGFR-Hfc from E16.5 to E18.5 reduced the numbers of terminal buds resulting in respiratory failure (Spry4) or non-inflammatory emphysema by PN21 (FGFR-Hfc). Early inactivation of FGFR2IIIb reduced lateral buds at E13.5 and enlarged epithelial tubules at E18.5. Inactivation after E14.5 resulted in reduced terminal buds at E18.5. Our experiments suggest that a FGF mediated signal at E12.5 and E16.5 is required to initiate lateral budding and to initiate expansion of dormant progenitor cells.

444. **A Significant Reduction of the Diaphragm in mdx:MyoD/-9th Embryos Leads to Pulmonary Hypoplasia.** Mohammad R. Inanlou and Boris Kablar. Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

To further investigate the role of MyoD during skeletal myogenesis, we backcrossed mdx mutant mice (lacking dystrophin) with MyoD knock-out mice. After nine generations of backcrossing, it was not possible to obtain a viable mdx:MyoD/- phenotype (designated as: mdx:MyoD/-9th). The compound-mutant

embryos were examined just before birth. Essentially normal Myf5-dependent and most of the MyoD-dependent musculature was observed. By contrast, the skeletal muscle compartment of the diaphragm was significantly reduced. The failure of mdx:MyoD/-9th diaphragm to develop normally was not caused by a reduced number of satellite cells, but from the inability of stem cells to progress through the myogenic program. In addition, lung hypoplasia was detected in mdx:MyoD/-9th embryos. In the hypoplastic lung, the number of proliferating cells was lower in comparison to the control littermates, while the TTF-1 gradient was not maintained. Surprisingly, no difference was observed in the apoptotic lung cells distribution and occurrence. Together, it appears that mechanical forces generated by contractile activity of the diaphragm muscle play an important role in normal lung growth and development by affecting cell proliferation and TTF-1 expression. Supported by an NSERC operating grant to B. K.

445. Abstract #445 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

446. **Wnt Signaling Is Required for Vertebrate Pancreas Formation.** Hyon J. Kim,*† Shuo Lin,* Stephen C. Ekkert. *University of California Los Angeles; and †University of Minnesota.

Wnt signaling is used in many different cell-cell interaction processes. The WNT family of secreted proteins signal through its receptors, the frizzled (FZ) family of transmembrane proteins. Recently, we have isolated the zebrafish homolog of fz-2 and have used morpholinos, sequence specific translational inhibitors, to study its loss of function phenotype. In addition to our previously published phenotype of an undulating notochord, FZ-2 morphants have defects in pancreas formation. Further analysis showed that pdx-1 is expanded at 14 somite stage, insulin and somatostatin expressing cells do not properly migrate, often forming more than 2 patches at 24 hpf stage, and glucagon expressing cells are reduced or absent at 24 hpf stage. WNT-5 morphants also show similar pancreas defects directly implicating a role for Wnt signaling in pancreas formation. Co-injection of low dose wnt-5 and fz-2 RNA in the ventral blastomeres of *Xenopus* at 4 cell stage induces secondary axis, implicating that wnt-5 and fz-2 can genetically interact. In addition, co-injection of fz-2 and wnt-5 morpholinos at low doses displayed synergistic effects on pancreas formation demonstrating a genetic interaction between wnt-5 and fz-2 in this process. These results suggest that a Wnt-5-Fz-2 interaction is involved in pancreas formation in zebrafish. We can rescue the effects of wnt-5 morpholino by injecting back synthetic RNA coding wnt-5 protein suggesting specificity of wnt-5 morpholino. Additional experiments to determine how wnt-5 signaling are involved in pancreas formation and if this pathway is also required in pancreas development of other vertebrates are underway.

447. **Gain-of-Function Screening to Identify Novel Genes Implicated in Early Pancreas Development in *Xenopus Laevis*.** Francesca M. Spagnoli and Ali H. Brivanlou. The Rockefeller University, New York, New York.

The pancreas originates from distinct embryonic outgrowth of the dorsal and ventral regions of the duodenal portion of the endoderm. The importance of reciprocal interactions of the

prepancreatic endoderm with the surrounding tissues in the development of the pancreas has been demonstrated. In addition, a number of transcription factors operating at various levels in pancreas development have been identified. However, the early steps that control the commitment of a region of localized gut epithelium toward a pancreatic fate are still not well understood. We investigated the molecular mechanisms underlying the early stages of pancreas development in *Xenopus laevis*. To identify novel genes involved in this process we used an expression cloning strategy. Pools of transcribed cDNAs from an early gastrula stage expression library were injected into vegetal blastomeres of 8-cell stage embryos, which were then allowed to develop and analyzed for changes in pancreas organogenesis. Embryos were analyzed at tadpole stage by whole mount *in situ* hybridization for the expression of two specific markers: 1) the homeobox transcription factor *Xlhb-8* (a.k.a. *Pdx1* in mouse), and 2) the endocrine hormone insulin. Following this gain-of-function screening we identified a positive pool of 100 cDNAs which induced ectopic expression of insulin along the gut. By fractionating the pool we isolated two individual clones that possessed this activity. Following their injection into the embryos, patches of ectopic insulin-expressing cells were further induced along the gut. Both candidate genes are being characterized.

448. **Development of the Dorsal Pancreatic Endoderm Requires Vascular Function.** Josefina Edsbacke,* Jenny K. Johansson,* Farzad Esni,*† Glenn L. Radice,** Henrik Semb*. *Department of Medical Biochemistry, Box 440, Göteborg University, S-405 30 Göteborg, Sweden; †Department of Surgery, Johns Hopkins University, 720 Rutland Avenue, Ross 743, Baltimore, Maryland 21205; and **Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, 1355 Biomedical Research Building II/III, 421 Cuire Boulevard, Philadelphia, Pennsylvania, 19104.

Early growth and differentiation of the pancreatic endoderm requires soluble factors derived from the pancreatic mesenchyme. Whereas the ventral pancreatic mesenchyme encloses the presumptive ventral pancreatic endoderm before gut closure, the dorsal pancreatic mesenchyme is recruited by an as yet unidentified mechanism from the lateral plate mesenchyme. Previously, we demonstrated that N-cadherin-deficient mice lack a dorsal pancreas, and that the mechanism appeared to involve an important role for N-cadherin in survival of the dorsal pancreatic mesenchyme. In this study, we show that when the cardiac and circulatory function of N-cadherin^{-/-} mice is rescued by cardiac-specific expression of N-cadherin, the dorsal pancreas formation is also rescued, indicating that the lack of dorsal pancreas formation in N-cadherin deficient mice is secondary to defects related to cardiac/vascular function. In our search for the underlying cause for this observation, we identified a novel mechanism, which is distinct from the recently demonstrated role for endothelial cells in organogenesis of the pancreas, for how vascular function regulates dorsal pancreatic morphogenesis. Mechanistic data will be presented for how vascular function affects development of the dorsal pancreatic endoderm.

449. **Notch Signaling Inhibits Pancreatic Endocrine and Exocrine Development.** L. Charles Murtaugh, Ben Stanger, and Douglas Melton. Harvard University, MCB Department,

We are interested in the signaling pathways that govern pancreas development. Previous studies showed that mice lacking components of the Notch pathway exhibit accelerated differentiation of pancreatic endocrine cells, suggesting that Notch is necessary for self-renewal of endocrine progenitor cells. To further determine whether Notch activity is sufficient to prevent precursor differentiation, we used conditional transgenesis to express activated Notch1 (NotchIC) throughout the pancreatic endoderm. We targeted NotchIC to the ubiquitously-expressed *Rosa26* locus, with transcription blocked by a ϕ loxed ϕ upstream STOP sequence. When *Rosa26*^{NotchIC} mice are crossed with mice expressing Cre recombinase in the pancreas, their double-transgenic offspring will activate expression of NotchIC throughout the early pancreatic epithelium. Consistent with loss-of-function data, we find that Notch activation in the pancreas is sufficient to inhibit endocrine cell differentiation; there is a similar block to exocrine differentiation, a result not predicted by previous studies. This effect is dose-dependent, such that mice with two copies of the *Rosa26*^{NotchIC} allele have a more severe phenotype than those inheriting one copy. The affected pancreata are dominated by largely-unbranched cystic epithelium, which may represent cells trapped in an early progenitor fate. Preliminary results suggest that activation of *Rosa26*^{NotchIC} specifically in committed endocrine progenitors does not inhibit their differentiation, suggesting that the effects we observe reflect the action of Notch signaling on multipotent, early-stage progenitors, possibly stem cells.

450. **The Role of HNF6 in Pancreatic Islet Differentiation and Mature Islet Function.** Elizabeth Tweedie, Heidi Scrabble, and Maureen Gannon. Vanderbilt University and University of Virginia

HNF6 has a single CUT-like DNA binding domain and a divergent homeodomain. As a transcriptional regulator, HNF6 has been implicated as a transactivator of the proendocrine gene *ngn3*, a transcription factor common to all pancreatic endocrine cells. HNF6 is initially expressed throughout the pancreas during embryogenesis, but becomes downregulated specifically in murine islet endocrine cells at e18.5. HNF6^{-/-} mice show a severe reduction in *ngn3* and differentiated endocrine cells. Maintained expression of HNF6 in endocrine cells of transgenic past e18.5, when endocrine cells organize to form islets, results in abnormal islet architecture and diabetes. These transgenic mice, which also overexpress HNF6 through embryogenesis, develop larger islets than controls. Thus, HNF6 action appears to be differentially involved in multiple steps of islet endocrine cell differentiation and maturation. We will employ a reversible inducible repression system to manipulate HNF6 transgene expression at controlled timepoints during pancreatic endocrine development. We hypothesize that HNF6, through activation of *ngn3*, acts early in endocrine development to promote commitment of select cells of the prepancreatic endoderm to an endocrine cell fate. HNF6 will be transiently overexpressed in transgenic embryos, and the effect on endocrine cell formation will be analyzed. Immunohistochemistry will be used to examine the number of committed endocrine cells after HNF6 induction, as well as the ability of these cells to organize into islets. Physiological tests will be used to assess islet function.

451. **Transcriptional and Functional Analysis of Genes Required for Mouse Endocrine Islet Development.** Guqiang Gu*†

and Doug Melton†. *Department of Cell Developmental Biology, Developmental Biology Program, Vanderbilt Medical Center, Nashville, Tennessee 37232; and †Department of Molecular and Cell Biology, Harvard University, Cambridge, Massachusetts 02138.

The vertebrate pancreas contains two glands. Exocrine acini and pancreatic ducts form exocrine pancreas that produces enzymes for food digestion. Four endocrine cell types (B, A, D, and PP) comprise endocrine islets of Langerhans that produce hormones (insulin, glucagon etc.) to regulate carbohydrate metabolism. Failure of endocrine function results in diabetes mellitus, which inflicts over 16 million individuals worldwide. In order to define the regulatory genes and transcriptional networks that direct endocrine development, we separated islet progenitors from exocrine precursors in mouse embryos by FACS and examined the gene expression profiles in these cell pools respectively. These analyses have identified a set of known and novel genes that might define endocrine or exocrine cell fates. We have verified the expression of several candidate genes by RT-PCR and *in situ* hybridization. In addition, we performed functional analysis of one candidate transcription factor, Myelin Transcription factor 1 (Myt1). Myt1 is exclusively expressed in endocrine progenitors. When a dominant-negative form of Myt1 was introduced into endocrine progenitor cells, it inhibits generation of mature islet cells, in both chicken and mouse embryos. These results suggested that Myt1 function was necessary for endocrine islet development and our transcriptional analysis could provide a rich list of candidate genes that participate in endocrine islet development.

452. **Analysis of Murine Endoderm Fate from Early Somite Stages through Organogenesis.** Kimberly D. Tremblay and Kenneth S. Zaret. Cell and Developmental Biology Group, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111.

The goal of this study is to identify the progenitors of endoderm-derived organs in the early mouse embryo. The definitive endoderm, one of the three primary germ layers to arise during gastrulation, emerges as an epithelial sheet that lines the entire ventral surface of the pre-somitic mouse embryo. The cells of this epithelial sheet eventually become internalized and form the digestive tract and associated digestive organs, including liver and pancreas, through a series of inductive interactions with the underlying mesoderm. Although many studies have focused on the endoderm after the onset of organ-specific gene expression, very little is known about the endoderm prior to this stage of differentiation. The fate of the endoderm of 0-6 somite mouse embryos is studied here by DiI labeling small groups of definitive endoderm, culturing the embryos for 1-2 days and determining the location of the labeled cells in the gut-tube and associated organs of the resulting embryo. The generation of an early endoderm fate-map will elucidate the mechanisms leading to organogenesis.

453. **Functional Analysis of FGF10 Expressed in the Embryonic Chicken Stomach.** M. Shinand and S. Yasugi. Department of Biology Science, Tokyo Metropolitan University, Tokyo Japan.

Fibroblast growth factors (FGFs) play important roles in cell growth, movement, extension and differentiation in many developing organs. For example, FGF10 is involved in the formation of

several organs such as lung, spleen, limb and feather. Little is known, however, about the expression and function of FGFs in the digestive organ development. To reveal the functions of FGFs and the relations between FGFs and other genes involved in the cytodifferentiation and the morphogenesis of the digestive organs, we first examined the expression patterns of FGF4, 7, 8, 9 and 10, and FGFR1-4 by *in situ* hybridization in the embryonic chicken, esophagus, proventriculus (glandular stomach), gizzard (muscular stomach), small intestine, caecum and large intestine of days 4 to 12 of incubation. FGF10 was expressed in the mesenchyme of the proventriculus and gizzard, and FGF7 in the gizzard mesenchyme while expression of FGF4, 8 and 9 was not detected in the digestive tract. Expression of FGFRs was seen throughout digestive tract. We then analyzed the function of FGF10 in the proventriculus. The proventriculi of 6-day embryo were cultured in the presence of recombinant FGF10. FGF10 repressed the expression of Shh and cSP (markers of luminal epithelium) in a dose-dependent manner. With higher concentration of FGF10, ECPg (marker of gland epithelium)-expressing cells in the proventriculus decreased. This result suggests the possibility that FGF10 plays important roles in the morphogenesis of the embryonic chicken stomach.

454. **Gradient of JAK/STAT Signaling, Established by *drm/lin/bowl* Hierarchy, Is Required for Cell Rearrangement in the *Drosophila* Hindgut.** K. A. Johansen, D. D. Iwaki, R. B. Green, Xi, R. D. A. Harrison, and J. A. Lengyel. UCLA, University of Kentucky.

During embryogenesis, the *Drosophila* hindgut elongates by cell rearrangement, without increase in size by cell proliferation or molding by apoptosis. By analysis of both fixed and living material, we show that this cell rearrangement occurs in only a few hours by oriented cell protrusions that proceed in an anterior-to-posterior "wave". Characterization of mutants allows identification of genes that control this process. drumstick (*drm*) and bowl mutants have similar, short and wide hindguts, with defects in cell rearrangement. Related but distinct defects are seen in embryos mutant for the segmentation gene lines (*lin*). Epistasis analysis reveals that *drm* acts to relieve repression by *lin*; this relief-of-repression then allows bowl to promote spatially localized expression of genes controlling hindgut morphogenesis. A key target of the *drm/lin/bowl* regulatory hierarchy is the gene unpaired (*upd*) which encodes the *Drosophila* ligand for the JAK/STAT pathway. Several lines of evidence indicate that the *Upd* ligand and its target STAT are present in gradient in the elongating hindgut. These observations, taken together with the "wave" of rearrangement and the fact that uniform *Upd* is not sufficient for elongation, suggest that a gradient of JAK/STAT activation is required to orient the cell rearrangement driving elongation of the hindgut epithelium.

455. **Characterization of *gob-1*: a Gene Required for Intestine Development in the Nematode *Caenorhabditis elegans*.** Jay D. Kormish and James D. McGhee. Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada.

We wish to identify transcription factors and downstream effectors that direct the development and differentiation of the intestinal cells of *Caenorhabditis elegans*. We have developed a genetic screen in *C. elegans* to isolate zygotically expressed genes

that, when mutated, result in a gut obstructed "gob" defect. The Gob phenotype results from a malformation in the intestine that prevents the entry of bacteria into the gut lumen and is the same lethal phenotype caused by a null mutation in the intestine specific GATA factor *elt-2*. We have identified the lesion causing the Gob phenotype in one particular strain isolated in this screen. dsRNA-mediated interference (which causes a loss of function-like effect) against the gene H13N06.3 phenocopies the gut obstructed phenotype, thereby identifying the *gob-1* gene. RNAi affected animals exhibit varying degrees of lumen malformation, excess dark crystal-like structures in the intestinal cells and the presence of lumen twists. *gob-1* is predicted to encode a novel protein of possible ancient origin. cDNAs corresponding to two alternately spliced transcripts have been sequenced. Two fusion proteins and a small peptide have been generated for antibody generation. *gob-1* promoter driven reporters are being used to describe *gob-1* expression patterns. Progress in the characterization of *gob-1* function and expression will be described.

456. **Fgf-8 is a Key Molecule to Liver Development of *Xenopus laevis*.** Yong-Hwan Kim, So-Young Ko, Jang-Woo Lee, and Won-Sun Kim. Department of Life Science, Sogang University.

Previous studies in chick and mouse have shown that FGF-8 from cardiac mesoderm plays an important role in specifying the fate of the liver precursor cells from the endodermal cells during the liver development. In the present study, we have examined whether FGF-8 is also involved in the development of amphibian liver using *Xenopus* as a model system. Whole mount *in situ* hybridization analysis has revealed that Fgf-8 is expressed in the *Xenopus* heart region from stages 26/27. When the Fgf-8 expression was inhibited by retinoic acid treatment, the expression of FOR [farnesoid X related (FXR) like orphan receptor], a liver specific molecular marker, was also inhibited. Since Fgf-8 is known to be an important signaling molecule for hepatogenesis, it was hypothesized that the inhibitory effect of RA on hepatogenesis was due to the insufficient Fgf-8 signaling. To verify the hypothesis, a series of rescue experiments has been done by supplying FGF-8, endogenously or exogenously. The inhibitory effect of RA on the liver formation could be alleviated by the transplantation of tail bud that strongly expresses FGF-8, or the implantation of bead conditioned in the tail bud. In addition, implantation of an FGF-8 coated bead in the heart region could restore the FOR expression. These results show that Fgf-8 signaling is an important component in the early process of amphibian liver development.

457. **BMP4 is Essential for Normal Development of the Enteric Nervous System.** Allan M. Goldstein, Adele M. Doyle, Jerrell A. Williams, and Drucilla J. Roberts. Massachusetts General Hospital.

Abnormalities of the enteric nervous system (ENS) account for many congenital intestinal motility disorders of childhood. The most common of these, Hirschsprung's disease, results from the absence of ganglion cells in a variable length of distal bowel. Using retroviral-mediated misexpression of various genes during gut morphogenesis, we have produced an avian model of intestinal aganglionosis. Replication-competent RCAS viral vector containing the Bapx1 homeobox transcription factor was injected into the presumptive hindgut of avian embryos. Embryos were

allowed to develop and the hindgut subsequently harvested and processed for immunohistochemistry. Bapx1 overexpression resulted in aganglionosis of the distal gut, with giant ganglia and ectopic ganglia in the lamina propria proximally, a phenotype seen in intestinal neuronal dysplasia, a condition often associated with Hirschsprung's disease. Moreover, expression of BMP4 and of activated Smad1 were both decreased in these specimens, suggesting an essential role for BMP signaling in normal ENS development. This hypothesis was confirmed by targeted overexpression of the BMP inhibitor, noggin, which also resulted in the absence of normal enteric ganglion cells. This avian model of abnormal enteric neuronal development positions BMP4 as a critical component of ENS development and serves as an excellent model for further investigations into enteric neuronal development.

458. **Knockdown of Gene Function during Early Mouse Thymus Organogenesis Using Morpholino Antisense Oligonucleotides.** Julie Gordon, Nancy R. Manley, and C. Clare Blackburn. Institute for Stem Cell Research, University of Edinburgh, EH9 3JQ, United Kingdom; and Department of Genetics, University of Georgia, Athens, Georgia 30602.

Morpholino antisense oligonucleotides have become established as effective tools with which to investigate the physiological roles of specific gene products in a range of organisms. To date, however, their use in mid-gestation mouse embryogenesis has been limited to studies of the central nervous system. We have therefore investigated the possibility of applying the technique to pharyngeal development by targeting a specific population of cells within the relatively inaccessible embryonic pharyngeal endoderm. The thymus develops from the third pharyngeal pouches, outpocketings of the foregut endoderm that arise around embryonic day nine (E9.0) in the mouse. The subsequent development of all mature thymic epithelial cell types is dependent upon the transcription factor Foxn1, whose expression is initiated at E11.25. In the proof-of-concept experiments described here, Foxn1-specific morpholinos were delivered to cells of the third pharyngeal pouch at E10.5 by electroporation. Embryos were then allowed to develop to E11.5 in whole embryo culture, after which time thymus development and Foxn1 expression were assessed. These data demonstrate that delivery of Foxn1, but not control nonsense morpholinos, to the third pharyngeal pouch results in the inhibition of Foxn1 gene function in the prospective thymic epithelium. This technique thus provides a rapid and efficient means of assaying gene function during thymus organogenesis, and in principle could be applied to any tissue in the mid-gestation mouse embryo.

459. **Rectal Formation in *C. elegans* Embryos Requires a Lag (Notchlike) Pathway Signal to Initiate *Pal-1* (Caudal/Cdx) Expression in Two AB Cells.** Lois Edgar and Bill Wood. MCD Biology, University of Colorado, Boulder Colorado 80309.

Laser ablation of a single cell late in gastrulation can abolish rectal formation in *C. elegans*. This cell is one of two ABpl lineage cells expressing *pal-1*, the Caudal/cdx homolog, at that stage (Edgar et al, Dev Biol 229: 71, 2001). Rescue of a *pal-1* null mutation (a deformed Nob phenotype) with a short (1 kb 5') genomic plasmid produces normally shaped larvae without a rectum, resembling mutants with defects in the Lag (*lin-12-glp*-

1Notchlike) signaling pathway (Lambie and Kimble, Dev 112: 231,1991). In *lag-1* and *lag-2* mutants, a *pal-1::GFP* reporter (7 kb 5' sequence) is not expressed in the AB lineage, though normally in other lineages, suggesting that *pal-1* expression in this specific lineage requires a Lag signal. We identified 6 putative Lag-1 (the final transcription factor in the Lag pathway) binding sites upstream of *pal-1*, and 4 intragenic sites, including both the *C. elegans* (Christensen et al, Dev 122:1379,1995) and *Drosophila* homolog SuH (Bailey and Posakony, Genes and Dev 9:2609,1995) published consensus sequences. Deletions of the internal or most distal 5' (-5.5 kb) binding sites did not affect reporter expression in the AB lineage. However, site-specific mutagenesis of the other 5' binding sites, singly or in combination, weakened or completely eliminated AB reporter expression. Mutated reporters are also ectopically expressed in 4 embryonic head cells. We are currently identifying these cells, and addressing the questions of which of the possible ligands (Lag-2 or Apx-1) and receptors (Lin-12 and/or Glp-1) are the operative signaling molecules, and which cells are signaling.

460. **What Makes a Salivary Gland a Salivary Gland?: the Transcriptional Control of Secretory Pathway Genes in the Primary Secretory Organ of *Drosophila*.** Elliott W. Abrams and Deborah J. Andrew. Department of Cell Biology, The Johns Hopkins University School of Medicine, Baltimore, Maryland.

Our lab studies the development and function of the *Drosophila* salivary gland. Through the aid of the Berkeley *Drosophila* Genome Project (BDGP) expression study, we identified a number of secretory pathway component encoding genes (SPCGs) that are expressed to high levels in the salivary gland during embryonic development. These genes have strong homologies to their mammalian and yeast counterparts and include homologues to signal recognition particle (SRP) receptor subunits, signal peptidases, KDEL receptor and various proteins involved in vesicle transport. Here we show that the expression of these genes is either turned on or greatly enhanced by the transcription factor(s) Fork head (Fkh) and/or dCreb-A. In addition, we demonstrated that one of these SPCGs (*dmErd2*) functions in the *Drosophila* salivary gland to retain resident proteins in the endoplasmic reticulum. Hence, it appears that the function of at least one of these SPCGs is consistent with that of a *bona fide* component of the secretory pathway. This study implicates *fkh* and *dCREB-A* as having a major role in making the salivary gland an organ with a specialized and high level secretory capacity.

461. **Analysis of the Role of the GDNF/GFR Alpha-1 Signal Transduction Pathway in the Development of the Zebrafish Kidney.** Marcia L. O'Connell, Benjamin Levy, and Julie Drawbridge. The College of New Jersey, Rider University.

Investigations into the formation of the kidney in amphibians and mammals have indicated a role for the GDNF (glial cell line-derived neurotrophic factor) GFR alpha-1 (GDNF family receptor alpha-1) signaling pathway. Specifically, it has been demonstrated in mice that in the absence of either GDNF, GFR alpha-1, or Ret-the signaling component of this ligand/co-receptor pair-the metanephros fails to form. In addition, evidence from axolotl indicates that the migration of the pronephric duct during embryogenesis requires signaling by GDNF. In zebrafish, all three components of the signaling pathway are expressed in

the forming pronephros. Taken together these data indicate that this pathway is involved in kidney formation in both amniotes and anamniotes. While the evidence in axolotl indicates that the GDNF pathway mediates cell migration, data from studies on the role of GDNF in developing neurons indicates that it is also a survival factor. In zebrafish, lineage labeling studies suggest that the pronephric duct forms primarily through delamination, as opposed to migration. Therefore, it is possible that the GDNF pathway in zebrafish is mediating survival, or plays an as yet unidentified new role in pronephros formation. In order to investigate the role of the GDNF pathway in zebrafish pronephric duct formation, we have performed studies in which the pathway is inactivated during development by removing GFR alpha-1 and other GPI-linked proteins from zebrafish embryos through micro-injection of PI-PLC (phosphatidylinositol-specific phospholipase C) before involution is complete. Subsequent to inactivation of the pathway we have then investigated both the expression of Ret by pronephric duct cells, and the appearance of apoptotic cells by TUNEL assay.

462. **The Novel ras/MAPK Antagonist, Sprouty1, Is Essential for Normal Renal Development.** M. Albert Basson, S. Akbulut, J. Johnson, R. Simon, T. J. Carroll, F. Costantini, T. Lufkin, A. P. McMahon, P. D. Wilson, and Jonathan D. Licht. Department of Medicine and Brookdale Center for Cell and Developmental Biology, Mount Sinai School of Medicine, New York; Department of Molecular and Cellular Biology, Harvard University, Boston; and Department of Genetics and Development, Columbia University, New York.

The sprouty gene was initially identified as a novel FGF antagonist in *Drosophila* that regulates branching morphogenesis of the tracheal tubes. Four mammalian homologues have been cloned and Spry 1, 2 and 4 shown to be expressed in the embryo at sites of FGF activity. Sprouty proteins are localized intracellularly and antagonizes receptor tyrosine kinase signalling downstream of several growth factors. In order to investigate the role of the first mammalian homologue of this family, Sprouty1, during embryogenesis, this gene was inactivated by gene targeting in the mouse. Spry1 knockout mice exhibit severe defects in kidney and genito-urinary tract development, resembling the human condition CAKUT (congenital abnormalities of the kidney and urinary tract). These include duplex kidneys, multiple ureters, hydronephrosis and cystic dysplasia of the kidney. Immunohistochemical analyses indicated Erk hyperactivation, hyperplasia, misexpression of fetal genes and apical mislocalisation of transporters in the cystic epithelium, identical to those found in human cystic kidney diseases such as polycystic kidney disease. The characterisation of these phenotypes and their embryonic origin will be discussed. We conclude that Spry1 is essential for normal renal organogenesis.

463. **Molecular Characterization of Early Mammalian Kidney Development.** G. W. Yip* and A. P. McMahon*. *Harvard University; and National University of Singapore.

Development of the mammalian metanephric kidney involves reciprocal induction between the ureteric bud and the metanephric blastema. Ingrowth of the ureteric bud into the blastema leads to condensation of the loose mesenchymal tissues. Epithelial conversion of the mesenchyme produces renal vesicles, which develop into mature nephrons via intermediary stages of comma-

and S-shaped bodies. To screen for genes that are expressed during early kidney development, renal vesicles and S-shaped bodies were isolated from E14.5 SW embryos by microdissection based on morphological criteria. Antibody and lectin staining together with RT-PCR were used for identity verification and for exclusion of contamination by other tissues. RNA isolated from the collected structures was put through two rounds of T7-driven amplification to obtain the microgram quantities of cRNA needed for hybridisation to Affymetrix microarrays. Data analysis reveals 2316 present calls in renal vesicles and 3339 present calls in S-shaped bodies. There are 714 genes that are up- and 832 genes that are down-regulated by at least two-fold in S-shaped bodies relative to renal vesicles. These include transcription and signaling factors, as well as molecules involved in cell cycle, apoptosis and cellular adhesion. Detection of genes previously shown to be expressed in these early structures, such as *Wt1*, *Wnt4* and *Pax8*, validates the data from the screen. Work is currently underway to verify the microarray results using RT-PCR and *in situ* hybridization. Once it is completed, the data will form a useful resource and springboard for future studies on kidney development.

464. **Wnt11 and c-Ret/GDNF Pathways Cooperate in Regulating Ureteric Branching during Metanephric Kidney Development.** Arindam Majumdar,* Seppo Vainio,† Andreas Kispert,** Jill McMahon,* and Andrew P. McMahon*. *Harvard University; †University of Oulu; and **Medizinische Hochschule Hannover.

Reciprocal cell-cell interactions between the ureteric epithelium and the metanephric mesenchyme are needed to drive growth and differentiation of the embryonic kidney to completion. Branching morphogenesis of the Wolffian duct derived ureteric bud is integral in the generation of ureteric tips and the elaboration of the collecting duct system. Wnt-11, a member of the Wnt superfamily of secreted glycoproteins, which have important regulatory functions during vertebrate embryonic development, is specifically expressed in the tips of the branching ureteric epithelium. In this work, we explore the role of Wnt11 in ureteric branching and use a targeted mutation of the Wnt11 locus as an entrance point into investigating the genetic control of collecting duct morphogenesis. Mutation of the Wnt11 gene results in ureteric branching morphogenesis defects and consequent kidney hypoplasia in newborn mice. Wnt11 functions, in part, by maintaining normal expression levels of the gene encoding Glial Cell Derived Neurotrophic Factor (GDNF). GDNF encodes a mesenchymally produced ligand for the c-Ret tyrosine kinase receptor critical for normal ureteric branching. Conversely, Wnt-11 expression is reduced in the absence of c-Ret/GDNF signaling. Consistent with the idea that reciprocal interaction between Wnt11 and c-Ret/GDNF regulates the branching process, Wnt11 and c-Ret mutations synergistically interact in ureteric branching morphogenesis. Based on these observations, we conclude that Wnt11 and c-Ret/GDNF cooperate in a positive autoregulatory feedback loop to coordinate ureteric branching by maintaining an appropriate balance of Wnt11 expressing ureteric epithelium and GDNF expressing mesenchyme to ensure continued metanephric development.

465. **Transcriptional Profiling of Tubulogenesis Using Wnt4 Mutant Mice.** M. Todd Valerius and Andrew P. McMahon. Harvard University.

The mature nephron forms from a simple epithelial vesicle into an elaborate structure with distinct regions of specialized physiological function. The molecular components driving this process of tubulogenesis are not well understood. To identify genes that may play a role we have conducted a transcriptional profiling screen using Wnt4 mutant kidneys. In Wnt4 $-/-$ homozygous mice condensates and pretubular aggregates are induced, however, epithelial renal vesicles fail to form and subsequent tubulogenesis is blocked. A transcriptional profile comparison between wildtype and Wnt4 $-/-$ mutant kidneys at E14.5 was performed using Affymetrix oligonucleotide microarrays. Over 200 genes with expression levels > 1.8 fold higher in wildtype versus mutant kidneys were identified. These included expected differences confirming the validity of the screen. These results were explored by whole mount *in situ* hybridization of E15.5 urogenital systems and section *in situ* hybridization on E15.5 and P1 kidneys. Two main classes of expression patterns were found: 1) renal vesicle derivatives \rightarrow structures absent in the mutant, 2) mesenchymal cell expression \rightarrow cells around the duct tips that wholly or partially overlap the Wnt4 expression domain. These may represent genes under more direct regulation of Wnt4 signaling. A small number of genes in areas not overlapping the Wnt4 expression domain were also identified (ductal and stromal regions). Several genes identified have additional, restricted domains of expression elsewhere in the urogenital system. Current efforts are to examine the expression of the mesenchymal class of genes in Wnt4 mutant kidneys. These included regulators of the Wnt signaling pathway as well as several ESTs.

466. **Fgf8 Plays a Fundamental Role in Kidney Development.** A. O. Perantoni,† O. Timofeeva,† C. Richman,* S. Pajni-Underwood,* and M. Lewandoski*. *CDBL, NCI-Frederick, NIH; and †LCC, NCI-Frederick, NIH.

An essential aspect of vertebrate metanephric development is a reciprocal inductive interaction between two mesodermal lineages: the nephric duct/ureteric bud (UB), which gives rise to the branched collecting duct network, and the metanephric mesenchyme (MM), which converts to the epithelia of the nephron. To investigate the role of *Fgf8*, which is expressed in the MM at mesenchymal-to-epithelial transition, we inactivated a conditional *Fgf8* allele in all mesodermal lineages using a Brachyury-Cre mouse line. Such mice manifest a severe renal hypoplasia and die at birth but otherwise appear normal. At E14.5, mutant metanephroi are significantly reduced in size and extent of branching, and the MM at branch termini does not form tubules and undergoes aberrant cell death. By E18.5, the UB completely loses its characteristic branching morphology, and the MM in the nephrogenic zone is deficient and devoid of tubules. At the molecular level, mesenchymal condensation occurs in E14.5 metanephroi as evidenced by appropriate marker expression (*Pax2*, *N-myc*, *Wt-1*) but *Lim1*, which is essential for nephrogenesis, is absent. At E16.5 there is no detection of the MM-specific expression of (*N-myc*, *pax-2*, *wnt-4*, *sim-1*, and *sfrp2*) nor the UB-specific expression of *Hnf-1 β* , *C-ret* and *Gata3*. In vitro, FGF8 cannot induce MM as can other FGFs, but mutant MM will form tubules in response to an exogenous inducer (embryonic spinal cord). Our data suggest a model in which MM-specific *Fgf8* expression is required in a crine fashion to sustain branching morphogenesis of and inductive signaling by the UB for nephronic differentiation.

467. **Fgf8 is Required for Nephrogenesis in the Developing Mouse Kidney.** Roger M. Ilagan and Erik N. Meyers. Duke University Medical Center.

Nephrogenesis is the process in which metanephric mesenchyme aggregates and organizes into nephrons, the filtering unit of the kidney. This process is dependent on several molecular signals that originate from the metanephric mesenchyme itself, as well as the expanding collecting duct system. During mouse kidney development, Fibroblast Growth Factor 8 (*Fgf8*) is transiently expressed in comma- and s-shaped bodies. *Fgf8* expression ceases before the nephron reaches maturity. The spatial and temporal expression pattern suggests that *Fgf8* may play a role in nephron maturation. Utilizing a Cre-loxP genetic strategy, we have eliminated *Fgf8* expression from the metanephric mesenchyme prior to the initiation of nephrogenesis. Embryonic kidneys that lack FGF8 are notably reduced in size by E14.5. We show that cells in the cortical-most region undergo excess apoptosis, which accounts, at least in part, for this size reduction. Nephron development proceeds to the s-shaped body stage, but fails to form mature nephron structures, such as the glomerulus, Bowman's capsule, and convoluted tubules in these mutants. We propose that *Fgf8* signaling is essential for nephron maturation, and may function either 1) by inducing specific epithelial cell fates in the comma- and s-shaped bodies, or 2) by acting as a necessary survival factor in the developing nephron.

468. Abstract #468 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

469. **A Novel Pathway Controlling Epithelial Tube Size Revealed by an Insertional Mutagenesis Screen for Cystic Kidney Mutants in Zebrafish.** Zhaoxia Sun,* Adam Amsterdam,* Gregory Pazour,† and Nancy Hopkins*. *MIT; and †University of Massachusetts Medical School.

A large fraction of genes have no known biological or even biochemical functions. Meanwhile, the mechanisms of many biological processes are poorly understood. One such process is the size control of epithelial tubes. Defects in control of tube size can lead to human diseases, including polycystic kidney diseases (PKDs). Autosomal dominant PKDs are among the most frequent human monogenetic disorders. Cysts are thought to result from over-proliferation and under-differentiation of epithelial cells. We have recently completed a large-scale insertional mutagenesis screen in zebrafish. This screen reached about 25% saturation and provides an invaluable resource to evaluate gene functions in the context of biological functions on a genome-wide scale. From this screen, we isolated 11 different genes that can cause cystic kidney when mutated. *Pkd2*, a gene responsible for a subgroup of ADPKD, and *vHnf1*, which is linked to glomerular cystic kidney disease, are among those identified, suggesting that genes identified in this screen are directly relevant to human PKD. The striking phenotypic similarity of the mutants suggests that the responsible genes function in the same pathway. Intriguingly, 7 of 11 identified genes are novel. Further analysis revealed that three of them encode proteins required for flagella formation in *Chlamydomonas*. Our results suggest a novel pathway in which channels on primary cilia of epithelial cells sense tube size, signal to transcriptional machinery, which eventually regulates differentiation and proliferation of epithelial cells.

470. **Conditional Inactivation of the Mouse *Lim1* Gene in the Nephric Duct.** Andrew Wilber, Sarah Wanner, and William Shawlot. University of Minnesota.

Abnormalities of the urogenital system are among the most common birth defects in humans. *Lim1* is a homeobox gene expressed in the nephric duct, ureteric bud, metanephric tubules and Müllerian duct during mouse embryogenesis. Although most *Lim1*-deficient embryos die at embryonic day (E) 10 of development due to defects in gastrulation, a few *Lim1* mutant mice have been delivered stillborn. These mice lacked kidneys and reproductive tract structures. To begin to understand the role of *Lim1* in urogenital development, we generated mice that lack *Lim1* function in the nephric duct. We first generated *Pax2-cre* transgenic mice that express Cre recombinase in the nephric duct and its derivatives. *Pax2-cre* transgenic carrying the *Lim1^{lacZ}* null allele were then crossed with mice carrying a "floxed" *Lim1* allele. At E14.5, mice with both *Lim1* mutant alleles had hypoplastic kidneys and altered mesonephros development. The nephric duct was absent or abnormally formed. Examination of earlier stage *Lim1* mutant embryos indicated that the caudal mesonephric tubules did not form and formation of the ureteric bud was delayed. In addition, the induction/development of the Müllerian duct, which forms adjacent to the nephric duct, was impaired. In postnatal mice, hydronephrosis and mega-ureter were observed and nephric (Wolffian) and Müllerian duct-derived reproductive tract structures were absent. These findings suggest that *Lim1* is critical for proper development and maintenance of the nephric duct and its derivatives. These mice provide a unique genetic resource for understanding the molecular and cellular events that control ureteric bud formation and Müllerian duct induction in mammals.

471. **Sexual Development of the *Caenorhabditis elegans* Gonad.** Kara Thoenke, Weiru Chang, Julie Illi, and David Zarkower. Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, Minnesota 55455.

The *C. elegans* gonad develops from a sexually ambiguous four-cell primordium that undergoes very different cell lineages, migrations, and cellular differentiation in the two sexes. These differences in development result in a symmetrical two-armed gonad in hermaphrodites, and an asymmetrical single-armed gonad in males. To better understand how such highly dimorphic organs develop, we performed an F2 screen for mutants with defective male gonads, aided by a GFP reporter expressed in the male vas deferens and seminal vesicle. From about 3,300 mutagenized haploid genomes, we have isolated 30 independent mutations. Mutants fall into three classes: i) feminized male gonads; ii) severely malformed male gonads; iii) male gonads with late differentiation defects. We are focusing on genes in the first two classes. *fkh-6*, a forkhead transcription factor, falls into the first class. In *fkh-6* mutant males early gonadogenesis resembles that of hermaphrodites, and the adult gonad is both disorganized and extensively feminized. We identified several genes in the second class, and describe two of them here. In each of these mutants, the adult gonad is severely disorganized, yet still expresses markers of terminal differentiation, including *lag-2::gfp*, a marker of the male leader cell. This preliminary characterization suggests at least partially correct cell fate specification despite

the lack of an organized structure. We will report our progress in characterizing and cloning these mutants.

472. **Germline-Soma Interactions in the Embryonic Gonad of *Drosophila melanogaster*.** Allison B. Jenkins and Mark Van Doren. Johns Hopkins University.

Throughout gonad development and gametogenesis, germ cells require intimate contact with specialized somatic cells in the gonad for their proper development into oocytes or spermatazoa. Using *Drosophila melanogaster* as a model system, we have analyzed the establishment of germ cell-soma interaction during embryonic gonad formation. We have found that during the process of gonad coalescence, the germ cells and mesodermally-derived somatic gonadal precursors (SGPs) interact intimately as they compact into a rounded organ, with cellular extensions of the SGPs individually ensheathing each germ cell in the gonad. The cell-cell adhesion molecule DE-cadherin is required for both gonad compaction and germ cell ensheathment, and is upregulated in the SGPs during gonad formation. This data suggests a model where differential cell adhesion contributes to cell sorting and the establishment of proper gonadal architecture. Our current work is focused on determining which factors provide the specificity necessary to establish interactions between germ cells and SGPs. We are undertaking a genetic screen to identify genes required for this heterotypic interaction, as well as any additional molecules that may be acting in other aspects of gonad coalescence. We will present the initial results of our screen, as well as our continuing analysis of the role of differential cell adhesion in gonad morphogenesis.

473. **Specification of the Indifferent Gonad in the Avian Embryo.** Jamil B. Scott and Thomas M. Schultheiss. Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts.

The indifferent gonad is a unique organ in that it has the potential to give rise to either an ovary or a testis, while in other organ systems, one specific tissue primordium gives rise to only one specific terminally differentiated organ. Much effort has gone towards understanding later time points in gonad development, such as sex determination and sexual differentiation, whereas relatively little attention has been given to understanding the events that cause the initial specification of the indifferent gonad. The goal of this research is to further clarify this issue by considering the molecular signals and tissue interactions that are required for indifferent gonad specification. We have examined the expression patterns of several genes known to play a role in gonad development such as LH-2B (Lhx9), DMRT-1, AMH, and Sox-9; as well as other genes expressed in the intermediate mesoderm such as WT-1, Osr-1, and Lim-1. Using whole mount *in situ* hybridization, we found that the earliest gonad specific marker, LH-2B (a LIM homeobox gene) is expressed at HH stage 22; around the same time that a morphological change can be seen in the urogenital ridge indicating that the indifferent gonad is beginning to form. We are assessing the requirement of the Wolffian duct and mesonephros for gonad specification, using classical embryological musurgery techniques. We have blocked the posterior migration of duct tissue and subsequent development of the mesonephros in stage 10 chicken embryos with the placement of an aluminum barrier posterior to the tenth somite,

and examined the effect on the expression of gonad markers, such as LH-2B.

474. **Molecular Basis of Sex Reversal in Mice Lacking Insulin Receptor Family Members.** Sunita Verma-Kurvari,* Serge Nef,* Argiris Efstratiadis,† Domenico Accili,** and Luis Parada*. *Center for Developmental Biology, University of Texas Southwestern Medical Center, Dallas, Texas; and †Department of Genetics and Development and **Naomi Berrie Diabetes Center, College of Physicians and Surgeons of Columbia University, New York, New York.

In the mammalian male gonad, testis determination is initiated by transient Sry expression in the genital ridge resulting in cell proliferation, male specific vascularization, and Sertoli cell differentiation. We have recently demonstrated that mice lacking three insulin receptor family members, show complete male to female sex reversal by E17.5. These receptors include the insulin receptor, insulin-like growth factor 1 receptor, and the insulin receptor-related receptor. We are now analyzing whether testis determination is initiated in the triple mutant male gonad, and if so, when male differentiation is derailed, at both histological and molecular levels. Our preliminary results show that the male differentiation pathway is initiated in the triple mutant gonads, however, complete male to female sex reversal is observed by E14.5. We hypothesize that changes in cell death and cell proliferation in the mutant male gonads contribute to sex reversal and we are now investigating this possibility in more detail.

475. Abstract #475 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.
476. **Nuclear Localization of FGFR2 Acts Downstream of Sry and Is Critical for SOX9-Induced Sertoli Cell Differentiation.** Blanche Capel, Yoo-Nha Kim, and Jennifer Schmah. Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710.

Sex determination provides a valuable system for studying how cell fate decisions are integrated with processes that govern growth in the embryo. The initiation of testis development in mammals depends on the presence of the Y chromosome-linked gene, Sry, and the nuclear localization of its immediate downstream gene, SOX9. We have shown that one of the earliest effects of Sry is the induction of cell proliferation in the XY gonad. DiI lineage tracing experiments have shown that this proliferating population gives rise to Sertoli cells, the cells that express Sry and orchestrate testis morphogenesis. If proliferation is inhibited at early stages, Sertoli cells and testis cord formation are also inhibited. In XY gonads from Fgf9^{-/-} mice on a C57BL/6 genetic background, proliferation is dramatically reduced resulting in a decrease in the number of Sertoli progenitor cells. In wild-type gonads, FGFR2 shows a sexually dimorphic pattern in which it is localized to the cell membrane in proliferating cells, and to the nucleus in Sertoli progenitors along with SOX9. Interestingly, in Fgf9 mutants, neither FGFR2 nor SOX9 is localized to the nucleus. We show that nuclear localization of the intact FGFR2 is dependent not only on Fgf9, but also on some element of the male pathway downstream of Sry. These results show that FGFR2 plays a critical role between SRY and SOX9 in the initial specification of the Sertoli cell and male sex determination. FGF9

is a good candidate for a growth factor that plays a role both in proliferation of Sertoli cell progenitors, and their fate commitment to the Sertoli pathway.

- 477. Local and Global Signaling in Development of the External Genitalia.** Claire Perriton,* Anita Petiot,† Clive Dickson,† and Martin J. Cohn***. *School of AMS, University of Reading, United Kingdom; †Cancer Research UK, Lincoln's Inn Field, London WC2A 3PX; and ***Department of Zoology, University of Florida, Gainesville, Florida 32611.

Morphogenesis of the urogenital system involves the integration of localized developmental signals with systemic, sex-specific endocrine signals such as androgens and estrogens. Congenital malformations of the external genitalia are common, with hypospadias, a failure of ventral urethral closure, affecting 1 in 125 live male births. The molecular mechanisms that regulate normal development of the external genitalia are not well understood. Recent work on mouse genital development suggests the involvement of several pathways that have been well characterized in other organ systems. We identified the urethral plate epithelium as a polarizing region in the genital tubercle and, together with Yamada and colleagues, found that Shh from the urethral plate epithelium is required for outgrowth and patterning of the mouse genital tubercle. Fgfs may play a role in outgrowth of the genital tubercle in a manner analogous to Fgf function in the apical ectodermal ridge (AER) of the limb bud. We now show that Fgf signaling is required for urethral tube closure. Loss of Fgf receptor function causes hypospadias and ventral genital aplasia. Genitalia of the mutants resemble those of androgen-compromised mice. We therefore went on to investigate the relationship between Fgf and androgen signaling in mouse external genital development. Our results highlight a novel mechanism by which localized growth factor signaling and global endocrine signaling are integrated during morphogenesis of the mammalian genitalia.

- 478. Outgrowth and Apoptosis for the External Genitalia Formation by Concerted Functions of Bmp Signaling.** K. Suzuki, D. Bachiller, Y. P. Chen, M. Kamikawa, H. Ogi, R. Haraguchi, Y. Ogino, Y. Mishina, K. Ahn, E. B. Crenshaw III, and G. Yamada. Center for Animal Resources and Development (CARD), Kumamoto University, Honjo 2-2-1, Kumamoto 860-0811, Japan.

Bone morphogenetic protein genes (Bmps) and their antagonists were spatio-temporally expressed during GT(genital tubercle) development. Exogenously applied BMP increased apoptosis of GT and inhibited its outgrowth. Such effects were restored by an application of secreted BMP antagonist, NOGGIN. It has been shown that the Distal Urethral Epithelium (DUE), distal epithelia marked by the Fgf8 expression, may control the initial GT outgrowth. Exogenously applied BMP4 down-regulated the expression of Fgf8 and Wnt5a, concomitant with decreased cell proliferation of the GT mesenchyme. To further address the function of Bmp signaling during external genital morphogenesis, Bone morphogenetic protein receptor type IA (BmprIA) conditional mutant mice were analyzed, which displayed hyperplasia of the external genitalia with decreased apoptosis. Consistent with the above findings for Bmps and BmprIA, Noggin mutant mice exhibited GT hypoplasia with decreased Fgf8 expression. These results may suggest that some of the BMPs could nega-

tively affect proximodistally-oriented outgrowth of GT with regulatory functions on apoptosis. The DUE region can be marked only until 14.0 dpc in mouse development while GT outgrowth continued thereafter. Study of gene expression and histogenesis revealed that the DUE is located adjacent to the outer-most epithelial layer aligned with the GT surface ectoderm.

- 479. The Role of Edar Signaling in Ectodermal Organogenesis.** M. L. Mikkola, T. Mustonen, J. Pispä, M. Ilmonen, J. Laurikkala, M. Pummila, A. Kangas, R. Jaatinen, and I. Thesleff. Developmental Biology Program, Institute of Biotechnology, University of Helsinki, Finland.

Organs developing as ectodermal appendages share similar molecular mechanisms and early morphogenesis. Edar and its ligand ectodysplasin-A1 (Eda-A1) are recently identified members of the tumor necrosis factor receptor (TNFR) and TNF superfamily, respectively. Signaling by Edar activates the NF- κ B pathway through the specific adapter protein Edaradd and is required for normal development of various ectodermal organs such as teeth, hairs, and sweat glands. Mutations in Edar or other molecules of the same signaling pathway cause ectodermal dysplasias characterized by the absence or abnormal shape of the affected organs. During organogenesis, expression of Edar becomes patterned to ectodermal placodes which are epithelial thickenings marking the initiation of all organs developing as ectodermal appendages whereas expression of Eda is confined to the flanking epithelium. In order to further elucidate the function of Edar, we have generated transgenic mice mis- and overexpressing Edar or Eda-A1 throughout the ectoderm using the keratin 14 promoter. Overexpression of Eda-A1 resulted in supernumerary organs such as teeth and mammary glands. Also, new hair placodes developed close to the previously existing ones, and the placodes were abnormally large and occasionally fused with each other. Similar effects could be obtained with recombinant Eda-A1 protein in tissue culture. On the contrary, misexpression of Edar resulted in inhibition of primary hair follicle formation suggesting that the patterning of Edar into developing placodes is essential for the epithelial cells to adopt follicular fate. Further studies are under way to reveal the cellular mechanisms regulated by Edar during placode formation. Together, our results suggest that Eda-Edar signaling has a central function in formation of ectodermal placodes.

- 480. BMPRI-IA Signaling is Required for Differentiation and Growth of Hair during Postnatal Hair Follicle Morphogenesis.** Kin-Ming Kwan,* Allen G. Li,† Yuji Mishina,‡ Xiao-Jing Wang,†** Dennis R. Roop,†** Wolfgang Wurst,§ and Richard R. Behringer*. *Department of Molecular Genetics, UT M.D. Anderson Cancer Center; †Department of Dermatology and **Department Molecular and Cellular Biology, Baylor College of Medicine; ‡Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences; §Department of Molecular Neurogenetics, Max-Planck-Institute of Psychiatry.

The molecular mechanisms regulating hair follicle morphogenesis are not fully understood. De-regulation of signaling pathways controlling hair morphogenesis and cycle lead to alopecia and neoplasias in the skin. Elucidating the signaling pathways controlling hair follicle morphogenesis is therefore important to our understanding of the pathogenic states in the skin. BMPs

(BMP2 and BMP4) and their receptor, type I BMP receptor BMPR-IA, are expressed in the postnatal hair follicle, suggesting that BMP signaling may be involved in postnatal hair morphogenesis. However, mice lacking BMP2, BMP4 and BMPR-IA die during early embryonic development, thus restricting their usage in studying the roles of BMP signaling in hair morphogenesis. We used the Cre/loxP system to induce tissue-specific null mutation of the *Bmpr1a* gene in the ventral limb ectodermal-derived tissue by using a mouse line expressing engrailed 1 (*En1*)-Cre. Mice harboring the tissue-specific inactivation of *Bmpr1a* show lacking of hair outgrowth from the skin of ventral limb region. Histological and immunohistochemical analyses of affected postnatal skin revealed defects in hair follicle morphology and hair shaft formation. Our results demonstrate that BMPR-IA signaling is required for the differentiation and growth of hair during postnatal hair morphogenesis.

481. Abstract #481 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

482. **Loss of Periderm: Tightness is Guaranteed.** M. Saathoff, T. Quast, G. Kirfel, and V. Herzog. Institute for Cell Biology and Bonner Forum Biomedizin, University of Bonn, D-53121 Bonn, Germany.

The periderm is an epithelial monolayer which covers the developing epidermis and forms the embryonic barrier during chicken embryogenesis until its detachment during the time of hatching. We recently observed that simultaneous cell death of all peridermal cells was followed by the detachment of the entire periderm. In order to see whether this process coincides with a transient loss of this barrier function we studied epidermal tightness during chicken embryogenesis. Immunoblot analyses suggested that tight junction (TJ) components such as occludin and claudin-1 were restricted to the periderm until its detachment but appeared to be absent from the epidermis. Electron microscopy and immunocytochemistry revealed that a thin stratum corneum had formed and that occludin and claudin-1 became detectable in the subperidermal cell layers shortly before detachment of the periderm. To determine whether the embryonic stratum corneum and the expression of TJ proteins at this stage were of functional significance we tested the tightness of the epidermis using HRP. Before day 16 the subcutaneously injected tracer diffused through the epidermis up to the TJs of the periderm. Later, beginning with day 16, HRP was retained by the newly formed TJs within the subperidermal cell layers. We conclude that the periderm is the main embryonic barrier but that the embryonic stratum corneum and TJ proteins in the subperidermal layers provide an additional barrier and ensure tightness during loss of the periderm. (Supported by DFG, Forschergruppe "Keratinocytes" FOR 367/1 and by Fonds der Chem. Industrie)

483. **Separate Nutrient-Sensitive and Nutrient-Resistant Physiology Revealed by Studies on Growth of the Zebrafish Caudal Fin.** M. I. Goldsmith, M. K. Iovine, and S. L. Johnson. Washington University and Lehigh University.

Understanding the mechanisms regulating growth remains a fundamental challenge facing biologists. We previously established that zebrafish caudal fins grow via successive cycles of

saltation (growth) and stasis (rest). While we have identified markers (genes) that delimit the segment addition phase of a fin ray growth cycle, signals that regulate initiation of the growth cycle remain poorly characterized. We are interested in how animals survey their nutritional status and integrate this information into a hierarchy of growth control signals. If deprived of food, ectothermic vertebrates stop growing, yet they remain healthy for extended periods of time. We show here that the mature, wild type (wt) caudal fin exits the fin ray growth cycle 5-7 days following nutritional deprivation. Interestingly, this rapid response to nutritional status is not uniform to all examples of caudal fin growth: (1) As the juvenile (wt) caudal fin transitions to a mature bi-lobed shape, it is resistant to the withdrawal of nutritional support; (2) The fin overgrowth mutant *rapunzel* (*rap*), but not long fin (*lof*), has a delayed response to nutritional deprivation; (3) Despite nutritional deprivation, regenerative growth precedes unabated following amputation of the wt caudal fin. Together, these results suggest that two separate physiologies exist vis-à-vis nutritional status and growth. A rapid cessation of growth follows nutritional deprivation in *lof* and in the mature wt caudal fin. In contrast, a delayed cessation of growth follows nutritional deprivation in *rap*, in juvenile wt caudal fins and in regenerating caudal fins.

484. **Ocelot Mutation Lengthens Rest Phase During Ontogenetic Fin Growth.** M. Kathryn Iovine and Caleb R. Scott. Lehigh University, Bethlehem, PA 18015.

Fin growth is achieved by the addition of bony segments to the distal ends of fins. Our model for fin growth is that segments are built during a discrete growth phase. Young, rapidly growing fins experience frequent growth phases flanked by short rest phases. Mature, slowly growing fins experience less frequent growth phases and long rest phases. Thus, the rate of segment addition is controlled by the duration of rest phase. Fin length mutations affect either the growth phase, which changes the length of segments, or the rest phase, which changes the number of segments. For example, in the asymmetric caudal fin of *long fin* (*lof*) mutants, the longer ventral fin rays add segments at the constant rapid rate of juvenile fins. Therefore, the ventral fin rays in *lof* mutants bypass rest phase. Recently, a mutant that may lengthen rest phase has been identified. Adult *ocelot* (*oce*) mutants have fewer segments than wild-type. However, juvenile *oce* mutants have similar number of segments as wild-type fins. The hypothesis that *oce* mutants regulate rest phase was tested by monitoring the *oce* phenotype in a *lof* background. Double mutants for *oce;lof* have an exaggerated asymmetric caudal fin when compared with *lof* single mutants. The reason for this difference is that ventral fin rays in *oce;lof* double mutants have the same number of segments as the ventral fin rays in the *lof* single mutant, but the dorsal fin rays have significantly fewer segments. Thus, the *oce* mutation affects only the shorter fin rays in *lof* mutants. This is consistent with the hypothesis that the *oce* mutation lengthens rest phase, and that young, as well as ventral fin rays in *lof* caudal fins, do not experience rest phase.

485. **Skin Melanoblasts Undergo Only Initial Stages of Differentiation in White Japanese Silky Embryos.** H. A. Castillo and C. D. Faraco. Department of Cell Biology, University of Fed. Paran, Curitiba, Brasil.

White Japanese Silky (WSK) chickens have dermal melanocytes while their feathers lack pigmentation. However, the feathers of the WSK embryo are pigmented. Melanoblasts, melanocyte precursors, derive from neural crest and migrate dorsolaterally during embryonic development reaching peripheral regions of the embryo and populating the skin. We used Smyth line serum (SL), a useful marker for melanoblasts in the early stages of melanogenesis, to demonstrate that at the stage 22, some melanoblasts that have detached from the dorsal surface of the neural tube and initiated the migration throughout the dorsolateral path, are already located in the embryo ectoderm. At stage 24 many melanoblasts populate this embryonic germ layer. Also using SL immunolabel, we detected these precursor cells in the twelve days embryo epidermis. Nevertheless, L-Dopa reaction, which labels tyrosinase in active melanosomes, wasn't effective to detect the presence of those cells. In earlier stages (22 and 24) SL positive cells showed no reaction to L-Dopa as well. The results show that SL serum labels cells in very early stages of melanogenesis and that melanoblasts populate the epidermis although they only undergo full differentiation in the feathers. The fate of epidermal melanoblasts is unknown. Maybe these cells go to the feathers and there, in an appropriate environment, melanogenesis is accomplished, or in later stages of development the population of epidermal melanoblasts is lost due to the lack of survival factors.

486. **Cellular Interactions during Adult Pigment Stripe Development in Zebrafish.** D. M. Parichy and J. M. Turner. Institute for Cellular and Molecular Biology, University of Texas, Austin, Texas 78712.

Pigment patterns of vertebrates often include alternating light and dark stripes consisting of neural crest-derived pigment cells. The adult pigment pattern of the zebrafish *Danio rerio* consists of alternating horizontal stripes of black melanophores and yellow xanthophores. Using cell transplantations, we show that interactions between melanophore and xanthophore lineages are essential for stripe formation. Furthermore, we use a conditional allele of the Fms receptor tyrosine kinase to show that melanophore-xanthophore interactions are essential from metamorphosis through adult stages both to generate and maintain the striped pattern. Our results suggest a pattern generating mechanism that may underlie stripe formation in a diverse array of ectothermic vertebrates.

487. **Withdrawn**

488. Abstract #488 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

489. **Direct Hedgehog Signaling in the Neural Crest Cells Is Essential for the Normal Craniofacial Development.** Juhee Jeong, Toyooki Tenzen, and Andrew P. McMahon. Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts.

Most of the vertebrate head skeleton is derived from the multipotent progenitor cells known as neural crest cells (NCC). Cranial NCC's first migrate into and proliferate within the mesenchyme of frontonasal prominence (FNP) and branchial arches (BA) of the head, where they differentiate into cartilage and

bone. The epithelium of FNP and BA's, which is of non-NCC origin, expresses signaling molecules such as Shh. Facial abnormalities in Shh mutants implicate the Hh pathway in craniofacial development, but it has been unknown whether direct Hh signaling within the NCC's is necessary. To address this question, we analyzed the phenotypes of mice in which Hh signaling has been removed specifically from the NCC, using Wnt1-Cre and conditional knockout allele of Smo, an obligatory component of the Hh pathway. At birth, Wnt1-Cre;Smo^{-/-} animals have severely truncated faces without jaws. Skeletal preparations indicate that most of the NCC-derived bones and cartilages are absent or reduced in size. While the initial formation of the facial processes is unaffected, by E11.5 facial structures in mutants are clearly smaller than their wild type counterparts, indicating defects in tissue growth. Molecular analysis revealed massive apoptosis in the mutant BA's. In addition, the expression of several members of the forkhead transcription factors, Foxc2, Foxd1, Foxd2, and Foxf1, is affected in the mutants. Since the mutation of Foxc2 results in skeletal defects, this, and possibly other forkhead proteins also, may mediate the role of Hh signaling in head skeleton development.

490. **Inactivation of the Alk-2 Gene by Wnt1-Cre-Mediated Deletion Results in Failure of Cranio-Facial Development.** Vesa Kaartinen, Andre Nagy, and Marek Dudas. Developmental Biology Program, Department of Pathology, Childrens Hospital, Los Angeles, California.

Neural crest (NC) cells, derived from lateral ridges of the neural plate during the early embryogenesis, migrate extensively and give rise to multiple organs including many craniofacial structures. Although bone morphogenetic proteins (BMPs) have been implicated in cranial NC development, many details are still poorly known. BMPs signal through a receptor complex composed of type II (BmprII, ActrII) and type I receptors (Alk2, Alk3 and Alk6). Type I receptors are the primary determinants of signaling specificity, and therefore understanding their function is of utmost importance in order to comprehend molecular signaling mechanisms regulated by BMPs during cranio-facial development. Unfortunately the early embryonal lethality at gastrulation has prevented the use of conventional Alk2 and Alk3 knockout mice in assessment of potential roles of these receptors later in development. To overcome this problem, we decided to use the Cre/Lox system. We generated mice carrying the floxed Alk2 allele in their genome and utilized Wnt1-Cre transgenic mice to conditionally abrogate Alk2 in the NC. Our results show that mice lacking Alk2 in NC cells consistently display numerous cranio-facial defects including cleft palate, hypomorphic mandible, hypomorphic frontal bones, malformed zygomatic arches and tooth defects. However, cranial nerves develop normally. These results indicate that BMP signaling via Alk2 is important for normal development of cranio-facial structures derived from the cranial NC.

491. **Cadmium Affects the Formation of the Notochord in Zebrafish Embryonic Development.** Shuk Han Cheng and Elly Suk Hen Chow. Department of Biology and Chemistry, City University of Hong Kong, Kowloon, Hong Kong.

We examined the morphological features of the notochord in cadmium treated embryos by immunohistochemistry with the antibody Znt-1 to stain for the notochord specifically. In un-

treated control embryos at 24 hpf, the notochord can be detected as a rod-like structure throughout the trunk and it extended into the tail region with a descending intensity of Znt-1 staining. In cadmium treated embryos with curvatures, the notochord looped off and failed to extend into the tail region as shown by the abrupt loss of Znt-1 staining in the caudal regions. The fate of axial mesodermal cells destined to become notochord was investigated. Where the notochord failed to form in the caudal region, the somitic cells simply committed to the myogenic lineage as they expressed the myoD and myogenin genes instead. Infringement by differentiated muscle cells in the notochord area could also be found in some cadmium treated embryos. In untreated controls, the fast muscle fibres appeared as bilateral rows flanking the notochord. In cadmium treated embryos, the fast muscle fibres could be found in the midline mesoderm. Taken together, it appears that the cell fate decision of axial mesodermal cells to notochord may be affected by cadmium and hence some of these cells take up the default pathway and become muscle cells.

492. **Identification of a Gamma A-Like Protocadherin in the Developing Chick.** Anthony A. Capehart and Christine B. Kern. Department of Biology, East Carolina University, Greenville, North Carolina; and Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, South Carolina.

The protocadherins are calcium-dependent cell adhesion molecules of the cadherin superfamily. Although less well characterized than classical cadherins, the protocadherins are also thought to facilitate critical cell-cell interactions during embryonic development. We have cloned a novel protocadherin from the embryonic chick utilizing a monoclonal antibody produced against a peanut agglutinin-binding fraction of cultured chick limb mesenchyme to screen a lambda ZAP cDNA expression library from the stage 25 chick limb. A 2.8 kb cDNA clone was obtained that encoded multiple cadherin-like ectodomains. Northern blotting showed a developmentally-regulated 4.6 kb RNA transcript that was highly enriched in the stage 43 chick brain. RT-PCR/RACE utilizing chick brain tissue identified the entire 2.4 kb reading frame. The chick protocadherin contained five cadherin-like repeats and a highly conserved cytoplasmic domain. Amino acid alignment of the extracellular domains revealed marked identity to the human gamma A protocadherin subfamily. In situ hybridization showed low levels of mRNA localization in several developing tissues, but stronger expression in the neural tube and dorsal root ganglia at stage 27. In the stage 43 chick brain, protocadherin transcripts were noted in discrete regions, particularly within the optic lobe. As for protocadherins described in other species, these results suggest that this novel gamma A-like protocadherin may also play a role in chick neural development.

493. **Role of Shh in Sensory Ganglion Formation.** Natalia Fedtsova* and Eric E. Turner*†. *Department of Psychiatry, UCSD, La Jolla, California 92093; and †VA Medical Center, San Diego, California 92122.

The sensory peripheral nervous system consists of cranial and dorsal root ganglia, which are derived from migratory cell populations. Neurons at cranial level arise from the ectodermal placodes and neural crest, while the dorsal root ganglia are differentiated entirely from the neural crest. It is not clear what signals determine the final position of the ganglia. At spinal

levels, neural crest cells avoid and do not enter perinotochordal mesenchyme. Sonic hedgehog (shh) is one of factors emanating from the notochord that could mediate this process. We examined gangliogenesis in Shh knockout mice, using transcriptional factors Brn3a and Islet as early neuronal markers for sensory neurons. We found that Shh is not required for the generation of early precursors of sensory ganglia, but it is necessary for the normal cessation of their migration. In normal embryos, sensory neurons condense into symmetrical ganglia along the neuroaxis. In mice lacking Shh neuronal precursors continue to migrate ventrally. At cranial levels they condense into a single midline ganglion. Dorsal root ganglia formation is also profoundly affected, but with greater variability. In chick embryos, implantation of Shh-beads at the site of the presumptive trigeminal ganglion creates a nonpermissive environment for neural migration, inducing expression of the proteoglycan PG-M/versican, which is thus a candidate for mediating the effects of Shh on sensory ganglia formation.

494. **Identifying Functional Interactions between the Retinal Transcription Factors Required for Specification of the Vertebrate Eye.** Michael E. Zuber,* A. S. Viczian,* G. Gestri,† B. Knox,* G. Barsacchi,† and W. A. Harris**. *Departments of Biochemistry and Molecular Biology and Ophthalmology, SUNY Upstate Medical University, Syracuse, New York; †Laboratorio di Biologia Cellulare e dello Sviluppo, Università di Pisa, Italy; and **Department of Anatomy, University of Cambridge, United Kingdom.

The eye field is that region of the anterior neural plate that will eventually give rise to most tissues of the eyes. Eight transcription factors (*Otx2*, *ET*, *Pax6*, *Six3*, *Rx1*, *tll*, *Lhx2* and *Otx2*) are required for normal eye formation and are expressed in the eye field. We show in the frog *Xenopus laevis* that these eye field transcription factors (EFTFs) are expressed co-ordinately in the eye field at the time of its specification. Using the ectodermal explant (animal cap) assay we have identified functional interactions among these genes. Our results suggested to us that coexpression of the EFTFs may be sufficient to pattern presumptive retina from the neural plate. This model is analogous to that proposed for the specification of the eye disk in *Drosophila*. By coordinating the ectopic expression of the EFTFs we induced ectopic eye fields and eyes both in and outside of the frog nervous system. Therefore, it appears that in vertebrates, as in the fly, a self-regulating hierarchy of transcription factors is responsible for eye formation. To further characterize the functional interactions between the EFTFs, we have now isolated the 5' flanking regions of the *Otx2* and *Lhx2* genes. Using transgenesis in frogs, we are currently characterizing these promoters in order to identify the elements responsible for their expression in the eye field and responsive to the other EFTFs.

495. **Rx is Required Both for Optic Vesicle Formation and Maintenance of Retinal Cell Fate.** Peter Mathers,* Vera Voronina,† Catherine Wilson,† Serguei Kozlov,† and Mark Lewandoski†. *Sensory Neuroscience Research Center, West Virginia University, Morgantown, West Virginia; and †Cancer and Developmental Biology Laboratory, National Cancer Institute, Frederick, Maryland.

Numerous model systems demonstrate an important role for the Rx homeobox gene in specifying early retinal cell fate. Mouse

and fish mutants show defects in the formation or growth of the optic vesicle, and we have isolated RX mutations in a patient with anophthalmia and microphthalmia. Germline defects in the Rx genes suggest a crucial role in early retinal patterning, and, particularly in mouse knockout mutants, demonstrate their requirement for the initiation of retinal development. These mutants, however, do not allow us to investigate what role Rx plays at later stages of retinal development and differentiation. Therefore, we developed a conditional allele of the mouse Rx gene. We used a Foxg1-Cre transgenic line to delete Rx expression in the developing optic vesicle from E9.0 onward. Rx conditional deletion causes degeneration of the optic vesicle and prevents lens placode formation, resulting in anophthalmic adults. In E9.5-10.5 mutant embryos where optic vesicles are still present, we find defects in the expression patterns of Rx, Pax6, Pax2, and Otx2 within the optic cup and stalk along the proximo-distal axis. These embryos also fail to activate Chx10 and down-regulate retinal proliferation markers, such as Six6 and Six3. These results are consistent with a failure to maintain the proliferative potential of retinal stem cells in embryos where Rx has been deleted. We conclude that the mouse Rx gene is required for both initiating and maintaining early retinal cell fate.

496. **Math5 Null Mice Have Abnormal Retinal and Persistent Hyaloid Vasculatures.** Joseph A. Brzezinski IV,* Sara M. Schulz,* Susan Crawford,† Emily Wroblewski,** Nadean L. Brown,** and Tom Glasert. *Departments of Human Genetics and Internal Medicine, University of Michigan. Ann Arbor, Michigan; †Department of Pathology, Northwestern University Medical School, Chicago, Illinois; and **Departments of Developmental Biology and Ophthalmology, Children's Hospital Research Foundation. Cincinnati, Ohio.

The bHLH transcription factor *Math5* is required to form retinal ganglion cells (RGCs) and optic nerves. In addition to intrinsic neuroretinal defects, the ocular vasculature is significantly abnormal in *Math5* mutants. First, the central retinal artery is variably absent or attenuated. Second, hyaloid (fetal) vessels persist in the vitreous and hemorrhage frequently. Third, we observed a variety of retinovascular defects, ranging from complete absence of blood vessels (rare) to vascular hypertrophy (common). In many mutants the normal radial pattern is lost, and vessels penetrate the photoreceptor layer into the subretinal space. During late gestation, astrocytes enter the retina via the optic stalk, migrate along RGC axons, and spread across the inner retinal surface, where they are thought to promote development of the retinal vasculature. In principle, the vascular defects in *Math5* mutants could result from aberrant astrocyte migration in the absence of RGCs. However, the number of GFAP immunopositive astrocytes is greatly increased. Therefore, RGCs are not necessary for astrocyte migration into and throughout the retina. RGCs may have important roles in regulating astrocyte number, regression of hyaloid vessels, and retinal vasculogenesis.

497. **Interactions during Inner Ear Induction.** Raj Ladher, Susan Boerner, and Gary Schoenwolf. RIKEN Center for Developmental Biology, Kobe, Japan; and Department of Neurobiology and Anatomy, University of Utah, Salt Lake City, Utah.

The inner ear of the chick embryo is derived from the otic placode, a thickened ectodermally derived disk induced from the non neural ectoderm, between stages 7 to 9. Recently we have

shown that the intersection of localized fibroblast growth factor signaling, provided by fgf-19, with wnt signals provided by wnt-8c constitute the molecular nature of this induction. In this poster we compared the expression of fgf-3 and fgf-8, with that of fgf-19 as both are expressed early enough to play a role in inner ear induction, and both have been shown to play some function in the development of the inner ear. We wished to order these molecules into a hierarchy of inductive interactions in the chick otic placode. We show that the endoderm fated to underlie the caudal cephalic paraxial mesoderm plays a role in otic induction and we hypothesize that this role is fulfilled by fgf-8. Fgf-8 in the endoderm, may act as a trigger, initiating both fgf-19 and fgf-3 in the paraxial mesoderm, allowing otogenesis to occur. The respective roles of fgf-19 and fgf-3 in inner ear induction will be discussed.

498. **Generation of Inner Ear-Specific Knockout Mice by Modification of A Pax-2 Bacterial Artificial Chromosome.** Taka Ohshima and Andy Groves. House Ear Institute.

To generate inner ear conditional knockout mice using The Cre-loxP system, we focused on the regulatory region of the Pax-2 gene to isolate a specific promoter for inner ear precursor cells. Pax-2 is expressed in the midbrain-hindbrain boundary, mesonephric organs, optic vesicle, spinal cord and otic vesicle during embryonic development. Pax-2 is also expressed in all inner ear precursor cells shortly after the otic placode appears at E8.5. To analyze the regulatory region of Pax-2, we isolated a BAC clone containing about 101kb of upstream sequence of the Pax-2 coding region and inserted IRES-Cre-polyA into the Pax-2 coding region using a BAC modification system. We established two transgenic lines with this Pax-2-Cre construct. Here, we show the analysis of these lines by mating with loxP reporter mouse strains and a lineage analysis of Pax-2 positive otic placodal cells. Reporter positive cells are detected in the otic placode from E8.5 (7-8ss). Interestingly, cells in epibranchial placodes at slightly later stages are also reporter positive. In chick, it has been shown that not all Pax-2-positive cells will ultimately contribute to the otic placode (Andrea Streit: Developmental Biology 249 p237-254, 2002). We are now exploring the lineage relationships between the otic + epibranchial placodes.

499. **Early Development of the Otic Placode in Zebrafish.** Andreas Fritz,* Keely S. Solomon,* Tetsuhiro Kudoh,† Melinda D. Mackereth,* and Igor B. Dawid†. *Department of Biology, Emory University, Atlanta, GA 30322; and †Laboratory of Molecular Genetics, NICHD, Bethesda, MD 20892.

The inner ear develops from the otic placode, a thickening of ectoderm that becomes visible during somitogenesis in most vertebrate species. The otic placode is a transient structure, giving rise to the otic vesicle, which proliferates and differentiates to form specialized cell types necessary for hearing and balance. Although inductive signals for otic placode formation have been characterized, less is known about the molecules that respond to these signals within otic primordia. We have identified a mutation in zebrafish, *hearsay* (*hsy*), which results in a reduction or loss of the otic placode and vesicle. We have shown that the *hsy* mutation disrupts *foxi1*, a forkhead domain-containing gene. Expression analysis of *foxi1* suggests a direct role for this gene in the development of the otic placode and branchial arch deriva-

tives. In addition, otic expression of the early marker *pax8* is absent in *hsy* mutants, and misexpression of *foxi1* can induce ectopic *pax8* expression. These data suggest that *foxi1* function is required within pre-otic cells for the earliest stages of otic placode formation. However, *hsy* mutant embryos display a variable loss of the otic placode, which is accompanied by a delay in the induction of the otic placode. Our results support a model in which an otic placode developmental program can be reinitiated in the absence of a proper early gene expression sequence. Furthermore, our studies of the roles of *foxi1*, *dlx3b*, *textitfgf3* and *fgf8* reveal complex interactions between these genes in the early development of the otic placode.

500. **Insertional Mutagenesis Screen for Genes Involved in Vestibular/Neural Development in *Xenopus tropicalis*.** Marcela Torrejon, Minh Nguyen, Rakhi Gupta, and Sigrid Reinsch. NASA-Ames Research Center, National Research Council, Moffett Field, California.

Sensitivity to gravity is essential for spatial orientation. Consequently, the gravity receptor system is one of the phylogenetically oldest sensory systems and is highly conserved. The main goal of this project is to use *Xenopus* to identify genes expressed during otic/neural development to gain a better understanding of the molecular mechanisms involved in vestibular function. We are using a gene-trap approach in *X. tropicalis* with the green fluorescent protein (GFP) gene as the transgene reporter. GFP expression occurs only when the GFP gene is correctly integrated in actively transcribed genes and thus allows visual screening for transgenic animals. Using the GFP as a molecular tag we can ultimately identify and clone the mutated gene using RACE-PCR. In addition, we can study the function of the mutated gene by analyzing the defects generated by insertion of the GFP transgene. To date we have identified transgenic animals with tissue-specific GFP expression in ear, neural tube, kidney, muscle, eyes and olfactory tissue. Current efforts focus on four lines of founders with expression of GFP in the developing nervous and otic systems. Each founder has generated a family (F1) in which there is Mendelian transmission of the transgene, and in which heterozygotes are phenotypically normal. Each family displays a unique GFP expression pattern that is constant within a given family. The F1 heterozygotes will be intercrossed to generate homozygous progeny for mutant analysis. The F1 have been used to purify RNA for RACE-PCR to clone the gene into which the GFP transgene is inserted.

501. Abstract #501 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

502. **Is the Early Hh Transcriptional Response Conserved Between Neural and Pituitary Tissue?** Burcu Guner and Rolf Karlstrom. University of Massachusetts, Amherst, Massachusetts.

During development of the pituitary gland, the posterior lobe (neurohypophysis) arises from neural ectoderm, while the endocrine secreting anterior lobe (adenohypophysis) arises from adjacent oral ectoderm. We recently showed that Hedgehog (Hh) signaling from neural tissue is essential for both the induction and the patterning of the zebrafish adenohypophysis (Sbrogna et al., 2003). In the spinal cord, Sonic Hh works as a morphogen to

guide neural differentiation, directing expression of a dorsal/ventral "code" of Hh responsive transcription factors (see Ingham and McMahon, 2001). Our observation that the ventral Hh responsive genes *ptc1* and *nk2.2* are expressed in the anterior region of the adenohypophysis (nearest the source of Hh signals) led us to speculate that the neural Hh response "code" might be conserved in this endocrine tissue. We therefore set out to determine whether neural Hh responsive genes might also play a role in pituitary development. We show that four genes regulated by Hh in the spinal cord, *ptc1*, *nk2.2*, *hlx1*, and *pax7* are expressed in the zebrafish adenohypophysis, with expression relative to the source of Hh being largely conserved. We also cloned the ventral Hh response genes *nk2.9* and *nk6.2* using sequence obtained from the zebrafish genome project. While both *nk2.9* and *nk6.2* are expressed in the spinal cord in the expected pattern, neither gene is expressed in the developing adenohypophysis. Finally, by examining the Hh pathway mutants *syu/shh*, *smu/smoothened*, and *yot/gli2*, we show that defects in Hh signaling affect precursor cell differentiation similarly in these two distinct tissue types.

503. **New Zebrafish Laterality Mutants That Have Altered Dorsal Forerunner Organization and Altered Localization of Inversin to Monocilia.** Patricia A. Sacayon and H. Joseph Yost. Center for Children, Hunstman Cancer Institute.

The mechanisms that induce and maintain asymmetric gene expression patterns preceding organogenesis in vertebrates are unresolved. Monociliated cells within the node in mammals and the shield in zebrafish are thought to play a central role in the early steps of left-right axis formation (Essner, 2002). In zebrafish, these monociliated cells are in Kupffer's vesicle (KV), derived from dorsal forerunner cells (DFCs). Four distinct classes of zebrafish laterality mutants have been characterized based on aberrant expression of left-sided markers *lefty1*, *lefty2*, and *pitx2* in brain, heart, and gut primordia: concordant bilateral expression, concordant absence, discordance (randomization), or brain-specific absence (Bisgrove, 2000). All of these laterality mutants also have midline (notochord or neural plate) defects. Here, we describe two new zebrafish laterality mutants that represent a novel class: reversed heart looping and concordant inverted expression of *lefty2* and *pitx2* and normal midline development (Sonic hedgehog and *lefty1*). Since mice *inversin* mutants have a similar phenotype with inverted expression markers (Yokoyama, 1993; Okada, 1999), we cloned INV in zebrafish (Morgan, 2002) and localized INV to monocilia in KV, analogous to the recently demonstrated localization of INV in mouse nodal cilia (Watanabe, 2003). In our laterality mutants, the organization of DFCs is affected and localization of INV to monocilia is decreased. Further characterization of these laterality mutants will lead to a better understanding of the conserved role of *inversin* and monocilia in vertebrate left-right development.

504. **Roles of the Zebrafish Nodal-Related Gene *Southpaw* in Visceral and Brain Left-Right Asymmetry.** Sarah Long, Nadira Ahmad, and Michael Rebagliati. Department of Anatomy and Cell Biology, University of Iowa College of Medicine, Iowa City, Iowa.

We have identified and characterized a new zebrafish nodal-related gene, *southpaw* (*spaw*), that is required for establishing the left-right asymmetry of the embryonic heart, other visceral

organs, and diencephalon. Southpaw is expressed within the left lateral plate mesoderm beginning at early somite stages. Analysis of southpaw expression in various genetic and morphant backgrounds clarifies the nature and location of the early midline structures/signals regulating embryonic left—right patterning. At late somite stages, left-side southpaw expression transiently overlaps the left-side expression domains of other genes that mark the developing heart, such as *lefty 2*. Consistent with its expression pattern, inhibition of southpaw function with morpholinos causes a severe disruption of both early (cardiac jogging) and late (cardiac looping) manifestations of cardiac left—right asymmetry. Expression of downstream left-side genes is severely down-regulated or abolished in *spaw*-deficient embryos. Since the left—right asymmetry of the pancreas and other organs is also affected, southpaw appears to regulate left—right asymmetry throughout a large part of the embryo. We have not detected any southpaw expression within the zebrafish central nervous system. Despite the fact that southpaw is not expressed within the brain, southpaw is also required for diencephalic left—right asymmetry. These observations suggest a possible model for the initiation of embryonic brain left—right asymmetry and its coordination with visceral organ left-right asymmetry.

505. **Subdividing the Embryo: A Role for Notch Signaling.** Stacey P. Coleman and Kelly A. McLaughlin. Tufts University, Medford, Massachusetts 02155.

The Notch pathway is highly conserved among many organisms, indicating its importance in cell decisions during development. Previous research has shown Notch signaling to be involved in the establishment of the borders of the germ layers in early sea urchin development. However, this question has never been examined in a vertebrate organism. To address this question, we injected inducible RNA constructs into early cleavage stage *Xenopus laevis* embryos to manipulate the Notch pathway. Constructs were activated just prior to the establishment of early organ fields. Resultant embryos were examined for misexpression of molecular markers for each germ layer using immunohistochemistry and in situ hybridization. Preliminary data suggest activation of the Notch pathway results in a loss of mesodermal derivatives and an expansion of the endoderm. Similar to what has been observed in invertebrate organisms, our results indicate that Notch may perform a similar role in establishing boundaries during early embryonic development in vertebrate systems as well.

506. **Control of A-P Identity in Endoderm by Lateral Plate Mesoderm Signals.** Maya Kumar, Nathalie Jordan, Doug Melton, and Anne Grapin-Botton. Harvard University, 7 Divinity Avenue, Cambridge, Massachusetts 02138; and ISREC, Chemin des Boveresses 155, Case Postale, CH-1066 Epalinges s/Lausanne, CH.

During development organs arise from the endoderm in a stereotyped anterior—posterior (A-P) pattern. An early sign of patterning is the expression of organ-specific genes in restricted endoderm domains. Using chick-quail chimeras and in vitro cultures, we have studied the interactions governing the induction and maintenance of organ identity, with particular attention paid to the establishment of the prepancreatic domain. We show that lateral plate mesoderm (LPM) from different A-P levels sends region-specific information to the endoderm. Instructive signals

from the LPM at the level of the duodenum/pancreas induce the expression of *Pdx1*, glucagon, and other pancreatic markers, while more posterior LPM induces *CdxA* characteristic of the small intestine. LPM patterns the endoderm in a posterior dominant fashion analogous to the patterning of the neural tube at the same stage. Taken together these findings argue for a global mechanism that coordinates the A-P patterning of all three primary germ layers. We further find that stimulation of the activin/BMP pathway elicits a posterior shift in endoderm A-P identity. Blocking the activin/BMP pathway prevents *Pdx1* induction by mesoderm. Moreover, BMP4 is expressed in an A-P gradient in LPM at the time of *Pdx1* and *CdxA* induction. Experiments are in progress to determine whether BMPs act directly on endoderm and whether other molecules that have posteriorizing effects on ectoderm also posteriorize endoderm.

507. **Step-wise Patterning of Trunk Mesoderm by Bmp Signals.** Richard G. James and Thomas M. Schultheiss. Molecular Medicine Unit, Beth Israel Deaconess Medical Center and Harvard Medical School, Cambridge, Massachusetts.

There are several models suggesting that a simple gradient of Bmp signal is responsible for patterning the trunk mesoderm. In our studies of the avian intermediate mesoderm (IM), we have found evidence implying that Bmp signal integration is far more complex. First, we have analyzed the embryonic distribution of phosphorylated *smad1*, a known marker of Bmp signaling, and found that it is detectable in the lateral mesoderm immediately after gastrulation. After the trunk is organized into somites, IM, and lateral plate, phosphorylated *smad1* becomes restricted to dorsal structures such as the dermamyotome and nephric duct. Second, experiments in which we have manipulated embryonic Bmp levels by placing cells that secrete Noggin or beads soaked in Bmp-4 have stage-specific effects. If Bmp levels are manipulated early, the entire mesoderm becomes dorsal- or ventralized, respectively. However, if levels are altered after somites have formed, gene expression in individual tissues such as the nephric duct and ventral IM is changed, but the location of somites, lateral plate, and IM is unaffected. Last, electroporation of a cell autonomous activator of Bmp signaling at early stages inhibits somite but not IM differentiation, demonstrating IM gene expression can be initiated in the presence of high levels of Bmp signal. We propose an alternative model in which Bmp signals are used as “on/off” switches at several different times to progressively pattern the mesoderm.

508. **tortuga, A Zebrafish Mutation Affecting the Segmentation Clock.** Kariena K. Dill, Jennifer L. Anderson, and Sharon L. Amacher. University of California, Berkeley, California.

Current models for vertebrate somitogenesis suggest that an oscillator, or segmentation clock, acts in the presomitic mesoderm (psm) to generate a metamer pattern. Many data indicate that Notch signaling is involved in regulating the segmentation clock. We have isolated an ENU-induced mutation in zebrafish, *tortuga*, which disrupts expression of the Notch pathway genes *her1*, *her7*, and *deltaC*. These genes are expressed in an oscillating pattern: waves of gene expression move through the cells of the psm in an anterior progression, but at fixed points in time, expression appears as distinct stripes. In *tortuga* mutant embryos, expression patterns of the oscillating genes are similarly disrupted: normal stripes of expression are apparent, but a lower

level of ectopic expression persists in the interstripe regions. Although the segmentation clock appears disrupted in *tortuga* mutant embryos, other genes that pattern the psm (including *papc* and *mesp* genes) are expressed in normal patterns and somitogenesis proceeds with only subtle disruptions. As somites mature, *tortuga* mutant embryos exhibit defects in somite boundaries; however, we show that these defects likely result from a defect in boundary maintenance rather than a defect in boundary formation. Analysis of the *tortuga* mutant phenotype indicates that the segmentation clock can be disrupted, at least partially, without disrupting somitogenesis. We are currently working to identify the gene mutated in *tortuga* and are hopeful that the gene will reveal new aspect(s) of the mechanism controlling vertebrate somitogenesis and the segmentation clock. *her1*.

509. Withdrawn.

510. **Developmental Regulation of Muscle Fiber Number in Zebrafish.** Stephen H. Devoto, Xuesong Feng, Joel D'Angelo, Frank Stellabotte, and Daniel Fernández. Department of Biology, Wesleyan University, Middletown, Connecticut 06459.

Zebrafish muscle development begins early in the segmentation period, with the differentiation of a remarkably consistent number of slow muscle fibers in each somite (22.5 ± 1.0 per somite in the posterior trunk). Hedgehog (Hh) signaling is both necessary and sufficient for the development of these embryonic slow muscle fibers. Using a combination of genetics and pharmacology, we have begun to ask whether the number of embryonic slow muscle fibers is regulated by the amount of Hh signaling. Cyclopamine is a plant-derived alkaloid that specifically binds to the Smoothened protein and blocks Hh signaling. We show that there is a quantitative relationship between the concentration of cyclopamine and the number of slow muscle fibers. Hh signaling is also reduced in embryos heterozygous for mutations in *smu* (which encodes the zebrafish Smoothened protein) or *yot* (which encodes the zebrafish *gli2* protein). The number of slow muscle fibers is more sensitive to low doses of cyclopamine in heterozygous embryos than in wild-type embryos, providing further evidence for a quantitative relationship between Hh signaling and the number of slow muscle fibers. During the early larval period, new slow and fast muscle fibers begin to appear at the dorsal and ventral extremes of the myotome. We propose that these postembryonic muscle fibers derive from proliferative myogenic germinal zones. The development of these myogenic germinal zones and their differentiation into either slow or fast muscle fibers does not depend on Hh signaling.

511. **Characterization of Lmbr1 during Chick Limb Development.** Sarah A. Maas* and John F. Fallon†. *Department of Biomolecular Chemistry and †Department of Anatomy, University of Wisconsin, 1300 University Ave. Madison, Wisconsin 53706.

Sonic Hedgehog (Shh) is a secreted signaling molecule required for proper development of many structures during vertebrate embryogenesis. In the developing amniote limb, anteroposterior (A/P) patterning is controlled by secretion of Shh protein by the zone of polarizing activity (ZPA) in the posterior mesoderm. In the chicken mutant *oligozeugodactyly* (*ozd*), Shh is expressed normally throughout the body but is entirely lacking in the

developing limbs, which results in the loss of specific bones in both wings and legs. Humans affected with acheiropodia (ACHR) have limbs that closely resemble *ozd* limbs. Recently, the ACHR mutation was mapped to the LMBR1 gene and consists of the deletion of one exon and several kilobases of intronic sequence. It is unclear whether the defect is due to disruption of protein function or to loss of regulatory elements in the intronic sequence. Although the role of the Lmbr1 protein has been investigated in the mouse, the results were inconclusive. We are using the developing chicken to more thoroughly characterize the role of Lmbr1 during vertebrate limb development. We have cloned the chick homolog of the mouse Lmbr1 and human LMBR1 genes, and we find a high degree of conservation among them. In addition, we have established that in chick, Lmbr1 expression is contained within the ZPA. A full-length Lmbr1 transcript is present at wild-type levels in *ozd* embryos by Northern analysis, and no mutations are found within the Lmbr1 coding region in *ozd*. At this time, it appears unlikely that Lmbr1 has a role in limb development. Lmbr1 overexpression experiments are in progress.

512. **Ephrin-A/EphA Interaction Involved in Position-Specific Cell Affinity and Cartilage Differentiation in the Limb Bud.** Tsutomu Nohno and Naoyuki Wada. Department of Molecular Biology, Kawasaki Medical School, Kurashiki 701-0192 Japan.

In the developing limb bud, mesenchymal cells show position-specific affinity, suggesting that the positional identity of the cells is represented as their surface properties. Since the affinity is regulated by glycosylphosphatidylinositol (GPI)-anchored cell surface proteins, and by EphA4 receptor tyrosine kinase, as revealed by inhibition of cell sorting with phospholipase C and EphA4 antibodies, we hypothesized that the GPI-anchored ligand, the ephrin-A family, contributes to the affinity. We examined the role of ephrin-A2 in the chick limb bud. Ephrin-A2 protein was uniformly distributed in the limb bud at early stages of development. As the limb bud grows, expression of ephrin-A2 was intense in its proximal to intermediate regions, whereas weak distally. The position-dependent expression was maintained in vitro during cell culture and was regulated by FGF protein produced in the apical ectodermal ridge. To investigate the role of ephrin-A2 in affinity and in cartilage morphogenesis of limb mesenchyme, we ectopically expressed ephrin-A2 in the limb bud using the retrovirus vector. Overexpressed ephrin-A2 caused malformation of cartilage elements of the autopod and modulated the affinity of the mesenchymal cells that differentiate into autopod elements. It also interfered with the cartilage nodule formation of the cells without inhibiting chondrogenesis. These results suggest that ephrin-A2 regulates the position-specific affinity of limb mesenchyme and is involved in the cartilage pattern formation of the limb.

513. Abstract #513 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

514. **Scleraxis Is a Major Regulator of Tendon Formation.** Ronen Schweitzer. Shriners Hospital, Portland, Oregon.

Little is known about the differentiation and patterning of tendons. We have recently found that Scleraxis, a bHLH tran-

scription factor, is a unique marker for tendons with expression that persists from early tendon progenitors to the mature adult tendons. To study Scleraxis function we targeted the Scleraxis locus in mice. We find that the previously reported early embryonic lethality was due to an effect of the Neo cassette on a neighboring gene. Mice homozygous for the Scleraxis mutation (ScxCR) are viable but have a severe tendon phenotype. All tendons appear affected in the ScxCR mutants ranging from inability to use the paws and tail to limited use of the upper limb, back, and jaw muscles. Using a tendon GFP reporter (ScxGFP), we find that Scleraxis transcription and early specification and patterning of the tendon progenitors are not dependent on Scleraxis. Tendon defects are first detected at E13.5, with the onset of distinct tendon formation and by E18.5, all tendons appear smaller, fragmented, and missing secondary patterning and articulation. The defects are most pronounced in the long tendons of the tail and limb and tail tendons are completely lost a few weeks after birth. The tendon matrix is also affected directly in ScxCR mutants. TEM reveals a severe loss of collagen bundles in mutant tendons and a severe disruption of the ultrastructure of the tendon matrix is revealed by TEM and staining for Tenascin and Collagen XII. Other matrix molecules are absent in mutant tendons, suggesting that they may be directly regulated by Scleraxis. Scleraxis is therefore the first major regulator of tendon formation to be identified, affecting both cellular patterning and the ability to generate the tendon matrix.

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516. **Multiple Roles of VEGF during Skeletal Development.** E. Zelzer, T. Kobayashi, H. M. Kronenberg, N. Ferrara, and B. R. Olsen. Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115.

Several studies demonstrated that VEGF is expressed in the hypertrophic zone of growth plates and suggested that VEGF is important for cartilage angiogenesis during the formation of endochondral bones. In limbs of early stage embryos VEGF is expressed in mesenchymal condensations, the nonvascularized tissue templates of the future cartilage elements. This supports a role for VEGF in cartilage development that is independent of its function as an angiogenic factor. To further study this role, we abolished expression of VEGF in developing limbs at the early stage of mesenchymal condensations or later when the condensations have differentiated into cartilage, using the P-lox-cre system. Lack of VEGF in limb mesenchyme resulted in severe defects in limb growth and cartilage formation. Interestingly, the vascularity of mutant limbs appeared unaffected. In mutant embryos, chondrogenic elements were formed after E16.5 but they were small and misshaped, and joint regions were fused. Central regions of the epiphyses showed signs of cell death. In the distal limbs, no vessel invasion into cartilage was observed and bone formation was inhibited. Eliminating VEGF expression in chondrocytes after cartilage differentiation in the skeleton had taken place and resulted in delayed vessel invasion into cartilage and reduced ossification. In addition, massive cell death occurred in central regions of epiphyses at about the same time as the cell death was seen in mutants lacking VEGF expression in condensed mesenchyme. In conclusion, we have demonstrated that interfering with VEGF expression in mesenchymal condensations dra-

matically affects cartilage and bone development. Later in development, VEGF is required for vascularization of cartilage during endochondral ossification and acts as a survival factor for chondrocytes.

517. **Deficiency for Dentin Matrix Protein 1, a Specific Gene for Mineralized Tissues, Causes Osteochondrodysplasia, Osteoarthritis, and Rickets/Osteomalacia during Postnatal Development.** Yuji Mishina,* Ling Ye,† Haiyang Huang,† Di Chen,‡ Sarah Dallas,† Tetsuo Kunieda,† Takeo W. Tsutsui,† Yongbo Lu,† Hua zhu Ke,‡ Lynda F. Bonewald,† and Jian Q. Feng†. *National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; †School of Dentistry, University of Missouri-Kansas City, Kansas City, Missouri; and ‡University of Texas Health Science Center at San Antonio, San Antonio Texas.

Dentin Matrix Protein 1 (Dmp-1), initially identified in dentin, is an acidic matrix phosphoprotein that is expressed in chondrocytes, osteoblasts, and osteocytes. To determine the role of this matrix protein, we generated Dmp1 null mice. These mice appeared normal at birth, but began to exhibit osteopetrotic changes in 3 days after birth. Amount of trabecular bone was dramatically increased to fill the bone marrow space. Interestingly, however, a dwarfism appeared by 3 weeks of age that became more profound by 3 months. Long bones were shortened, and bone mineral density was reduced. Growth plates were extremely disorganized in which the columnar organization of the hypertrophic zone was disrupted and replaced with clusters of hypertrophic chondrocytes. This disorganized growth plate appeared to be responsible for the presence of nonmineralized, hyperostotic matrix in place of the normal trabeculae. These animals also exhibited osteoarthritic changes including narrowing of the joint spaces and erosion of the joints. These studies show that Dmp1 is a major regulator in bone and cartilage that is critical for growth plate formation and function, for coupling of chondrogenesis with osteogenesis, for mineralization, and for bone remodeling.

518. **Belly Spot and Tail: A Mouse Minute.** Edward R. Oliver, Thomas L. Saunders, Susan A. Tarle, and Tom Glaser. University of Michigan, Ann Arbor, Michigan.

In *Drosophila melanogaster*, the *Minutes* are a large class of mutants with similar phenotypes. All cloned *Minute* loci to date encode ribosomal proteins. *Minutes* were instrumental in defining concepts of cell autonomy, compartmental development, and cell competition. While these mutations are abundant in *Drosophila*, almost nothing is known about mammalian riboprotein gene defects. *Belly spot and tail* (*Bst*) is a semidominant, homozygous lethal mutation that arose spontaneously. Heterozygotes have a white midventral spot, vertebral anomalies (kinked tail), and a profound deficiency in retinal ganglion cells (RGCs). We mapped *Bst* to a 0.5 cM genetic interval on mouse chromosome 16 and identified a mutation in *Rpl24*, a gene encoding a ribosomal protein component of the large ribosomal subunit. The mutation deletes 4 bp from the first intron splice branchpoint of *Rpl24*. It impairs splicing of the first and second exons, resulting in premature truncation of the L24 peptide. Approximately 25% of *Bst* transcripts are correctly spliced, indicating a partial loss of function mechanism. An *Rpl24* BAC rescues the *Bst*/+ phenotype. *Bst* impairs ribosome biogenesis and causes a growth

disadvantage at both the cellular and the organismal levels. *Bst/+* MEFs (mouse embryonic fibroblasts) have decreased rates of protein synthesis and cell proliferation compared to wild-type cultures. The differential growth rate is evident in chimeras derived from ROSA26 (*lacZ*+) ES cells and *Bst/+* or *+/+* blastocysts, with significantly greater R26 contribution occurring in *Bst/+* \ll R26 adult chimeras compared to *+/+* \ll R26 adult chimeras. Our findings establish *Bst* as a mouse *Minute*.

519. **Cited1 Is Required for Normal Placental Development in Mouse.** Duncan B Sparrow,* Sarah L Withington,* Annabelle N Scott,* Jost Preis,* Tristan Rodriguez,† Rosa S. P. Beddington,† and Sally L. Dunwoodie*,†. *Developmental Biology Program, Victor Chang Cardiac Research Institute, Sydney, Australia; and †Division of Mammalian Development, National Institute for Medical Research, London, United Kingdom.

Cited1 is a 27-kDa protein that binds Smad4 and in vitro potentiates TGF β -dependent transcriptional activity. We generated a null mutant allele by deleting the entire coding region of *Cited1* on the X chromosome. This allele leads to lethality as 85% of null individuals die perinatally, and surviving null pups are significantly smaller than wild-type littermates. Low birth weight is presaged by reduced embryo weight at 18.5 dpc, but not at earlier stages. This suggests that a lack of Cited1 may affect the placenta, resulting in intrauterine growth restriction. Backcrosses of heterozygous females with wild-type males results in 47% fewer heterozygous female offspring than expected. However if heterozygous females are mated with null males, the expected numbers of heterozygous female offspring are observed. In the first cross, extraembryonic tissues from heterozygous females will not express the *Cited1* wild-type allele due to inactivation of the paternal X chromosome, suggesting that the defect lies in these tissues. Immunohistochemical analysis reveals high levels of Cited1 protein in the labyrinthine spongiotrophoblast and giant cell layers of the placenta. Thus it seems likely that Cited1 is required for normal placental development. To further investigate the nature of the defect in *Cited1* null placentas, we have used a combination of histology, marker gene expression, and resin casting of embryonic and maternal blood vessels.

520. **Hoxa5 Regional Expression along the Developing Antero-posterior Axis Involves CDX Proteins as Transcriptional Regulators.** Sébastien Tabariès,* Jérôme Lapointe,* Terry Besch,† Christopher K. Tuggle,† and Lucie Jeannotte*. *Centre de Recherche de L'Hôtel-Dieu de Québec, Québec, Canada G1R 2J6; and †Department of Animal Science, Iowa State University, Ames, Iowa 50011, USA.

Hox gene function is intimately linked to their correct developmental expression. Using transgenic mice, we have pursued our study of the regulatory elements involved in the control of the specific spatiotemporal *Hoxa5* developmental expression. We have identified a 2.1-kb mesodermal enhancer sequence, MES, located in 3' of the *Hoxa5* coding sequences. The MES region targets transgene expression in mesodermal derivatives of the cervical region. Deletion analyses have revealed that the MES region contains several DNA regulatory elements among which two appear essential for the establishment of the posterior boundary at the level of prevertebra 10. This posterior boundary corresponds to the normal posterior limit of expression of the

major *Hoxa5* transcript. The two DNA elements, identified as the 169 and the 331 bp fragments, seem to act in synergy to define the posterior limit of expression of *Hoxa5* transgenes. Moreover, the *Cdx* gene products, which are known candidates to set up the correct expression domain of *Hox* genes, interact specifically with the 169-bp DNA fragment via two caudal consensus binding sites. Among the three CDX proteins present in murine embryos, CDX4 appears to be one potential candidate involved in the regionalization of *Hoxa5* gene expression. (Supported by the FRSQ (Canada) and the NIH (USA)).

521. **Withdrawn**

522. **Dissecting the Regulatory Elements of Tbx6 Expression during Mouse Development.** D. R. Farkas, P. H. White, and D. L. Chapman. Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania.

The transcription factor Tbx6 is absolutely required for the development of posterior paraxial mesoderm in the mouse: it is expressed in the primitive streak and presomitic mesoderm. In Tbx6 mutant embryos, posterior somites are replaced by ectopic neural tubes. We are taking a transgenic approach to identify and characterize the *cis*-acting regulatory regions responsible for directing Tbx6 expression during embryogenesis and ultimately the factors directing paraxial mesoderm specification and patterning. We have identified a number of elements capable of driving reporter gene expression in a Tbx6-specific pattern. Several of these transgenes can direct expression in both the primitive streak and the paraxial mesoderm, while others direct expression in a subset of these tissues. Using this approach, we have detected the presence of both positive and negative regulatory elements directing Tbx6 expression. We have also identified the classic mouse mutant rib-vertebrae as a mutation in the regulatory region of Tbx6 (White et al., Development 2003, 130, 1681-1690). This spontaneous mutation results in decreased levels of Tbx6 expression producing a hypomorphic mutation that affects somite patterning and results in rib and vertebral fusions. Mutations in the regulatory region of human TBX6 that decrease expression below heterozygous levels could therefore result in birth defects characterized by vertebral and rib fusions. (Sponsored by NIH NICHD Grant RO1-HD38786.)

523. **The Homeobox Gene caudal Is Involved in Anterior—Posterior Patterning and Development of Posterior Structures in Zebrafish.** Isaac Skromne,* Nancy Hopkins, † and Robert Ho*. *University of Chicago, Department Organismal Biology and Anatomy, 1027 E 57th Street R107, Chicago Illinois 60615, USA; and †MIT, Department of Biology, 31 Ames Street Room E17-341, Cambridge Massachusetts 02139, USA.

To date, little is known about the molecular mechanisms controlling cell proliferation and patterning in the tailbud, the region of the vertebrate embryo which gives rise to the animal's posterior trunk and tail. Knockout experiments in mice and overexpression experiments in *Xenopus* have implicated the transcription factor Caudal in controlling posterior body formation. It is not clear, however, if the posterior malformations observed in these experiments are caused by defects in anterior—posterior patterning or by a tailbud-specific function of Caudal. Here we address the problem of tail formation and the role that

caudal plays in this process in the zebrafish embryo. We show that embryos lacking *caudal* function develop severely truncated tails that lack notochord and blood forming mesenchyme. These defects can be partially rescued in a non-cell-autonomous manner by transplanting wild-type cells into the notochord-forming region of the tailbud. In addition, loss of *caudal* shifts the expression of the Hox genes posteriorly, suggesting that posterior regions of the embryo (i.e., tail) may have acquired more anterior (i.e., trunk) characteristics. These results suggest that *caudal* regulates posterior body formation in zebrafish by (1) specifying different cell types in the tailbud such as notochord and (2) properly patterning the anterior–posterior axis of the embryo.

524. **bHLH Factor pMesogenin1 Is a Transcriptional Repressor Involved in Paraxial Mesoderm Development.** Kyoung-sook Park,¹ Ju-suk Nam,¹ and Jeong K. Yoon. Center for Molecular Medicine, Maine Medical Center Research Institution, Scarborough, Maine 04074, USA.

bHLH class transcription factor pMesogenin1 is specifically expressed in the posterior presomitic mesoderm (unsegmented paraxial mesoderm) during vertebrate embryogenesis. Previous studies indicated that pMesogenin1 may be involved in both cell specification and morphogenetic cell movement of the presomitic mesoderm. However, it is unclear how pMesogenin1 displays its function at the molecular level during presomitic mesoderm development. Here, we provide the evidence that pMesogenin1, together with E12/47 bHLH proteins, acts as a transcriptional repressor. In addition, a candidate target gene for pMesogenin1 has been identified. Our data further indicate that pMesogenin1, indeed, negatively regulates the expression of the identified candidate gene in the presomitic mesoderm. This study brings new insight into the molecular regulation of presomitic mesoderm development. ¹These authors contributed equally to this work.

525. **Assigning Pbx Function in Lateral Plate Mesoderm Patterning.** Jeffrey Schoenebeck,* Jessica L. Feldman,* Cecilia B. Moens,[†] and Deborah Yelon*. *Developmental Genetics Program, Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, New York; and [†]HHMI, Division of Basic Science, Fred Hutchinson Cancer Research Center, Seattle, Washington.

The lateral plate mesoderm (LPM) is a patterned lineage source that gives rise to many tissues including blood, vasculature, limb mesenchyme, and heart. A number of factors are known to promote the development of LPM derivatives; however, the precise roles of these factors in patterning the LPM remain unclear. For example, the bHLH transcription factor *Hand2* is essential for forelimb and heart development, but it is not yet established how *Hand2* function is regulated or complemented during subdivision of the LPM. We are interested in identifying additional factors that contribute to LPM gene expression and patterning. Hox proteins and their cofactors, Pbx and Meis proteins, are appealing candidates. Zebrafish *lazarus/pbx4* (*lzx*) mutants lack pectoral fins and exhibit a dysfunctional heart characterized by a small, poorly contractile ventricle and a dilated atrium. These observations raise the intriguing possibility that Pbx4 may be involved in patterning the LPM. Initial analysis of zygotic *lzx* mutants reveals substantial upregulation of *hand2* and supports the idea that regulation of LPM gene expression may

involve Pbx function. To eliminate maternal loading and assess possible functional redundancies, we are currently analyzing the LPM phenotypes in maternal-zygotic *lzx* mutants produced from germline chimeras, with or without *pbx2* morpholino injections. We will present an overview of Pbx function in the LPM based on our compiled results.

526. **Identifying *spadetail*- and *no tail*- Dependent Factors in Posterior Floor Plate Formation.** T. M. Han and S. L. Amacher. Molecular and Cell Biology, University of California, Berkeley, California.

While the zebrafish T-box genes *spadetail* (*spt*) and *no tail* (*ntl*) are well known to regulate mesodermal differentiation individually, in combination they also impact posterior floor plate development. In addition to completely lacking trunk and tail mesoderm, *spt*^{-/-} mutants also lack posterior floor plate. Because cells that form floor plate and notochord likely arise from common precursors around the shield, we are investigating *spt*- and *ntl*-dependent inductive events during gastrulation. Preliminary results from transplant studies suggest that the most anterior involuting axial mesoderm, the prechordal plate, can induce posterior floor plate formation in *spt*^{-/-} embryos. Based on this observation, we hypothesize that defects in prechordal plate differentiation in *spt*^{-/-} embryos results in failure of posterior floor plate to develop. Thus far, we have found that the types of disruptions in expression of prechordal plate markers such as *hatching gland 1*, *H2.0-like homeobox 1*, and *goosecoid* in both *spt*^{-/-} embryos and morpholino phenocopies to be consistent with convergence/extension defects. We are also investigating the role of *cyclops*, a gene that encodes a TGF- β signaling molecule, in floor plate formation in *spt*^{3-/-} morpholino phenocopies. Finally, we are examining gene expression patterns in morpholino phenocopies using microarray analysis.

527. **Hoxb1 Is Required in Neural Crest for Proper Development of the VIIth Cranial Nerve.** Benjamin Arenkiel, Petr Tvrdik, and Mario Capecchi. University of Utah, Salt Lake City, Utah.

Vertebrate cranial neural crest gives rise to many complex derivatives of the head, neck, and face, including neuronal and glial cells that act in concert for the proper development of the anterior nervous system. Several genes have been implicated in processes of neural crest specification and differentiation; among these are the Hox genes. Hoxb1 null mutants exhibit distinct facial paralysis phenotypes, attributed to the selective loss of facial branchiomotor neurons that innervate crest-derived, second branchial arch tissues. Due to the transient expression and pleiotropic nature of the Hox genes in development, it has been difficult to address specific roles for Hoxb1-expressing neural crest cells in the null mutant. By conditionally targeting the deletion of the Hoxb1 locus in neural crest, we obtained genetic evidence that Hoxb1 functions in r4-derived neural crest during the development of the branchiomotor component of the VIIth cranial nerve. To determine the fates of Hox-expressing cranial neural crest, we conducted a lineage analysis utilizing the Cre/loxP system to drive the activation of ROSA26 reporters under the regulation of the Hoxb1 locus. In addition to forming cartilage, bones, and muscle of the ears and neck, a significant population of r4-derived neural crest is fated to generate the glial component of the VIIth cranial nerve. Taken together, our results

demonstrate that the molecular program governing the development of the VIIth cranial nerve is dependent upon *Hoxb1* not only in the facial branchiomotor neurons, but also in the neural crest-derived glia that contributes to the VIIth nerve fascicle.

528. **Zic2 and Zic3 Proteins Together Regulate Proneural and Neural Crest Domains in Zebrafish.** Michael Keller, Di Jiang, and Ajay Chitnis. National Institute of Child Health and Human Development.

The Zic subfamily of zinc finger proteins have been implicated as candidate patterning genes in the vertebrate neural plate. Experiments with *Xenopus* have shown that the different Zic orthologues are involved in the determination of neural crest and they have implicated Zic2 in preventing formation of neurogenic domains, where neurons differentiate. We used an antisense morpholino translational knockdown strategy to investigate the possible roles of Zic2 paralogues and Zic3 on patterning neurogenic domains in the zebrafish neural plate and adjacent placodes. The expression of these genes is initially very broad in the anterior neuroectoderm but becomes increasingly restricted to the lateral edges of the neural plate and specific domains of the prospective forebrain, midbrain, and hindbrain. We show that the Zic2 paralogues are at least partially redundant and function to limit neural and neuronal gene expression in the anterior forebrain and trigeminal ganglia and enhance recruitment of neural crest. Furthermore Zic3 acts synergistically with the Zic2s in these domains. At least one of these proteins is required for proper hindbrain development, as coincident knockdown of all three severely impairs hindbrain development and recruitment of primary interneurons. These results suggest that zebrafish Zic proteins are important regulators of the size and character of domains within the neural plate but their effects and degree of redundancy or synergy vary among domains.

529. **Transcription Factor ap2a and Patterning of Cranial Neural Crest in Zebrafish.** Robert D. Knight, Sreelaja Nair, Sarah S. Nelson, Yashar Javidan, and Thomas F. Schilling. Department of Developmental and Cell Biology, University of California, Irvine, California.

The neural crest gives rise to much of the craniofacial skeleton, pigment cells, and peripheral nervous system, yet its specification during embryogenesis is poorly understood. Zebrafish homozygous for the lockjaw (low) mutation show defects in all crest derivatives and we show that low encodes a zebrafish relative of ap2a, one of a small family of transcription factors implicated in epidermal and neural crest development. Analysis of early ectodermal markers revealed that neural crest specification and migration are disrupted in low mutant embryos. TUNEL labeling of dying cells in mutants revealed a transient period of apoptosis in crest cells prior to and during their migration that could account for many of the crest defects. Defects in the cranial neural crest, however, are more restricted than the patterns of cell death. Neural and glial derivatives of the crest, for example, are less affected than cartilage or pigment. Gene expression in the mandibular arch is unaffected in low mutants, in contrast to the hyoid arch, which shows severe reductions in *dlx2* and *hoxa2* expression. These studies demonstrate that ap2a is required for early steps in neural crest development that appear to specify certain subpopulations. Similar restricted requirements for other

transcription factors expressed in the crest have been identified and their interactions with ap2a will be presented.

530. **A Novel Chick spalt Gene Expressed in Branchial Arches Reduces Neurogenic Potential of the Cranial Neural Crest.** Meyer Barembaum and Marianne Bronner-Fraser. Division of Biology, 139-74 California Institute of Technology, Pasadena, California 91125.

Cranial neural crest cells differentiate into diverse derivatives including neurons and glia of the cranial ganglia and cartilage and bone of the facial skeleton. Here, we explore the function of a novel transcription factor of the spalt family that may be involved in early cell lineage decisions of the avian neural crest. A chicken spalt gene, most closely related to *Xenopus sal3*, is expressed in the neural tube, migrating neural crest, branchial arches, and transiently in the cranial ectoderm. After overexpression by electroporation into the cranial neural tube and neural crest, we observed a marked redistribution of electroporated neural crest cells in the vicinity of the trigeminal ganglion. In control-electroporated embryos, numerous labeled neural crest cells (>80% of the population) entered the ganglion and differentiated into neurons and glia. In contrast, few (<30% of the population) spalt-electroporated neural crest cells entered the trigeminal ganglion. Instead, they localized in the mesenchyme around the ganglionic periphery or continued further ventrally to the branchial arches. Interestingly, no spalt-electroporated neural crest cells differentiated into neurons. The results suggest that spalt negatively biases the ability of neural crest cells to populate peripheral ganglia and adopt a neuronal fate.

531. **Identification of Sox9 Partner Molecules in *Xenopus* Neural Crest.** Xiao Huang and Jean-Pierre Saint-Jeannet. Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia, Pennsylvania 19104, USA.

Sox proteins form a large class of transcriptional regulators implicated in the control of a variety of developmental processes. They are characterized by a conserved DNA binding domain (HMG-box), through which Sox proteins interact with the promoter region of specific target genes. This interaction allows them to function as transcriptional activator or repressor. However, all Sox factors appear to recognize a similar motif on the DNA, suggesting that Sox proteins may regulate their target genes through interaction with specific partner molecules, thereby allowing Sox proteins to act in a cell-specific manner. One member of this family of gene, Sox9, has been implicated in neural crest formation in *Xenopus*. Depletion of Sox9 protein in developing embryos, using morpholino antisense oligos, causes a dramatic loss of neural crest progenitors. Later during embryogenesis, morpholino-treated embryos have a specific loss or reduction of neural crest-derived craniofacial skeletal elements. To further our understanding of Sox9-mediated gene regulation in the context of the neural crest, we performed a yeast two-hybrid screen to identify potential partners of Sox9 in neural crest tissue. Different portions of Sox9 protein fused to the GAL4 DNA-binding domain were used as baits to screen a *Xenopus* neurula stage cDNA library fused to the GAL4 activation domain. The identification and characterization of such partner molecules may provide important information on the target genes of Sox9 in the developing neural crest.

532. **The Functional Role of the Six3 in Murine Development.** Oleg V. Lagutin, Wei A. Liu, Jacek Topczewski, Lilianna Solnica-Krezel, and Guillermo Oliver. Department of Genetics, St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, Tennessee 38105-2794, USA; and Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee 37232, USA.

Suppression of posteriorizing Wnt signaling is required for the formation of anterior neural structures in zebrafish and frog embryos. Previously we demonstrated that the development of the vertebrate forebrain requires the activity of the homeobox gene *Six3* to repress *Wnt1* expression in the anterior neuroectoderm. In *Six3*^{-/-} mice, the prosencephalon was severely truncated, and the expression of caudal diencephalic markers such as *Pax3* was rostrally expanded, a finding that indicates that the mutant head was posteriorized. These results, together with those of in vitro and in vivo DNA-binding assays and phenotypic rescue of *headless/tcf3* zebrafish mutants by mouse *Six3*, indicate that *Six3* promotes vertebrate head development by direct repression of *Wnt1* in the anterior neuroectoderm. Furthermore, these results support the hypothesis that a Wnt signal gradient specifies posterior fates in the anterior neural plate. Progress in the study of the *Six3* function in the murine forebrain and eye development will be presented.

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535. **Bar-like Homeobox Genes Play a Critical Role in Prepat-
tern the Anterior Neural Plate through Regulation of
BMP Signaling.** Béatrice Durand,^{*}† Heithem El-Hodiri,^{*} and Milan Jamrich^{*}. ^{*}Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, 77030 Houston Texas USA; and †RTG Institut Pasteur, 28 rue du Dr Roux, Paris 75015 France.

In the anterior neural plate, patterns of homeobox gene expression subdivide the neuroepithelium into domains that give rise to the different parts of the forebrain. We have isolated and characterized a highly conserved homologue of the *Drosophila* *barH* genes, *barhl2*, in *Xenopus* and mouse, that appears to be one of the first genetic determinant in the dorsal diencephalic primordium. At early neurula stage *barhl2* expression is restricted to the dorsal diencephalic domain in *Xenopus* and mouse. No other homeobox protein is expressed so specifically and so early in this region of the developing forebrain. We have shown that the *Barhl2* protein acts as a transcriptional repressor and promotes dorsal diencephalic formation at the expense of the neighboring territories in the anterior neural plate. Moreover we provide evidence that *barhl2* mimics BMP signaling in the dorsal part of the embryo during gastrulation. These observations provide one of the first links connecting BMP signaling to genes that help pattern the dorsal part of the anterior neural tube.

536. **Change in BMP Signaling Affects the Nuclear Organization of the Developing Chick Diencephalon.** Youngshin Lim,^{*}

Jeremy Minarcik,[‡] and Jeffrey Goldent,[‡]. ^{*}Department of Biology, and †Department of Pathology, University of Pennsylvania, Philadelphia, Pennsylvania; and ‡The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania.

Bmp signaling is known to participate in dorsal specification of the spinal cord. Recent studies suggest it is also involved in forebrain development. To examine the role of BMP signaling in diencephalic development, we electroporated a constitutively active form of Bmp receptor type IB (ca-bmpr1b) into the neural tube of stage 8-9 chick embryos. Overexpression of ca-bmpr1b generated a range of brain phenotypes. We have classified the electroporated embryos into four different groups (types I–IV) depending on severity of the brain phenotype. Our data indicate that ca-bmpr1b overexpression affects the expression pattern of multiple transcription factors including cLhx2, Lim1/2, Pax6, and Zic, and increases apoptosis in the electroporated side. The changes in gene expression and apoptosis resulted in a later diencephalic phenotype of disorganized nuclear formation. Currently ongoing studies are in progress to examine the BMP loss-of-function using dominant negative forms of the BMP receptor IB.

537. **Systematic Identification of Elements of a Neocortical Pro-
tomap** S. N. Sansom and F. J. Livesey. Wellcome Trust/Cancer Research, UK Institute of Cancer and Developmental Biology, University of Cambridge, Cambridge, United Kingdom.

The neocortex is a highly specialized forebrain structure unique to mammals, which has expanded throughout mammalian evolution. In humans there are over 50 structurally and functionally distinct neocortical areas including visual, motor, somatosensory, and associative areas. Although area formation appears to independent of thalamic innervation, little is known about the genetic mechanisms underlying the specification of areas. Recent evidence has demonstrated the importance of the graded expression both of transcription factors across the neocortex and of extrinsic factors such as FGF8. The majority of differentially expressed factors identified so far show differences along the rostrocaudal axis of the neocortex. To systematically screen for genes differentially expressed along this axis, we used microarrays of over 22,000 oligonucleotides, covering over two-thirds of the mouse genome. We carried out our analysis at several developmental stages, both before and after innervation, and in both inbred and outbred strains. Robust statistical analysis led to the identification of more than 100 genes showing differential rostrocaudal expression. A secondary in situ hybridization screen distinguished between genes expressed in neurons and progenitor cells and enabled the definition of neocortical progenitor cell populations.

538. **The Role of the Transcription Factor Cux2 in Craniofacial Morphogenesis and Neurogenesis.** Angelo Iulianella and Paul Trainor. Stowers Institute for Medical Research.

The patterning of the craniofacial region involves signaling from surface ectoderm of the pharyngeal arches as well as the foregut endoderm underlying them. Members of the bone morphogenetic (bmp) and fibroblast growth factor pathways are active in the ectoderm overlying the arches and olfactory placode, while retinoids and sonic hedgehog signal primarily in the foregut endoderm. How these signaling molecules act to sculpt the

unique characteristics of the vertebrate head is currently poorly understood. A candidate bmp signaling effector we are analyzing is *cux2*, a cut domain and homeobox-containing transcription factor. Although the function of *cux2* is unknown, *cux1* acts as a transcriptional repressor and is implicated in cell-cycle control. We have characterized the early embryonic expression pattern of *cux2* in the mouse and found its transcripts to be distributed at or adjacent to sites of *bmp4* and *msx2* expression, including the distal fusing arch mesenchyme, lateral plate esoderm, urogenital system, limb buds, and interdigital mesenchyme. In addition, we report a highly dynamic pattern of *cux2* expression along the dorsal—ventral axis of the neural tube, with the strongest expression occurring in the roof plate and developing motor neuron pools. In the craniofacial region, limb buds and urogenital system *cux2* transcripts were found in regions undergoing extensive proliferation, while in the neural tube *cux2* was expressed in neurons exiting the cell cycle. This suggests that *cux2*, similar to *cux1*, may play an important role in regulating proliferation during development.

539. **Synergy between FGF Signaling and the Transcription Factor *vhnf1* Is Required during Gastrulation for Rhombomere Specification.** Elizabeth Wiellette and Hazel Sive. Whitehead Institute for Biomedical Research, Cambridge, Massachusetts.

The vertebrate hindbrain is specified early in development, during gastrulation. In zebrafish, cells which will contribute to the caudal hindbrain (rhombomeres 4 through 7) can be characterized at midgastrula stage by the broad expression of various molecular markers. By the end of gastrulation, pattern formation in the caudal hindbrain has been refined, as indicated by expression of *krox20* (r3+r5), *hoxB1a* (r4), and *valentino* (r5+r6) in future rhombomeres. Little is known about the molecular events that divide the early, broad region of the caudal hindbrain into rhombomere-specific domains. Recently, two genetic backgrounds were identified in zebrafish that result in loss of individual rhombomere identity in the caudal hindbrain. Mutation of the zebrafish homolog of *variant hepatocyte nuclear factor* (*vhnf1*) results in loss of r5+r6, with concomitant transformation of these domains toward r4 character. Loss of r5+r6 identity is also observed when Fibroblast Growth Factor (FGF) signaling is eliminated. Therefore, we have investigated interactions between *textitvhnf1* and FGF signals and have found that *vhnf1* functions in two ways to subdivide the zebrafish caudal hindbrain domain into individual rhombomeres. First, *vhnf1* promotes r5+r6 identity through an obligate synergy with FGF signals to activate *val* and *krox20* expression. Second, *vhnf1* functions independently of FGF signals to repress *hoxB1a* expression. We conclude that *vhnf1* promotes the specification of individual rhombomeres, in part by providing cellular competence to respond to FGF signals in a caudal hindbrain-specific manner.

540. **Expression of Zfh Transcription Factor in Differentiated Neurons of the Hindbrain.** Randi P. Stearman and Douglas S. Darling. University of Louisville, Louisville, Kentucky 40292.

Zfh is a member of the Zfh family of transcription factors having a homeodomain and zinc fingers. We previously demonstrated that, in the embryonic telencephalon, Zfh is restricted to progenitor cells of the ventricular zone and is not expressed in

differentiated cells. The goal of this work was to determine whether Zfh is similarly limited to proliferating progenitor cells in the developing hindbrain. During hindbrain development, the lower rhombic lip generates the precerebellar neuroepithelium (PCN), which produces three migratory streams of postmitotic neurons. Immunofluorescence of frozen or paraffin sections of embryonic day 14.5 embryos was used to study Zfh expression in the pons and medulla oblongata. The primary antibodies were anti-Zfh, anti-Proliferating Cell Nuclear Antigen (anti-PCNA), or anti-neurofilament 165. Zfh and PCNA are both strongly expressed in the highly proliferative PCN. In addition, Zfh (but not PCNA) is strongly expressed after the cells exit mitosis, form the anterior precerebellar extramural migratory stream, and migrate across the medulla. Examination of serial sections identifies a cluster of Zfh-positive cells as the trigeminal (V) nucleus of the pons. The Zfh-positive trigeminal cells are closely associated with neurofilament, confirming that these cells are differentiated neurons. Therefore, Zfh expression in the hindbrain involves postmitotic migratory neurons and specific differentiated brain nuclei, including the trigeminal. This is the first demonstration of Zfh expression in differentiated neurons. (Supported by NIDCR DE13614.)

541. **The Nlz Zinc-Finger Protein Acts As a Repressors to Control Segmental Gene Expression in the Zebrafish Hindbrain.** Charles G. Sagerstrom and Alexander P. Runko. Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts.

The zebrafish *nlz* gene, which belongs to a family of zinc-finger proteins related to *Drosophila* *nocA*, is expressed in the hindbrain primordium. *nlz* expression has a rostral limit at the presumptive rhombomere (r) 3/r4 boundary during gastrula stages and progressively expands rostrally to encompass both r3 and r2 by early segmentation stages, suggesting a role for *nlz* in hindbrain development. We find that Nlz associates with the corepressor Groucho, suggesting that Nlz may repress transcription. Consistent with a role as a repressor, misexpression of *nlz* causes loss of gene expression within r2 and r3, likely due to premature *nlz* action in this domain. This is accompanied by a partial expansion in the expression domains of r1- and r4-specific genes. To interfere with endogenous *nlz* function, we generated a form of Nlz that lacks the co-repressor binding site and demonstrated that this construct acts as a dominant negative. We find that interfering with endogenous Nlz leads to expansion of *hoxd4* expression, normally observed caudal to the r6/r7 boundary, into more rostral rhombomeres. We also observe a disruption of segmental gene expression in the caudal hindbrain, e.g., *krox20* expression (normally found in r5) intermingles with *hoxb1a* expression (normally found in r4). We conclude that Nlz is a transcriptional repressor that controls segmental gene expression in the caudal hindbrain.

542. ***vhnf1* and *fgfs* Synergize to Drive *val* Expression in the Zebrafish Hindbrain.** R. E. Hernandez, H. A. Rikhs, and C. B. Moens. Fred Hutchinson Cancer Research Center and HHMI.

The developing vertebrate hindbrain is transiently divided along its anterior—posterior axis into a series of segments, or rhombomeres (r). The individual rhombomeres can be distinguished both morphologically and by segment-specific domains

of gene expression. *valentino* (*val*), the zebrafish *kreisler/mafB* homolog, encodes a bZip transcription factor that is required cell-autonomously for the specification of r5 and r6. We are interested in elucidating the temporal and spatial regulation of *val* expression. Three key inputs are known to contribute to *val* expression. *val* initiation is dependent both on *fgf3/8* expression in r4 and on *vhnf1*, a transcription factor expressed posterior to r4. Maintenance of *val* requires Val protein in a positive autoregulatory loop. We aim to better understand how these three inputs are integrated to induce and maintain *val* expression. Here we show that although *fgf3/8* and *vhnf1* are themselves regulated independently, the ability of *vhnf1* to drive *val* expression is dependent on *fgf3/8*, suggesting that Fgf signals may be required to promote Vhnf1 protein function. Indeed, *fgf* and *textitvhnf1* synergize to drive *val* expression; however, this *vhnf1-fgf* synergy is itself dependent on Val activity, suggesting that Vhnf1 and/or Fgfs may also promote *val* autoregulation. Consistent with this possibility, Val contains several consensus Map Kinase (MapK) sites and is phosphorylated in vitro by MapK, a known downstream effector of Fgf signaling. We present a model in which Fgf signals from r4 function both to promote the Vhnf1-dependent initiation and the Val-dependent maintenance of *val* expression within the developing hindbrain.

543. **Meis Dominant-Negative Approach Reveals a Mechanism during Early Hindbrain Patterning.** Seong-Kyu Choe and Charles G. Sagerstrom. Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605.

In the present study, we reexamined the effect of reduced Meis function on hindbrain development by using a dominant-negative construct, *CPbx4. Both gene expression and neuronal specification in hindbrain region demonstrate that caudal part of the hindbrain transforms into rhombomere (r) 4 in response to reduced Meis function. We show that Hox paralog group 1 (PG1; Hoxb1b, and Hoxb1a) induces a transient r4-like identity in the caudal hindbrain, whereas vHnf1 counteracts it. These observations suggest that the caudal hindbrain undergoes a transient r4-like identity before proper patterning in this region occurs. We then used a morpholino-based approach to examine a combined effect of knock-down of Hox PG1 and Meis function on hindbrain patterning. We find that gene expression in the hindbrain is completely lost with the exception of r1-like weak ephA4 expression throughout the hindbrain. Consistent with this, segment-specific neurons are largely lost and disorganized. This suggests that Hox PG1 is required for the segmentation of rostral as well as caudal hindbrain. Finally, we combined two different Meis dominant-negative molecules to further reduce Meis function and find that expression of the combined Meis dominant-negative molecules generates a similar phenotype from knock-down of both Hox PG1 and Meis function. This indicates that Meis proteins are essentially required for the proper hindbrain patterning. Taken together, we suggest a model in which a presegmented hindbrain is initially transformed into two-segment hindbrain and this process is Meis-dependent.

544. **Class I HDAC Proteins Interact with Hox, Pbx, and Meis In Vitro.** Sigalit Zchut and Charles Sagerstrom. The Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Massachusetts.

We are examining the mechanism whereby Hox proteins and their cofactors, Pbx and Meis, activate transcription of target genes in the zebrafish developing hindbrain. We have shown that Hoxb1b, Pbx4, and Meis cooperate to induce posterior gene expression rostrally and cause extensive transformation of forebrain and midbrain fates to hindbrain fates. However, it is likely that other, as yet unknown, proteins are part of this transcription regulating complex. One possible candidate is Histone deacetylases (HDACs). Using GST pull-down assays we find that Pbx4, Meis3, and Hoxb1b interact with HDAC 1, 2, and 3 in vitro. We demonstrate that HDACs interact with Pbx4 N-terminus, the same domain that binds Meis3. The interaction of HDACs with Meis is through the Meis3 Meinox domain, although not through the residues that bind Pbx4, since Meis mutants that can no longer bind Pbx4 still bind HDACs. To test the effect of reduction in HDAC function on hindbrain segmentation, we used the very potent HDAC inhibitor Trichostatin A (TSA). Embryos were soaked 5-10 hpf for at least 18 hours in 1 μ M TSA, fixed at 24 hpf, and subjected to in situ hybridization. The results show loss of Hoxb1a expression in r4 and of Krox20 expression in r3, but not in r5, suggesting a role of HDACs in hindbrain development. To confirm that HDACs are being expressed in zebrafish hindbrain, we analyzed HDAC 3 expression. The results demonstrate that HDAC 3 mRNA appears at 75% epiboly (8 hpf), and is expressed throughout the embryo from head to tail. Our data so far suggest that there is an involvement of HDAC protein in hindbrain development; however, more work is required to show that HDACs function in vivo with Hox/Pbx/Meis.

545. **Neural Patterning and Retinal Specification in a Rx Knock-out Model.** Elena Kozhemyakina and Peter Mathers. Sensory Neuroscience Research Center and Department of Otolaryngology, West Virginia University, Morgantown, West Virginia.

The mouse Rx homeobox gene is expressed in the eye and ventral forebrain from E7.5 through to adult. Targeted deletion of the Rx gene results in ablation of the optic vesicle and leads to anophthalmia. This deletion shows that Rx is absolutely required to form the optic vesicle. Rx expression is also required for proper anterior neural development. Rx-deleted newborns show altered ventral brain morphology, with defects varying from D-V axis truncation with agenesis of the hypothalamus and pituitary to the complete absence of forebrain and midbrain. We analyzed a series of retinal and ventral forebrain markers in Rx-deleted embryos to elucidate the function of Rx in forebrain development and retinal specification. Consistent with a complete loss in optic vesicle morphogenesis, no optic-specific expression is observed in mutant embryos for the early retinal markers Lhx2, Six6, Chx10, Vax2, or Rx itself. These results suggest that Rx is absolutely required for the initiation of retinal cell fate in the mouse. On the contrary, when mutant embryos were analyzed for ventral forebrain markers from E9.5-11.5, little to no disruption in patterning was observed. Probes included Six6, Vax1, Pax6, and Rx. The fact that Rx expression continues even after its functional deletion suggests that this gene is not required for the initial specification of hypothalamic or posterior pituitary cell fate, unlike the case for the optic vesicle. We are currently examining gene expression patterns of these and other markers at later stages to determine the time at which defects in the ventral neural tube start to appear in this mutant.

546. **The Sonic Hedgehog Signaling Pathway in Ocular Development: Specific and Complementary Roles of the Gli Transcription Factors.** Marosh Furimsky and Valerie A. Wallace. Ottawa Health Research Institute, Ottawa, Ontario, CANADA

Sonic hedgehog (Shh) is a secreted molecule involved in patterning the vertebrate central nervous system, including the eye. Two distinct Shh signaling events are involved at different times during ocular development. During early embryogenesis, Shh from the ventral forebrain is required to form bilaterally symmetrical optic vesicles and to determine vesicle polarity. Shh from the retinal ganglion cells is later involved in the organization and maintenance of the retina and optic nerve. The Shh signaling pathway is mediated by the Gli transcription factors, of which three are known in vertebrates (Gli1, Gli2 and Gli3). The purpose of this study was to determine the unique and complementary roles of the three vertebrate Glis during eye development. We examined the phenotypes in the developing eyes of Gli mutant mice and monitored the expression of genes involved in optic vesicle and retinal patterning using *in situ* hybridization. Results showed that Gli3 is necessary for early optic vesicle patterning, specifically the determination of nasal and ventral polarities, and that Gli2 plays a complementary role in this process. In the absence of Gli3, the optic cup/optic stalk interface was poorly defined and the optic fissure did not close. The activator form of Gli3 is also the primary mediator of the Shh signal in the developing retina. Gli2 plays a complementary role to Gli3 in the retina and may, in association with Gli1, have a more important role in patterning the postnatal retina. During murine ocular development, Gli3 appears to play the principal role in mediating the Shh signaling pathway.

547. **Dlx1 and Dlx2 Homeobox Genes Regulate Vertebrate Retinal Ganglion Cell Differentiation.** J. de Melo, G. Du, M. Fonseca, and D. D. Eisenstat. Departments of Pediatrics, Anatomy, and Physiology, University of Manitoba, Winnipeg, Canada.

Regulation of retinal progenitor differentiation is not fully understood. Dlx1 and Dlx2 transcription factors are expressed in retinal ganglion, amacrine, and horizontal cells in the developing murine retina. Expression begins at E12.5 and is maintained until P0 for Dlx1, while Dlx2 expression extends to adulthood. Dlx2 is coexpressed with Brn3b, Pax6, and Chx10 in overlapping domains at E13.5. Brn3b precedes Dlx2 expression in the nascent ganglion cell layer (GCL). We have assessed the retinal phenotype of the Dlx1 and Dlx2 double knockout mouse. The Dlx1/2 null retina displays a distinct loss of cellularity in the GCL, with a 25% decrease in GCL cell number at P0. There is a corresponding 23% decrease in optic nerve thickness. At E18.5, there is a 34% loss in retinal ganglion cell (RGC) number as determined by quantitation of Brn3B expressing cells in the GCL. The numbers of amacrine and horizontal cells remain relatively unaffected in the Dlx1/2 double mutant. RGC-specific markers are expressed in the double mutant, suggesting that the differentiation of remaining retinal ganglion cells is intact. There is increased activated caspase-3 expression, suggesting increased cell death in Dlx1/2 null retinas at E18.5. We have also identified morphologic abnormalities in horizontal cells in the double mutant. These cells have greater process arborization than littermate controls as determined by NF165 expression. Our results suggest that Dlx1/

Dlx2 are necessary for RGC differentiation and survival. We propose a model where late born RGC progenitors are dependent on Dlx1 and Dlx2 transcriptional regulation.

548. **Wnt/ β -Catenin Signaling in Xenopus Retinal Development.** Terence J. Van Raay,* Milan Jamrich,† and Monica L. Vetter*. *Department of Neurobiology and Anatomy and Program in Neuroscience, University of Utah, Salt Lake City, Utah; and †Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas.

Eye development is regulated by a network of signaling components and their transcriptional effectors. While numerous transcription factors are known to influence retinal development, much less is known about the cell signaling mechanisms that regulate proliferation and differentiation. Here, we show that various components of the Wnt pathway are expressed in the developing retina. Tcf3 and β -catenin are expressed early in eye development and as development proceeds become restricted to the margins of the retina, a region of ongoing proliferation and differentiation. Utilizing *Xenopus* transgenics with a Wnt/ β -catenin reporter, we show that the Wnt/ β -catenin pathway is active during retinogenesis and this activity is coincident with proliferation of retinal progenitors. Further, we have generated *Xenopus* transgenics with the Rx promoter to drive expression of dominant-negative Tcf3 specifically in the developing eye. We show that inhibition of this pathway results in a reduced eye phenotype, supporting our hypothesis that this pathway is involved in retinal progenitor proliferation. This may be mediated by Xfrizzled5 which is specifically expressed in the early eye vesicle, during a period of high proliferation. Currently, we are looking at how inhibition of this Wnt/ β -catenin pathway influences proliferation and differentiation of retinal progenitors.

549. **The bHLH-Zip Transcription Factor Mitf Is Conserved in Drosophila and Is Expressed in the Developing Eye.** Jón H. Hallsson,*† S. Haflidadó,*‡ Chad Stivers,§ Ward Odenwald,§ Francesca Pignoni,‡ Heinz Arnheiter† and Eiríkur Steingrímsson*. *Biochemistry and Molecular Biology, Faculty of Medicine, University of Iceland; †Laboratory of Developmental Neurogenetics, National Institute of Neurological Disorders and Stroke, NIH, Baltimore, Maryland; ‡Harvard Medical School/MEEI, Cambridge, Massachusetts; and §Laboratory of Neurochemistry, NINDS, NIH, Baltimore, Maryland.

The MITF protein is a member of the MYC family of basic Helix-Loop-Helix Leucine Zipper (bHLHZip) transcription factors and is most closely related to the TFE3, TFEC, and TFEB proteins. In the mouse, MITF is required for the development of several different cell types including the retinal pigmented epithelial (RPE) cells of the eye. In *Mitf* mutant mice, the presumptive RPE cells hyperproliferate, abnormally express Pax6, and form an ectopic neural retina. Here we report the structure of the *Mitf* gene in *Drosophila* and demonstrate expression during embryonic development and in the eye. In addition, we show that transcriptional regulation by *Drosophila* Mitf, similar to its mouse counterpart, is modified by the Eyeless (*Drosophila* Pax6) transcription factor. Our results suggest that the *Mitf* gene is the original member of the *Mitf-Tfe* subfamily of bHLHZip proteins. Its expression in the peripodial membrane of the developing fly

eye further supports the common origin of the vertebrate and invertebrate eyes.

550. **Mechanism of Pitx Gene Regulation During Pituitary Development.** M. A. Charles,* H. Suh,† P. J. Gage,* I. Naso-nkin,* C. Liu,† J. Martin,† J. Drouin‡ and S. A. Camper*. *Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan; †Alkek Institute of Biosciences and Technology, Texas A&M System Health Science Center, Houston, Texas; and ‡Institut de Recherches Cliniques de Montreal, Québec, Canada.

Pitx2 is necessary for the development of Rathke's pouch as well as specification of gonadotropes and expansion of the *Pit1* lineage. Without *Pitx2*, the pouch undergoes excessive cell death and fails to proliferate. Gonadotrope development fails in mice with two hypomorphic alleles of *Pitx2* because a low level of PITX2 is not sufficient for expression of several lineage-specific transcription factors. Conversely, transgenic mice that overexpress PITX2 have a dramatic increase in gonadotropes, suggesting that the concentration of *Pitx2* is important for normal pituitary development. Gonadotropes are also deficient in mice when specific isoforms of *Pitx2* are disrupted, indicating that multiple isoforms are important. Unlike single mutants, double knockouts of *Pitx1* and *Pitx2* fail to express the transcription factor *Lhx3*, indicating that they are upstream of *Lhx3* and have compensatory roles during early development. Colocalization studies demonstrate that PITX1 and PITX2 are detectable in almost all adult gonadotropes and thyrotropes, but in very few corticotropes and somatotropes, suggesting an overlap in maintaining pituitary function. Our studies suggest that *Pitx* genes may be important in regulation of gonadotropin and thyrotropin production but may not have a major role in maintaining growth hormone fabrication in mature mice. We are currently using a *Pitx2* floxed allele and a cre recombinase under the control of the GH promoter to investigate this possibility.

551. **Identifying Modifier Genes that Suppress the *Engrailed-1* Cerebellar Phenotype.** Crystal Murcia, Natalie Bilovocky, Rita Romito-DiGiacomo, and Karl Herrup. Department of Neuroscience, Case Western Reserve University, Cleveland, Ohio.

The mouse *Engrailed* genes (*En1* and *En2*) are homeobox containing transcriptional repressors essential to the development of the midbrain and cerebellum. Mice homozygous for a targeted deletion of *En1* (*En1^{hd}*) show a variety of abnormalities including agenesis of the cerebellum and caudal midbrain. The initial characterization of *En1^{hd/hd}* was performed on mice of an inbred 129/Sv or mixed (C57BL/6J x 129/Sv) genetic background. We have found that when this mutation was bred onto the C57BL/6J background, the phenotype was "rescued" in that the cerebellar phenotype is suppressed and the mice are viable. All cell types are present and arranged properly in both cortex and deep nuclei, and cell counts reveal no significant absence of cerebellar Purkinje cells. Folial patterns are nearly normal, although an apparent fusion of lobules IV and V is consistently noted. Significantly, no change in the *Engrailed-2* mutant phenotype occurs after a similar background switch. We are in the process of mapping modifier genes through F1 intercross and F1 backcross strategies. The F1 backcross has yielded candidate intervals that require homozygosity of the C57BL/6J allele. Interestingly, the F1

intercross data excludes these loci as being candidate modifiers, instead requiring a combination of C57BL/6J and 129/Sv alleles. These data reveal locus heterogeneity of the *En1* modifiers in that there is more than one complement of C57BL/6J and 129/Sv alleles that are capable of mediating the rescue phenotype.

552. **Motor Neuron Progenitor Specification Depends on Retinoid Receptor-Mediated Activation of the bHLH Factor Olig2.** B. G. Novitch,* H. Wichterle,* S. Sockanathan,*† and T. M. Jessell*. *HHMI, Department of Biochemistry and Molecular Biophysics, Center for Neurobiology and Behavior, Columbia University; and †Department of Neuroscience, Johns Hopkins School of Medicine.

The generation of motor neurons (MNs) in the developing spinal cord depends on Shh signaling and a strategy of transcriptional derepression in which the emergence of the MN fate is achieved through the repressor activities of Nkx6 and Olig2 proteins. A key unresolved question in the MN differentiation pathway is the nature of the molecular steps that activate the expression of target transcription factors in the derepressed context provided by Nkx6 and Olig2 function. In this study, we provide evidence that MN generation depends on retinoid signaling and the function of retinoic acid receptors as transcriptional activators in MN progenitors. This retinoid-mediated program of transcriptional activation converges with the Shh-triggered program of transcription repression at steps downstream of Nkx6 protein function, first to induce Olig2 expression and then to direct the expression of motor neuron transcription factors downstream of Olig2. We also demonstrate that a third class of signals, FGFs, can mimic the ability of Shh to establish a derepressed cell context in which retinoids can induce Olig2 expression and promote motor neuron differentiation. Together, these findings reveal that a retinoid-triggered program of transcriptional activation intersects with the Shh-triggered regulation of transcriptional repressors to elicit motor neuron specification and differentiation.

553. **The Role of runx Genes in Rohon-Beard Sensory Neuron Development in Zebrafish.** Mariya Rzaszutak,† Pete Simpson,* Dawn Riedel,* and Kristin Bruk Artinger*. *Department of Craniofacial Biology and †Cell and Developmental Biology Graduate Program, University of Colorado Health Sciences Center, Denver, Colorado.

We hypothesize that Runt-domain transcription factors play a role in cell fate decisions between Rohon-Beard sensory neurons and neural crest cells in zebrafish. Both these populations of cells arise at the junction between the neural plate and non-neural ectoderm. Based on evidence from the zebrafish mutation narrowminded (*nrd*), we propose that these cell types may be related by lineage and/or respond to the same set of inductive cues. Runt-domain transcription factors are involved in cell fate decisions in the developing nervous system in *Drosophila*, suggesting that these genes may act similarly in vertebrates. Of the two known runt genes in zebrafish, *runxa* and *runxb*, only *runxb* is expressed in the nervous system. *Runxb* is expressed in Rohon-Beard sensory neurons and neural crest derivatives, such as trigeminal ganglia. *Runxb* is not expressed in the dorsal spinal cord of *nrd*, since the Rohon-Beard sensory neurons are absent, but is present in the trigeminal ganglia and lateral line primordia. Overexpression of *runxb* in wildtype embryos causes an increased

number of Rohon-Beard sensory neurons, although overexpression has not been shown to rescue the *nrd* mutation. Antisense Morpholino knockdown of *runxb* shows an absence of Rohon-Beard sensory neurons at the 2s stage, partially phenocopying the *nrd* mutation. Thus, *runxb* may act downstream of *nrd* and may be required for the specification of Rohon-Beard sensory neurons. Ongoing experiments aim to determine the role runt genes in Rohon-Beard neuron and neural crest cell specification. Further, we are cloning novel and candidate runt genes in zebrafish to test candidacy of these genes for the *nrd* mutation. Supported by the NIDCR K22 DE14200.

554. **Sp8, a New Sp Family Member Crucial for Limb Outgrowth and Neural Tube Closure.** Sheila Bell, Claire Schreiner, Steven Potter, and William Scott. Division of Developmental Biology, Cincinnati Children's Hospital Medical Center.

Legless (lgl) transgene insertional mutants are characterized by the absence of hindlimb structures distal to the femur, a variable loss of anterior forelimb structures, randomized visceral situs, and craniofacial malformations. The *Lrd* and *Sp4* genes are known to be deleted from the *textitlgl* genome however targeted ablation studies indicated that neither were responsible for the limb and craniofacial malformations. We have identified a new member of the zinc finger transcription factor gene family, *textitSp8* located telomeric to the *Sp4* gene. In situ hybridization assays defined localized *Sp8* expression in the limb AER precursor population and mature AER, dorsal and ventral limb ectoderm, tail bud, isthmus, lateral and medial nasal processes, and regions of the telencephalon. Expression at these sites in the *lgl* mutant was dramatically diminished. Progeny resulting from crossing *lgl* heterozygotes with *Sp8* (+/-) animals exhibited an increased incidence of forelimb and craniofacial malformations compared to *lgl* homozygotes. The *Sp8*(-/-) phenotype was more severe. All progeny possessed exencephaly with frontal facial clefts, missing tail, spina bifida, and are missing structures distal to the humerus in the forelimb and to the femur in the hindlimb. *Fgf8* expression is initiated in the developing *Sp8*(-/-) limb bud but is rapidly lost by the AER precursor cells and an AER fails to form. We conclude that *lgl* is a hypomorphic allele of the *Sp8* gene that functions in a secondary phase of AER development and in other sites in the embryo that are also co-incident with localized FGF8 signaling.

555. **Microarray Analysis of limb Patterning and Development.** Iaroslava Rouzankina, Allison Tobey, and Lee Niswander. Sloan-Kettering Institute and WGSMS of Cornell University.

The vertebrate limb is patterned along the anterior-posterior (A/P), dorsal-ventral and proximal-to-distal axes. Patterning along the A/P axis is regulated by the gradient of Shh signaling with the maximal activity in the posterior limb. Shh is necessary to inhibit Gli3 transcriptional repressive activity that is highest in the anterior of the limb. Even though several other factors have been identified to regulate limb development along the A/P axis, many questions related to A/P patterning are still unresolved and our knowledge of molecules involved in this process is far from being complete. To identify genes differentially expressed along the A/P axis of the mouse limb at 9.5 and 10.5 dpc. when the A/P patterning is being established, we have used DNA microarray technology. In the course of the screen we have identified about 200 differentially expressed sequences, some of which represent

genes that have been previously described as having a role in A/P patterning of the limb. More than 80 identified sequences represent known and novel genes whose function in limb development has not been investigated. We have begun to characterize the embryonic expression of these genes and examine their function in the limb by rapid misexpression analysis in chick embryos, since major processes of early limb patterning and development are conserved in the chick and mouse systems. Further functional analysis will be carried out for selected genes in the mouse system.

556. **Homeotic Transformation of First Branchial Arch and Cleft Limbs in *Dlx5/Dlx6* Double Mutant Mice.** Giorgio Merlo,* Stefano Mantero,* Michela Maggioni,* Annemiek Beverdam,† Laura Paleari,† Francesca Genova,† Giovanni Levi+**. *Dulbecco Telethon Institute CNR-ITB Segrate, Italy; †Laboratory of Morphogenesis IST, Genova, Italy; and **Laboratoire de Physiologie, CNRS UMR 8572, MNHN, Paris, France.

Craniofacial patterning results from complex interactions between neural crest cells and the mesoderm, endoderm and ectoderm of branchial arches (BA). Anterior Hox genes play a fundamental role in this process. This is not true, however, for the first BA where Hox genes are not expressed. *Dlx* genes are vertebrate homologues of *Drosophila* distalless (*dll*). They are expressed in the limb buds, in the basal forebrain and in the BAs. Inactivation of individual *Dlx* genes in the mouse leads to multiple defects in BA derivatives, but no obvious homeotic transformations. We have generated *Dlx5/Dlx6* double mutant mice. *Dlx5/Dlx6* homozygous mutants die perinatally with limb and craniofacial defects. The hindlimbs show deep clefts, revealing for the first time a conserved role of *dll/Dlx* genes in limb development from insects to vertebrates and confirming their involvement in the human Split Hand/Foot Malformation. Moreover most BA-derived structures are severely affected in these mice. The mandibular process has acquired the identity of a maxilla, causing a mirror image transformation of the lower jaw into an upper jaw-like structure. Moreover, vibrissae are present in both the upper and lower jaws, consistent with the acquisition of symmetry of mouth structures. This is the first evidence of a homeotic transformation in a Hox-free region of the developing head caused by gene inactivation. Interestingly, a *Dlx5* and *Dlx6* gene dosage effect can be demonstrated both in the limbs and in the mandibular arch. These data further suggest that *Dlx* genes are major actors in determining the form and evolution of the vertebrate face.

557. **Evidence in Support of a New Model of Epibranchial Placodes as Dynamically Shrinking and Segementing Regions of Neurogenic Ectoderm.** Yasuo Ishii and Paul J. Scotting. CBTRC, Institute of Genetics, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH, United Kingdom.

The epibranchial placodes are specialized areas of surface ectoderm that make vital contributions to the peripheral nervous system, producing neurons of the cranial sensory ganglia. They have long been characterized as a series of patches of thickened ectoderm in the vicinity of each pharyngeal cleft. We have recently demonstrated that *Sox3* is not only expressed in all of these structures but also marks an earlier larger post-otic domain.

In the present study, we demonstrate that neurons are produced from the *Sox3* positive ectoderm that lies outside of the prospective regions of the classically-defined epibranchial placodes. Fate mapping studies show that these regions of early neurogenesis contribute neurons to the cranial ganglia but then cease producing neurons as they lose *Sox3* expression, with coincident thinning of the ectoderm. Thus, the epibranchial placodes should be redefined as dynamically shrinking and segmenting structures, marked by *Sox3* expression.

- 558. Sonic Hedgehog Regulates Patterning in the Pharyngeal Region by Maintaining Pharyngeal Pouch Identity and Morphology.** Billie A. Moore-Scott,* Nancy R. Manley*†. *Medical College of Georgia Institute for Molecular Medicine and Genetics, Augusta, Georgia; and †University of Georgia Department of Genetics, Athens, Georgia 30602.

Correct patterning within the pharyngeal region is required for the specification of cell types that give rise to multiple organs, vascular and skeletal components in the head and neck. Sonic hedgehog is a secreted glycoprotein required to establish polarity within multiple tissues. Recent data has implied a broad role for Shh in the regulation of genes throughout the pharyngeal region weighted mostly towards the more caudal portions. We have determined that the impact of Shh expression in pharyngeal region patterning is in the first and second pouches and to a lesser extent the third. Although the null phenotype is severe, all of the pharyngeal pouches and arches form by E9.5. Pax1, Fgf8, Hoxa3 and Hoxb1 are expressed properly confirming the formation and patterning of the first through fourth pouches. However by E10.5 the arches begin to atrophy and the first pharyngeal pouch is missing. Fgf8 expression is upregulated in the second pouch. Pax1 is expressed equally in the second and third pouches instead of mostly in the third, and Gcm2 is undetectable in the third. Both Hoxa3 and Hoxb1 expression remain unaffected. This indicates that identity of the more caudal fourth pouch is not Shh dependent while expression of some but not all third pouch markers are affected by Shh expression. In summary, Shh is not required for the initial formation and patterning of the pharyngeal region, but is essential for maintenance of the first pouch and the subsequent patterning of the second and third pharyngeal pouches.

- 559. Functional Analysis Of Zebrafish *Dlx* Gene Expression in the Pharyngeal Arches.** Steven Sperber and Marc Ekker. Ottawa Health Research Institute, Department of Cellular and Molecular Medicine, University of Ottawa, Ontario Canada.

The vertebrate *Dlx* genes are a family of homeodomain containing transcription factors expressed in tissues that include the forebrain, branchial arches, olfactory and otic placodes and limb/fin buds. Generally organized into convergently transcribed bigene clusters, paired genes exhibit overlapping expression patterns attributed to shared *cis*-acting regulatory elements. However, the mouse *Dlx2* gene and its zebrafish ortholog, *dlx2a*, are exclusively expressed in the migrating cranial neural crest cells that contribute to the branchial arches. *dlx2a* arch expression is followed by the linked *dlx1a* gene and other *dlx* pairs that include *dlx3b/dlx4b* and *dlx5a/dlx6a* suggesting the existence of familial cross-regulation. In mice, knockouts have revealed that *Dlx* genes are responsible for the proximodistal polarity of the jaws and participate in a homeobox gene code that specifies development

of hard tissue derived from the branchial arches. To investigate the function of zebrafish *dlx2a* in the neural crest cells and subsequent arch formation we knocked down its gene activity using an antisense morpholino oligonucleotide. Significant morphological changes were observed including malformation of the arch cartilages as well as delays in melanocyte pigmentation. Morphants revealed a loss of *dlx* gene and other arch marker expression patterns. These results suggest combinatorial expression of *dlx* genes in the branchial arches is necessary for proper arch formation and achieved through cross-regulation between family members. Supported by the March of Dimes Birth Defects Foundation, NSERC and the OGSST program.

- 560. Understanding the Interaction Between MEIS and AbdB-Like HOX Proteins: Nature of Interaction and *in vivo* Applicability.** Thomas M. Williams, Melissa E. Williams, and Jeffrey W. Innis. Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48109.

HOX proteins specify different developmental fates by controlling expression of downstream target genes. One model for target-site specificity at *cis*-acting regulatory sequences involves interactions of HOX proteins with other DNA-binding cofactors, including MEIS homeodomain proteins from the TALE-class. Previous work has shown that AbdB-like HOX proteins form DNA-binding complexes with both MEIS1A and MEIS1B. In addition, trimeric complexes have been identified *in vivo* that includes a second TALE-class protein, PBX. Yet the critical residues involved in these interactions are not fully understood. In the developing mouse limb bud members of the Hox paralog group 13, *Hoxa13* and *Hoxd13*, are expressed in the distal most region while *Meis1-3* are restricted proximally preventing interaction. Outside of the developing limb, *Hoxa13* is expressed in the developing Müllerian duct as well as the mature female genital tract, two regions of documented *Meis* expression. Using yeast two-hybrid methods, we have demonstrated that each group 13 HOX paralog can interact with MEIS1A and MEIS1B. We have localized critical amino acid residues within MEIS and group 13 HOX proteins, necessary for interaction. In addition we have demonstrated that MEIS2 and MEIS3, unlike other TALE-class proteins, can interact with AbdB-like HOX proteins. The work here explores the nature, extent and location of MEIS/HOX interactions to advance our understanding of where these interactions are of function *in vivo*.

- 561. Genetic and Nutritional Modifiers of Hox Gene Function.** Claudia Kappen. University of Nebraska Medical Center, Omaha, Nebraska 68198-5455.

Hox genes are crucial for patterning of the vertebrate body plan. They are highly conserved, and changes in the expression of Hox genes cause developmental defects that may be incompatible with survival. This raises the question how mutations in Hox genes can successfully lead to variations in body plan. We have found genetic differences between inbred mouse strains in phenotypic expression of a Hox mutation. Hoxb-6 mutants exhibit homeotic transformations of vertebrae in the cervico-thoracic region. On a 129Sv background, this leads to lack of the first pair of ribs; on a C57Bl/6 background, this pair of ribs develops. Backcrosses demonstrate that this trait is strain-dependent, evidence for modifier loci for the phenotypic expression of Hox gene function. In C57Bl/6 Hoxb-6 mutants, the first pair of ribs does not articulate properly to the vertebral body. We found that

articulation in *Hoxb-6* mutant mice is restored to normal by administration of folate. This essential nutrient is known to prevent birth defects in humans. These results establish nutrition as a second modifier of *Hox* gene function. The important implication for evolution is that with sufficient supply of nutrients, *Hox* gene mutations may accumulate without detrimental effects. Such phenotypic masking would allow for variations that only come under selection when conditions drastically change. Similarly, genetic modifiers may prevent a mutation from becoming detrimental, and thus may allow for variations that only manifest phenotypically with concomitant changes in the modifiers. The coalescence of mutations and gene-environment interactions may be involved in body plan changes.

562. **Using Transcription Activation Mutants to Identify *Hox* Gene Targets *In Vivo*.** *Erin K. Engelhardt, †Sarah E. Bondos, and †Kathleen S. Matthews. *Department of Bioengineering and †Department of Biochemistry and Cell Biology, Rice University, Houston, Texas 77005.

Hox transcription factors specify position along the anterior-posterior axis during the development of all metazoans. Misexpression of these proteins can cause severe morphological deformities and loss of function mutations are often lethal. The variation in DNA binding sites with similar binding affinity complicates identification of the downstream gene targets for the *Hox* protein family. To identify new *Hox* gene targets, we created two mutants of a *Drosophila melanogaster* *Hox* protein, Ultrabithorax (*Ubx*), that specifically alter transcription regulation by *Ubx* by modulating stability of an alpha-helix critical for transactivation. The inactive proline mutant binds DNA and inappropriately represses transcription in promoter-reporter assays in *Drosophila* S2 cells, while the alanine mutant exhibits increased activation. These constructs, along with wild-type *Ubx*, have been expressed in *Drosophila* using the GAL-4-UAS system, enabling us to compare transcription activation and repression by wild-type and mutant *Ubx* transcription activation and repression in a time and tissue specific manner in embryo and larval development. We will specifically investigate known gene regulation by *Ubx* in the embryonic visceral mesoderm, antenna, and wing imaginal discs to verify altered activity of these mutants *in vivo*. DNA chip analysis will reveal novel downstream gene targets that are potentially regulated by *Ubx*.

563. ***Hoxc13b* Expression Pattern and Function in Early Zebrafish Development.** R. Thummel, * L. Li, † M. Sarras, Jr., † and A. R. Godwin*. *Department of Molecular and Integrative Physiology, †Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, Kansas 66160.

Homeobox containing (*Hox*) genes are a group of evolutionarily conserved transcription factors which are involved in specifying the vertebrate body plan. In addition, as cell- and region-specific selectors, they often play specialized roles in organ systems, or in organogenesis. Considerable progress has been made in understanding the role of individual *Hox* genes in the mouse and fruit fly. Recently, however, effort has been made to study *Hox* genes in different species in order to determine the evolutionary conservation of *Hox* gene functions. We have focused our studies on the *Hox* genes in zebrafish. Due to a duplication of four ancestral clusters, zebrafish contain a large number of *Hox* genes. After

millions of years in divergence, 47 *Hox* genes on 7 clusters have been maintained. Such a duplication event leads to the interesting possibility that two zebrafish orthologs have diverged in structure, expression pattern, and function. We have chosen to analyze the two *Hoxc13* orthologs in zebrafish for such divergence. We have cloned and sequenced both genes and find regions of protein divergence, especially in the N-terminal region. Moreover, we have found that both *Hoxc13* orthologs are differentially expressed. In this regard, special emphasis will be given to an unexpected expression pattern of *Hoxc13b*. Finally, morpholino-mediated gene knockdown experiments point to a role of *Hoxc13b* in early embryonic development.

564. **Using Microarray Analysis to Search for *Hoxd* Targets in the Developing Limbs and Genitalia of Mice.** John Cobb, József Zákány and Denis Duboule. Department of Zoology, University of Geneva, Geneva, Switzerland.

Despite the importance of *Hox* genes in patterning the mouse embryo, few target genes of the *Hox* transcription factors have been identified. To search for *HoxD* targets we contrasted gene expression profiles in the presence and absence of the *HoxD* genes in two tissues where these genes are important in embryonic patterning: the genital bud and the autopod domain of the limb bud. The *Del9* mutant, in which all nine of the *HoxD* genes are deleted, shows perturbed digit and genital morphogenesis. Therefore we used Affymetrix GeneChip arrays to compare gene expression in limb and genital buds from wild-type and *Del9* E12.5 embryos. Of the 12,000 probe pairs on the U74Av2 chip, only 31 genes showed differential expression (>1.5-fold) between wild-type and *textitDel9* limb buds. In genital buds, 25 genes showed differential expression between wild-type and *Del9*. There were no common candidate genes found in both limb and genital buds, and no genes showed greater than 2.5-fold change in expression. In limb buds, several genes known to be involved in limb development showed the most statistically significant differences (*Aldh1a2*, *Shox2*, *Papss2*, *Cbx2*, *textitStra6*). In contrast, in genital buds the candidates include another *textitHox* gene (*Hoxa11*) and genes involved in insulin (*Grb10*, *Igfbp3*), glucocorticoid (*Sgk*, *Anxa1*) and Fgf signaling (*textitSox2*, *Tead4*). Both groups include genes involved in neuronal pathfinding: *EphaA3* in limb buds and *Gfra2* in genital buds. Remaining candidates include many uncharacterized cDNAs and genes of diverse function. We shall confirm these findings by real-time PCR or *in situ* hybridizations.

565. **Paralogous *Hox10* and *Hox11* Genes Are Required for Global Patterning of the Mammalian Skeletal System.** Deneen M. Wellik and Mario R. Capecchi. Howard Hughes Medical Institute and University of Utah, Salt Lake City, Utah 84112.

The spectrum of perturbations of the mammalian skeleton resulting from either gain or loss of function mutations in individual *Hox* genes has been difficult to interpret in terms of a coherent model of how these genes participate in the patterning of the axial skeleton. Typically, these morphological changes involve perturbations in one or a small number of vertebrae. By generating mice in which all members of the *Hox10* or *Hox11* paralogous group are disrupted, we provide evidence that the mammalian *Hox* genes are involved in global patterning of the axial skeleton. In the absence of *Hox10* paralogous function, no lumbar vertebrae are formed. Instead, ribs project from all verte-

brae extending caudally from the last thoracic vertebrae to beyond the sacral region, and these elements assume a thoracic morphology. In the absence of Hox11 paralogous group function, sacral vertebrae are not formed and instead, these vertebrae assume a lumbar identity. Despite global changes in the vertebral identity of the posterior axial column, the anteroposterior (AP) position of the hindlimbs is not affected by these mutations, but loss of Hox10 paralogous function does severely affect the patterning of the hindlimb stylopod, while Hox11 paralogous mutants display disrupted zeugopod patterning in both the forelimb and the hindlimb.

566. **Direct Interaction between Gli3 and Hoxd Proteins Alters the Sonic Hedgehog Pathway and Skeletal Patterning during Limb Development.** Yuting Chen, Vlado Knezevic, Valerie Ervin, and Susan Mackem. Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.

The 'pattern' of different skeletal elements of the limb that form from anterior to posterior (eg. different digits: thumb to little finger) is regulated by secreted Sonic hedgehog (SHH) signals from the posterior edge of the limb bud. The Gli3 transcriptional regulator is a major direct mediator of the SHH pathway in the developing limb. Active SHH signaling prevents cleavage of the full length Gli3 activator to a repressor form that is otherwise predominant and prevents Shh target genes from being expressed. Hoxd genes are thought to be among key targets of Sonic hedgehog (Shh) signaling in the limb. We have found that increasing the level of Hoxd12 by transgenic expression and decreasing the level of Gli3 in mouse embryos has a synergistic effect on Shh expression and produces multiple digit duplications. Hoxd12, as well as certain other 5'Hoxd genes, interact directly with Gli3 *in vitro*, suggesting a possible mechanism for this synergy. In transfected cells, Hoxd12 converts the Gli3 repressor form into a transcriptional activator, thereby reversing its function on Shh target genes and activating the Shh pathway. Several 5'Hoxd genes and Gli3 are expressed at different levels along the distal limb bud, and we propose that different Hoxd:Gli3 ratios could lead to differential activation of Shh target genes across the limb bud. This interaction may be particularly relevant to the late regulation of digit identity, when Shh expression has declined, but 5'Hoxd genes and Gli3 are still highly active.

567. **GLI2 and GLI3 are Required for SHH Dependent Sclerotome Induction.** L. Buttitta,* C. C. Hui,† and C. M. Fan*. *Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210; and †Program in Developmental Biology, The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada.

SHH signaling is essential for sclerotome development in mammals. SHH activates expression of target genes through the family of GLI transcription factors, GLI1, GLI2, and GLI3. GLI2 and GLI3 are thought to be the primary mediators of SHH signaling, however their roles in SHH induction of sclerotomal genes have not been investigated. Using a combination of mutant analysis and *in vitro* explant assays, we demonstrate that GLI2 and GLI3 are required for SHH dependent sclerotome induction. This requirement is tissue autonomous, as somitic mesoderm from *Gli2*^{-/-}*Gli3*^{-/-} mutant mice cannot activate sclerotomal genes in response to exogenous SHH. While these mutants

exhibit a severe loss of sclerotomal gene expression, we find that one copy of the *Gli3* gene is sufficient for SHH induction of sclerotomal markers *Pax1* and *Pax9* *in vivo* and *in vitro*, revealing an activator function for GLI3. We further investigated GLI functions in the somite by overexpression in somitic mesoderm explants. Our overexpression studies reveal that different GLIs activate different SHH target genes, thereby suggesting that the functions of SHH in patterning, growth, and negative feedback are divided preferentially amongst different GLIs.

568. **The Role of WNT/Beta-catenin in Mammalian Myogenesis.** A. E. Chen and Chen-Ming Fan. Carnegie Institution of Washington, Department of Embryology, Baltimore, Maryland 21210.

Vertebrate skeletal muscle originates from somites, segmented mesodermal tissue that develops on both sides of the embryonic axis. Muscles of the deep back arise from the medial somite, whereas muscles of the limb and body wall arise from the lateral somite. *Myf5* and *MyoD* are essential genes that confer commitment to the medial and lateral fates, respectively. Myogenesis is dependent upon signals from adjacent tissues, namely, the axial structures (neural tube and notochord), and the surface ectoderm. Co-culture experiments have demonstrated that members of the WNT family, expressed in the neural tube and surface ectoderm, provide the inductive cues for *Myf5* and *MyoD* expression. However, the mechanism by which WNT does so remains unknown. Recent work has demonstrated that WNT proteins can be subdivided into two functional classes based on preferential signaling through either a canonical WNT/ β -catenin pathway or a non-canonical pathway. To investigate the contribution of the WNT/ β -catenin pathway to myogenesis, we generated adenoviruses carrying constitutively active β -catenin, *Axin*, and dominant-negative *Tcf-1* for overexpression in the mouse pre-somitic mesoderm. We provide evidence that canonical WNT signaling via β -catenin is necessary but not sufficient to activate myogenesis.

569. **Expression of Tendon-Related Gene *Six1* and *Six2* in Normal and *Lmx1b* Knockout Mice during Limb Development.** Takuji Naruse,* Charmaine U. Pira,* and Kerby C. Oberg*†. *Department of Pathology, Division of Human Anatomy and †Pediatric Pathology, Loma Linda University, Loma Linda, California.

The reported expression of *Six1* and *Six2* localized to developing dorsal and ventral tendons, respectively, may indicate a role in specifying tendon attachment. The Lim-Homeodomain transcription factor, *Lmx1b*, is known to specify dorsal fates during development and in knockout (KO) mice, limbs display distal ventral-ventral digit, joint and soft tissue symmetry. Thus, we examined the expression of *Six1* and *Six2* (by ISH) during digital joint formation (E12.5 - E18.5) in wild type (WT) and *Lmx1b* KO mice. In the autopods of WT mice, *Six1* localizes to the dorsal extensor hood (DH), lateral stabilizing ligaments of digital joints, and tendons of several extensors, lumbricals (LM) and interossei (IO). *Six2* is expressed in the major extrinsic flexors and extensor, but weakly in joint-associated ligaments. In *Lmx1b* KO mice, LM and IO are symmetrically duplicated with their tendons fusing laterally as they attach to the proximal phalanx; further no DH forms. *Six1* expression persists in the fused tendons and lateral digital ligaments. The expression of *Six2* is symmetrical in the

duplicated flexor tendons. The pattern of expression suggests a role for *Six1* in the development of tendons/ligaments that support and stabilize fine motor movement of the digits, while *Six2* may play a role in specifying tendons associated with gross motor movement. Although *Lmx1b* may regulate the dorsal attachment of *Six1* expressing tendons and ligaments, *Six1* does not appear to be a direct target of *Lmx1b* regulation since *Lmx1b* KO mice have persistent *Six1* expression.

570. **Identification of Gli3 Target Genes by Microarray Analysis of the Polydactylous Mouse Mutant, Extra-Toes.** E. C. McGlinn, K. Lammerts van Bueren, A. M. Poh, B. J. Wainwright, and C. A. Wicking. Institute for Molecular Bioscience, University of Queensland, St. Lucia, Queensland, Australia.

Recent evidence suggests that early limb patterning results from mutual antagonism between the two transcription factors dHand and Gli3. Gli3 also prevents anterior limb bud expression of 5' HoxD genes, Gremlin and later, of Sonic hedgehog (Shh). Loss of Gli3, as seen in the natural mouse mutant extra-toes (Xt) results in Shh-independent polydactyly, presumably by anterior expansion of patterning molecules. We sought to identify novel targets of Gli3 that are dysregulated in the mutant and thus are potentially involved in limb patterning and polydactyly. This was achieved by microarray analysis of anterior limb buds at various embryonic stages. Samples were used to interrogate the National Institute of Aging 15K cloneset as well as a 2K embryonic branchial arch custom cloneset. We have identified a range of known and novel transcripts which, by whole mount *in situ* hybridisation, show altered expression in the Xt limb. *In vitro* Shh signalling assays were then utilized to identify which of these genes are directly influenced by the Shh signalling pathway.

571. **Regulation of Chondrocyte Maturation by the Transcription Factor Nkx3.2/Bapx1.** Sylvain Provot,*† L. Charles Murtaugh,* Hervé Kempf,* Ung-Il Chung,† Henry Kronenberg,† and Andrew B. Lassar*. *BCMP Department, Harvard Medical School, Boston; and †Endocrine Unit, Massachusetts General Hospital, Boston.

The proper formation and growth of limb bones requires a tight control over the rate at which cartilage cells divide and mature. A feedback loop between Indian hedgehog (Ihh) and Parathyroid hormone related protein (PTHrP) is essential to maintain a pool of dividing, immature chondrocytes at the ends of growing long bones. Here we identify the transcription factor *Nkx3.2/Bapx1* as a potential key participant in this pathway. In chick and mouse, expression of *Nkx3.2/Bapx1* is restricted to the proliferative zone and is downregulated as maturation begins. Artificially preventing this downregulation, by retroviral misexpression of chick *Nkx3.2* inhibits chondrocyte maturation and traps the chondrocytes in an immature state. *Nkx3.2* acts as a transcriptional repressor and this activity is essential for its ability to inhibit chondrocyte maturation. Moreover, we show that a reverse function form of *Nkx3.2*, converted into a transcriptional activator, accelerates chondrocyte maturation. In addition, we demonstrate that retroviral misexpression of *PTHrP* in limb buds maintains *Nkx3.2* expression and leads to a maturational arrest of developing cartilage. Conversely, *Nkx3.2/Bapx1* expression is lost in the growth plate of either *PTHrP*^{-/-} or *PTHrP-receptor*^{-/-}

mice. While these results suggest that expression of *Nkx3.2* in immature chondrocytes acts to repress the expression of genes normally required for chondrocyte maturation, the precise relationship between *PTHrP* signaling and *Nkx3.2/Bapx1* expression requires further analysis.

572. **Smad-Dependent Transcriptional Repression by Nkx3.2.** Dae-Won Kim and Andrew Lassar. Harvard Medical School.

We have previously shown that *Nkx3.2*, a transcriptional repressor that is expressed in the sclerotome and developing cartilage, can activate the chondrocyte differentiation program in somitic mesoderm in a BMP-dependent manner. In this work, we elucidate the molecular mechanisms of which the BMP- Smad pathway activates transcriptional repressor function of *Nkx3.2*. We have found that *Nkx3.2* forms an *in vivo* complex with HDAC1 and Smads 1/4 in a BMP-dependent manner. The homeo and NK domains of *Nkx3.2* support the interaction with HDAC1 and Smad1, respectively, and both of these domains are required for the repressor activity of *Nkx3.2*. Furthermore, the recruitment of an HDAC/Sin3A complex to *Nkx3.2*, which is essential for *Nkx3.2* to repress transcription, requires the interaction with Smads 1/4. Consistent with this, *Nkx3.2* is completely unable to repress transcription in a Smad signaling null background. Taken together, while prior work has indicated that BMP-dependent Smads can support transcriptional activation, our findings suggest that BMP-dependent Smads can also potentiate transcriptional repression depending upon the identity of the Smad interacting partner.

573. **Expression Screen of Transcription Factors in the Mouse Metanephric Kidney.** Jing Yu and Andrew P. McMahon. Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138.

The metanephric kidney is formed through reciprocal interactions between the metanephric mesenchyme and the ureteric bud. The metanephric mesenchyme undergoes condensation, aggregation and epithelialization to form the renal vesicle. The renal vesicle gives rise to the nephron, the functional unit of the metanephric kidney. How and when the spherical renal vesicle is patterned to form the nephron of multiple segments and cell types is largely unknown. To dissect this nephrogenesis process, we are undertaking a systemic approach to screen all transcription factors in the mouse for those that are expressed in the renal vesicle derivatives. The data derived from the screen will allow us to describe the molecular anatomy of, and to provide with molecular markers for, the renal vesicle derivatives at different nephrogenesis stages, to construct tools for genetic analysis of nephrogenesis, and ultimately to understand the mechanisms governing nephron patterning and renal physiological functions. Our pilot screen showed that 84 percent of transcription factors were present in the E15.5 metanephric kidney by RT-PCR. With a combination of wholemount *in situ* hybridization with E15.5 kidneys and section *in situ* hybridization with the newborn kidneys, we are in the process of characterizing the expression patterns of transcription factors in the kidney. Acknowledgement: The transcription factor clones were generated and provided by the Ma, Stiles, and Rowitch groups at Dana-Farber Cancer Institute.

574. Withdrawn

575. **FGFR1 Signalling Regulates Intermediate Mesoderm Patterning and Subsequent Urogenital Development.** M. Hytönen, N. Trokovic, H. Sariola, J. Partanen, and K. Sainio. Developmental Biology Program, Institutes of Biomedicine and Biotechnology, University of Helsinki, Helsinki, Finland.

FGFR1 is one of the four receptor tyrosine kinases that bind more than 20 different FGF ligands. A null mutant of FGFR1 is early embryonic lethal and to dissect genetically the functions of *fgfr1* during development, two hypomorphic mutant lines of mice were generated. The reduced amount of the receptor leads to somitogenesis and neural tube defects, posterior homeotic vertebral transformations, and pharyngeal development defects (Partanen et al., 1998; Trokovic et al., 2003). We have now analyzed the possible defects in the urogenital organs of these animals. First, in stronger *n15* hypomorphs, the kidneys were hypoplastic or absent. However, when present, the renal tissue differentiates, since the few nephrons had normal tubules and glomeruli. Moreover, all the essential tubulogenic factors were present. Also the mesonephroi differentiated. Occasional hydronephroses or large cysts in kidneys suggested ureteric branching morphogenesis defect. Indeed, whole-mount immunohistochemistry showed delayed or blocked ureteric branching, but no obvious defects in the forming nephrons. In milder *n7* hypomorphs, ectopic ureteric buds, even multiple kidneys were produced and mesonephric area was posteriorly expanded. FGFR1 has been shown to regulate Hox gene activity during A/P patterning (Partanen et al., 1998), and it was recently reported that Hox genes are involved in the regulation of ureteric branching morphogenesis (Patterson et al. 2001). Indeed, posterior Hox genes but also Fox transcription factors show altered patterns in the hypomorphic urogenital tissues. Our results suggest that FGFR1-mediated signalling regulates early intermediate mesoderm patterning and subsequent positional information of the urogenital tissues. Later, it regulates ureter branching morphogenesis in the metanephric kidneys.

576. **Transcriptional Control of Terminal Epithelial Cell Differentiation: Insights from the Bradykinin B2 Receptor Gene.** Zubaida Saifudeen, Jessica Harrell, Susana Dipp, and Samir S. El-Dahr. Department of Pediatrics, Tulane University, New Orleans, Louisiana.

Differentiation in the kidney proceeds centrifugally, with the youngest nephrons displacing the older ones at the periphery. Thus, the developing kidney can be unequally divided into the outer proliferating zone and the inner differentiation zone. Terminal differentiation is characterized by cell-cycle arrest and expression of cell function proteins, such as the bradykinin B2 receptor (B2R). The purpose of this study was to elucidate the transcriptional mechanisms that regulate the induction of B2R during terminal nephron differentiation. We show here that expression of B2R is controlled by an evolutionarily conserved cis-acting enhancer element consisting of contiguous binding sites for CREB, p53 and KLF4. The spatio-temporal expression of CREB, p53 and KLF4 proteins in the differentiation zone mimics that of B2R. Chromatin immunoprecipitation assays demonstrated that assembly of CREB, p53 and KLF4 as well as the co-activator CBP on the endogenous B2R promoter is upregulated during the differentiation process (newborn) and attenuated once

renal epithelial cells are fully differentiated (adult). Transient transfection assays revealed the CRE-p53-KLF4 element is sufficient to drive reporter gene expression, and mutagenesis of either the p53 or CRE-response elements or disruption of phasing abrogates the transcriptional response to all three transcription factors. Our findings suggest that differentiation specific gene expression in the kidney is determined by combinatorial and synergistic interactions of developmentally regulated transcriptional activators and coactivators.

577. **Transcriptional Activation of the BMP-Responsive Chick Nkx-2.5 Homeobox Gene in Developing Heart.** Kyu-Ho Lee, Samuel Evans, and Andrew B. Lassar. Cardiology Department, Children's Hospital Boston, and BCMP Department, Harvard Medical School.

The Nkx-2.5 homeobox gene plays a vital role in several aspects of vertebrate heart formation: mesoderm induction, cardiac patterning or segment determination, and myocyte sub-specification. A homolog of the *Drosophila* tinman gene, Nkx-2.5 is one of several NK-homeodomain transcription factors expressed in early cardiac and foregut mesendoderm. At later stages of heart development, Nkx-2.5 regulates cardiac patterning and morphogenesis: Nkx-2.5 null mice form a small, single-chambered heart whose development is arrested shortly after the onset of cardiac looping. Human genetic studies have shown that expression of hypomorphic Nkx-2.5 isoforms in the heart results in the generation of atrial septal defects, AV conduction delay and eventual cardiac failure, evidence supporting a differential requirement for Nkx-2.5 function in some heart cells. Using a combination of in vitro response assays, transgenic enhancer analysis and embryonic gel shift techniques, we have found some intriguing correlations between Nkx-2.5 regulation and the various functions of the BMP-inducible Nkx-2.5 gene in heart formation. We have characterized a BMP responsive enhancer that regulates expression in early cardiac precursor populations. At later stages of cardiac development, the Nkx-2.5 BMP enhancer regulates segment-specific expression in the outflow tract and right ventricular segments of the looping heart. At least two other enhancers co-regulate additional and distinct left ventricular and atrial segment-specific gene expression at these stages. We present work characterizing the central BMP response mechanism activating Nkx-2.5, and the mechanisms by which other factors like the GATA4/5/6 zinc finger and Smad transcription factors restrict this response to the developing heart.

578. **Tbx-5 Is Required for the Development and Maturation of the Murine Cardiac Conduction System.** Ivan P. G. Moskowitz,* A Pizard,* V. Patel,† B Bruneau,** J. Kim,* S. Kupersmidt,‡ D. Roden,‡ C Berul,† CE Seidman,* and Jon G Seidman*. *Department of Genetics, Harvard Medical School and HHMI, Boston, Massachusetts 02115; †Department of Cardiology, Children's Hospital, Boston, Massachusetts; **Program in Cardiovascular Research, The Hospital for Sick Children, Toronto, Canada; and ‡Departments of Medicine Pharmacology, Molecular Physiology & Biophysics, Vanderbilt University Medical School, Nashville, Tennessee.

The cardiac conduction system is essential for the coordinated contraction of the multi-chambered vertebrate heart. Haploinsufficiency of the T-box transcription factor gene Tbx-5 causes

functional cardiac conduction system abnormalities in humans and mice (Bruneau et al., Cell 2001). We demonstrate that Tbx-5 plays a role in maturation and patterning of the developing murine conduction system. We show that Tbx-5 is transcribed in the atrioventricular node, atrioventricular bundle, and bundle branch conduction system at higher levels than in the surrounding myocardium. We find that the murine conduction system matures both morphologically and functionally between the neonatal and adult periods, and that haploinsufficiency of Tbx-5 causes a maturation defect of the atrioventricular ring (canal) conduction system. Furthermore, Tbx-5 haploinsufficiency causes a patterning defect of both the left and right bundle branches. Tbx-5 haploinsufficiency causes a complete failure of right bundle branch development in most animals. Absence of the right bundle branch by morphologic criteria correlated with right bundle branch block by EKG. These findings suggest a cell-autonomous role for Tbx-5 in the developing murine conduction system and identify Tbx-5 as the first known transcription factor required for the maturation and patterning of the mammalian atrioventricular canal and bundle branch conduction system.

579. **Structure/Function Analysis of Mix.3/Mixer and Endoderm Development.** Joanne R. Doherty, Haiqing Zhu, and Paul E. Mead. Department of Pathology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105.

The Mix/Bix family of transcription factors are PAX-homeodomain proteins that are involved in mesoderm and endoderm development in *Xenopus*. Mix.3/mixer and Mix.1 are expressed in the presumptive mesendodermal region from mid-blastula to late gastrula stage and both have been shown to be involved in mesoderm patterning. Mix.3/mixer but not Mix.1 induces endoderm specific genes when expressed alone in explanted ectoderm. This dramatic activity of Mix.3/mixer in vitro suggests that it is playing a critical role in endoderm formation in vivo. To begin to decipher the molecular pathway of Mix.3 endoderm formation we generated mutants of Mix.3 and Mix.1 and tested their ability to induce endoderm in explanted ectoderm. The amino termini, homeodomains and carboxyl termini were individually swapped between the two proteins. Mix.3 contains a Smad 2 binding domain (SBD) that is not present in Mix.1. We mutated the SBD in Mix.3 and added it to Mix.1. To ensure that all of these mutants were functional we tested their DNA binding activity by gel-shift assay and ability to pattern mesoderm in explanted ectoderm. Our results indicate that both the homeodomain and part of the carboxyl terminus of Mix.3 are necessary for endoderm induction. Further studies using these domains will allow us to characterize the molecular determinants of endoderm formation.

580. **Sox9, a Novel Pancreatic Marker in *Xenopus*.** Young-Hoon Lee and Jean-Pierre Saint-Jeannet. Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Like most endodermal organs, the development of the pancreas depends on reciprocal interactions between the mesoderm and the endoderm. The pancreas arises initially from dorsal and ventral evaginations of the foregut that will subsequently fuse into a single organ. Here we report the expression pattern of Sox9, a member of the Sox family of transcription factors, during development of *Xenopus* pancreas and compare its expression to

a well characterized pancreatic marker, PDX1. By whole-mount in situ hybridization Sox9 and PDX1 are first detected at stage 25 in the presumptive pancreatic tissues: dorsally in the prospective foregut and ventrally on each side of the liver diverticulum. As development proceeds, both markers can be used to trace the development of the dorsal and ventral pancreatic buds and their repositioning associated with the dynamic movements of the gastrointestinal tract. Interestingly, while PDX1 is expressed in both the pancreatic buds and the duodenum, Sox9 appears to be exclusively restricted to ventral and dorsal pancreatic buds. A recent study indicates that Sox9 is also expressed in the pancreas during human development. As Sox family members have been implicated in the control of a variety of developmental processes including cell fate specification, studies are currently in progress to address the role of Sox9 during development and differentiation of the pancreas.

581. Withdrawn

582. Abstract #582 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

583. **Structure and Functional Analysis of Novel Cell Death Promoting Protein Jpk Using Murine Embryonic Cell Line.** Kyoung-Ah Kong, Hye-Sun Kim, Hyoungwoo Park, and Myoung Hee Kim. Department of Anatomy, Embryology Laboratory, Brain Korea 21 Project for Medical Sciences, Yonsei University College of Medicine, Sodaemoongu Shinchondong 134, Seoul 120-752, Korea.

A novel gene Jpk has been originally isolated as a trans-acting factor associating with the PSRE of murine Hoxa-7. During subcloning, Jpk turned out to be toxic to both prokaryotic and eukaryotic cells. In order to decipher the mechanism of cell death, we used several analysis such as flow cytometry, RT-PCR and confocal microscope. When Jpk was transfected into F9 and p19 cells, it induced cell death in time-dependent manner and seemed to regulate Bcl-2 family at the transcriptional level with concomitant reduction of mitochondrial transmembrane potential ($\Delta\psi$) and generated ROS. When Jpk was expressed as a fusion proteins with EGFP, it was detected mainly in both mitochondria and endoplasmic reticulum. Sequence analysis revealed that Jpk contains one transmembrane domain, a plausible ER retention and nuclear localization signals along with several putative phosphorylation and glycosylation sites. Therefore, we constructed several deletion mutants containing one or several domains. When the localization of deletion protein was tested, the N-term possessing a TM domain expressed at the similar region to that of Jpk, whereas the expression of C-term was detected in the whole cell. The N-term of Jpk seemed to be less toxic generating less ROS and less reduction of $\Delta\psi$ compare to those of C-term. These results altogether suggest that Jpk induces ROS production and reduces $\Delta\psi$ during cell death, and the toxic domain seemed to be located at the C-term of Jpk.

584. **Expression of a Notch-Class Gene in Teloblasts and Blasts Cells of the Leech *Helobdella robusta*, a Segmented Lophotrochozoan.** Ajna S. Rivera and David A. Weisblat. University of California, Berkeley, California.

To understand how segmentation arose in each of the three major clades of Bilateria (Deuterostomia, Ecdysozoa, and Lophotrochozoa) it is necessary to investigate the developmental mechanisms by which it occurs in the segmented members of all three clades. We are studying segmentation in the leech, *Helobdella robusta*, an annelid member of Lophotrochozoa. *Helobdella* is an attractive system in which to study segmentation because its embryos are relatively large, hardy and undergo stereotyped cell divisions. In particular, segmental mesoderm and ectoderm arise from five pairs of large, identifiable stem cells (teloblasts) each of which produces a coherent column of segmental founder cells (blast cells). Previous experiments have shown that the expression pattern of *Hro-hes*, a hairy/enhancer-of-split homolog, is linked to the cell cycle during cleavage and in teloblasts and blast cells (Song et al., in preparation). We have now begun to examine the expression and function of a notch homolog (*Hro-notch*), during segmentation in *Helobdella*. In situ hybridization analysis suggests the presence of lineage-specific differences in the expression of *Hro-notch*. In particular, mesodermal blast cells exhibit a diffuse cytoplasmic distribution of *Hro-notch* transcripts in interphase (S and G2 phase) cells, plus a strong signal associated with the mitotic apparatus during M phase that is suggestive of the mitotic expression seen for *Hro-hes*. In contrast, ectodermal blast cells exhibit a strong nuclear signal in interphase (S and G2 phase) cells that is indicative of active transcription during interphase in the ectodermal lineages, plus diffuse cytoplasmic staining throughout the cell cycle.

585. Structure/Function Analysis of SHORTROOT (SHR) Identifies a Domain Required for SHR Movement. Kim Gallagher, Alice Paquette, and Philip Benfey. Duke University and New York University.

Studies in plant and animal systems have shown that signaling centers within developing organs play important roles in the control of cell division and differentiation. Recently the SHORTROOT (SHR) protein, a member of the GRAS family of putative transcription factors, has been shown to be an organizing signal in patterning of the *Arabidopsis* root. SHR is required for division of progenitor cells within the root apex via activation of another GRAS family member, *SCARECROW* (SCR). Analysis of the patterns of SHR protein and mRNA expression indicates that the protein moves in a highly specific manner from the internal cell types of the root, where SHR is transcribed, into adjacent cells. While SHR is not the only transcription factor that has been shown to move intercellularly during plant development, very little is known about the mechanisms of this movement. Through examination of a new *shr* allele in parallel with a more targeted structure/function analysis we have identified sequences that are required for its movement, subcellular localization and activation of downstream genes.

586. Searching for Direct Downstream Targets of APETALA3 and PISTILLATA, Arabidopsis Homeotic Regulators of Petal and Stamen Development. Naomi Nakayama, Moriyah Zik, and Vivian Irish. Department of MCDB, Yale University, New Haven, Connecticut 06520.

In the model plant *Arabidopsis*, a heterodimer of the MADS domain-containing transcription factors APETALA3 and PISTILLATA (AP3/PI) is necessary for specification of petal and stamen identities throughout organ differentiation. However, little is

known about how AP3/PI achieves this task; only two genes have been shown as immediate targets of AP3/PI thus far. In order to identify as yet unknown genes directly regulated by AP3/PI, we are conducting three complementary screens. Since loss-of-function forward mutageneses have been unsuccessful in recovering genes downstream of the floral homeotic proteins, we have used an activation-tagged mutant collection in a screen for gain-of-function mutations causing abnormal flower morphology. Several mutants have been recovered, and genes responsible for the phenotypes are being identified. We have also screened approximately 1,800 gene-trap lines for gene expression patterns suggestive of regulation by AP3/PI. Several dozen genes showing such petal and/or stamen-specific expression patterns were recovered. The promoters of the genes showing similar expression patterns are being compared and analyzed for possible transcription factor binding sites. In addition, chromatin immunoprecipitation is being used for genome-wide isolation of *in vivo* AP3/PI binding sites. Transgenic lines containing C-terminally-tagged AP3/PI have been constructed for immunoprecipitation with commercially available antibodies against Myc and HA tags. Progress on identifying direct AP3/PI targets using chromatin immunoprecipitation will be reported.

587. The MADS Box Gene AGL42 is Expressed in the Arabidopsis Root Meristem and Maintains Its Organization. Tal Nawy,* Jocelyn E. Malamy,† and Philip N. Benfey**. *New York University; †University of Chicago; and **Duke University.

Most growth in plants is mediated by apical regions termed meristems. The root meristem is a highly organized structure consisting of a well-defined central stem cell population surrounded by rapidly dividing derivatives. We have used an enhancer trap approach to probe the molecular mechanism of patterning in the meristem, detecting expression of one line in a gradient from less to more differentiated cells. Cloning of the insertion locus has uncovered *AGL42* (*AGamous-Like 42*), a novel member of the MADS box transcription factor family. RTPCR and GFP reporter fusions confirm the high expression of *AGL42* in the central cells of primary and lateral roots, with lower expression in surrounding cells. Independent hypomorphic alleles and plants expressing *AGL42*(RNAi) reveal a low penetrance defect in root meristem organization, and genetic studies of potential redundancy are underway. Preliminary results assign a role for *AGL42* in maintaining the organization of the root meristem.

588. Rapid Production of Temperature Sensitive Mutants for Functional Analysis In Vivo. Y. Eileen Shi,* Erin Engelhart,† Sarah Bondos*. *Department of Biochemistry and Cell Biology, and †Department of Bioengineering, Rice University, Houston, Texas 77005.

Temperature sensitive mutations allow *in vivo* analysis of protein function, including placement within a biochemical pathway and identification of the temporal requirements for protein activity. However, the difficulty in generating such mutants hinders their utility and ultimately the progression of related research. The goal of this study is to develop an efficient method to generate temperature sensitive mutants for *in vitro* and *in vivo* study. Site-directed and random mutageneses were used to disrupt protein function and stability, consequently

increasing loss of activity at high temperature. The activity of the resulting proteins is rapidly screened in yeast to identify temperature sensitive proteins. For a model system, we employed LIM-Domain-Only 4 (LMO4), a transcription regulator whose overexpression *in vivo* prevents retinal development in zebrafish. LMO4 functions in part through interactions with the protein LDB1. Therefore, the activity of thousands of LMO4 mutants can be simultaneously screened in yeast two-hybrid assay. Temperature sensitive LMO4 mutants will then be introduced into zebrafish for functional analysis at different stages of development. This methodology can be employed to generate temperature sensitive mutants of any protein with a rapid functional screen.

589. **Blood-Specific Expression of a Novel Zebrafish Zinc Finger Protein.** Saulius Sumanas, Rujuan Dai, and Shuo Lin. University of California, Los Angeles, California.

A zebrafish cDNA clone corresponding to a novel zinc finger protein was isolated from the blood-specific embryonic cDNA library. This cDNA encodes a novel 478 amino acid protein (ZF15) containing 15 sequential C2H2 type zinc finger domains. We characterized the expression pattern of ZF15 by Northern blotting and *in situ* hybridization. ZF15 displays strong ubiquitous early zygotic expression reaching maximum at the sphere stage. During somitogenesis its expression becomes gradually restricted to the two posterior stripes within lateral mesoderm, where other genes involved in hematopoiesis are known to be expressed. By 20-24 hpf, ZF15 is localized exclusively to the intermediate cell mass (ICM) region, where primitive red blood cells originate. At 24 hpf, ZF15-expressing cells enter circulation. Expression of ZF15 in ICM is completely eliminated in the cloche mutant embryos. We are currently using RNA overexpression and protein knockdown by the antisense morpholino oligonucleotides to investigate the biological role of ZF15.

590. **Estrogen Receptor Beta a (ER β a) and the Estrogen Pathway in Zebrafish.** Christopher S. Lassiter and Elwood Linney. University Program in Genetics, Duke University Medical Center, Durham, North Carolina 27710.

Estrogen is a prevalent steroid hormone in the adult life of all vertebrates, male and female. The embryonic uptake of estrogen also suggests use in development. Estrogen is now known to act through two receptors in mammals, estrogen receptors alpha and beta (ER α and ER β). These receptors function as ligand-dependent transcription factors, binding to estrogen response elements (ERE) and enhancing transcription. The two receptors can act as homodimers or as a heterodimer. Primers constructed from a highly conserved region in all vertebrate estrogen receptors were used to search a cDNA library of 1 month old zebrafish for the estrogen receptors. The cDNA sequence for estrogen receptor beta a was obtained. RT-PCR data indicate ER β a mRNA is maternally loaded into the oocyte, degraded, and then upregulated 24-48 hours post fertilization. The genomic structure of ER β a consists of eleven exons, with the coding region starting in exon 3. The intron/exon boundaries are highly conserved between zebrafish ER β a and human ER β at the amino acid level, and in some cases at the nucleotide level. A 3 kb promoter region of the gene has been cloned and shows no estrogen responsiveness. Currently, other genes in the estrogen pathway, including those for estrogen synthesis, are being examined.

591. **Feedback Mechanisms Regulate Retinoic Acid Production and Degradation in the Zebrafish Embryo.** B. Dobbs-McAuliffe,* K. Yacisin,† Q. Zhao,† and E. Linney†. *Duke University. Department of Cell Biology, †Department of Molecular Genetics and Microbiology.

Regulating the spatial distribution of retinoic acid (RA) activity is crucial to the development of the vertebrate embryo, as RA in excess or in inappropriate tissues is teratogenic. While RA cannot be directly regulated by transcription or translation, the enzymes that produce and degrade RA can be. Here, we report expression analysis in zebrafish of the degrading enzyme, *cyp26*, and the relationship of its expression to one of the enzymes that produce RA, retinaldehyde dehydrogenase (*raldh2*). *Cyp26* expression follows three phases: in presumptive anterior neur ectoderm and in a circumblastoporal ring during gastrulation, in the somites throughout somitogenesis, and in multiple specific tissue types beginning mid-somitogenesis and continuing through 72hpf. This expression pattern proved to be highly complimentary to that of *raldh2*. We determined that the RA free areas created by *cyp26/raldh2* boundaries are necessary for proper tail development. RA challenged embryos lose expression of tail somitic markers and have truncated tails. One possible regulator of enzyme expression is RA itself. We find that *cyp26* expression is strongly up-regulated by exogenous RA in a concentration, time, and tissue dependent manner in the zebrafish embryo. We have also isolated the zebrafish *raldh2* promoter region found it to be repressed by RA in cell transfection experiments. Similarly, *in vivo*, *raldh2* expression is strongly down-regulated by exogenous RA. Together these data strongly support a feedback mechanism as one of the regulators of enzyme expression. (Funding: PHS ES11375)

592. **Beyond Genetics: How to Purify Soluble Protein.** Sarah E. Bondos and Alicia Bicknell*. Department of Biochemistry and Cell Biology, Rice University, Houston, Texas 77005; *Current Address: UCSD Biology Student Affairs 038, 9500 Gilman Dr., La Jolla, CA 92093-0348.

Purified proteins allow delineation of a molecular mechanism for an observed phenotype as well as the generation of specific antibodies to identify protein expression patterns *in vivo*. However, protein structure and stability are derived from non-covalent interactions, which are extremely sensitive to buffer conditions. As a result, proteins often aggregate during purification, rendering them useless for biochemical experimentation or antibody preparation. While a large number of additives are available that influence protein solubility, iterative determination of appropriate buffer conditions may take weeks. We have developed a solubility assay to simultaneously screen multiple buffer conditions. This assay can be completed within a few hours and applied to purified protein, partially purified protein, and even crude cell lysates. Soluble and insoluble aggregates are separated from native protein by filtration, and native and aggregated proteins are detected by SDS-PAGE or western blotting. This assay is demonstrated using prokaryotic and eukaryotic proteins that range in size from 17 to 150 kDa, including monomeric and multimeric proteins. Potentially stabilizing cosolvents are listed with appropriate concentration ranges. Finally, a strategy for optimizing buffer conditions for a particular protein prior to purification is presented.

593. **A Screen to Find Signal Dependent Transcription Factor(s) Activating *knirps* Expression in the L2 Vein Primordia.** Jennifer L. Trimble and Ethan Bier. University of California San Diego, San Diego, California.

The second longitudinal wing vein (L2) of *Drosophila* forms along the anterior border of the broad domain of *spalt*-expressing cells in the 3rd instar wing imaginal disc. The related zinc-finger transcription factors *knirps* (*kni*) and *knirps related* (*knrl*) are expressed in the L2 primordia and are required for all gene expression in the L2 primordium, such as activation of the vein promoting gene *rhomboid* (*rho*) and downregulation of the intervein gene *blistered* (*bs*). *radius incompletus* (*ri*) mutations, which result in truncation of the L2 vein in adult wings, are regulatory alleles of *kni* and disrupt sequences within a 1.4 kb minimal *kni* L2 enhancer. One such allele, *ri*^{53j}, is a single point mutation within a region of the L2 enhancer required for activation specifically in the L2 primordium. We reasoned that this could be a critical binding site for a transcription factor mediating a vein inductive signal emanating from *spalt*-expressing cells. To find this putative factor, we used the surrounding sequence (23 bp) as bait in a yeast one-hybrid screen to find proteins that can bind to the wild-type sequence but not the *ri*^{53j} point mutation-containing sequence. This screen has identified several known and predicted transcription factors which bind selectively to the wild-type but not mutant 23 bp element. The expression patterns of these genes are being evaluated for overlap with the L2 primordia. Candidate genes expressed in L2 will be tested functionally to determine whether they play a role in initiating *kni/knrl* expression in the L2 primordium.

594. **Molecular Control of Vertebrate Neural Tube Closure.** John B. Wallingford, Saori L. Haigo, and Richard M. Harland. University of California, Berkeley, California.

The closure of the vertebrate neural tube requires the coordinated action of several independent morphogenetic engines. Two such engines are the bending of the neural plate and convergent extension of the midline. These two mechanisms are of interest because they are thought to rely primarily on apicobasal polarity and planar polarity, respectively. We will present data demonstrating that Dishevelled controls planar polarity during neural convergent extension and that this process is critical for normal neural tube closure in *Xenopus*. Targeted injection revealed that *Xdsh* function is required specifically in the midline for normal neural tube closure. We suggest that the movement of the neural folds by plate bending can accomplish only a finite amount of medial progress and that convergent extension of the midline is necessary to reduce the distance between the nascent neural folds, allowing them to meet and fuse. In mammals, distinct mechanisms are thought to drive and control closure of the cephalic and spinal neural tubes. We will present data from axolotl embryos that suggests a similar organization in amphibia; convergent extension plays a critical role in spinal neurulation and is less important, but not dispensable, for cephalic neurulation. Finally we will present new molecular data on the mechanisms of anterior neural plate bending.

595. **FRL-1 is Essential for Neural Differentiation in *Xenopus* Early Development.** Shin-Ichiro Yabe,* Kousuke Tanegashima,† Yoshikazu Haramoto,† Shuji Takahashi,† Tomoyuki Fujii,* Siro Kozuma,* Yuji Taketani,* and Makoto

Asashima†**. *Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tokyo; †Department of Life Sciences, University of Tokyo; and **SORST Project, University of Tokyo, Tokyo, Japan.

Recent studies indicate an essential role for the EGF-CFC family in vertebrate development, particularly in the regulation of nodal signaling. Biochemical evidence suggests that EGF-CFC genes can also activate certain cellular responses independently of nodal signaling. Here, we show that FRL-1, a *Xenopus* EGF-CFC gene, suppresses BMP signaling to regulate an early step in neural induction. We generated morpholino-antisense oligonucleotides against FRL-1 (FRL-1MO) and performed the study by microinjecting FRL-1MO into *Xenopus* embryos. Histological analysis showed that the embryos injected with FRL-1MO had neural tissue defects without the loss of mesodermal tissue. These results indicate that FRL-1 can directly occur the neural induction without the mesodermal induction. Next we examined how FRL-1 act to neural induction. Whole-mount immunohistology showed that FRL-1 activated the MAPK pathway, so we tested whether FRL-1 inhibits BMP signaling via the activation of MAPK signaling. RT-PCR analysis showed that the suppression of BMP responsive genes, *Xmsx-1* and *Xvent-1*, by injection of FRL-1 in animal caps was rescued by treatment with MAPK inhibitor. Furthermore, we showed that FRL-1 inhibited the Phosphorylation of Smad1 to cause the suppression of BMP signaling. These data indicates that inhibition of BMP signaling by FRL-1 is required for MAPK activation.

596. Abstract #596 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

597. **Role of SHH in the Initiation of Pituitary Development in Chick.** Lynn Dufresne and Aimee K. Ryan. Montreal University Health Center.

The primordium of the anterior pituitary gland, Rathke's pouch, is formed by the invagination of somatic ectoderm that lines the roof of the oral cavity. The molecular mechanisms that control the earliest aspects of pituitary organogenesis leading to the formation of Rathke's pouch are poorly understood, but several signaling pathways have been implicated in this process. We are examining the role of Sonic Hedgehog (Shh) in the formation and the thickening of the hypophyseal placode, which appears before Rathke's pouch, and in the transition from the rudimentary to the definitive pouch in chick embryos. Shh is expressed in the ventral diencephalon and the oral ectoderm but is excluded from the pouch. In mice, the overexpression of SHH in RP affects cell type differentiation, while overexpression of a SHH antagonist within the pouch leads to a hypomorphic pouch. Varying concentrations of a diffusible form of SHH or a SHH blocking antibody are being implanted adjacent to the hypophyseal placode in the ventral diencephalon or in RP. Embryos are treated at stages 10-14, collected at stage 17-20 and subjected to whole mount in situ hybridization to define the effects on Rathke's pouch morphogenesis. The results from these experiments will be presented.

598. **Genetic Analysis of a Neural Tube Defect Mutant from ENU Mutagenesis.** Tae-Hee Kim, Kathryn Anderson, and Lee Niswander. Developmental Biology Program, HHMI,

Sloan Kettering Institute/Cornell University, New York, New York 10021.

Neural tube defects (NTDs), in particular exencephaly and spina bifida, are common birth defects, that occur in 1 out of 1000 human births. To better understand the genetic networks of NTDs, we have used an ethylnitrosourea (ENU) mutagenesis approach to identify recessive mutations causing NTDs in mice. One line identified from this screen is G2E; it has an exencephalic phenotype and dies between E14 and E18. We have examined patterning, apoptosis, cell proliferation, cytoskeleton, and head mesenchyme defects. G2E mutant appears to have abnormal proliferation in the developing neural epithelium. We have mapped the G2E mutation to a 4 cM region on chromosome 4. Further characterization and mapping are being conducted to determine the genetic lesion responsible for the G2E phenotype.

599. **Cordon-bleu is a Conserved Gene Involved in Neural Tube Formation.** Elizabeth Carroll,* Dianne Gerrelli,† Stéphan Gasca,** Elizabeth Berg,* David Beier,‡ Andrew Copp,<939 and John Klingensmith*. *Department of Cell Biology, Duke University Medical Center, Durham, North Carolina; †Neural Development Unit, Institute of Child Health, London, United Kingdom; **Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada; ‡Division of Genetics, Brigham and Women's Hospital, Boston, Massachusetts.

The axial midline is an important source of patterning information in the vertebrate embryo. The midline derives from a small group of cells in the gastrulating embryo, known as "the organizer" in recognition of its ability to organize an entire body plan. Only some of the genes that direct midline development are known. One gene potentially important for this process is the novel mouse gene *cordon-bleu* (*cobl*), expressed specifically in the mammalian organizer and its derivatives until organogenesis stages. Here we present the cloning and analysis of the *cobl* cDNA. The sequence of *cobl* does not resemble any gene of known function. However, *cobl* is widely conserved: apparent orthologs and paralogs are found in many vertebrate species, with several sequence domains of high conservation but unknown function. We find that chicken *cordon-bleu* is similarly expressed in the node and its derivatives, suggesting functional conservation. We also report the sequence and non-overlapping expression of a homologous mouse gene. We present evidence that *cobl* interacts with the neurulation gene *Vangl2*, disrupted in the mouse mutant *loop-tail*, to facilitate midbrain neural tube closure, demonstrating roles for both *cobl* and *Vangl2* in midbrain neurulation. Finally, we are investigating the regulation of *cobl*'s dynamic expression in the neural tube using an explant system.

600. **Analysis of *Limulus* and Wing-Shaped Neural Plate, Two Mouse Mutants That Display Morphogenesis Defects during Late Gastrulation.** Jeffrey D. Lee and Kathryn V. Anderson. Department of Developmental Biology, Sloan-Kettering Institute.

The genetic components that govern mammalian embryogenesis are largely unknown. Mesoderm arises when cells of the early epiblast first involute through the primitive streak and then migrate to form mesodermal tissues, such as the heart, notochord and somites. To investigate these morphogenetic events, we are performing a phenotypic and molecular characterization of two

recessive mutants, named *limulus* (*lulu*) and textitwing-shaped neural plate (*wsnp*), that were isolated in a screen for ENU-induced mutations affecting embryogenesis. *lulu* and *wsnp* embryos both appear shortened along the AP axis, with severe deficits of posterior tissue; both die at 9.5 dpc. Mesodermal patterning at 7.5 dpc appears normal, but by 8.5 dpc both *lulu* and *wsnp* exhibit severe abnormalities in the morphogenesis of mesodermal derivatives. Some axial mesoderm forms, but does not segment into somites. Expression of *textitShh* and *T* in the axial mesoderm is initially normal but later becomes discontinuous. Despite these defects, AP patterning of the neural plate appears normal, although the neural tube does not close. Similar phenotypes have been described for mutations that affect extracellular matrix (ECM) signaling, particularly the *fibronectin*, *integrin $\alpha 5$* , *FAK* and *paxillin* mutants. *lulu* and *wsnp* may alter the motility or adhesive properties of nascent mesodermal tissue, or of anterior epiblast tissue that migrates through the primitive streak. Both mutations have been mapped to within 5 Mb; each interval is predicted to contain several candidate genes that could participate in ECM-integrin signaling.

601. Abstract #601 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

602. **Programmed Cell Death is Required for Inner Ear Morphogenesis and Growth.** Francesco Cecconi,* Kevin A. Roth,† Oleg Dolgov,** Eliana Munariz,* Konstantin Anoukhin,** Peter Gruss,‡ and Marjo Salminen§. *Dulbecco Telethon Institute, Department of Biology, University of Rome "Tor Vergata", Rome, Italy; †University of Alabama at Birmingham, Birmingham, Alabama; **P. K. Anokhin Institute of Normal Physiology RAMS, Moscow, Russia; ‡Department of Molecular Cell Biology, Max-Planck Institute of Biophysical Chemistry, Goettingen, Germany; and §Institute of Biotechnology, University of Helsinki, Helsinki, Finland.

Programmed cell death is known to be a critical event for normal morphological development of many organs. Focal apoptosis occurs in the otic epithelium during inner ear development. The significance of this localized cell death and the molecules involved have remained unclear. To get more insights into the role of apoptosis during inner ear morphogenesis we have undertaken the analysis of mouse mutants in which genes encoding for proapoptotic or antiapoptotic factors have been inactivated. The inactivation of the *apaf1* gene led to a dramatic decrease in apoptosis in the inner ear epithelium and to severe morphogenetic defects demonstrating that an Apaf1 dependent apoptotic pathway is necessary for normal inner ear development. The caspase-9 mutant mice suffered from similar defects. In addition to morphogenetic defects, a size reduction of the *apaf1* and caspase-9 mutant membranous labyrinth was observed. Inactivation of the *bcl-X* gene led to an overall increase in the number of cells undergoing apoptosis in the otic epithelium due to the lack of the antiapoptotic Bcl-XL isoform. However, the excess of apoptosis did not have a major impact on the general morphogenetic development.

603. **Regulating Dorsoventral Polarity within the Inner Ear.** Martin Riccomagno, Lenka Martinu, Shinji Takada, Doris Wu, and Douglas Epstein. University of Pennsylvania.

The principal components for hearing and balance are formed from ventral and dorsal outgrowths, respectively, of a common bilateral structure, the otocyst. Organization of the inner ear into auditory and vestibular components is established under the influence of surrounding tissues. The proximity of the otocyst to the hindbrain suggested that extracellular signals that pattern the CNS might also polarize the otic epithelium along its dorsoventral (d/v) axis. Our studies identify Sonic hedgehog (Shh) secreted by the notochord as a primary regulator of auditory fates within the mouse ear. Ventral otic derivatives including the cochlear duct and cochleovestibular ganglia failed to develop in the absence of Shh. The origin of the ear defects in Shh^{-/-} embryos was attributed to alterations in the expression of genes previously implicated in the specification of cochlear, neuronal and chondrogenic lineages. Several of these genes are targets of Shh signaling given their ectopic activation in transgenic mice misexpressing Shh in the otocyst. Interestingly, in the absence of Shh, some dorsal otic genes including *Dlx5* and a Wnt responsive TOPGAL reporter expand ventrally indicating 1) that their expression is normally antagonized by Shh and 2) that a Wnt signal(s) possibly secreted from the dorsal CNS impacts on dorsal otic fates. To test this hypothesis we assessed *Dlx5* expression in various Wnt mutants. Whereas, *Dlx5* was detected in the otic vesicles of Wnt1 and Wnt3a single mutants, its expression was lost in Wnt1;Wnt3a double mutants. Our data supports a model whereby d/v compartments in the ear are initiated through the opposing actions of Shh and Wnt signaling pathways.

604. **The Roles of Mouse Fgfs and Fgfrs during Early Inner Ear Development.** T. J. Wright, E. Hatch, H. Karabagli, P. Karabagli, R. Ladher, G. C. Schoenwolf, and S. L. Mansour. Human Genetics and Neurobiology and Anatomy, University of Utah, Salt Lake City, Utah.

The inner ear, which contains the sensory organs specialized for audition and balance, develops from an ectodermal placode adjacent to the hindbrain subsequent to inductive signals arising from the underlying mesenchyme and adjacent neuroectoderm. The placode invaginates, delaminates otic neuroblasts and forms a closed vesicle. The otic vesicle then initiates cellular differentiation and morphogenesis, which ultimately results in the inner ear. Several intercellular signalling molecules, including the fibroblast growth factors (FGFs) are involved in these processes. We have previously shown that Fgf3, expressed in the hindbrain, and mesenchymal Fgf10 are required to initiate otic development. We have used genetic and expression analyses to assess the potential roles of other Fgfs and Fgfrs in early otic development. Recently, zebrafish Fgf8 has been shown to play roles in both otic placode induction and vesicle morphogenesis. We will show that depletion of mouse Fgf8 in Fgf3 null mutants leads to an exacerbation of the otic phenotype seen in Fgf3 mutants. In addition, we have examined inner ear development in mice that lack Fgf15, the putative homologue to chick Fgf19 and will present evidence that Fgf15 and Fgf19 play different roles in otic development. An expression screen identified two members of the Fgf family, Fgf4 and Fgf16, whose roles in otic development have not previously been determined. Fgf4 is expressed in preplacodal and placodal otic ectoderm and Fgf16 is localized to the posterior otic cup and vesicle. The roles of these genes in otic development are currently being investigated.

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606. **Elucidating the Timing of Endothelin-A Receptor Function in Neural Crest Cells Using Conditional Gene Inactivation.** Louis-Bruno Ruest and David E. Clouthier. Birth Defects Center, University of Louisville, Louisville, Kentucky 40292.

During craniofacial development, neural crest cells migrate to the pharyngeal arches, giving rise to the facial skeleton. Signaling through the endothelin-A (ETA) receptor is crucial in crest cell development, as ETA^{-/-} mice exhibit severe craniofacial defects. While loss of ETA signaling disrupts the expression of transcription factors involved in crest cell patterning, the exact timing of ETA signaling during crest cell development is still not clear. To address this question, we used a conditional knockout strategy, in which the ETA gene, flanked by loxP sites (̐flox&̐), was specifically inactivated in neural crest cells using the Wnt1-Cre and dHAND-Cre transgenic mouse strains. Both strains direct Cre recombinase expression to neural crest cells. In E18.5 ETAflox/flox;Wnt1-Cre embryos, craniofacial defects resembling those in ETA^{-/-} embryos are observed, including transformation of the mandible into a maxilla-like structure. Strikingly, a few ETAflox/flox;Wnt1-Cre embryos develop more severe craniofacial defects than those in ETA^{-/-} embryos, including loss of all lower jaw tissue between the two mandibular halves. In contrast, E18.5 ETAflox/flox;dHAND-Cre embryos do not show any defects. Wnt1 directs Cre expression in migrating crest cells whereas dHAND directs Cre expression in post-migratory crest cells. Therefore, ETA function appears to be required during late neural crest migration or early post-migratory proliferation. Further, the increased severity of defects in ETAflox/flox;Wnt1-Cre embryos suggests that the timing of ETA loss is crucial in dictating the severity of facial deformities.

607. **Ectopic Fgf4 Alters Neural Tube and Neural Crest Development in the Mouse Embryo.** Noah A. Byrd,* Graham Smyth,* George Minowada,† and Erik N. Meyers*. *Department of Pediatrics and Cell Biology, Duke University Medical Center, Durham, North Carolina 27710; †Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106.

Neural tube defects (NTDs) are among the most common birth defects observed in neonates. Previous studies in mice have demonstrated that both gain and loss of Fibroblast Growth Factor (FGF) signaling can result in neural tube closure defects along the anterior-posterior axis. Several FGF ligands and FGF receptors are expressed in and around the developing neural tube during neurulation. To further investigate the effect of FGF signaling on neural tube development, we have utilized a Cre-loxP approach to drive *textitFgf4* expression (*Fgf4OE*) in the dorsal neural tube and subsequently in migratory neural crest cells. Using Wnt1-Cre and *textitPax3-Cre*, we have ectopically expressed *Fgf4* in the rostral and caudal dorsal neural tube, respectively. Ectopic *Fgf4* in the *textitWnt1* domain results in exencephaly at E9.5 and later induces a dramatic change in the morphology and patterning of the more caudal neural tube. *Pax3-Cre::Fgf4OE* embryos exhibit abnormalities in the caudal neural tube and tail region as early as E9.5, and later demonstrate a severely dysmorphic neural tube

lumen. In both the *Wnt1* and *Pax3* domain, *Fgf4* overexpression leads to morphological defects in cranial nerves and dorsal root ganglia, both derivatives of neural crest. In support of these observations, *Crabp1* and *Ap2α*, markers of neural crest, are reduced in the dorsal midline in *Pax3-Cre::Fgf4OE* embryos. These studies elucidate the effect of ectopic FGF signaling during neural tube and neural crest development.

608. Dissimilar Regulation of Cell Differentiation in Cranial and Trunk Neural Crest Cells. Arhat Abzhinov and Clifford J. Tabin. Department of Genetics, Harvard Medical School.

During vertebrate development neural crest cells give rise to a wide variety of specialized cell types in response to cytokines from surrounding tissues. Depending on the cranial-caudal level of their origin, different populations of neural crest cells exhibit differential competence to respond to these signals as exemplified by the unique ability of cranial neural crest to form skeletal cell types. Our *in vitro* data show that in addition to differences whether they respond to particular signals, mesencephalic cranial neural crest cells differ dramatically from the sacral trunk neural crest cells in how they respond to specific extracellular signals, such that under identical conditions the same signal induces dissimilar cell fate decisions in the two populations *in vitro*. In turn, the same differentiated cell types can be induced by different signals in the two populations. We believe that these results obtained *in vitro* are relevant to normal head development. For example, FGF8 and SHH are both expressed in distinct domains in the developing face and are known to be important for craniofacial development. We found that these two molecules have strong synergistic effects on chondrogenesis *in vitro* and are sufficient to promote significant cranial cartilage outgrowth *in vivo*, suggesting a specific role for these molecules to produce the elongated beak structures during chick facial development. We also provide evidence that these differences in responsiveness are modulated, at least in part, by differential expression of Hox genes within the neural crest.

609. Zebrafish *touchtone* Is Selectively Required for Neural Crest-Derived Melanophore Development. B. Arduini and P. Henion. Ohio State University, Columbus, Ohio.

The development of neural crest-derived pigment cells has been established as a tractable system for studying cell fate specification, differentiation, proliferation and survival. The recessive lethal zebrafish mutation *touchtone*^{b508} was identified in a mutagenesis screen based on altered pigmentation. *tct*^{b508} homozygotes exhibit a severe phenotype in crest-derived melanophores, the majority of which are absent. Those present are smaller and have fewer processes than wild-type melanophores. In *tct* mutant embryos, NC and melanoblast cell populations appear normal prior to 25 hpf. Subsequently, as melanoblast numbers increase in wild-type embryos, they are reduced in *tct*^{b508} mutant embryos. This indicates that melanoblasts may die in *tct* mutants. Visual inspection and analysis with pigment sublineage-specific markers reveal that iridophores and xanthophores develop normally. NC-derived neuronal cell types investigated also appear normal. Mosaic analysis revealed that *tct* acts cell autonomously within melanoblasts. Three other alleles have been identified. The alleles differ in severity of melanophore phenotype, *b508* being the strongest. However, all four alleles are lethal, and embryos are transiently insensitive to tactile stimuli.

The *tct* locus is located on linkage group 18. Taken together, these data suggest that *tct* is required specifically for melanophore development within the NC lineage. In addition, since *tct* mutations are lethal even though melanophores are not required for viability, *tct* must be required in at least one other cell type.

610. Wnt Regulation of Myogenic Differentiation in the Developing Avian Limb and Face. Laurent Antoni,* Kelly Anakwe,* Julia Hadley,* Darrell J. R. Evans,** Lesley Robson,† and Philippa Francis-West*. *Department of Craniofacial Development, King's College, London, SE1 9RT, United Kingdom; †Department of Neuroscience, Queen Mary's School of Medicine and Dentistry, London, E1 4NS, United Kingdom; and **School of Biosciences, Cardiff University, Cardiff, CF10 3US, United Kingdom.

During development myogenic cells come within range of Wnt signals, both in the limb and face. We show that Wnts regulate myogenic differentiation, controlling both the number of terminally differentiated myogenic cells and the ratio of slow and fast fibers determining the intricate patterning and function of the musculature. Of particular note, Wnt5a and Wnt11 promote and decrease the number of slow myoblasts respectively in the wing. In contrast, Wnt5a has no effect on fibre type specification in mandibular micromass cultures whereas, like in the wing, Wnt11 decreases the number of slow myoblasts without altering the total number of differentiating myoblasts. Loss of function studies confirmed the endogenous role of Wnt11 signalling as a regulator of fibre type differentiation in both the wing and face. We also show that in the limb bud Wnt3a and the Wnt antagonist Sfrp-2 reduces the number of terminally differentiated cells while Wnt7a and -14 have the converse effect. To determine whether Wnts signal directly to myogenic cells, we are currently analyzing the expression of Frizzled receptors in the developing face.

611. Role of Tbx-1 in Chick Craniofacial Muscle Development. Rebecca Walker, Julia Hadley, Laurent Antoni, and Pip Francis-West. Department of Craniofacial Development, King's College, London, SE1 9RT, United Kingdom.

We are interested in how craniofacial muscles are specified from the unsegmented paraxial mesoderm which gives rise to the majority of head muscles. Here we analyze the expression of Tbx-1, a T-box transcription factor which is the candidate gene for DiGeorge Syndrome (DGS), in the developing chick embryo. We show that Tbx-1 is expressed in a discrete location in the unsegmented paraxial mesoderm adjacent to the caudal hindbrain at stage 7. Later expression is found within the branchial arches. Comparison with the myogenic regulatory factor (MRF), MyoD shows that Tbx-1 is expressed by developing myogenic cells. Of interest Tbx-1 is not expressed in extraocular muscles which have distinct origins. This implies extraocular muscles and branchial arch muscles are differentially specified. However, Tbx-1 expression is not restricted to the developing myogenic cells, suggesting that it alone does not determine myogenic fate. We have also compared the timing of expression with that of capsulin and MyoR, two related MRFs which gene inactivation studies in mice have shown these are required for the development of some lower jaw muscles. We have found that Tbx-1 is expressed prior to these factors. We are currently investigating the role and regulation of Tbx-1 expression during craniofacial myogenesis.

- 612. Pitx2 Is Involved in Regulation of Chick Craniofacial and Limb Myogenesis.** Julia Hadley, Laurent Antoni, Lesley Robson, and Pip Francis-West. *Department of Craniofacial Development, King's College, London, SE1 9RT, United Kingdom; and †Department of Neuroscience, Queen Mary's School of Medicine and Dentistry, London, E1 4NS, United Kingdom.

We are interested in the regulation of craniofacial and limb muscle development. Here we show that these developing muscles express the transcription factor, Pitx2. We have investigated the function of Pitx2 by gain- and loss-of-function studies, using the replication competent retrovirus RCAS(BP) *in vitro* in limb and mandibular primordia micromass culture. We show that Pitx2 controls the number of terminally differentiated myoblasts in a stage dependent manner. Loss of Pitx2 function in the wing and leg decreases and increases the number of terminally differentiated myoblasts as assessed by MyHC staining at stage 19 and 21 respectively. In the wing overexpression of constitutively active Pitx2 increases the number of terminally differentiated myoblasts, while in the leg Pitx2 overexpression decreases the number of differentiated myoblasts. We have also investigated how Pitx2 expression regulates differentiation of slow and fast fibrotypes, and again found stage specific effects on slow versus fast myoblast differentiation. In the mandibular primordia Pitx2 also regulates the number of myoblasts. Loss-of-function of Pitx2 decreases while gain-of-function of Pitx2 increases the number of myoblasts. Therefore, we have shown a role for endogenous Pitx2 function in both the head and limb.

- 613. Function of Endogenous Retinoids in Craniofacial Patterning.** Y-P. Song and J. M. Richman. Department of Oral Health Sciences, Faculty of Dentistry, UBC, Vancouver, Canada.

In this study, we locally inhibit retinoid synthesis specifically at the time and place when important events in facial patterning are taking place. Citral soaked beads were applied to the nasal pit of stage 20 chicken embryos, a stage we previously showed is sensitive to exogenous retinoids. Concentrations from 0.001 g/ml to 0.89 g/ml of citral were tested with 0.1 g/ml being the minimum dose at which consistent defects were induced. Most of the maxillary and lateral nasal prominence derivatives were either abnormal or absent while frontonasal mass derivatives were largely normal (11/11). We observed that Citral caused blistering of epithelium immediately after contact and that often the nasal pit epithelium sloughs off, however only limited programmed cell death was induced around the nasal pit compared to the contralateral side (5/5 at 16h). Expression of the following genes was decreased 24h following treatment with 0.1 gm/ml Citral: *Fgf8*(4/4), *Msx1* (5/5), *Msx2*(4/4). *Shh* was ectopically induced around the nasal pit (16h, 5/5). In contrast, in embryos in which epithelium remained intact *Shh* was not induced (6/6). We hypothesize that it is important for Citral to be in contact with the mesenchyme, the main source of endogenous retinoids in order to exert its effects. In summary, endogenous RA is required for maxillary and lateral nasal prominence development and required to maintain *Fgf8*, *Msx1* and *Msx2* expression. Furthermore we suggest that endogenous RA represses *Shh*. The contrast between the effects of exogenous RA and the effects of Citral on face development suggests that exogenous and endogenous reti-

noids may act by different mechanisms. This study was supported by CIHR.

- 614. The Orofacial Cleft Mutation *Dancer* Disrupts T-Box Gene, *Tbx10*.** Jeffrey O Bush, Kathleen Maltby, and Rulang Jiang. University of Rochester

We have chosen to use the *Dancer* mutant mouse as a model to study the molecular genetic basis of cleft lip and palate. *Dancer* (*Dc*) is a semi-dominant spontaneous mutation that causes vestibular defect in heterozygotes, and cleft lip and palate in the homozygotes. Using a backcross mapping strategy, we mapped the *Dc* mutation to a 1 cM region on proximal chromosome 19. A candidate gene screening approach, identified a T-box gene, *Tbx10* with tight linkage to the *Dc* mutation. By using *in situ* hybridization, we found that *Tbx10* is expressed highly specifically in a unique pattern during hindbrain development. While direct mutation screening did not identify any mutation in the exons or exon-intron boundaries in the *Tbx10* gene, we found that *Dc* mutant embryos display ubiquitous ectopic expression of *Tbx10*, particularly strong in the facial primordia. We found that the ectopic expression of *Tbx10* in the *Dc* mutants is caused by a translocation insertion of a Chromosome 10 segment containing the promoter-exon1 region of the *p23* gene into the first intron of the *Tbx10* gene. This insertion results in the expression of a chimeric message containing the first exon of the Chromosome 10 derived *p23* gene, spliced to the second exon of *Tbx10*. The *p23-Tbx10* chimeric message is predicted to encode a truncated *Tbx10* protein lacking the 41 N-terminal amino acid residues. The *Dc* mutant phenotype most likely results from this insertion event and the resultant ectopic expression of a chimeric *Tbx10* molecule. Analysis of endogenous genetic pathways disrupted by this mutation provides insight into general mechanisms of craniofacial development and reveals possible causes for cleft lip and palate in humans.

- 615. Activin Receptors IIa and IIb Exhibit Distinct Roles in Craniofacial Development.** R. Craig Albertson, Tracie L. Payne-Ferreira, and Pamela C. Yelick. Department of Cytokine Biology, The Forsyth Institute and Department of Oral and Developmental Biology, Harvard School of Dental Medicine, Boston, Massachusetts 02115.

We explored the roles of two closely related activin type II receptors, Actr2a and Actr2b, during craniofacial development in the zebrafish (*Danio rerio*). Protein depletion studies, using antisense morpholino oligomers, suggest that both receptors are required for normal development of the zebrafish craniofacial complex, although each may be acting independently. Both Actr2a and Actr2b deficient animals lack posterior pharyngeal arch cartilages, while Actr2a deficient animals also exhibit severely reduced or absent anterior arch cartilages. Animals with reduced levels of both receptors lack posterior arch cartilages, and exhibit a novel anterior arch phenotype characterized by a medial-laterally compressed Meckel's cartilage. Molecular characterization of *actr2a* and *actr2b* morphants demonstrates that both genes function in neuroepithelial patterning and cranial neural crest cell induction. In summary, our results demonstrate critical roles for both receptors in craniofacial development, and reveal distinct roles in establishing anterior-posterior patterning of craniofacial tissues.

616. **A Somatic Boundary Is Formed by Two Unidirectional Signals Acting Posterio-Anteriorly and Vento-Dorsally.** Yuki Sato and Yoshiko Takahashi. RIKEN, Center for Developmental Biology (CDB), 2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe, 650-0047, Japan.

The somites are the bases of antero-posteriorly reiterated structures of the vertebrate body. The somites undergo periodical segmentation from the anterior end of the unsegmented mesoderm. By embryological surgeries with chicken embryos, we previously found that the cells posteriorly juxtaposed to a prospective boundary possess a novel boundary-inducing activity, which we designated as a β segmenter" (Sato, Y., Yasuda, K., and Takahashi, Y. (2002) Morphological boundary forms by a novel inductive event mediated by Lunatic fringe and Notch during somitic segmentation. *Development*, 129, 3633-3644). We also showed by combining in ovo electroporation and tissue transplantation techniques that the segmenter is mediated by Notch/Lunatic fringe. The segmenter acts only on the anterior cells but not the posterior ones. We here demonstrate another novel activity important for a boundary formation which acts along the dorso-ventral axis of the presomitic mesoderm. When transplanted into a non-segmentation site, a small portion of the next-boundary tissue induced an ectopic boundary only dorsally but not ventrally. Our findings of the two distinct unidirectional signals acting along the A-P and D-V axes imply a cellular polarity present in individual presomitic cells. Furthermore, the D-V acting signal appears to be important for the A-P identity of a formed somite since a formation of an ectopic boundary is accompanied with rearrangement of the A-P marker expression. We present a model in which the V-to-D acting signal and the segmenter (P-to-A) establish the segmentation fissure.

617. **Characterization of a Zebrafish Mutant That Affects Segmentation.** Wendy Durst,* Clarissa Henry,* and Sharon Amacher. University of California Berkeley, California.

The Notch pathway plays a crucial role in segmentation of the paraxial mesoderm into somites and we have undertaken an *in situ* based screen to identify upstream regulators of Notch signaling. We have identified a cold sensitive mutation that disrupts expression of *her1*, a downstream transcriptional repressor in the Notch pathway. We find that *textit*her1 expression is disrupted in *b562* embryos at all temperatures examined. A related gene, *her7*, as well as the Notch ligands *textit*deltaC and *deltaD* are also misexpressed in the presomitic mesoderm. However, the expression of downstream genes such as *textit*myoD and *paraxial protocadherin* is only mildly disrupted. Thus, there is residual segmental patterning in *b562* mutant embryos that is clearly seen when somite morphology is analyzed at the cellular level. Furthermore, F59 staining, which outlines muscle cells, indicates that muscle cells do not frequently cross segmental boundaries in *b562* embryos demonstrating that there is some segmental patterning in these embryos. However, proper somite boundary formation in these embryos is disrupted. Whereas there is a large accumulation of phosphorylated (active) Focal adhesion kinase (FAK) at wild-type somite boundaries, Fak does not accumulate at somite boundaries in *b562* mutant embryos. Furthermore, staining for *titin*, which delineates somite boundaries in wild-type embryos, also indicates that boundary formation is disrupted in *textit*b562 mutant embryos. Thus, these experiments indicate

that segmentation and boundary formation/maintenance are genetically separable and *b562* plays a role in the latter.

618. **Notch and Hedgehog Signaling in Zebrafish Somite Morphogenesis.** C. A. Henry, and S. L. Amacher. University of California, Berkeley, California.

The formation of myotome during development underlies much of the segmentation in the vertebrate adult. Myotome formation is a 2 step process. First, epithelial somites form. Next, somitic cells undergo muscle cell elongation where they elongate to span the entire anterior to posterior width of the somite. We have shown that embryos deficient for 2 downstream transcription factors of the Notch pathway, *her1+7*, form robust albeit imperfect somite boundaries. We currently show that muscle cells lacking *textit*her1+7 respect wild-type somite boundaries. Wild-type cells in a *textit*her1+7-deficient background do not rescue normal boundary formation, suggesting a non cell autonomous action for *her1+7*. To further understand muscle cell elongation, we have analyzed the dynamics of muscle cell elongation in wild-type embryos. Wild-type embryos show both an anterior to posterior and a medial to lateral gradient of muscle cell elongation. This pattern is preserved in all Notch pathway mutants examined thus far. However, Hedgehog signaling is necessary for the normal medial to lateral progression of muscle cell elongation. When Notch pathway mutant embryos are analyzed at 24 hours, robust albeit imperfect boundaries are delineated with phosphorylated Fak. The formation of these boundaries is dependent upon Hedgehog signaling. Therefore, we hypothesize that the specification of medial adaxial cells through Hedgehog signaling is a morphogenetic signal that instructs the medial to lateral pattern of muscle cell elongation. Furthermore, as has been found with *fused somites/tbx24* mutants, we suggest that the specification of adaxial cells in Notch pathway mutants is necessary for the recovery of boundary formation.

619. **Inhibition of the Cell Cycle Is Required for Convergent Extension of the Paraxial Mesoderm in Xenopus.** W. F. Leise and P. R. Mueller. The University of Chicago, Chicago, Illinois.

During embryogenesis, there must be balance between cell proliferation and other developmental events. While cell division is required to generate a sufficient number of cells for the functional organization of an embryo, the process of cell division is incompatible with other events such as cell specification, cell differentiation, and some types of cell movements. In *Xenopus*, cell divisions are rapid and synchronous early in development, but then slow and become spatially restricted with the onset of gastrulation. One tissue that transiently stops dividing at this point is the paraxial mesoderm, a dynamically mobile tissue that eventually forms the somites and body musculature of the embryo. We have found that inhibition of the cell cycle is required for the proper positioning and segmentation of the mesoderm. The cell cycle and rate of cell division is controlled by the activity of the Cdks. Wee2, a Cdk inhibitory kinase, is expressed in the paraxial mesoderm and is required for the low mitotic index of the paraxial mesoderm. Morpholino mediated depletion of Wee2 raises the mitotic index of the paraxial mesoderm to levels that match the surrounding tissues. Furthermore, in the absence of Wee2, convergent extension and somitogenesis of the paraxial mesoderm are severely disrupted. This defect can

be rescued by replacing the depleted, endogenous *Wee2*. In addition, other mechanisms that advance the cell cycle cause the same defects as *Wee2* depletion. Thus, the low mitotic index of the paraxial mesoderm plays an essential function in the integrated cell movements and patterning of this tissue.

620. Abstract #620 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

621. **Retroviral Expression of IGF Binding Protein 2 in Chick Embryo Limbs Suggests Involvement in Endochondral Bone Formation.** M. C. Fisher, C. Meyer and C. N. Dealy. University of Connecticut Health Center, Farmington, Connecticut.

Insulin-like growth factor binding protein 2 (IGFBP-2) has been implicated in regulating chondrocyte maturation and bone formation in the developing chick limb. IGFBP-2 is primarily thought to act as an inhibitor of IGF-I or IGF-II, but it may also act independently of IGF signaling. During endochondral bone formation, IGF-I and IGFBP-2 are colocalized in the precartilaginous condensations and perichondrium. As chondrogenic differentiation proceeds, IGFBP-2 is present in prehypertrophic and hypertrophic chondrocytes whereas IGF-I is only expressed in terminal hypertrophic chondrocytes. In this study, we found that IGF-II is also present in the condensing cartilage and perichondrium and, at later stages, in the proliferating and hypertrophic chondrocytes. To further investigate the role of IGFBP-2 in endochondral bone formation, an avian replication competent retrovirus, RCASBP(A), was used to express human IGFBP-2 in stage 18-21 chick embryo forelimb buds. By day 13 (stage 39), hIGFBP-2 was strongly expressed throughout the skeletal elements of the infected limb. Overexpression of IGFBP-2 resulted in a 10 percent reduction in length in the humerus and radius in embryos collected at day 13 and day 18. The mechanisms contributing to this phenotype are currently under investigation using molecular and cellular biology techniques to determine if overexpression of IGFBP-2 results in altered chondrocyte differentiation, proliferation, cell death or remodeling. Supported by NIH grants 5T32-DE07302 and HD22610.

622. **Role of EGF Signaling in Chick Limb Development.** M. Omi,* N. J. Maihle,† and C. N. Dealy*. *Department of BioStructure and Function, University of Connecticut Health Center, Farmington, Connecticut; and †Department of Biochemistry and Molecular Biology, Tumor Biology Program, Mayo Clinic, Rochester, Minnesota.

Epidermal growth factor (EGF), transforming growth factor- α (TGF- α) and their receptor (EGFR) are expressed in chick limb buds and have been implicated in limb morphogenesis. To investigate further the role of EGFR signaling in limb development, we expressed a constitutively active form of the chicken EGFR in chick limb buds *in ovo* using an RCAS retroviral vector. We found that constitutive EGFR signaling results in dramatic limb patterning abnormalities such as pre- and post-axial polydactyly including mirror-image-type digit duplication, syndactyly, and abnormal ventral limb curvature. Consistent with these phenotypes, we found that constitutive EGFR signaling induces ectopic *Fgf-8* expressing apical ectodermal ridges (AERs) and ectopic anterior *Shh* expression; inhibits cell death and stimu-

lates cell proliferation; and suppresses expression of the dorsal marker, *Lmx-1*. Our results suggest endogenous roles for EGFR signaling in limb digit patterning, AER formation and ventral identity. (This work was supported by NIH grant HD22610 to C.N.D.)

623. **Tbx Genes Specify Posterior Digit Identity through Shh and BMP Signaling.** Takayuki Suzuki, Jun Takeuchi, Kazuko Koshihara-Takeuchi, and Toshihiko Ogura. Nara Institute of Science and Technology, Japan.

Despite the extensive studies on the AP axis formation of limb bud, molecular mechanism that specifies the distinct digits along the AP axis has been remained unsolved. Mutations of human *TBX3* gene cause the ulnar-mammary syndrome, which is characterized by the absence of digit 4 and/or 5. Since *Tbx3* is expressed in the posterior side of limb bud, we speculate that *Tbx3* specifies the identities of posterior digits, which develop in the *Tbx3*-positive area. To examine this hypothesis, we first studied expression patterns of *Tbx2* and *Tbx3* genes in developing chick limb bud. *Tbx2* is expressed in interdigit (ID) 4. In contrast, *Tbx3* is expressed in ID3 and ID4. When examined in retinoic acid (RA)-treated limb buds, expression patterns of *Tbx2/3* genes correlate well with the identities of RA-induced digits. Furthermore, misexpression of *Tbx2* and *Tbx3* induced posterior homeotic transformation of digit III to digit IV and digit II to digit III, respectively. Conversely, misexpression of *VP16 Δ Tbx2* and *VP16 Δ Tbx3* induced anterior transformation. In both cases, alterations of expression of several markers (e.g. *BMP2*, *Shh* and *HoxD* genes) were observed. In addition, *Tbx2* and *Tbx3* rescued *Noggin*-mediated inhibition of the interdigital BMP signaling, which is pivotal to establish the digit identities (Dahn R.D., and Fallon J. F., (2000) *Science* 289, 438). These results strongly suggest that the identities of posterior digits are specified by *Tbx2* and *Tbx3* genes, highlighting the pivotal roles of *Tbx* genes in the specification of digit identities of vertebrate limbs.

624. **Limb Development in Doubleridge, a New *Dkk1* Hypomorph.** Maja Adamska, Bryan T. MacDonald, and Miriam H. Meisler. Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48109-0618.

Development of the vertebrate limb depends on establishment of three axes: proximal/distal, anterior/posterior, and dorsal/ventral. The apical ectodermal ridge (AER), a distinct stripe of stratified epithelium on the distal margin of the limb bud, regulates proximal/distal growth of the limb by secretion of FGF signals. Dorsal/ventral polarity is determined by the non-ridge limb bud ectoderm. Expression of *En1* in the ventral ectoderm represses *Wnt7a*, which is therefore restricted to the dorsal ectoderm. The AER forms on the border between ventral cells expressing *En1* and dorsal cells expressing *Wnt7a*. Molecular markers of the AER are first expressed in a broad ventral region of the mouse limb bud. It has been suggested that the AER forms by compaction of this region (Loomis et al., Development 1998). In *En1* null mice, the ventral ectoderm fails to compact, leading to expanded ventral expression of AER markers such as *Fgf8*. Similar expansion of the AER is seen in mice null for *Dkk1*, an inhibitor of *Wnt* signaling (Mukhopadhyay et al., Dev. Cell 2001). We recently described a new mouse mutant, doubleridge, with abnormal AER that is strikingly similar to *En1* and *Dkk1* null mice (Adamska et al., Dev. Biol. 2003). doubleridge appears to be a

Dkk1 hypomorph, with sufficient expression to permit head development and viability but not normal limb development (MacDonald et al. Abstract, this meeting). We are investigating interactions between Dkk1, En1, and Wnt7a by combining mutant alleles of these genes. The results will contribute to understanding Wnt signaling mechanisms in specification and maturation of the AER.

- 625. Hypomorphic Expression of *Dickkopf-1* in the *Doubleridge* Mouse Mutant.** Bryan T. MacDonald, Maja Adamska, and Miriam H. Meisler. Department of Human Genetics, University of Michigan, Ann Arbor, MI 48109-0618.

Dickkopf-1 (*Dkk1*) is a secreted Wnt antagonist required for head induction and limb morphogenesis. Mice homozygous for a targeted null allele of *Dkk1* die at birth, have abnormal limbs and lack anterior head structures. We report the cloning and characterization of *doubleridge* (*dbl*), a viable, transgene-induced limb mutant with postaxial polydactyly and syndactyly (Developmental Biology, (2003) 255: 350-362). The *dbl* transgene insertion site was isolated from a cosmid library of genomic DNA. A 60 kb genomic deletion on chromosome 19 was identified, consistent with previous physical and genetic mapping of the mutant. The insertion site is located 160 kb downstream of *Dkk1*. The *Dkk1* gene sequence and intron/exon structure are normal. In a cross between *Dkk1*^{+/+} and *dbl/dbl* mice, the *dbl* mutation failed to complement the *Dkk1* null allele, indicating *doubleridge* is an allele of *Dkk1*. *Dkk1*^{dbl/dbl} mice display a limb phenotype similar to *dbl/dbl* and *kk1*^{-/-} mice. Reduced expression of *Dkk1* was detected by whole-mount *in situ* hybridization of *dbl/dbl* embryos. To quantitate the expression of *Dkk1* from the *dbl* allele, we used an allele-specific primer extension/chain termination assay based on a SNP in the 3' UTR to determine the ratio of mutant to wild type transcripts. The *doubleridge* mouse provides a model for studying the dosage requirements for *Dkk1* during mammalian embryonic development.

- 626. Dach1 is a Novel Nuclear Bmp Antagonist Required for AER Maintenance and Transcriptional Repression of *Meis2* during Proximodistal Axis Formation of Limb Bud.** Yasuyuki Kida and Toshihiko Ogura. Nara Institute of Science and Technology, Nara, Japan.

Several factors are secreted from apical ectodermal ridge (AER) that controls outgrowth and patterning of the vertebrate limbs. Formation of this key structure is regulated by several factors. For example, a secreted factor Gremlin is involved in formation and maintenance of AER. Nonetheless, the precise molecular mechanism of these processes is still remained unclear. Here we show that Dach1, vertebrate homologue of *Drosophila* Dachshund, is one of key factors of these processes. Dach1 is expressed in the mesenchyme of limb bud, with an expression profile similar to Bmp-4. In biochemical analyses, we have found that Dach1 blocks the BMP signaling by interaction with Smad1 and recruitment of co-repressor complex. To explore the roles *in vivo*, we used a DD2 construct that acts as a dominant-negative form of Dach1. Overexpression of DD2 in the pre-AER region induced disruption of AER and distal truncation of limb. Additionally, DD2 construct misexpressed in the limb mesenchyme also caused disruption of AER. These results indicate that the intracellular BMP antagonism by Dach1 is directly involved in both the formation and the maintenance of AER. Moreover, overex-

pression of a VP16 fused Dach1, which acts as a constitutively active BMP agonist, up-regulated *Meis2* expression, whereas the DD2 cancelled the BMP-4 mediated repression of *Meis2*. Taken together, we conclude that the intracellular BMP antagonism by Dach1 regulates the formation and the maintenance of AER. In addition, Dach1 controls the pattern formation of limb bud along its PD axis mediated through *Meis2*.

- 627. Out of Bounds, a Mouse Mutant with Defective Apical Ectodermal Ridge (AER) Compaction Resulting in Patterning Defects along the AP, DV and PD Axes.** Scott D. Weatherbee, Kathryn V. Anderson, and Lee. A. Niswander. Developmental Biology Program, HHMI, Sloan Kettering Institute, New York, New York.

Out of bounds (*oob*) is a recessive ENU-induced mutant with abnormal autopod morphology. *oob* mutants consistently exhibit syndactyly of the central digits in all limbs, dorsal polydactyly and ossification defects in carpal/tarsal elements and phalanges. Morphologically, the phenotype first manifests at E10.5 when *oob* mutants do not show a visible AER, due to a lack of compaction of AER cells. Marker gene analyses reveal that *oob* mutants display defects in patterning along all three axes of the developing limb at this stage. These defects appear to result from a failure to properly set the dorsal and ventral boundaries of the AER. The AER marker *Fgf8*, shows expanded expression in E10.5 mutant ectoderm. The *En1* expression domain is also expanded, indicating that much of the AER expansion occurs in the ventral compartment. The dorsal AER also appears to contain more cells than normal, as the broadened domain of *Fgf8* expression is coincident with a retraction of dorsal *Wnt7a* from distal limb ectoderm. Despite this loss of distal *Wnt7a* (and *Lmx1b*), dorsal and ventral identity in *oob* mutant limbs is largely normal. However, the *Shh* expression domain is reduced, which may account for the altered limb morphology along the AP axis in *oob* limbs. Further analyses have identified patterning defects as early as E9.25, suggesting that the *oob* mutation affects very early stages of AER formation. Studies of the BMP and WNT signaling pathways are currently underway to further define the function of the gene disrupted by the *oob* mutation.

- 628. Cilia and Limb Patterning.** Qihong Zhang,* Noel S. Murcia,† and Bradley K. Yoder*. *University of Alabama at Birmingham and †Case Western Reserve University.

Mice lacking the Tg737 ciliogenic gene exhibit pathologies in multiple tissues along with developmental abnormalities including random left-right axis specification, neural tube closure defects, polydactyly, cleft palate, and supernummary teeth. A hypomorphic and null Tg737 allele has been generated that result in the formation of an extra anterior digit or as many as eight digits per limb, respectively. Here we confirm a role for Tg737 in skeletal patterning through transgenic rescue, analyze the pattern of Tg737 expression during development, and assess whether the loss of Tg737 alters the expression of limb bud markers activated in other polydactyly mutants. Surprisingly, there were no overt changes in *shh*, *ihh*, *patched*, *glis*, *bmp2*, *bmp4*, *fgf8*, or *hoxD* genes in the limbs of either of the Tg737 mutant lines. The only gene that showed an ectopic domain of expression was *fgf4*, the level of which correlated with the severity of the limb phenotype. Collectively, the data argue for a dosage affect of Tg737 on the limb pathology in the two Tg737 alleles that is independent of the

shh pathway. Due to the function of Tg737, we explored whether cilia are present on cells in the limb. Our analysis indicates that cilia are found on both the ectoderm and mesenchyme and that these cilia are aberrantly formed or absent in the mutants.

629. **Indistinguishable Forelimb Dysmorphogenesis Induced by Ethanol and by Retinoic Acid (RA) Signaling Antagonism or RA Synthesis Inhibition Provide Clues Regarding Ethanol's Teratogenic Mechanism.** C. S. Johnson, * K. K. Sulik, *† E. S. Hunter, III*. *Department of Cell and Developmental Biology, UNC-Chapel Hill; †Bowles Center for Alcohol Studies, UNC-CH; and **NHEERL, ORD, US EPA, RTP, North Carolina.

Administration of ethanol to pregnant C57BL/6J mice on embryonic day 9.25 causes distal forelimb reduction defects. The mechanism(s) of these and other ethanol-induced defects remains unclear. Several lines of evidence have linked ethanol's teratogenicity to hypovitaminosis A. Ethanol exposure decreases RA concentrations in mouse embryos and targeted double mutations of both RAR- α and - γ or of retinaldehyde dehydrogenase 2 cause limb dysmorphogenesis. Here we demonstrate in vivo that disulfiram (D), a known RA synthesis inhibitor, BMS-189453(B), a pan-RAR antagonist, and ethanol (E) all result in a comparable spectrum of limb malformations. Postaxial forelimb ectrodactyly in which 1 to 4 digits were absent was commonly observed in all three treatment groups: 64%, 67%, and 45% of affected limbs from D-, B-, and E-treated fetuses, respectively. Intermediate digit reductions were also observed: 7%, 23%, and 14% of D-, B-, and E-treated fetuses, respectively. Within 8 hrs of maternal drug treatment, cell death was observed in the distal ectoderm of limb buds following both E and D exposure. B exposed embryos exhibited the same pattern of cell death although less frequently and at a lower magnitude than ethanol or disulfiram-exposed specimens. These results demonstrate morphological and pathological consistency among E, D, and B treatment groups, supporting the hypothesis that RA depletion is a mechanism underlying ethanol-induced limb defects. Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

630. **Functional Analysis of BMP3 in Mice and Frogs.** Laura Gamer, John Nove, and Vicki Rosen. HSDM and Forsyth Institute, Boston, Massachusetts.

BMP3 is the most abundant BMP in bone, but unlike many other BMP family members, cannot induce bone formation at ectopic sites. In vitro, BMP3 inhibits osteoblast differentiation and blocks the osteogenic activity of BMP2 and BMP7. During skeletogenesis, BMP3 localizes to the forming perichondrium and later is found in perisoteal cells and differentiating osteoblasts of developing bone. In mice, loss of BMP3 results in a significant postnatal increase in trabecular bone mass without any effects on skeletal patterning. Despite these data, nothing is known about the mechanism of action of BMP3-how it signals, what its target genes are, or how it's regulated. We have used *Xenopus* embryos, a well established system for understanding BMP ligand, receptor, and antagonist function to address some of these questions. Unlike BMP4, which is a strong ventralizing factor, BMP3 is a dorsalizing molecule in *Xenopus* embryos. Overexpression of BMP3 in the marginal zone dorso-anteriorizes embryos, causing them to have shortened, curved axes, and enlarged notochords, as

well as reduced eye and brain tissue and expanded cement glands. When BMP3 is injected ventrally, it induces partial secondary axes. In animal caps, BMP3 induces neural tissue and cement gland without mesoderm induction. These effects of BMP3 on embryos and ectodermal explants are similar to what has been reported for factors that block BMP signaling. Interestingly, BMP3 appears to be antagonized by BMP signals, as BMP4 blocks the induction of cement gland markers in animal caps. Our data suggest that BMP3 is a novel regulator of patterning in *Xenopus* embryos and bone formation in mice and may be acting by inhibiting BMP signals.

631. Abstract #631 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

632. **Beyond Vertebrates: Distribution and Differentiation of Cephalopod Cartilage.** A. G. Cole and B. K. Hall. Dalhousie University.

Cartilage has long been thought to be a tissue restricted to vertebrates. However, tissues that are histologically indistinguishable from vertebrate cartilage are found in most cephalopod species. We examined the onset of development and distribution of these cartilaginous tissues in five cephalopod species. Of these, *Octopus bimaculoides* (octopus), *Sepia officinalis* (European cuttlefish), *S. pharionis* (pharaoh cuttlefish) and *Euprymna scolopes* (bob-tail squid) were found to develop most of their cartilaginous tissues during embryogenesis so that many of these structures were present when the juveniles hatched. In contrast, *Loligo pealeii* (common squid) hatched with only the scleral and nuchal cartilages present. To determine whether or not these tissues develop in a similar manner to vertebrates, the differentiation of cartilaginous tissues in *S. officinalis* was investigated using histological and immunohistological techniques. *S. officinalis* has many distinct cartilaginous tissues which we categorize into two different developmental modes: 1) direct development from loose mesenchyme, and 2) development from a condensation of mesenchymal cells. Either of these developmental modes may be preceded by the formation of a distinct overlying epithelial layer, although this feature does not appear to be necessary for all cartilages. The development of cephalopod cartilages was found to be remarkably similar to cartilage development in vertebrates.

633. **FGF/FGFR2 Signaling in Mandibular Morphogenesis.** B. Havens, A. Liddell, P. Murthy, B. Rodgers, and M. Mina. School of Dental Medicine, University of Connecticut Health Center, Farmington, Connecticut.

The alternative splicing of *Fgfr2* results in the generation of IIIb (containing exon 8) and IIIc (containing exon 9) isoforms with different ligand-specificities and tissue distributions. FGFR2B is activated by FGF-1, -3, -7 and -10 which are predominately synthesized by mesenchyme, whereas FGFR2C is activated by FGF-2 and FGF-4, which are predominately synthesized by epithelium. Despite extensive information on the patterns of expression and functions of *FGFR2b*, *FGFR2c*, and *Fgf10* during morphogenesis of many organs, there is a lack of detailed analysis of their expression and functions in the developing chick mandible. To address functions of FGF/FGFR2 in mandibular morphogenesis we have analyzed the expression of *FGFR2b*, *FGFR2c* and *Fgf10* in

developing chick mandibles. *FGFR2c* is predominantly expressed in the mandibular mesenchyme, and *FGFR2b* is predominantly in the epithelium. Our results also show expression of *Fgf4* in the mandibular epithelium and a transient expression of *Fgf10* between stages 18-20 in the mandibular mesenchyme. These observations suggest roles for FGF10/FGFR2b signaling in epithelial-mesenchymal interactions regulating mandibular morphogenesis and are further supported by the hypoplastic mandibles in mutant animals specifically deficient for the *FGFR2b* isoform. Our preliminary observations indicate that over-expressing a RCAS-DN-*FGFR2c* in the mandibular mesenchyme results in abnormalities in mandibular skeletogenesis. These observations suggest that reciprocal paracrine signaling loops between different FGFs and different isoforms of FGFR2 function during mandibular morphogenesis. Supported by DE08682 DE07302.

634. **RAR Alpha Regulation of Distal Lung Differentiation.** C. Wongtrakool, S. Malpel, J. Gorenstein, J. Sedita, M. Ramirez, M. Underhill, and W. V. Cardoso. Pulmonary Center, Boston University School of Medicine; and School of Dentistry, University of Western Ontario, London, Ontario.

Although retinoic acid (RA) has been shown to be critical for lung development, previously reported data from a RA responsive element *RARE-lacZ* reporter mouse show that when epithelial tubules are branching and differentiating RA signaling is dramatically downregulated in the epithelium. Little is known about why this downregulation occurs and what role it plays in the developing lung epithelium. Here we analyze the effects of preventing potential progenitors of the distal lung from turning off RA signaling in a genetic model. We generated transgenic mice expressing constitutively activated RAR α or β chimeric receptors (RARVP16) targeted to the distal lung epithelium with a surfactant protein-C (*Sp-C*) promoter. RAR α VP16 lungs were immature and expressed the early markers of distal epithelial cell fate *Sp-C* and *Ttf1* (thyroid transcription factor-1) and showed high levels of fibroblast growth factor *Fgf10* in the mesenchyme. Distal saccules and type I cells did not form and differentiation appeared to be arrested in a stage reminiscent of that of a primordial lung. In contrast RAR β VP16 lungs underwent later stages of differentiation and showed less severe changes. Our data suggest a model in which activation of RAR α signaling at the onset of lung development assigns distal cell fate to the prospective lung epithelium. RAR α activation, however, is incompatible with subsequent stages of this program which should be carried out by a retinoid-independent network of regulators in the distal lung during branching morphogenesis.

635. **Heparan Sulfates Expressed in the Distal Lung Are Required for Fgf10 Binding to the Epithelium and for Airway Branching.** Konstantin I. Izvolsky,* Lin Zhang,† Lin Wei,* Qiang Yu,* Matthew A. Nugent,† and Wellington V. Cardoso*. *Pulmonary Center, Department of Medicine, and †Department of Biochemistry, Boston University School of Medicine.

Fibroblast growth factor (Fgf) 10 is a critical regulator of bud formation during lung morphogenesis. Fgf10 is expressed in distal lung mesenchyme at sites of prospective budding from the earliest developmental stages and signals through its epithelial receptor Fgfr2b. Experiments in intact lung organ cultures dem-

onstrate that distal but not proximal epithelium responds to exogenous Fgf10 by growing toward the Fgf10 source. This differential response suggests the involvement of an additional mechanism regulating Fgf10-Fgfr2b interactions, since Fgfr2b is uniformly expressed throughout the respiratory tract. Here we use an immunohistochemistry-based binding assay to show that O-sulfated heparan sulfates (HS) are critical for Fgf10 binding to the distal epithelium. We show that altering endogenous gradients of HS sulfation with sodium chlorate or over-O-sulfated synthetic heparin in lung organ cultures dramatically decreases Fgf10 binding. Moreover we show that under these conditions epithelial binding is not improved by providing exogenous FGF10. Our data suggest that, not only ligand availability, but also the presence of specific patterns of HS modification in the distal lung epithelium are critical determinants of Fgf10 binding to the epithelium and Fgf10-Fgfr2b signaling.

636. **Cathepsin H, a FGF10 Target in the Developing Lung.** J. Lu, K. Izvolsky, X. Qi, D. Keppler, and W. Cardoso. Pulmonary Center, Boston University School of Medicine, Boston, Massachusetts; and Department of Cellular Biology and Anatomy, LSU-HSC, School of Medicine, Shreveport, Louisiana.

Cathepsin H, a FGF10 target in the developing lung signaling by FGF10-FGFR2b is essential for primary lung bud formation and branching morphogenesis. To identify epithelial targets activated by the FGF10 pathway when lung buds are forming, we performed DNA microarray analysis of FGF10-treated mesenchyme free epithelial cultures. Among the genes whose expression was altered, we found several members of the cathepsin family of cysteine proteases differentially induced at 8 and 24h. Cathepsin H (CTSH) showed the highest levels of induction at 8h ($p > 0.01$, 35 x folds). *In situ* hybridization of E11-14 lungs confirmed strong CTSH signals in distal epithelium. CTSH expression is markedly up-regulated by grafting of FGF10-soaked beads in lung organ cultures. Treatment with Ser (O-Bzl)-CHN2, a CTSH-selective inhibitor, reduces branching activity and the epithelial response to exogenous FGF10 in vitro. Our data suggest a role for CTSH in FGF10 induced migration and proliferation during lung bud morphogenesis.

637. **The Interaction of a Novel Xin Protein with β -catenin at the Adherens Junction of the Intercalated Discs in Developing Hearts.** Elisabeth Gustafson-Wagner, Shannon Jaacks, Haley Sinn, Jenny L-C. Lin, and Jim J-C Lin. Department of Biological Science, University of Iowa.

We have previously demonstrated a likely role for a novel, striated muscle specific gene, *Xin*, in cardiac morphogenesis (Wang et al., (1999) *Dev.* 126: 128). *Xin* co-localizes with both N-cadherin and β -catenin at the adherens junction of the intercalated disc throughout embryogenesis and adulthood. Co-immunoprecipitation experiments demonstrated that *Xin* is detected in a complex with N-cadherin/ β -catenin (Sinn et al., (2002) *Dev. Dyn.* 225: 1). The potential interaction of *Xin* with this complex suggests that *Xin* may play an important role in the formation of cell-cell contacts at the adherens junctions and myofibrillogenesis. To explore this interaction, an *in vivo* yeast 2-hybrid analysis was employed. Using the full length mouse *Xin* (mXin) fused with the binding domain of Gal4, and β -catenin fused with the activation domain of Gal4, we demonstrated a

direct protein-protein interaction between mXin and β -catenin. The amino acid sequence of Xin is highly conserved between chicken and mouse, and contains proline rich regions at its C-terminus, 16 amino acid repeats in its middle region, and a DNA binding domain at its N-terminus. To determine more specifically where mXin is interacting with β -catenin, several deletion constructs lacking portions of these domains were generated. Preliminary data using these constructs suggests that the novel 16 amino acid repeat region is required for this interaction. Furthermore, a minimum number of these repeats may be necessary to construct the β -catenin binding domain on the mXin protein.

638. **Morphogenesis of the Zebrafish Liver and Pancreas.** Holly A. Field, Elke A. Ober, Duc Dong, and Didier Y. R. Stainier. Department of Biochemistry and Biophysics, University of California, San Francisco, California.

Using a unique transgenic zebrafish line that expresses GFP throughout the endoderm (Field et al., (2003) *Developmental Biology* 253, 279-290), we are able to follow the morphogenesis of the zebrafish digestive system, focusing specifically on the development of the liver and the pancreas. Since the liver has been relatively understudied in the zebrafish, our primary work defined its spatiotemporal development from the first signs of organogenesis through the establishment of a well formed organ. The zebrafish pancreas has received greater attention, with previous studies suggesting that it develops from a single bud arising dorsally from the developing intestine. Our analysis of the gutGFP line revealed for the first time a second, more anterior bud that also contributes to the pancreas. We used gene expression and mutant analyses to show that pancreatic endocrine and exocrine tissues originate from the posterior and anterior buds, respectively. Since recent studies have demonstrated a role for vascular endothelium in the development of the liver and pancreas (Lammert et al., (2001) *Science*, 294, 564-567; Matsumoto et al., (2001) *Science* 294, 559-632), we outlined the process of liver and pancreas vascularization and use mutant analyses to investigate the role of vascular endothelium in the development of these organs in zebrafish. This research is supported by a grant from the NIDDK.

639. **Analysis of *Fear of Intimacy*: a Novel Gene Involved in Gonad Formation.** W. Mathews and M. Van Doren. Johns Hopkins University, Baltimore, Maryland.

Formation of the embryonic gonad is a unique developmental process essential for the propagation of a species. This bi-potential organ provides a nurturing environment for developing germ cells as they mature into egg or sperm. Despite the importance of this process, very little is understood about how the germ cells interact with specialized somatic cells. We discovered a *Drosophila* gene, *fear of intimacy* (*foi*), that is required for the proper coalescence of these two distinct cell types into a single organ. In *foi* mutants, somatic gonad precursors are properly specified but fail to undergo the morphological movements required for gonad formation. *foi* mutants also have tracheal defects, where neighboring segments fail to fuse, preventing the formation of a continuous tracheal network. Our work indicates that FOI, a member of a novel family of transmembrane proteins conserved from yeast to humans, is a cell surface glycoprotein with extracellular N- and C-termini and a cytoplasmic central

domain. To ascertain FOI's role in gonad formation, we are determining which regions of FOI are essential for its function using an *in vivo* structure-function analysis. Our data indicates that the specific sequence of the transmembrane domains and the highly conserved HELP domain are essential for FOI function, suggesting FOI may act molecularly as a channel and interact with other proteins. Possible cellular roles for FOI include cell-cell adhesion, interactions with the cytoskeleton or transduction of a signal.

640. **Disturbed Expression of Sox9 in PreSertoli Cells Underlies B6.Ytir Sex-Reversed Mouse Gonads.** Norma Moreno-Mendoza, * Leda Torres-Maldonado* and Horacio Merchant-Larios†. *Department of Cell Biology and Physiology, Instituto de Investigaciones Biomédicas, UNAM, México, D.F. México 04510.

The Sry of some varieties of *Mus musculus domesticus* fails to form normal testis when introduced into the C57BL/6J (B6) strain. Here, we studied the developmental pattern of preSertoli cells expressing Sox9 by immunofluorescence and the profile levels of Sox9 transcripts by semiquantitative RT-PCR in developing gonads of B6.Ytir mice. Sox9 positive cells (preSertoli cells) appeared in all B6.Ytir genital ridges at 11.5 and 12.5 dpc. However, at 13.5 dpc, Sox9 positive cells were undetected in around 50% of the B6.Ytir gonads. From 14.5 dpc onwards, preSertoli cells formed the seminiferous cords in the medial region of the gonad, while at the cranial and caudal regions ovarian tissue was formed. Surprisingly, Sox9 transcripts were detected in all B6.Ytir gonads regardless of their phenotypic sex. However, B6.Ytir ovaries have lower levels of Sox9 than ovotestes at all stages. Present results suggest that the preSertoli cell lineage appears in B6.Ytir genital ridges but their further differentiation as Sertoli cells is prevented. The cause may be the low levels of Sox9 and down-regulation of its product. Results suggest that inhibitory signals of Sox9 acting along the whole genital ridge or only at its cranial and/or caudal regions underlie formation of B6.Ytir ovaries or ovotestes, respectively. We hypothesize that failure of Sox9 expression is due to disturbed positional cues in B6.Ytir gonads. Furthermore, current results suggest that infertility of B6.Ytir females is due to the abnormal presence of Sox9 transcripts in their ovaries.

641. **MEK1 Signaling Is Primordial for Normal Murine Placental Morphogenesis.** Bissonauth Vickram and Charron Jean. Centre de Recherche de L'Hôtel Dieu de Québec, CHUQ, QC, Canada.

To study the role of MEK1 in murine development, we have generated a mouse line in which *Mek1* has been disrupted by insertional mutagenesis. *Mek1*^{-/-} embryos die between E9.5-10.5 but do not present any major malformations. A marked reduction in labyrinthine placental vascularisation is observed. Starting at around E9.5, mutant placentae show abnormal morphogenesis of the syncytiotrophoblasts (SCT) that separate the foetal blood vessels from the maternal sinuses. SCT precursors (*Gcm1* positive cells) are present in mutant placentae but do not seem to migrate in the labyrinthine region, hence interfering with normal angiogenesis in the labyrinthine region. Western analyses on placental extracts show that the ERK/MAPK pathway is much less activated in the *Mek1*^{-/-} specimens. Immunohistochemical analyses show that in *Mek1*^{+/-} placentae, MEK1/2 are ubiquitary

and highly activated in the labyrinthine region while ERK1/2 are activated around maternal sinuses and in labyrinthine trophoblasts. In mutant placentae, MEK2 is activated in the chorio-allantoic junction and in some cells of the chorion while ERK1/2 are activated exclusively in the allantois. Our results suggest that ERK1/2 are not activated in the chorion in absence of MEK1 despite the presence of activated MEK2. We also show that MEK1 is necessary for normal vascular network formation in the labyrinthine region and might participate in the migration and differentiation of SCT. We have designed a conditional mutant *Mek1* allele which will allow us to generate *Mek1*^{-/-} embryos with wild type placentae. This would help us determine the importance of *Mek1* in trophoblast morphogenesis. (Funded by the CIHR)

642. **Formation of the Chorioamniotic Membrane (Choram) in Chick Embryos.** Y. Evrard, R. Pulver, and B. Holton. Department of Biology/Microbiology, University of Wisconsin, Oshkosh, Wisconsin.

In avians, the ChorAm begins as a fold in the anterior blastodisc. It rises above and envelopes the head, then moves posteriorly. Later, a second fold forms in the posterior blastodisc and moves anteriorly. The two folds merge and eventually close like a drawstring purse. We are interested in mechanisms of ChorAm formation. 1) The anterior fold of the ChorAm may progress posteriorly by a) zipping closed or b) tractoring. We marked the ChorAm, with charcoal or DiI, near its center and close to its leading edge. The markers aggregated and moved with the leading edge as it progressed down the embryo. This suggests that neither mechanism (above) is responsible for ChorAm expansion. Rather, both layers (overlying chorion and underlying amnion) may move down the embryo simultaneously. 2) The anterior fold of the ChorAm, as it forms, is under tension. Apoptotic cells (that exist in the ChorAm) may maintain equal tension across the membrane. Thus, we stained embryos, at different developmental stages, for apoptotic cells. Before embryos turned, dying cells were equally distributed at the leading edge of the ChorAm. When the embryos bulged as they turned to the right and when the ChorAm dropped below the heart of the embryo, cell death increased about two-fold. Significantly, 2-3X more dying cells were on the right side of the membrane (in right-turned embryos). Cell death may eliminate excess cells on the right to equalize tension across the membrane. Finally, when the anterior and posterior folds met and as they closed, like a string pouch, cell death decreased by at least half, perhaps because other mechanisms (contraction of cytoskeletal elements) maintained even tension. We have begun experiments to alter tension across the membrane and to observe resulting changes in cell death patterns.

643. **COBRA, a GPI-Anchored Membrane Protein, Regulates Anisotropic Cell Expansion during *Arabidopsis* Development.** François Roudier, Anita Fernandez, and Philip N. Benfey. Duke University; New York University.

In the absence of cell movement during plant morphogenesis, cell expansion is one of the key parameters that determines cell shape and ultimately the form organ takes. The regulation of the extent and orientation of cell expansion is directly linked with the ability of the plant cell wall to dynamically modulate its mechanical properties to resist osmotic pressure. COBRA, a

GPI-anchored membrane protein, plays a critical role in this fundamental process. *cobra* mutants were originally identified by their dramatic and conditional expansion defect during root development. They were also characterized by a deficiency in crystalline cellulose deposition. Cellulose microfibrils are the main load-bearing component of plant walls and their oriented deposition is critical to set the direction of expansion. Phenotypic analysis of a knock-out mutant revealed that COB is a key regulator of anisotropic expansion throughout post-embryonic morphogenesis and will be presented in detail. The developmentally regulated expression of *COB* as well as the subcellular localization of the protein will be shown. Possible functions for COBRA in regulating the deposition or re-arrangement of cellulose microfibrils will be discussed. Functional analysis of the ten *COB-like* homologues present in the *Arabidopsis* genome will also be presented.

644. **Electrical Controls of Regeneration in Planaria.** Taisaku Nogi and Michael Levin. The Forsyth Institute.

Planarians possess remarkable powers of regeneration. Morgan and Child proposed a gradient of morphogen along the anterior-posterior (AP) axis that controls which structures are formed at each position, as a cut fragment regenerates a head and tail at the correct end. However, no endogenous morphogens are yet identified and this fascinating process is still poorly understood. Interestingly, the little-known work of Marsh and Beams suggested that externally-applied voltage gradients can control the AP polarity of regenerating fragments. Thus, we propose a new model: that an endogenous electrical gradient formed by ion channels and pumps is aligned with the AP axis, and controls the identity of regenerating tissues. Because the electrical characteristics of tissue depend crucially on gap-junctional communication (GJC), and because electric fields can direct the movement of small signaling molecules through gap junctions, we performed a pharmacological screen to determine whether electrogenic proteins and/or gap junctions are involved in patterning during regeneration. Exposure of cut fragments to many blockers of various ion channels, pumps, and gap junctions revealed that while most targets were not implicated in regeneration, inhibition of GJC results in a bipolar head phenotype, and inhibition of the H⁺/K⁺-ATPase results in a no-head phenotype. In vivo use of DiBAC4(3), a membrane voltage potential-sensitive dye, showed that while there is no electrical gradient in the intact worms, a striking AP voltage gradient appears within 6 hours of cutting. We are now pursuing cloning of the genes identified by our screen, analysis of their expression, and molecular gain- and loss-of-function approaches.

645. **The Role of Microtubules in *Xenopus* Convergent Extension.** Kristen M. Kwan and Marc W. Kirschner. Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115.

Lane and Keller (1997) discovered, via nocodazole treatment, that microtubules are required for the initial polarization of cells of the vegetal alignment zone, the first cells that exhibit mediolateral polarity in the dorsal marginal zone of *Xenopus* gastrulae. We are interested in understanding the cellular and molecular basis of the nocodazole effects, and the specific role that microtubules play during the polarization process. We are also interested in understanding the developmental switch at stage 10.5

which renders convergent extension insensitive to nocodazole. To this end, we are performing timelapsed spinning disk confocal microscopy, and we can simultaneously image both actin and microtubules in live explants. We have found that nocodazole treatment prior to stage 10.5 inhibits lamellipodial and filopodial protrusions. However, nocodazole treatment of explants at stage 10.5 or later does not inhibit active protrusions. There does not appear to be a large scale change in the structure or dynamics of the microtubule cytoskeleton at stage 10.5, as microtubules are still extremely dynamic and can be fully depolymerized by nocodazole. Lane and Keller reported that taxol has no effect on convergent extension, and we observe that taxol has no effect on active protrusions, although microtubules are indeed stabilized. This suggests that microtubule mass, and not some aspect of polarized dynamic growth, may be the crucial aspect of the microtubule requirement. We are currently exploring aspects of actin-microtubule crosstalk as the explanation for the nocodazole results, and if so, how this is regulated by the developmental switch at stage 10.5.

646. **Characterization of Chicken NF2/Merlin Indicates Potential Roles In Growth Regulation and Cell Migration.** Y. Chen,* D. H. Gutmann,† C. Haipek,† M. Bronner-Fraser,** and C. E. Krull*. *Division of Biological Sciences, University of Missouri-Columbia; †Department of Neurology, Washington University, School of Medicine; **Division of Biology, California Institute of Technology.

Merlin, the protein product of a tumor suppressor gene, Nf2, acts as a negative regulator of cell growth. Merlin is required for embryonic development, since Nf2 null mice die before gastrulation due to defects in extraembryonic ectoderm. Recently, we isolated and characterized chicken Nf2 (cNf2) and its protein product, merlin (c-merlin). RT-PCR results show that cNf2 produces a full-length version and two truncated isoforms. C-merlin shares over 90% homology with mammalian merlins and contains similar protein motifs. C-merlin is expressed in a variety of cell types during avian embryonic development, including neural crest cells, muscle precursors, and cells in the heart and lens. Specifically, c-merlin protein labels myotomal cells that give rise to axial muscle but is not detected on migratory muscle precursors in the limb. As limb muscle forms, c-merlin expression appears to be reinitiated. To examine the role of merlin, we have taken a gain-of-function approach and ectopically expressed c-merlin in muscle precursors in the lateral dermomyotomes of multiple somites at limb levels, using *in ovo* electroporation. Few muscle precursors enter the limb when ectopically expressing c-merlin. Analyses to date indicate that ectopic merlin expression does not elicit cell death but reduces cell proliferation. Experiments are in progress to examine whether ectopic expression of c-merlin alters the delamination or migration of limb muscle precursors.

647. **Defective Convergence in Half-Baked Gastrulae.** D. A. Kane, K. N. DelKanic, and R. M. Warga. Department of Biology, University of Rochester, Rochester, New York.

The gene *half-baked* (*hab*) is required for zebrafish epiboly, the first morphogenetic movement of the teleost embryo. In *hab* mutants, epiboly of the blastoderm is arrested shortly after the beginning of gastrulation, although epiboly of the enveloping layer and the yolk occurs normally. Using a combination of

morphology, double mutant analysis, and cell transplantation, we have examined the dominant phenotypes of *hab* that affect the development of the neural tube and other ectodermally derived structures. These studies have supported the idea that *hab* is acting within the ectodermal epithelium during these later developmental events. To examine the hypothesis that *hab* is acting within the spreading epiblast of the deep cells during epiboly, we monitored cell movements in the epiblast before and during the period when the epiboly arrest phenotype occurs. We found that *before* the arrest of epiboly *convergence* of the mutant epiblast is reduced to 25% of the wild-type rate. Such reductions arise neither in the speed of cell movement in the hypoblast nor in the rate of involution into the hypoblast. In an analysis of cell shape, we found that epiblast cells are lacking their normal epithelial morphology and polarity, suggesting that *hab* is necessary for the organization and function of the converging epiblast. Hence, *hab* is necessary both for the epiboly of the epiblast and the convergence of the epiblast, providing the first genetic evidence linking the two processes. Could it be that convergence of cells to the midline of the embryo, an ancient process found in all vertebrates, has been co-opted to help drive the extreme epiboly of the teleosts?

648. **PAR Proteins are Required for Cell Adhesion and Gastrulation in *C. Elegans* Embryos.** Jeremy Nance and James R. Priess. FHCRC and HHMI, Seattle, Washington.

The morphogenesis and migration of cells depends on the polar localization of proteins to specific membrane domains. The first cell migrations within the *C. elegans* embryo occur during gastrulation, when cells destined to form internal tissues move from the embryo's surface into a central cavity, the blastocoel. We are interested in learning how early embryonic cells are polarized to allow processes such as blastocoel formation and gastrulation to occur. There is a well-described requirement for a group of conserved proteins called PAR proteins in cell polarity, including in the one-cell embryo of *C. elegans*. PAR proteins that show anterior-posterior cortical asymmetries at the one-cell stage are redistributed at later stages to adopt an apical-basal asymmetry. As an approach to studying the role of PAR proteins in apical-basal polarity, we have tagged PAR proteins that normally are restricted to the apical domain (PAR-3, PAR-6) with a sequence (the ZF1 domain) that promotes their degradation in early embryonic cells. We find that PAR::ZF1 proteins rescue the anterior-posterior defects of *par* mutant embryos, and are then degraded. After PAR::ZF1 protein degrades, PAR proteins normally restricted to basolateral surfaces spread to the apical surfaces of cells. *par* mutant embryos expressing PAR::ZF1 develop defects in the adhesion of early embryonic cells, and the ingress of cells during gastrulation is delayed. We propose that 'apical' PAR proteins regulate the localization or activity of proteins involved in cell migration and/or adhesion.

649. Abstract #649 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

650. **Hedgehog Signaling Regulates Cell Proliferation and Differentiation in Pharyngeal Cartilages.** L. P. Hernández and S. H. Devoto. George Washington University and Wesleyan University.

Hedgehog (Hh) signaling regulates important developmental processes including growth and morphogenesis of teeth and chondrocyte proliferation in long bones. We have tested whether Hh signaling is involved in growth and differentiation of the cartilaginous pharyngeal arches in zebrafish. We examined the development of the pharyngeal arches in embryos with mutations in the sonic you (*syu*, encoding *Shh*), you too (*yot*, encoding *Gli2*) or slow muscle omitted (*smu*, encoding *Smoothed*) genes. We found a significant difference in growth rate of arches between mutants and WT. All arches have developed by 72hpf in WT, but branchial arches (arches 3-7) are missing at this stage in all mutants. While branchial arches in *syu* and *yot* mutants ultimately developed, they never did in *smu* mutants. Moreover, the mandibular and hyoid arches were greatly reduced in *smu* mutants. Cyclopamine specifically blocks Hh signaling. To test whether the loss or reduced growth of arches in mutants was an indirect result of early disruption of head structures, we allowed WT embryos to develop until 12h and 24h before treating with cyclopamine. Larvae sampled after treatment for 3d and 4d showed defects in both the pharyngeal arches and neurocranium reminiscent of those characterizing midline mutants. While these larvae all had cartilaginous elements associated with arches 1 and 2, cartilaginous elements associated with arches 3-7 were missing. We conclude that while Hh is involved in cell proliferation within all pharyngeal cartilages it plays a unique role in differentiation of branchial cartilages since in the absence of Hh signaling, these cartilages fail to develop.

651. Position-Specific Responsiveness to Jaw Identity Signals RA and Noggin. Joy M. Richman. Department of Oral Health Sciences, UBC, Vancouver, Canada.

Jaw identity is, at least in part controlled by secreted signals, retinoic acid (RA) and Bone Morphogenetic Proteins (BMP). We have demonstrated that the implantation of beads soaked in Noggin, a BMP antagonist and RA in the stage 15 chicken embryo leads to transformation of the side of the face (maxillary region) into a second facial midline (frontonasal mass). The previous study placed the beads only into one location, the proximal first branchial arch (BA1). The objective of the present study was to determine whether low BMP and high RA levels could specify the midline in other regions of the face or whether only the proximal BA1 was competent to respond. We implanted Noggin/RA beads 1) in BA2, 2) midway between the eye and first arch, the presumptive maxillary region or 3) between the eye and nasal pit, the presumptive lateral nasal prominence. Noggin/RA beads implanted into position 2, induced complete duplications of midline skeletal elements (8/8). Noggin/RA beads placed in the presumptive lateral nasal prominence gave no defects (4/4), nor did beads placed in the second branchial arch (2/2). A re-examination of skulls that had beads implanted in the first branchial arch revealed that duplicated elements lay lateral to an almost intact palatine bone. In contrast, beads in position 2 gave rise to duplicated elements that completely replaced all maxillary prominence derivatives. We conclude that precise RA and BMP concentrations are sufficient to completely respecify identity of the presumptive maxillary region and that the region of competence does not extend medial to the eye or inferior to the second branchial arch. Funding provided by the CIHR.

652. Apoptosis Plays A Role in Removal of the First and Second Aortic Arch Arteries, in Remodeling of the Thyroid Rudi-

ment and in Rupture of Closing Plates in Chick Embryos. S. J. Klempner and S. A. Miller. Biology Department, Hamilton College, Clinton, New York.

Extensive modification is an important part of avian pharyngogenesis. Aortic arch arteries form and are then removed or altered, pharyngeal pouches are remodeled as their closing plates rupture, and the thyroglossal duct is lost after the thyroid evaginates from the pharyngeal floor. Differential cell proliferation is part of pharyngeal remodeling (Miller, et al, 1993), but involvement of apoptosis has not been conclusively demonstrated in chick embryos. We used the TUNEL technique (ApopTag^o, Serologicals) to determine if apoptosis correlated with these morphogenetic events. Apoptotic cells are present in the mesenchyme around aortic arch arteries 1 and 2 at the time they regress via a capillary plexus (Hiruma and Hirakow, 1995). Extensive apoptosis is apparent in the thyroid rudiment (HH18-20) and later in the thyroglossal duct area of HH22-25 embryos. Apoptotic cells were also marked in closing plates of pharyngeal pouches 2 and 3. Our results suggest that programmed cell death acts in conjunction with cell proliferation, cell shape changes and possible cell migration during remodeling of the chick embryo pharynx. [Supported by a Hamilton College Senior Fellowship and a Sigma Xi Grant-in-Aid of Research to SJK].

653. Moe, a Novel FERM-Domain Containing Protein Required for Tight Junction Formation. Abbie Jensen and Monte Westerfield. Biology, University of MA-Amherst and Institute of Neuroscience, University of Oregon.

Moe embryos have small brain ventricles, edema, abnormal retinal pigmented epithelium, and a disorganized retina (Jensen et al., Dev.128:95-105, 2001). Our recent analyses show that *Moe* is required for tight junction (TJ) formation, either directly as a component of the TJ or by acting upstream in the cell polarity pathway. *moe* encodes a novel FERM-domain containing protein. Two point alleles display the same phenotype as a *moe* deficiency and are predicted to be nulls that truncate *Moe* in the middle of the FERM domain. Fly, worm, and human genomes contain conserved *moe* homologs. In zebrafish, another FERM-domain containing gene, *protein tyrosine phosphatase non-receptor type 4* (*ptpn4*), lies adjacent to *moe*. This relationship is conserved in humans, KIAA1548, a *moe* homolog on 2q21, is adjacent to *PTPN4*, and KIOOO388, a second *moe* homolog on 9q22 is adjacent to *PTPN3*. In worms, the *moe* homolog (U80955) and *ptp-1* are on chromosome III and in flies, the *moe* homolog (CG9764) and *ptpmeg* are on chromosome 3. Phylogenetic analysis groups all the *moe*-like genes together and all the *ptp*-like genes together, with a common ancestral gene in the distant past. We propose that a tandem duplication in an ancestor to flies, worms, and vertebrates generated a *moe*-like gene and a *ptp*-like gene and a chromosomal duplication in the vertebrate lineage generated the second copies of *moe* and *ptpn* genes.

654. Abstract #654 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

655. Genetic Mapping and Characterization of the m196 Mutation in Zebrafish. R. McBride,*† M. Montero,** S. Sachdev, E. Knapik,** M-A. Akimenko,*†. *Ottawa Health Research Institute and †Department of Cellular and Molecular Medi-

cine, University of Ottawa, Ontario; and **Institut fuer Biologie I, Universitaet Freiburg, Germany.

The m196 mutation, a result of an ENU mutagenesis screen in zebrafish, disrupts embryonic development resulting in the loss of gill arches of the craniofacial cartilage, median and pectoral fin folds, as well as causing defects in the heart and a shortened stature. A high variability can be seen among mutant phenotypes, though a lack of median and pectoral fin fold development is consistently observed. Mutant phenotypes arise at 24 hours post fertilization (hpf) with the onset of fin fold development and lead to early mortality between 30 hpf and 3 weeks from unknown causes. The branchial arches, heart, and fin mesenchyme are all contributed to in part by cells of neural crest origin. However early and late neural crest markers, such as *dlx2*, *fgf8*, *msxC*, *sox9b*, and *foxd3*, show wildtype expression patterns in 10-32 hpf with two exceptions. *Dlx2* expression is disrupted in the posterior median fin epithelial layer. *MsxC* expression is also perturbed in the pectoral fin mesenchyme, which may be due to the varying extent of pectoral fin development in these mutants. Currently we have mapped the m196 mutation to a 0.99cM interval on LG17 and are screening large genomic insert libraries for clones spanning this interval. These results as well as the candidate gene approach will lead to the identification of a novel or known genetic factor essential for craniofacial and fin development in zebrafish. Supported by a NSERC grant to MAA.

656. **Functional Analysis of Zebrafish c-Met Receptor Tyrosine Kinase during Embryogenesis.** Jason R. Jessen and Lilianna Solnica-Krezel. Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee 37232.

Deregulation of c-Met activity contributes to the formation and progression of many human cancers. During normal vertebrate development, engagement of the c-Met receptor tyrosine kinase by its ligand, hepatocyte growth factor (HGF)/scatter factor, stimulates a variety of cellular responses including migration and proliferation. To better understand the role of the HGF/c-Met signaling system in development and disease, we have cloned a zebrafish homologue of the *c-met* proto-oncogene and initiated its functional analysis during embryogenesis. Zebrafish c-Met has 50% overall amino acid identity with human c-Met and 81% identity in the cytoplasmic domain encompassing the kinase catalytic domain and the multi-substrate docking site located near the C-terminus. Whole-mount *in situ* hybridization showed *c-met* expression in tissues undergoing morphogenetic cell movements including endoderm, anterior neural plate, and posterior lateral line primordium. Ectopic overexpression of *c-met* mRNA in zebrafish embryos caused effects ranging from shortened embryonic axes to formation of partial secondary axes. To specifically address how c-Met influences morphogenesis, we have constructed putative dominant-negative constructs that are either missing the entire cytoplasmic domain or possess specific amino acid changes that disrupt protein function. Embryos injected with these constructs are currently being analyzed for disruptions in cell movements and tissue patterning.

657. **Functional Analysis of a Zebrafish PDZ-RhoGEF.** Jennifer L. Ray, Jason R. Jessen, and Lilianna Solnica-Krezel. Department of Biological Sciences, Vanderbilt University, Nashville, TN 37232.

Wnt signaling regulates many aspects of animal development. In vertebrate systems, there are two major classes of Wnt signaling pathways: the canonical, which mediates cell fate specification via beta-catenin, and the non-canonical, which influences morphogenesis through regulation of RhoA and calcium. During vertebrate gastrulation, non-canonical Wnt/Planar Cell Polarity (PCP) signaling mediates convergence and extension (C&E) cell movements, which aid in formation of the embryonic body plan by narrowing its mediolateral axis and lengthening its anteroposterior axis. Given that RhoA is activated in response to Wnt/PCP signaling, and Rho guanine nucleotide exchange factors (GEFs) facilitate Rho activation by stimulating the exchange of GDP for GTP, it is likely one or more RhoGEFs act in this pathway. We have isolated a novel zebrafish gene that encodes a PDZ domain-containing RhoGEF. Sequence analysis revealed this gene contains an evolutionarily-conserved site of alternative splicing and that the encoded protein is similar to human PDZ-RhoGEF. Whole mount *in situ* hybridization indicated *pdz-rhogef* RNA is present ubiquitously during early development. Loss of function experiments produced shorter and broader embryos, while overexpression of *pdz-rhogef* RNA also resulted in an impairment of C&E movements, along with a notable epiboly delay. These observations suggest a role for PDZ-RhoGEF in mediating zebrafish gastrulation movements. Our current experiments investigate the involvement of PDZ-RhoGEF in non-canonical Wnt signaling.

658. **Death Is the Major Fate of Medial Edge Epithelial Cells of Secondary Palate Shelves and the Cause of Basal Lamina Degradation during Their Fusion.** L. Covarrubias and R. Cuervo. Department of Developmental Genetics and Molecular Physiology, UNAM, Cuernavaca, Mor., México.

A pair of shelves fuses to form the secondary palate, a process requiring the degeneration of the medial edge epithelial tissue (MEE) that separates them. It has been reported that epithelial-mesenchymal transformation (EMT) occurs during shelf fusion and is considered a fundamental process for MEE degeneration. On the other hand, we recently found that cell death detected in the fusion line is required for shelf fusion. We used different labeling procedures to follow the fate of MEE cells in whole cultured palates, or in a novel slice culture system. Very few labeled cells were found in the mesenchyme compartment, and almost all were undergoing cell death. Inhibition of metalloproteinases, which prevents EMT in other systems, had no effect on MEE degeneration and cell death. Remarkably, independently of shelf fusion, activation of cell death promoted the degradation of the basal lamina underlying the MEE. Finally, by specific labeling of periderm cells (i.e., the superficial cells that cover the basal epithelium), we observed that epithelial triangles at oral and nasal ends of the epithelial seam do not appear to result from MEE cell migration but rather from migration of periderm cells. Inhibition of migration or removal of these cells suggests that they have a transient function controlling MEE cell adhesion and survival, and ultimately died within the epithelial triangles. We conclude that MEE degeneration occurs almost uniquely by cell death, and for the first time we show that this process can activate basal lamina degradation during a developmental process.

659. **Mechanisms Underlying the Robustness of Morphogen Gradients.** Arthur D. Lander, Sean Kim, Qing Nie and Frederic

Y.M. Wan. University of California, Irvine, California 92697.

Morphogen gradients are established by an interplay between opposing processes: transport of morphogens through tissues, and binding and subsequent degradation of morphogens by their receptors. The dynamics of this interplay are sufficiently complex as to yield unexpected properties and constraints. For example, when equations are written and analyzed that describe gradients formed by diffusion as the sole morphogen transport mechanism, many experimental results are predicted, including most of those that have been taken as evidence against morphogen transport by diffusion [Lander et al. [2002] *Dev. Cell* 2:785]. Although diffusion and receptor-interaction alone can generate morphogen gradients of appropriate shapes in appropriate time periods, such gradients are not at all robust—where robustness is defined as the ability of a system to produce the same, or nearly the same, output in the face of substantial changes in input or internal parameters. The fact that development and patterning are very robust suggests that some molecular interactions that occur within morphogen gradients have evolved to improve robustness. Here we consider two processes that are observed in most morphogen gradients: feedback regulation of receptor synthesis by morphogen signaling, and morphogen 'stickiness' to abundant non-receptor binding sites. We show computationally that regulation of receptor synthesis, by itself, adds little to gradient robustness, whereas morphogen stickiness can add a great deal. Interestingly, these two phenomena can act synergistically to provide greater improvement in robustness than either process alone. Supported by NIH grants NS26862 and HD038761.

660. **Genetic Integration of Semaphorin/Ephrin Signaling Controls Cell Sorting and Guidance during Epidermal Morphogenesis in *C. Elegans*.** R. Ikegami and J. Culotti. Mt. Sinai Hospital.

Semaphorins and ephrins are two conserved families of repulsive guidance cues. In *C. elegans*, *sema-2a/mab-20* and *ephrin/mab-26* regulate guidance during ventral enclosure and sorting of sensory ray precursor cells during male tail morphogenesis. We present genetic data for an integrated semaphorin/ephrin signaling control. Several *erf* (enhancer of ray fusion) mutations were identified in an enhancer screen for the ray fusion defect of a weak allele of *mab-20*. Plexin/*plx-2* was also identified, consistent with the function of plexins as specific receptors for semaphorins. The expression pattern of *plx-2* confers the cell-specific function of ubiquitous MAB-20. Our genetic analysis shows that: (1) *plx-2* functions in the same genetic pathway as *mab-20*. (2) MAB-20 also signals in parallel to PLX-2 through a functionally redundant non-homologous receptor. (3) *mab-26* signals in the same genetic pathway as *mab-20*, but in parallel to *plx-2*. (4) *erf-1* and *erf-2* signal in the *mab-20* pathway parallel to *plx-2* as shown by a synthetic ray fusion defect (Syn-Mab). (5) *erf-2* functions in the same pathway as *mab-20* and *mab-26*. We present a genetic and cellular model for *mab-20*-dependent guidance of pocket cell migration and show that the Eph receptor *vab-1* functions in parallel to *plx-2* to sort out and position a *plx-2*-expressing neuroblast domain that serves as a substratum for the guidance of the overlying pocket cell migration.

661. **Investigating the Role of Eph/ephrin-A during Trigeminal Ganglion Axon Guidance.** C. S. Jayasena and S. A. Koblar.

ARC Centre for the Molecular Genetics of Development (CMGD), School of Molecular and Biomedical Science, The University of Adelaide, SA, 5005, Australia.

The trigeminal ganglion (TG) is composed of sensory neurons that innervate the ophthalmic (Op), maxillary (Mx), and mandibular (Md) processes of the developing vertebrate face. To date, the expression of a number of axon guidance cues such as Semaphorin3A¹ and Neuropilin in the TG and its target fields has been demonstrated. An additional family of axon guidance molecules, the Eph receptor tyrosine kinases and ephrins (their ligands) are likely to participate during patterning of the TG peripheral nerve branches. We investigated the global expression of EphA and ephrin-A at stages 13, 15 and 20, by probing with ephrin-A5 and EphA3-Fc fusion proteins respectively in the avian embryo. EphA and ephrin-A were expressed in a complementary and overlapping manner during early TG development. RNA *in situ* hybridisation revealed the expression of *ephrin-A5* in the TG, and both *ephrin-A2* and *-A5* in the first branchial arch (Mx and Md processes). Immunofluorescent staining demonstrated the presence of EphA4 in all TG target fields. The functional significance of target EphA4, and ephrin-A5 at stage 20 was explored using an *in vitro* substratum choice assay². Based on the *in vitro* results, we hypothesise that ephrin-A5-EphA mediated repulsive signaling guide TG axon growth to the Op, Mx and Md processes respectively. 1. Taniguchi *et al.*, *Neuron* (1997) **19** :519-30. 2. Birgbauer *et al.*, *Development* (2000) **127** :1231-41.

662. **Axon Guidance during Neural Regeneration in Planarians: Characterization of Netrin and Netrin Receptor Homologues.** Francesc Cebrià,* Kiyokazu Agata,† and Phillip A. Newmark*. *Department of Cell and Structural Biology, University of Illinois at Urbana-Champaign, Illinois; and †RIKEN Center for Developmental Biology, Kobe, Japan.

Well-orchestrated axon guidance is a fundamental step towards the proper patterning and wiring of the nervous systems of both vertebrates and invertebrates. Several evolutionarily conserved families of molecules (e.g. netrin, slit, semaphorin and ephrin) are involved in guiding axons to their proper targets. Whereas most studies have focused on how these different attractive or repulsive cues operate during embryogenesis, much less is known about their roles during neural regeneration. Freshwater planarians can regenerate a complete, functional central nervous system (CNS) *de novo*. Recently, the characterization of a large number of neural genes has revealed the complexity of the planarian CNS at both the molecular and structural levels. The availability of conserved neural markers, together with planarian CNS plasticity, make these animals attractive models in which to study axon guidance during neural regeneration. Here, we show the isolation of planarian homologues of netrin and netrin receptor (DCC/frazzled) genes, as well as their expression patterns in intact and regenerating organisms. The netrin receptor homologue is expressed broadly in the CNS; planarian netrin, however, shows more limited expression in the brain and in the ventral muscle fibers. Functional analyses of these genes using RNAi indicate that they are involved in axonal guidance during the regeneration of the planarian nervous system.

663. **Analysis of Molecular Guidance Cues Directing Axon Extension from the Ventral Cochlear Nucleus.** David M. Howell, Albert S. Berrebi, George A. Spirou, and Peter H.

Mathers. Sensory Neuroscience Research Center and Department of Otolaryngology, West Virginia University School of Medicine, Morgantown, West Virginia.

A goal of developmental neuroscience is to identify molecular factors necessary for the orderly formation of neural connections. In contrast to other sensory systems, little is known about the cues guiding the development of auditory circuits. Our model system, the calyceal projection, originates from globular bushy cells in the ventral cochlear nucleus (VCN) whose axons terminate within the contralateral superior olivary complex (SOC) as calyces of Held. We have visualized developing VCN projections by placing DiI in the VCN of mouse brains between embryonic day (E) 12.5 and postnatal day 3. We observed that as early as E14.5, axons exiting the VCN have already bypassed the presumptive ipsilateral SOC and crossed the midline. Branches into the contralateral SOC were first detected on E18.5. To identify candidate molecules directing the initial extension of this projection, we analyzed the expression patterns of netrin-1 and its receptor, deleted in colorectal cancer (DCC). Netrin-1 expression was restricted to the midline of the auditory brainstem (E11.5 û E17.5) and DCC protein was present in fibers exiting the VCN (E13.5 to E15.5). Thus, the spatial and temporal distributions of netrin-1 and DCC are consistent with attracting developing VCN axons to the midline. Moreover, initial analysis indicates that the calyceal projection is disrupted in DCC-deficient mice. These results suggest that the netrin-DCC signaling system plays an important role in the establishment of calyceal projections in the developing mouse auditory system.

664. Hypomorphic and Normal Expression of a Point Mutated NMHC II-B Results in Distinct Phenotypes in Mice. X. Ma, S. Kawamoto, and R. S. Adelstein. LMC/NHLBI/NIH.

In an effort to produce a mouse model for a putative human disease, we introduced a single amino acid mutation (R709C) into the nonmuscle myosin II-B heavy chain (NMHC II-B) in a conserved area of the motor region. Presence of Neo-cassette resulted in decreased expression of the mutated myosin. Hypomorphic homozygous mice, expressing 35% of the mutated myosin, showed defects in hearts and brains. These included a ventricular septal defect and binucleation and enlargement of the embryonic cardiac myocytes. The brain defects included delayed migration of the cerebellar granule cells, pontine and facial neurons. Removal of the Neo cassette elevated the mutant NMHC II-B expression to the level in the wild types. Both heterozygous and homozygous mutants showed novel abnormalities in addition to some of those found in the hypomorphic mice. Most heterozygous mice develop an umbilical hernia containing liver and intestine, due to a failure in closure of the abdominal wall. In homozygous mutants a more severe herniation develops that mislocates the heart, liver and intestine, outside the body wall. Defects in cardiac structure, including septal defects and origin of both aorta and pulmonary artery from the right ventricle were consistently seen. The homozygous mutants also developed generalized edema, and died around E14. Brain defects in these mice include protrusion of neural cells through the pial surface of the neocortex, in addition to the abnormal migration of pontine and facial neurons found in the mutant hypomorphic mice. In summary, NMHC II-B R709C mutant mice are providing a useful model for exploring myosin II-B function in the mouse and ultimately in humans.

665. Slit1a is Required for Optic Tract Development in the Zebrafish. Lara D. Hutson,* Sang-Yeob Yeo,† Hitoshi Okamoto,† and Chi-Bin Chien*. *Neurobiology and Anatomy, University of Utah Medical Center, Salt Lake City, Utah; and †Brain Science Institute, RIKEN, Wako-Shi, Japan.

Slits are secreted proteins that guide migrating cells and growth cones. While they are repulsive in many contexts, Slits are able to promote axon outgrowth and branching in others. Slit2 has been demonstrated to repel retinal ganglion cell (RGC) axons in several systems, and it appears that multiple Slits combine to direct RGC axons across the ventral midline of the diencephalon so that the optic chiasm forms at the appropriate location. In the zebrafish mutant *astray/robo2*, a presumptive Slit receptor, RGC axons make pathfinding errors throughout the pathway between the eye and the optic tectum. Thus, it is likely that one or more Slits are important for RGC axon pathfinding not only in the optic chiasm, but also later in the optic tract. Our laboratories have cloned cDNAs for four zebrafish *slits*, *slit1a*, *slit1b*, *slit2*, and *slit3*. *slit2* and *slit3* are expressed in patterns consistent with their mediating pathfinding in the ventral diencephalon, while only *slit1a* is expressed in the optic tract region. We have injected morpholino oligonucleotides (MO) to *slit1a*, which were designed to prevent translation of mRNA into protein, as well as an MO designed to disrupt splicing of *slit1a*. As predicted, *slit1a* MOs disrupt optic tract formation at doses which do not affect patterning or differentiation of the developing CNS. Specifically, MO injections cause delays in optic tract formation and misrouting of axons in the optic tract. These results suggest that, unlike Slits in the ventral diencephalon, Slit1a may be attractive for RGC axons in the optic tract. Alternatively, Slit1a may act to modulate responses to other guidance cues.

666. Defining the Cellular and Molecular Architecture of the Zebrafish Chiasm Region. Barresi,* E. Swindell,† N. Hopkins,† J. Shin,** H. Park,** B. Appel,** and R. Karlstrom*. *University of Massachusetts, Amherst, Massachusetts; †M.I.T.; and **Vanderbilt University.

We use zebrafish genetics and embryology to further define the cellular identity and guidance functions of radial glial cells at the midline of the forebrain. We show that prior to commissural axon pathfinding, GFAP positive glial cells occupy the midline where crossing occurs. Furthermore, AC and POC axons cross the midline in association with radial glial cells that specifically express GFAP and Olig2. There is a reduction in this specific population of forebrain glial cells in the Hedgehog pathway mutants (*you-too* and *detour*), and this may in part be responsible for the midline pathfinding errors also seen in these mutants. We have characterized several molecular cues in the chiasm region. Both *sema3D* and *slit2* are expressed locally at the diencephalic midline prior to POC/RGC axon crossing. In *yot* mutants, *sema3D* expression is reduced and *slit2* is expanded at the diencephalic midline. The correlation between the loss of *sema3D* and expansion of *slit2* expression with the loss of midline axon crossing in *yot* suggests that *Sema3D* may act as an attractant and *Slit2* as a repellent for retinal and commissural growth cones. Finally, we are conducting an antibody screen of retroviral induced mutations for defects in axon pathfinding and glial development. We have now screened 50 of 300 mutant lines and identified three axon guidance mutants and three glial mutants. This screen is extremely sensitive and promises to

reveal both novel and known genes that are important for CNS development.

667. **Zebrafish *Topped* Is Functioning in a Subset of Fast Muscle Cells to Allow Stereotyped Axon Outgrowth.** Louise Rodino-Klapac and Christine E. Beattie. Neurobiotechnology Center, The Ohio State University, Columbus Ohio.

Zebrafish primary motor axons extend along stereotyped pathways punctuated by characteristic intermediate targets. In a screen conducted to elucidate genes that control motor axon pathfinding, we isolated a mutation in the *topped* gene. In *topped* mutants, CaP (Caudal Primary) motoneurons are specifically affected. The axons stall at the first intermediate target, the future horizontal myoseptum, eight times longer than wild-type CaP axons, and fail to enter the ventral myotome at the proper time. *In vivo* labeling of individual CaP cell bodies in *topped* mutants revealed that the axons begin to recover and enter the ventral myotome by 30 hours post fertilization. To test the autonomy of *Topped* with respect to CaP motoneurons, we generated genetic mosaics by blastula transplants. These data revealed that *Topped* is non-cell-autonomous for CaP motoneurons. To elucidate the exact location of *Topped* function, we used blastula transplantation to introduce wild-type cells into *topped* mutant embryos. Interestingly, ventromedial fast muscle was the only subset of cells able to rescue the CaP axon defect in *topped*. Moreover, the extent of rescue was limited to the dorsoventral position of the transplanted muscle. This data suggests that *Topped* is acting as a ventral cue to allow CaP axons to navigate to their final target in the distal ventral myotome.

668. Abstract #668 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

669. **Identification of Neurotactin as a Dominant Enhancer of The Ablason Tyrosine Kinase Mutant Phenotype.** M. A. Seeger,* R. G. Rowe,† D. J. Forsthoefel,* A. Stammer,* E. Bishop,* X-Y. Liu,* and E. C. Liebl†. *The Ohio State University; Denison University.

We have previously reported identifying a strong dominant enhancer of the Abl mutant phenotype as a missense allele of amalgam (ama-M109). Wild-type amalgam encodes a secreted protein, composed of three immunoglobulin-like domains, that binds to the transmembrane receptor Neurotactin (Nrt), mediating cell:cell adhesion (EMBO J. 19: 4463-4472). We now show that ama-M109 encodes a secreted protein that binds to Nrt, but does not mediate efficient cell:cell adhesion. This altered biological activity is responsible for ama-M109's dominant enhancement of the Abl-mutant phenotype, as we have recovered an ama-null allele in screens for reversion of ama-M109's dominant enhancement of the Abl-mutant phenotype. The genetic interaction between ama and Abl led us to ask whether there is any interaction between nrt, and Abl. Previously, RNAi experiments in embryos had shown disruption of nrt enhances the Abl mutant phenotype, resulting in strong CNS phenotypes. We now report that mutations in nrt are strong dominant enhancers of the Abl-mutant phenotype as assayed by either viability or CNS architecture. We have identified this effect in three null alleles and two missense alleles of nrt. Furthermore, we have found that all five mutations originally attributed to disabled are in fact

alleles of nrt. Additional genetic and cell biological characterization of the nrt:Abl interaction will be presented. These results are all consistent with a model whereby cell:cell adhesion mediated by Nrt engagement of Ama forms part of an signaling network involving Abl during axon pathfinding.

670. **Theseus, an Orphan G-Protein Coupled Receptor, Is Required for Transepithelial Migration of *Drosophila* Germ Cells.** Rabhat S. Kunwar,* Michelle Starz-Gaiano,* Roland Bainton,† Ulrike Heberlein,† and Ruth Lehmann*. *Skirball Institute, HHMI, Developmental Genetics Program, NYU Medical Center, 540 First Ave, New York, NY-10016; and †Neuroscience Program, University of California, San Francisco.

In *Drosophila*, the primordial germ cells (PGCs) are the first cells to form during embryogenesis. Subsequently, they undergo a complex migratory route through and along different tissues to form a gonad. During gastrulation, germ cells are carried into the blind pocket of the posterior midgut pocket. They have to cross through the posterior midgut epithelium and migrate towards gonadal mesoderm to form embryonic gonad. The migration of germ cells is directed by repulsive and attractive cues expressed by different somatic tissues along their route. Previous work has identified several guidance molecules such as the phosphatidic acid phosphatases, (Wunen and Wunen-2) and HMGCoA reductase necessary for germ cell migration. Little is known about the genes that act within the germ cells required for migration. Here we report the identification of *theseus*, which encodes an orphan G protein coupled receptor and is expressed in the germ cells. Embryos deficient for the receptor are defective in transepithelial migration through the posterior midgut. In the *theseus* mutant embryos, most germ cells do not exit from posterior midgut. But those that leave the midgut migrate normally to the gonad suggesting this gene is not required of normal motility and directionality of germ cells. Its phenotypic characterizations, interaction with other known guidance molecules and downstream signaling pathways using multiple approaches will be presented.

671. **Signalling Downstream of Guidance Receptors in Border Cell Migration.** Carlos M. Luque, Tudor Fulga, Peter Ducheck, Pamela Klingbeil, and Pernille Rorth. European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.

Border cells (BC) are a cluster of 6-10 specialized cells present in *Drosophila* egg chamber. At stage 9 of oogenesis migrate towards the oocyte in a stereotypic way. Studies in the lab have identified two receptor tyrosine kinases (RTKs) that guide BC; the epidermal growth factor receptor (EGFR) and the PDGF/VEGF receptor (PVR). RTK signalling involves receptor dimerization, phosphorylation of Tyr and docking of proteins. We aim to determine which pathways downstream of EGFR and PVR translate guidance information into directed BC movement by 1) identifying in the receptors the residues relevant for signal transduction used in BC guidance, 2) defining the set of molecules that are able to bind to the activated receptors via the residues identified above and 3) determining whether those proteins are required for BC guidance *in vivo*. Using point mutagenesis and constitutively active receptors, we identified two cytoplasmic Tyr on EGFR that are both necessary and sufficient to block BC

migration. PVR has a different behaviour, 16 cytoplasmic Tyr are not necessary, though PVR kinase activity is still required. In a Two-Hybrid screen, 22 proteins were found to interact with PVR. Eight of them display SH2 domains: PI3K (isolated 10 times), Shc (9), Drk (3), Vav (3), Dock (3), Spt6 (2), Stat92E (1) and CG13289 (1). We are testing their ability to bind either PVR or EGFR through the residues relevant for guidance. In this approach we will include as well all SH2, PTB or PTBI proteins predicted in the *Drosophila* genome. Finally, we will look for migration defects on BC mutant for the partner proteins identified above.

672. Regulation of Border Cell Migration by Effectors of the EGFR Pathway. L.-M. Pai, P.-Y. Wang, and I.-C. Lee. Department of Biochemistry and Molecular Biology, Chang Gung University, Taiwan.

Cell migration is a fundamental process during development in various organisms. During *Drosophila* oogenesis, a group of epithelial cells called border cells migrate through nurse cells to reach the oocyte. Border cell migration requires at least two signals. 1, The JAK-STAT activation stimulates the transition of epithelial cell to mesenchymal cell. 2, Gradient signals from the oocyte attract border cells to move posteriorly then dorsally. Signals from the oocyte have been identified as EGF and PDGF, and both receptors for these signals share common downstream effectors in cell migration. Hyper-activation or inactivation of either receptor in border cells results in migration defects. *D-cbl*, a negative regulator of the EGFR pathway, had been previously identified in our laboratory. When a dominant negative mutant of *D-cbl* was expressed in border cells, the cell migration was interrupted. Over-expression of wild-type *D-cbl* also displayed similar phenotypes. Using these backgrounds, a screen for second mutation, which either enhances or suppresses the migration defect, is undertaken. Further elucidation of the role of these mutated genes will lead to understanding the mechanism regulating cell migration. In addition, we have identified a protein with a Calponin homology domain and a LIM domain, which acts as a suppressor in extra vein phenotype generated by expression of dominant negative *D-cbl* in the wing. Ectopic expression of the gene in border cells markedly affected cell migration. To which point of the EGFR signaling is influenced by this gene will be reported.

673. Abstract #673 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

674. Expression of a Human Caldesmon Fragment Defective in Ca^{2+} Calmodulin Binding Sites Interferes with the Dynamics of Actin Filaments and Affects Motile Behaviors. Yan Li, Jenny L.-C. Lin, Rebecca S. Reiter, and Jim J.-C. Lin. Department of Biological Sciences, University of Iowa.

Calmodulin (CaM) regulates many aspects of cell movement, yet the mechanism remains unclear. Caldesmon (CaD) is an actin-binding protein whose functions can be regulated by Ca^{2+} /CaM. We have previously shown that the carboxyl-terminal fragment of human fibroblast CaD (CaD39) retained the ability to bind actin and CaM, and to participate in the Ca^{2+} -sensitive regulation of actomyosin ATPase activity. To study the regulation of CaD by Ca^{2+} /CaM, we generated a CaD39 mutant, CaD39/CaM, in which the two critical tryptophan residues

required for Ca^{2+} /CaM binding (W461 and W494) were mutated into alanines. In vitro, the mutations drastically reduced Ca^{2+} /CaM binding but did not impair the ability to bind to actin. Stable expression of CaD39/CaM in the Chinese Hamster Ovary (CHO) cells led to increased cell size. Force-expressed CaD39/CaM exhibited a diffuse staining throughout the cell body. However, it concentrated in the lamellapodia protrusions, colocalizing with cortical actin meshwork. The fact that CaD39/CaM does not incorporate into stress fibers suggests CaD39/CaM may disrupt the formation of actin bundles, possibly by interfering with the bundling proteins like fascin. In wound healing assays, cells overexpressing CaD39/CaM exhibited defects in both speed and persistence of movement. Such motile defects were not observed in cells that overexpress the wild type CaD39 fragment. All together, our data suggests CaD is a key downstream effector that confers the regulation of Ca^{2+} /CaM on the dynamics of cytoskeleton.

675. The p120 Catenin Regulates Morphogenetic Movements in Early *Xenopus* Embryos by Modifying the Activity of the Rho-Family Small Gtpases. Malgorzata Ciesiolka, Mieke Delvaeye, Griet van Imschoot, Frans van Roy, and Kris Vlemminkx. Ghent University-VIB Department of Biomedical Research, Developmental Biology Unit, Ghent, Belgium.

In order to understand the function of Xp120ctn in early development we either overexpressed Xp120ctn or mutants of E-cadherin that no longer bind Xp120ctn. In both cases the head structures were affected as reflected by malformations of the eyes and the craniofacial skeleton. Interestingly, as has also been documented in vitro, we observed that overexpressed Xp120ctn modulates the activity of endogenous RhoA and increased the amount of GTP-bound Rac. Moreover, Xp120ctn overexpression is phenocopied by dominant-negative RhoA or wild type Rac. The morphological defects obtained by Xp120ctn overexpression can be fully rescued by coinjection of wild type or constitutive active RhoA or dominant-negative Rac. In addition, coinjection of XE-cadherin with Xp120ctn could rescue the embryos and relieved the RhoA inhibition/Rac activation, possibly by sequestering the excess of cytosolic p120ctn. These results indicate that Xp120ctn is critically involved in regulating morphogenesis in the early *Xenopus* embryo both through action on the cytoskeleton and by regulating the activity of the cadherins.

676. Isolation and Characterization of Reticulon (RTN) and Nogo Proteins in *Xenopus laevis*. Sangwoo Shim, Edmond C. Park, Gun-Sik Cho, Jin-Kwan Han. Division of Molecular and Life Sciences, Pohang University of Science and Technology, San 31 Hyoja-Dong, Pohang, 790-784, Republic of Korea.

Reticulon (RTN) is a gene family possessing a conserved Reticulon domain and consists of two main groups: Nogo and Reticulon (RTN1, 2, 3). Although Nogo has been implicated for the axon outgrowth and neuronal regeneration, the nature of Reticulon function remains elusive. We isolated *Xenopus* homologues of Reticulon family, XRTN1, 2, 3, and XNogo-A, B, C. They all encode proteins that contain a Reticulon domain, two putative membrane-spanning domains and an ER retention motif at the C terminus. For RTN1, we showed that XRTN1 is localized to the ER membrane when overexpressed in the embryos or mammalian cells. Northern blot analysis and whole-mount in

situ hybridization showed that XRTN1 is not expressed in early neural precursors but rather in differentiating neuronal populations including olfactory nerve, optic nerve, dorsal root ganglia, and trigeminal ganglia. Moreover, we showed that 3' UTR RNA sequences in XRTN1 have evolutionarily conserved motifs that may have a role in localization of XRTN1 RNA and protein in specific regions of the neuron. As for XNogos, the transcripts are expressed from the early neurula stage in the neural regions and localized to the neural tissues and somites at the tailbud stages. To further explore the roles of Nogos in axon outgrowth and neuronal regeneration, or putative additional role in the early embryogenesis, overexpression and loss-of-function study using morpholinos against XNogos are underway. We are also investigating the roles of Rho-family GTPases and their effectors in Nogo signaling pathway.

- 677. PINCH Mediates A Syndecan-2: β 1-Integrin Complex That Drives Fibronectin Fibrillogenesis and Cell Migration during *Xenopus* Gastrulation.** K. L. Kramer, E. W. Prewitt, J. E. Barnette, M. McGrail, M. C. Beckerle, and H. J. Yost. Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah.

PINCH is a cytoplasmic adapter protein that has recently been shown to regulate fibronectin fibrillogenesis in cell culture by mediating integrin inside-out signaling. Here we demonstrate a novel role for PINCH in mediating fibronectin fibrillogenesis through the cell surface heparan sulfate proteoglycan syndecan-2. We show that endogenous syndecan-2, PINCH, and β 1-integrin are required for fibronectin fibrillogenesis on the blastocoel roof of *Xenopus* embryos; loss of function of any of these proteins by antisense morpholino depletion prevents fibril formation. PINCH co-immunoprecipitates with both endogenous syndecan-2 and endogenous β 1-integrin, and expression of a dominant negative PINCH blocks co-immunoprecipitation of endogenous β 1-integrin with endogenous syndecan-2. Furthermore, PINCH functionally interacts with syndecan-2 because ectopic co-expression of syndecan-2 and PINCH in embryonic cells that do not normally form fibers resulted in ectopic fibrillogenesis. Interestingly, we observed that ectopic fibronectin fibrillogenesis on the blastocoel floor of blastula-stage embryos resulted in ectopic mesoderm migration along the blastocoel floor. This redirection of cell migration suggests that the syndecan-2: β 1-integrin:fibronectin matrix is sufficient to direct the migration of developing mesoderm. Thus, PINCH mediates the function of both syndecan-2 and β 1-integrin, integrating them into an inside-out signaling complex that regulates fibronectin fibrillogenesis and the direction of cell migration.

- 678. Roles of WASp Related Proteins in *Dictyostelium discoideum* Development.** Diana Caracino, Cheryl Jones, Julia Steiner, and Charles L. Saxe III. Emory University, Atlanta, Georgia.

The WASp (Wiskott-Aldrich Syndrome protein) family proteins are involved in rearrangement of the actin cytoskeleton and cell motility. A subset of WASp-related proteins, the Scars, was first discovered in *Dictyostelium* in our lab in a developmental genetic screen for rescue of a signaling receptor mutant (Bear et al., 1998). Both the Scar and WAS proteins have orthologues in metazoans including humans, mice, *C. elegans*, zebrafish, and *Drosophila*. We have examined the expression and localiza-

tion of Scar and WASp in wild type cells; both proteins localize to the leading edges of cells but exhibit different patterns. The human Scar1/WAVE1 protein was recently found to exist in a heteropentameric complex (Eden et al., 2002). The protein complex is involved in regulation of actin dynamics via Rac GTPase signaling and orthologues of all complex partners are found in our tractable model system *Dictyostelium discoideum*. Protein expression of Scar does not change dramatically over the 24-hour developmental time course of this organism; therefore regulation of Scar at the post-translational level is likely. We are investigating the role of the protein partners as regulators of Scar. We are performing *in vitro* transcription/translation assays to determine which regions of Scar are most important for interaction with complex partners. We are concurrently using an *in vivo* rescue screen with our Scar null cell line to establish the fidelity of the *in vitro* results. These combined assays will help further clarify the roles and regulation of Scar.

- 679. Cleavage at the S2 Site of BMP-4 Is Regulated in a pH-Dependent Fashion.** C. Degnin,* G. Thomas, G.† and J. Christian*. *Department of Cell and Developmental Biology and †Vollum Institute, Oregon Health and Science University, Portland, Oregon.

Bone Morphogenetic Protein-4 (BMP-4) is a highly regulated protein that acts in a concentration dependent manner. The BMP-4 precursor is activated by a two-step proteolysis. The initial cleavage occurs after an optimal furin consensus motif that separates the pro- and mature domains of the protein (the S1 site), and this allows for cleavage at an upstream minimal furin consensus motif within the prodomain (the S2 site). While we have shown that these sequential cleavages regulate the activity and signaling range of mature BMP-4, the mechanism regulating cleavage at the S2 site remains undefined. Here we show that cleavage at the S2, but not the S1 site, is enhanced at reduced pH. This pH-dependent cleavage is mediated by a histidine residue at the P6 position in the S2 site, that inhibits cleavage at neutral pH. Mutation of this residue to either a basic or polar residue oblates the pH-dependent rate enhancement and allows for efficient cleavage of the S2 site at neutral pH. In addition, whereas mutation of the S1 site to a non-consensus motif completely blocks *in vivo* processing of proBMP-4, furin can still cleave this purified protein *in vitro*, in a pH dependent fashion. This is consistent with the hypothesis that cleavage of the BMP-4 precursor occurs in two distinct sub-cellular compartments, with cleavage at the S1 site occurring first, within the more neutral TGN. This initial cleavage may be required for transport to a more acidic, post-TGN compartment, where the S2 site is cleaved by furin or possibly by a distinct protease that is expressed in a tissue-specific fashion.

- 680.** Abstract #680 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.
- 681. Crossveinless 2 Is Required for BMP Signaling during *Drosophila* Crossvein Development.** A. Ralston,* M. Serpe,† D. J. Olson,* M. C. Halloran,* M. B. O'Connor,† and S. S. Blair*. University of Wisconsin, Madison, Wisconsin; and †University of Minnesota, Minneapolis, Minnesota.

Drosophila Crossveinless 2 (Cv-2) contains cysteine rich (CR) domains similar to those of invertebrate and vertebrate BMP signaling antagonists Short gastrulation (Sog) and Chordin. However, Cv-2 is required for BMP-mediated phosphorylation of Mad during *Drosophila* crossvein (CV) development, indicating that CR domain-containing proteins can act as BMP signaling agonists. Ectopic Sog abrogates Mad phosphorylation within the presumptive CVs, but concomitant overexpression of Sog and Cv-2 rescues this phenotype. A truncated variant of Cv-2, containing only the CR domains, is largely capable of rescuing both Sog overexpression and *cv-2* mutant phenotypes, confirming the importance of these domains for Cv-2 activity. CR domains are thought to bind BMP ligands directly, and injection of full-length *cv-2* mRNA into zebrafish and frog embryos results in phenotypes consistent with reductions in BMP signaling. These data suggest that in some contexts Cv-2 may bind BMP ligand, and that a regulated physical interaction between Cv-2 and ligand could be important during CV patterning. Furthermore, we have identified two BMP ligands that are required for Mad phosphorylation during CV development. Cv-2 also contains a partial Von Willebrand Factor D (VWFD)-like domain. Since proteins containing both CRs and VWFD domains are predicted to exist in frogs, mice, and humans, Cv-2-like proteins may be important for BMP signaling in a variety of developmental contexts. We will also discuss our efforts to understand the role of the VWFD domain in Cv-2 function.

682. **Modulation of Wnt and BMP Signals by Hip (Hedgehog Interacting Protein-Mediated Inhibition of Hedgehogs in Murine Intestine.** Blair Madison, Katherine Braunstein, and Deborah Gumucio. University of Michigan.

Epithelial-mesenchymal crosstalk via secreted small molecules dictates the molecular development of many organs. In the intestine, both Sonic (Shh) and Indian (Ihh) hedgehog have been implicated as critical signaling molecules secreted by the epithelium. Deletion of Ihh or Shh in the mouse results in both mesenchymal and epithelial phenotypes consistent with the role of both molecules in tissue crosstalk. Mice doubly deficient in Ihh and Shh die prior to gut formation. To circumvent this, and to examine the combinatorial role of the Hh signal, we inhibited Hh signaling by over-expression of the hedgehog interacting protein (Hip) using a mouse villin promoter characterized in our laboratory (JBC 277:33275, 2002). Inhibition of Hh signaling was confirmed by quantitative RT-PCR analysis of known Hh target genes. Phenotype severity correlated with transgene expression levels. In severe cases, smooth muscle was reduced. Consistent epithelial abnormalities included bizarre branched villi and epithelial hyperplasia. Microarray analysis revealed up-regulation of Wnt target genes, and immunostaining confirmed increased cytoplasmic and nuclear β -catenin in epithelium. Several aspects of the epithelial phenotype of VillinHip mice were similar to the phenotype observed in the *Nkx2.3*^{-/-} mice, and we confirmed significant reductions in *Nkx2.3* expression. BMPs, implicated as possible *Nkx* targets in *Nkx2.3*^{-/-} mice, were also down-regulated. Thus, a combinatorial Hh signal from the epithelium acts to keep Wnt signals in check, and may simultaneously stimulate BMP expression. Increased Wnt signals in combination with decreased BMP signals likely result in the epithelial hyperplasia observed in VillinHip mice.

683. Abstract #683 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

684. **Mechanism of Dickkopf Anti-Wnt Action.** Misha Semenov and Xi He. Division of Neuroscience, Enders 370, Children's Hospital/Harvard Medical School, 61 Binney Street, Boston, MA 02115.

Wnt signaling is controlled by extracellular modulators that bind either Wnt proteins or Wnt receptors. These modulators, in most cases, function to antagonize Wnt signaling and in concept, define the range, amplitude, and duration of Wnt signaling. Four conserved but structurally distinct families of Wnt antagonists are currently known to exist in species from lower vertebrates to humans: SFRP (secreted frizzled related protein), WIF1 (Wnt-inhibitory factor 1), Cerberus, and Dickkopf (DKK). SFRP proteins, WIF1 and Cerberus have been shown to bind Wnt molecules and may inhibit multiple signaling pathways activated by Wnt molecules. DKK proteins bind to the Wnt co-receptor LRP5/LRP6 and specifically inhibit β -catenin/canonical signaling. There are two models of the mechanism of DKK anti-Wnt action. One proposes that DKK binds to LRP5 or LRP6 and prevents LRP from Wnt induced interaction with Frizzled (Curr Biol 11:951-61(2001)). According to another model, DKK provokes the internalization of LRPs that results in the depletion of cell surface of these Wnt co-receptors (Nature 417:664-7(2002)). We are conducting experimental testing of both models and will present our findings.

685. **Using TAP Technology and Proteomics to Identify Novel Modulators of the Notch Signaling Pathway.** Alexey Veraksa,* Ashim Mukherjee,* Andreas Bauer,† and Spyros Artavanis-Tsakonas*. MGH Cancer Center, Harvard Medical School, Charlestown, Massachusetts 02129; and †Cell-zome AG, Heidelberg, Germany.

Signaling events involving the Notch receptor constitute a mechanism for generating cell fate changes that is widely used in animal development. We have undertaken a systematic biochemical approach to identify novel proteins that modulate different steps in the Notch signaling cascade. Several known Notch pathway components (e.g. Notch, Deltex, Mastermind) have been fused with a TAP (Tandem Affinity Purification) module and expressed in *Drosophila* cultured cells and embryos. Protein complexes containing these baits and associated subunits were purified, and individual subunits identified by mass-spectrometry. In addition to confirming known interactions, such as the existence of a stable trimeric complex between Notch, Su(H) and Mam proteins, this approach identified several novel potential Notch modulators. The range of predicted functions of these proteins includes control of trafficking of the Notch receptor to and from plasma membrane, nuclear localization and protein stability. These results are further supported by the discovery of some of the same interactors in a large-scale yeast two-hybrid analysis. We present an overview of results to date and evidence for the involvement of a non-visual arrestin (Kurtz) in modulating the function of Deltex.

686. **What Does a Notch1 Residual Processing Allele Teach Us about Notch Signaling Mechanisms?** Stacey Huppert, Amy Nichols, Deeangelee Pooran, and Raphael Kopan. Washington University School of Medicine.

The Notch genes encode single-pass transmembrane receptors that transduce extracellular signals responsible for cell fate determination. We have demonstrated that Notch1 intracellular pro-

cessing is required for embryonic viability in the mouse vertebrate system by generating a processing deficient allele (V1744G). The V1744G mouse embryos display many phenotypes associated with loss of Notch1. In somitogenesis, Notch signaling is thought to act as a component of the segmentation clock via PS-dependent proteolysis and CSL interaction. Paradoxically, the V1744G allele, which should be unable to participate in the segmentation clock, has no phenotype in the posterior somites. Three mechanisms could explain this observation. First, residual processing is sufficient for somitogenesis. Second, another protease or cleavage site exists for Notch1 in presomitic mesoderm (PSM). Third, Notch1 may have a proteolysis independent function in PSM. Genetic experiments have uncovered a dominant negative character of the V1744G allele in all tissues but the somite. These results argue against a simple residual processing explanation. Furthermore, embryos carrying combinations of the Presenilin null alleles (PS1 and PS2) with V1744G display an enhanced somite phenotype, and previously unrealized functional differences between PS1 and PS2. Finally, to compare the ability of null Notch1 and V1744G processing deficient cells, we derived embryonic stem (ES) cells and have begun the analysis of chimeric PSM. Based on our analysis of the V1744G allele, the role of Notch1 signaling in the PSM may be more complicated than the current models suggest.

687. **Induction of Neural Crest in *Xenopus* Embryos by Notch and Xiro1.** A. Glavic, F. Silva, M. J. Aybar, R. Mayor. Millennium Nucleus Developmental Biology, Facultad de Ciencias, Universidad de Chile.

Neural crest are cells that originates at the interface between the neural plate and non-neural ectoderm. We have analyzed the molecular mechanisms through which Notch signaling and Xiro1 regulate the generation of the neural crest. We show that while Notch and Hairy2A are both expressed in the neural crest, the Notch ligands Delta1 and Serrate are expressed in the cells that surround this tissue. We show that expression of Xiro1 overlaps with Xslug at the early gastrula stage in the neural crest region. We have used inducible dominant negative and activator constructs of both Notch signaling and Xiro1 to analyze the role of these genes in neural crest specification. Activating these constructs at the end of gastrulation, we could analyze their influence specifically on the neural crest without interfering with neural plate development. Activation of Xiro1 or Notch signaling led to an enlargement of the neural crest, while blocking their activity inhibited the expression of Xslug. We show that Xiro1 and Notch signaling upregulates Hairy2A and inhibits Bmp4 transcription during neural crest specification. Furthermore we show that Delta1 expression at the borders of the neural crest is positively controlled by Xiro1 and repressed by Snail. These results, allow us to propose a model where Xiro1 lies upstream of the cascade regulating Delta1 transcription, and the interaction of Delta1 with Notch induces Hairy2A expression at the border of the neural plate. Here, Hairy2A acts as a repressor of Bmp4 transcription ensuring that the optimal levels of BMP are maintained to specify Xmsx1 expression and the genesis of neural crest cells.

688. **Notch Signaling in the *C. elegans* Embryo: Suppression and Enhancement of the aph-1 Mutant Phenotype.** Shormeh O. Yeboah, Esmerelda M. Guzman, Valerie A. Hale, and Caroline Goutte. Amherst College, Amherst, Massachusetts 01002.

Activation of the Notch signaling pathway involves a conserved set of membrane-associated proteins: Presenilins, APH-1, APH-2/Nicastrin, and PEN-2. These proteins are all involved in the presenilin-mediated intramembranous cleavage of Notch, that occurs in response to ligand-receptor interaction. In *C. elegans*, removing any one of these components causes a fully penetrant maternal-effect embryonic lethality, due to the failure of Notch signaling between early blastomeres. We have been studying the effects of a leaky allele of aph-1 that was isolated in the laboratory of Jim Priess, and causes only 85% embryonic lethality. We show that this leaky aph-1 mutant phenotype can be enhanced (to 100% lethality) by slightly reducing the amount of Presenilin present in the embryos. We have isolated extragenic suppressors of the leaky aph-1 phenotype, and report here our progress with one of these suppressors, ik1. The recessive ik1 mutation suppresses the amount of embryonic lethality from 85% to 15% and appears to have no other phenotype. The ability of ik1 to suppress the leaky aph-1 mutation is dependent on the presence of normal levels of Presenilin in the embryo. ik1 is unable to suppress more severe alleles of aph-1, suggesting that this mutation does not bypass the need for APH-1 activity, but rather must increase or stabilize the activity of the mutant APH-1 protein. Our data are also consistent with a model in which the ik1-mediated suppression of aph-1 is indirect, and acts by increasing the levels or stability of Presenilins in the embryo.

689. **Nodal, an Early Micromere Signal that Affects Endomesoderm Specification.** Vera Lynn Flowers,* Girard Courteau,* Dave McClay† and Judith M. Venuti*. *LSU Health Sciences Center, New Orleans, Louisiana; and †Duke University, Durham, North Carolina.

Overexpression of in vitro transcribed RNAs encoding the TGF- β , activin, altered patterning along the sea urchin embryonic DV (also called the aboral/oral) axis. Since many of functions originally attributed to activin in other species are mediated by the closely related TGF- β , Nodal, we searched for this ligand in the sea urchin. Using degenerate PCR and macroarrays, we cloned Nodal homologs from *S.purpuratus* and several other sea urchin species. RT-PCR and RNase protection analyses revealed that *S.purpuratus* Nodal (SpNodal) RNA is expressed at low levels throughout development. However, *in situ* hybridization revealed that SpNodal message is enriched in mumeres at the 16 cell stage. When we overexpressed SpNodal in the embryo, ectopic guts were induced. In contrast, SpNodal antisense morpholino oligonucleotide injection suppresses endomesoderm development. Classic micromere transplantation and removal experiments have shown that mumeres signal to neighboring cells and function as an early organizer. We are therefore testing whether eliminating or overexpressing Nodal affects the micromeres ability to function as an organizer and where in the micromere signaling hierarchy Nodal is positioned. Our data suggest that Nodal is not the endogenous factor responsible for the effects observed with activins, but rather Nodal is an important component of the mumere signaling cascade.

690. **Extracellular Regulation of Nodal Signaling during Mouse Embryogenesis.** M. Shen, Canhe Chen, Jianhua Chu, Jixiang Ding, Sandy Price, and Yu-Ting Yan. Center for Advanced Biotechnology and Medicine and Department of Pediatrics, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey 08854.

Signaling by the transforming growth factor- β (TGF β) related protein Nodal is required for several fundamental aspects of establishment of the vertebrate body plan, including patterning of the anterior-posterior and left-right axes, as well as specification of embryonic mesoderm and definitive endoderm. At the extra-cellular level, the activity of Nodal is regulated by its interaction with co-receptors of the EGF-CFC family, including *Cripto* and *Cryptic*, and is inhibited by members of the Lefty subfamily of divergent TGF β molecules. In our laboratory, we have been investigating these regulatory interactions by functional analyses using mutant mice, as well as by biochemical studies utilizing a cell culture-based assay for Nodal signaling. We will present our current findings on: 1) the partially redundant functions of *Cripto* and *Cryptic* at pre-gastrulation stages of embryogenesis, particularly with regards to the interactions between the epiblast and extraembryonic visceral endoderm; 2) the subsequent requirement for *Cripto* in formation of the axial mesendoderm during gastrulation, as revealed by phenotypic analysis of a hypomorphic allele; and 3) the molecular mechanisms by which Lefty proteins inhibit Nodal signaling. These and related studies are providing insights into how the activity of a potent morphogenetic signal is modulated to provide precise temporal and spatial control of patterning.

691. Abstract #691 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

692. Withdrawn.

693. **Ext1 Regulates Chondrocyte Differentiation.** L. Koziel, M. Kunath, O. Kelly, B. Skarnes, and A. Vortkamp. Max-Planck-Institute for Molecular Genetics.

Hereditary multiple exostoses (HME) syndrome is an autosomal dominant inherited human disorder, which is characterized by the formation of multiple cartilaginous capped benign tumors (exostoses) that develop from the growth plate of endochondral bones. So far HME has been linked to missense or frameshift mutations in the tumor suppressor genes *Ext1* and *Ext2*. Both are glycosyltransferases involved in heparan sulfate (HS) biosynthesis. It has been shown that in *Drosophila* the homolog of *Ext1*, *tout velu* (*ttv*), is required for transport of hedgehog (Hh). One of the vertebrate homologs of Hh, Indian hedgehog (*Ihh*), is a key regulator of endochondral ossification. *Ext1* knockout mice are embryonic lethal due to gastrulation defects. We are analyzing a mouse line carrying a hypomorphic allele of *Ext1* obtained from a genetrap screen (*Ext1-GT*). Since *Ext1* mutations lead to skeletal deformations in human patients we started to analyze the role of *Ext1* and HS-chains during endochondral ossification focussing on the potential role of *Ext1* in mediating the *Ihh* signal. Analysis of the *Ext1-GT* mutants revealed a severe delay in chondrocyte differentiation. We could show that less amounts of HS in these mutants allow further diffusion of *Ihh* whereas treatment of limb explants in a limb culture system with heparin leads to a restriction of the *Ihh* signal. These experiments implicate an important role of HS in establishing a gradient of *Ihh* signalling in cartilage thereby regulating chondrocyte differentiation.

694. **Role of Fat in Regulation of Cell Proliferation and Planar Cell Polarity.** Xuesong Zhao, Chung-hui Yang, and Michael

A. Simon. Department of Biological Sciences, Stanford University, Stanford, California.

Planar cell polarity is important for many epithelial cells. Genetic analyses from our lab and other labs have shown that two cadherin superfamily members, *Fat* and *Dachsous*, and the transmembrane/secreted protein *Four-jointed* play crucial roles in this process. In our model, *Fat*, *Dachsous* and *Four-jointed* generate a gradient signal, bridge wingless signal to *Frizzled* activity, and bias *Fz* signaling between the R3/R4 precursors, resulting in establishment of *Drosophila* ommatidia polarization along the epithelial plane. In addition to its role in planar polarity signaling, *Fat* has long been identified as a tumor suppressor. Loss of *fat* function leads to the hyperplastic overgrowth of imaginal disc. Despite the genetic evidence we obtained, little is known about the biochemical properties and mechanisms of *Fat* function in these processes. We are using *Drosophila* as a model organism to investigate the structure and function of *Fat* to understand its regulatory roles in cell proliferation and planar polarity signaling.

695. **The Zebrafish *Belladonna* Mutation Is Required for Axon Guidance and Cell Differentiation in the Forebrain.** Anandita Seth, James A. Culverwell, Mitchell Walkowicz, and Rolf Karlstrom. University of Massachusetts, Amherst, Massachusetts.

The zebrafish *belladonna* (*bel*) mutation causes defects in axon guidance, patterning of the diencephalon, and eye growth. In homozygous *bel* embryos, retinal ganglion cell (RGC) axons can grow towards but not across the ventral midline before projecting to the incorrect, ipsilateral tectal lobes. Analysis of the embryonic axon scaffold in *bel* mutants reveals that the *bel* mutation specifically affects formation of the anterior commissure, post-optic commissure and optic nerve. The regions where axons make mistakes also have defects in gene expression and cellular differentiation. Expression of some axon guidance cues, such as *netrin* and *Semaphorin 3D*, is defective in *bel* mutants. Expression of *nk2.1b*, *dlx2* and *fgf8* is reduced at or near the forebrain midline prior to axon outgrowth, suggesting that the ventral diencephalon is improperly patterned and may not provide the proper molecular environment for growth cones. Midline glial cells are disorganized in *bel* mutants. Although *bel* retinas are properly stratified and patterned, mutants exhibit several phenotypes starting at 4 days. These phenotypes include reduction in Müller cell processes, ectopic lens material, and failure of the pigmented epithelium to contact the lens. In rare cases, homozygous *bel* animals are viable, underscoring the relative specificity of the mutation. The *bel* locus has been localized on the zebrafish genetic map and positional cloning is underway. Analysis of the *bel* mutant promises to illuminate the molecular mechanisms underlying midline axon guidance, forebrain patterning and growth of the eye.

696. **Dysregulated Expression of p21(CIP1) and PCNA in Lungs Lacking the Epithelial Type I Cell Gene *T1alpha* Links Type I Cell Differentiation to Cell Cycle Control in Distal Lung during Alveolus Formation.** G. Millien, A. Hinds, J. Wang, Y. X. Cao, M. C. Williams, and M. I. Ramirez. Pulmonary Center, Department of Medicine, Boston University School of Medicine, Boston, Massachusetts.

The extensive distal lung gas-exchange surface that supports respiration at birth forms in the last 2-3 days of gestation in mice.

Distal air spaces form by differentiation of epithelial type I and type II cells and thinning of the mesenchyme. Proliferation studies in normal lung at late gestation show a progressively reduced number of distal cells in S-phase before term. We reported (Dev. Biol. 256, 61-72, 2003) that mice lacking the type I cell gene *T1 α* (-/-) die at birth of respiratory failure. Term (-/-) lungs have smaller air spaces, fewer attenuated type I cells and normally differentiated type II cells and secreted surfactant. PCNA staining shows abnormal sustained proliferation of mesenchymal and epithelial cells in (-/-) lungs. At embryonic day 18.5 (E18.5), lung H&E staining shows no morphological differences in (-/-) compared to wild type (+/+). PCNA staining patterns at E18.5 are similar in (-/-) and (+/+) and the levels of PCNA and the cdk inhibitor p21 are not different. However, at term PCNA protein is upregulated 1.8-fold. Conversely, p21 mRNA is reduced 3.6-fold and p21 protein 2-fold. Microarray gene expression analyses at E18.5 indicate that several other genes that regulate cell cycle are altered in (-/-) lungs. Data are being validated by real-time PCR. Thus, loss of *T1 α* and/or abnormal differentiation of type I cells likely interfere with a cell-cell signaling pathway/s that results in suppression of p21 cell cycle inhibition in distal lung. HL47049.

697. **The Signaling Protein Jelly Belly Specifies Visceral Muscle Progenitors through the Receptor Tyrosine Kinase *Alk* and the Ras/MAPK Pathway in *Drosophila*.** H-H. Lee,* A. Norris,† J. B. Weiss,† and M. Frasch*. *Department of Molecular, Cell and Developmental Biology, Mount Sinai School of Medicine, New York, New York; and †Molecular Medicine and Cardiology, Oregon Health and Sciences University, Portland, Oregon.

The circular visceral muscles are derived from metamereric primordia in the dorsal mesoderm that are defined by the expression of two essential regulators, *bagpipe* and *textitbinou*. Within these primordia, an important subdivision occurs that generates two distinct cell populations: (1) A ventral row of founder cells, which function as patterning pioneers for visceral muscles; (2) dorsally-located fusion-competent cells, which lack intrinsic differentiation potential and need to fuse with founders in order to differentiate. Here we define a novel signaling pathway that controls the subdivision of visceral mesoderm into founders and fusion-competent cells. Genetic and biochemical evidence shows that founder cell specification requires the activities of Jelly belly (Jeb), a secreted molecule from ventral-lateral mesoderm, and a tissue-specific high affinity receptor, the receptor tyrosine kinase *Alk*, which is expressed in the visceral mesoderm primordia. Jeb binding to *Alk* activates the Ras/MAPK cascade, which induces founder cell markers, including the myoblast fusion gene *dumb-founded* and the Tbx-1-related T-box gene *org-1*, in presumptive founder myoblasts. Furthermore, Notch activity antagonizes Jeb/*Alk* signaling to restrict the range of Jeb signals to a ventral row of *Alk* expressing cells. Altogether, our data suggest that Jeb acts as a spatially-restricted ligand of the RTK *Alk* and triggers visceral muscle founder specification via a Ras/MAPK cascade.

698. **Cloning and Characterization of a Novel *Drosophila* PDZ Domain Protein.** Sabrina Y. Kim, Maia Renihan-McLaren, and Gabrielle Boulianne. Programme in Developmental Biology, Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8; and Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada.

A GAL4 enhancer trap line, C96, was identified whose expression pattern includes the developing embryonic and adult peripheral nervous systems (PNS). Based on cDNA sequences, information from the *Drosophila* genome project, and Northern blot analysis, the C96 gene spans over 100 kb in the genome and encodes for at least seven different transcripts produced by alternative splicing. The predicted protein products all have in common several protein-protein interaction motifs including PDZ domains, coiled-coils, and proline-rich regions. Since PDZ domain proteins classically act as scaffolding molecules, we propose that C96 may be involved in the assembly of a protein complex required for one or more signalling pathways during development. Using an antibody which recognizes all the predicted protein products, the expression pattern was examined at different developmental stages. In the embryo, expression is observed in the developing gut and pharynx, as well as the developing PNS. In the third instar larva, expression is observed along the presumptive wing margin in the wing disc, in the developing photoreceptors and cone cells of the eye, and along the ventral cord of the central nervous system. Isoform-specific *in situ*'s have also been carried out to examine the expression pattern of the different transcripts. To further characterize the function of the C96 gene, mutants have been generated by imprecise P element excision, and are presently being analyzed.

699. **Proteoglycan Synthesis and Function during Early Development of the Vertebrate Embryo.** Juan Larraín, Héctor Carrasco, Andrés Leschot, Juan Pablo Guzmán, Gonzalo Olivares and Mauricio Moreno. Center for Cell Regulation and Pathology, Department of Cell and Molecular Biology, P. Universidad Católica de Chile, Santiago-Chile.

Cell-cell signaling plays an important role during early patterning of the vertebrate embryo. The Spemann organizer is one of the best studied signaling centres in the vertebrate embryo. The organizer is a source of secreted growth factors antagonists, like chordin, noggin, follistatin, cerberus, dkkopf, frzb-1. We have recently noted that most of the organizer molecules contain putative heparin binding domains (HBD). It is known that the HBD contained in noggin and Shh are indeed necessary for its interaction with Heparan sulphate proteoglycans (HSPG). Our aim is to understand the role of PGs during early development of the vertebrate embryo. For this purpose we are currently working in two objectives: 1) Biochemical studies on the biosynthesis of PGs during early development of the *Xenopus* embryos, and 2) To determine the possible role of HSPG in Chordin and Shh function. For the first objective we have labelled *Xenopus* embryos with 35S-[SO₄] and measured the incorporation of sulphate to PGs during gastrula. We have found that the dorsal blastopore lip incorporates higher levels of 35S-[SO₄] than the ventral side. We are currently studying the possible function of this polarization in proteoglycan synthesis in dorso-ventral patterning. For our second objective we have point mutated the putative HBD of chordin and we found that it abolishes its ability to bind to heparin but has no effect on the ability of Chordin to interact with BMP and Tsg. We are studying the effect of this mutation on chordin activity in the embryo and its possible role in regulating chordin diffusion. We are also trying to identify a Shh-interacting HSPG using a biochemical approach; results on the function of this HSPG in Shh signaling will be discussed.

700. **Developmental Expression of Perlecan in the Chick Embryo.** Nikolitsa Soultzi, Anastasia Giakoumaki, and Nikolaos Zagris. Division of Genetics and Cell and Developmental Biology, Department of Biology, University of Patras, Patras, Greece.

Perlecan (Pln) is a heparan sulfate proteoglycan that interacts specifically with secreted growth factors and extracellular matrix (ECM) components and participates in signaling pathways. We studied the spatiotemporal distribution of Pln by immunofluorescence and immunoprecipitation in the early chick embryo from stages X (morula) to HH17(29-32 somites). Perlecan was first detectable at the late morula stage and its presence may be fundamental in the assembly of the ECM and the first basement membrane organized during the blastula stage. The accumulating ECM displayed intense Pln fluorescence during the gastrula (stHH3-4) and neurula (stHH5) stages. Presence of Pln in the neural tube was dynamic and was decreased by stage HH17. In the developing heart, Pln fluorescence was strong in the dorsal mesocardium, myocardium, endocardium and in the ECM (cardiac jelly) between them. The dermamyotome showed intense Pln fluorescence but the sclerotome was not labeled in the somites. Perlecan immunoreactivity was intense in the liver rudiment and in the mesonephric duct and tubule rudiments. We also studied the role of Pln using blocking antibodies in a set of functional studies. When embryos at stages X to XIII (blastula) were treated with Pln antibodies, the embryos stopped development at the neural plate stage. Perlecan showed distinct developmental and tissue expression patterns and seemed to act an organizational role in differentiation and morphogenesis of tissues and organs in the developing embryo. This work was supported by the "K. Karatheodoris" grant 2435 from the University of Patras.

701. **Inhibitory Effect of Sertoli Cells on Murine Teratocarcinoma Cell Growth.** P. Vecino, J. P. Gaillard, and J. Arechaga. Department of Cell Biology-University of the Basque Country, 48940 Lejona, Spain.

Given the regulatory activity of Sertoli cells on the male gametogenesis, we have hypothesized that teratocarcinomas might arise from loss of bona fide controls provided by the seminiferous tubule microenvironment to the ever-remodelling subsets of spermatogonia. Indeed, murine spermatogonia can be maintained *in vitro* pending on physical contact with a monolayer of Sertoli cells. Nevertheless, cell growth remains poor despite addition of growth factors, suggesting a tight control operated by Sertoli cells upon the survival vs. growth balance. With a view to testing our hypothesis, we have focused on various aspects of the co-culture of murine teratocarcinoma cell lines with murine primary or tumoral Sertoli cells. Along with a significant reduction of tumoral growth, subconfluent layers of Sertoli cells reproducibly established physical contacts with colonies of the teratocarcinomas tested. More strikingly, confluent monolayers of Sertoli cells totally abolished teratocarcinoma cell growth. Furthermore, this effect could be reversed upon co-culturing in the presence of a polyclonal antiserum against the murine CE44 teratocarcinoma cell line. Of note is the observation that the rescuing effect from the antiserum was found to be interchangeable among the teratocarcinoma cell lines tested. Lastly, we took advantage of this straightforward *in vitro* assay for the functional screening of monoclonal antibodies raised against the CE44 cell line. Several supernatants were found to recapitulate the

rescuing effect observed with the polyclonal. Molecular identification of their cognate antigen(s) is currently underway.

702. **A Model to Study Stromal-Epithelial Sonic Hedgehog Signaling in Prostate Cancer.** Aubie Shaw and Wade Bushman. Departments of Surgery and Cancer Biology, University of Wisconsin-Madison.

During fetal prostate development, Sonic hedgehog (Shh) expressed by the urogenital sinus epithelium activates Gli expression in the adjacent mesenchyme and induces ductal budding and growth. While Shh expression is downregulated when prostate development is completed, Shh is expressed at abundant levels in the majority of human prostate cancers. Shh signaling was shown to be important for prostate tumor growth in previous studies which showed: (1) that Shh expressed by LNCaP tumor cells activates Gli expression in the tumor stroma, (2) that genetically engineered Shh overexpression in LNCaP tumor cells increases stromal Gli expression and (3) that Shh overexpression dramatically accelerates tumor growth. To elucidate the mechanisms by which stromal cells accelerate tumor growth, we have developed a bi-clonal system in which the interactions between LNCaP tumor cells and an immortalized stromal cell line made from the mouse prostate (DLP) can be studied *in vivo* in xenografts made by co-injection of the two cell types and *in vitro* by co-culture of the cells. Our studies have shown that (1) co-injection of DLP cells with LNCaP cells produce tumors of typical LNCaP xenograft histology but in which the stroma stains positive for LacZ expressed by the DLP cells and (2) co-culture of the DLP cells with LNCaP cells overexpressing Shh showed Shh-induced up-regulation of Gli-1. This system will provide a novel opportunity to genetically manipulate both tumor and stromal cells independently, to examine the functional effect of targeted perturbations on stromal-epithelial signaling, and to link these to specific effects on prostate tumor growth.

703. **Disruption of Maternal-Fetal Communication during Pre-Implantation Embryogenesis in Mice Over-Expressing the Short Form of p53.** Wendy Gluba,* Ann Sutherland,† and Heidi Scrabble*. *Departments of Neuroscience and †Cell Biology, University of Virginia, Charlottesville, Virginia 22908-1392.

During the preimplantation period of mammalian embryogenesis, maternal-fetal communication utilizes components of the insulin-like growth factor (IGF) signaling cascade. We have generated mice that over-express the short isoform of the tumor suppressor, p53 (p44), and have demonstrated that IGF signaling is impaired in cells from p44-transgenic embryos. Irreversible growth deficits beginning as early as E6.5 are linked to disturbances in the IGF receptor that affect Akt-mediated pathways downstream of receptor activation by IGF. Pre-implantation embryos derived from homozygous p44 females (irrespective of paternal genotype) reach the blastocyst stage with fewer than normal cells, indirectly demonstrating that cleavage is also retarded by p44. This maternal effect can be rescued by transplanting embryos from p44 females into non-transgenic females. We find evidence of increased IGFBP-3 in the zona pellucida of non-transplanted embryos compared to embryos transplanted into non-transgenic mothers. Over-activity of the IGF signaling cascade in maternal tissues could result in the secretion of excess IGFBP-3 by oviductal cells into the lumen, where it could

interfere with the IGF1-receptor on the embryo, reducing the IGF1 signal and limiting the growth of the inner cell mass. As the zona pellucida is the first site of interaction between maternally-secreted IGFBP-3 and the embryo, retarded cleavage appears to be the result of disruption of the IGF signaling cascade in maternal tissues, rather than a defect inherited in the ooplasm.

704. **Lrp5 and Ang2 are Critical for Developmentally Programmed Macrophage-Induced Cell Death.** Savita Kurup,* Ivan Lobov,* Jefferson E. Vallance,* Amit Korde,* Millan Patel,† Donald Glass,† Gerard Karsenty,† and Richard A. Lang*. *Division of Pediatric Ophthalmology and Developmental Biology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati Ohio 45229; and †Department of Molecular and Cellular Biology and Medicine, Baylor College of Medicine, Houston, Texas 77030.

Regression of the hyaloid vessels of the eye is a programmed event that is dependent on macrophage function. In recent analysis we have shown that it is also dependent on Lrp5 and Ang2. Lrp5 is a close homologue of *Drosophila* Arrow, a transmembrane molecule that functions as a Frizzled coreceptor. Lrp5-deficient mice have two phenotypic characteristics; osteoporosis and failure of embryonic eye vasculature to regress. These features recapitulate human Osteoporosis-Pseudoglioma Syndrome, caused by LRP5 inactivation. Ang-2 is a ligand for the Tie2 receptor in endothelial cells and also the natural antagonist for Ang-1. Ang-2 is expressed by endothelial cells at site of vascular remodeling and acts by destabilizing the contacts between endothelial cells and surrounding cells. Lrp5^{-/-} and Ang2^{-/-} deficient mice have similar phenotypes characterized by reduced levels of capillary cell apoptosis; these mice retain the hyaloid vasculature throughout life. Since the similar phenotypes suggested Lrp5 and Ang2 might function in the same pathway, we performed a genetic test of interaction by assessing the capillary regression defect in single and double heterozygotes. This showed that Ang2^{+/-} and Lrp5^{+/-} animals had no regression defect while Ang2^{+/-} Lrp5^{+/-} animals did. We are currently investigating the mechanism of action of Lrp5 and Ang2 in this system.

705. **How Cells Sense Chemoattractant Gradients.** Peter Devreotes, Elaine Huang, Miho Iijima, Chris Janetopoulos, and Lingfeng Chen. Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

The ability to sense and respond to shallow gradients of extracellular signals is remarkably similar in *Dictyostelium discoideum* amoebae and mammalian leukocytes. Consideration of the features of a chemotactic response presents several fascinating and unique challenges. Shallow external gradients must generate sharply localized internal responses at the leading edges of the cells. Moreover, cells at different points in the gradient sense equally well so there is a powerful mechanism for background subtraction or adaptation. We have suggested that a balance between local excitatory and global inhibitory processes controls the response to chemoattractants. An extensive series of studies in the last several years have indicated that the upstream components and reactions in the signaling pathway are uniformly localized in cells exposed to a chemoattractant gradient. However, downstream responses such as PI[3,4,5]P₃ accumulation and actin polymerization are sharply localized towards the high side of the edge. We have recently found that uniform stimuli tran-

siently recruit and activate PI3Ks while PTEN is released from the plasma membrane. Although chemoattractant receptors and G-proteins are evenly distributed along the cell surface, gradients of chemoattractant cause PI3Ks and PTEN to bind to the membrane at the front and the back of the cell, respectively. This reciprocal regulation provides robust control of PI[3,4,5]P₃ and leads to its sharp accumulation at the anterior. Interference with PI3Ks modifies chemotaxis while disruption of PTEN broadens PI localization and actin polymerization in parallel. Thus, counter-acting signals from the upstream elements of the pathway converge to regulate the key enzymes of PI metabolism, localize these lipids, and direct pseudopod formation.

706. **Guidance of Primordial Germ Cell Migration by Chemokine Signalling.** M. Doitsidou,* M. Reichman-Fried,* J. Stebler,* J. Doerries,* D. Meyer,* <939 C, V. Esguerra,** T. Leung,** and E. Raz*. *Germ Cell Development, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Goettingen, Germany; †Department for Developmental Biology, Institute for Biology I, University of Freiburg, Hauptstrasse 1, D-79104 Freiburg, Germany; and **Mermaid Pharmaceuticals GmbH, Falkenried 88, 20251 Hamburg, Germany.

Zebrafish PGCs are found at four locations randomly distributed with respect to the dorsoventral axis of the early blastula. From these positions they start their migration through the developing embryo to reach the region of the future gonad. We show that the chemokine SDF-1a and its receptor CXCR4b are involved in directing PGC migration in zebrafish. cxcr4b is expressed in the PGCs during their migration and sdf-1a is expressed where the PGCs are found in a dynamic fashion that prefigures their migration route. In mutant embryos where sdf-1a expression is altered, there is a strong correlation between the altered expression pattern of the chemokine and the abnormal path of PGC migration. Moreover, eliminating the activity of SDF-1a or CXCR4b using morpholino antisense oligonucleotides results in a severe PGC migration phenotype. Despite the migration defects, the ectopic cells retain their PGC identity and the surrounding somatic tissues differentiate properly. PGCs that do not receive the SDF-1a signal show lack of directional movement, although they retain their motile behaviour. Finally, we showed that the PGCs can be attracted toward an ectopic source of the chemokine, strongly suggesting that this molecule provides a key directional cue for the PGCs.

707. Abstract #707 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

708. **Differentiation and Genetic Manipulation of Human Embryonic Stem Cells.** Nissim Benvenisty. Department of Genetics, The Hebrew University, Jerusalem, Israel.

Human embryonic stem (ES) cells could help us understand early human development and serve as a source of cells for transplantation medicine. In culture human ES cells can differentiate into embryoid bodies comprising the three embryonic germ layers. This differentiation can be affected by addition of various growth factors. Using a DNA micro-array analysis we could analyze the differentiation of human ES cells in a genomic scale. To determine whether human ES cells might be rejected

after transplantation, we examined the expression of the MHC proteins in these cells. Our results show very low expression levels of MHC-I proteins that increase upon differentiation or cytokines treatment. In addition, we were able to genetically modify human ES cells and thus to label and sort out cells at different stages of their differentiation. Using genetically labeled human ES cells we have examined the differentiation of human ES cells in the developing chick. The human cells were shown to proliferate, differentiate and migrate into the chick tissues. Finally, our genetic manipulations allow us to mutate specific genes in human ES cells and thus to model for human genetic diseases in a cell-based fashion.

709. **Targeting of Dosage Compensation in *Drosophila* by Non-coding roX RNAs.** Mitzi I. Kuroda, Hyangyeon Oh, and Yongkyu Park. Baylor College of Medicine, Houston, Texas; and Harvard Medical School, Boston, Massachusetts.

MSL proteins and non-coding roX RNAs form complexes that bind along the length of the single *Drosophila* male X chromosome to make X-linked gene expression equal in males (XY) and females (XX). The roX1 and roX2 genes are located on the X chromosome and are thought to have dual functions. First, roX RNAs are components of the MSL complex, and the proteins cannot bind the X efficiently without them. Second, roX transgenes can function as nucleation sites for ectopic targeting and spreading of MSL complexes into flanking autosomes regardless of location. Ectopic spreading of MSL complexes is profoundly influenced by altering the ratio of MSL proteins to roX RNA. In protein excess, the MSL complex concentrates near sites of roX transcription and is depleted elsewhere, dramatically changing X chromosome morphology. These results support a model for distribution of MSL complexes in which local spreading in cis from roX genes is balanced with diffusion of soluble complexes in trans. Our results provide visual evidence that MSL complexes can spread locally on the X, the natural target of dosage compensation.

710. **DM Domains and Sexual Development.** David Zarkower. Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, Minnesota.

Sex-determining mechanisms are highly divergent, but the involvement in multiple phyla of genes related to the insect gene *doublesex* (*dsx*) suggests that sexual regulatory mechanisms may be conserved. The *C. elegans* male regulator *mab-3* shares with *dsx* a DNA binding motif we named the DM domain. *mab-3* performs multiple functions related to those of *dsx*, binds a similar DNA site, and can be functionally replaced in vivo by *dsx*. In mammals, the DM domain gene *Dmrt1* has multiple roles in testis differentiation but is dispensable in the ovary. We are using several approaches to address the functions of *Dmrt1* in testis development. Among vertebrates *Dmrt1* is widely conserved in male development, despite rapid evolution of sex determination. Apparently DM domain genes either play an ancient and conserved role in sexual development or have properties that promoted their frequent recruitment into this process during evolution. Mice have seven DM domain genes. At least three, in addition to *Dmrt1*, are expressed in the early gonad, consistent with roles in sexual development. One is expressed at similar levels in both sexes, one is testis-enriched, and one is ovary-enriched. Knockouts are underway. Are other types of sexual

regulators widely used? Genetic screens have found other genes that generate sexual dimorphism. One is a forkhead transcription factor that acts specifically in the gonad. Mutant male gonads are disorganized and feminized. A forkhead gene has been implicated in mammalian gonad development, though the relationship is unclear. We currently are cloning other male-specific *C. elegans* gonad genes and hope to find additional evidence of conserved regulation.

711. **Sex-Specific Apoptosis Regulates Sexual Dimorphism in the *Drosophila* Somatic Gonad.** T. De Falco, G. Verney, and M. Van Doren. Johns Hopkins University, Department of Biology, Baltimore, Maryland.

Sexual dimorphism is a critical aspect of development, especially in the gonad, where male- or female-specific differentiation is crucial for forming functional gametes. The somatic gonad, which supports and protects the germline, is therefore an essential cell type for sexual reproduction, and we are interested in studying sexual dimorphism in the somatic gonad to better understand its role in ovary and testis development. We have identified a group of somatic gonadal precursor cells (SGPs) that is initially specified in both sexes but is incorporated only into the posterior of the male *Drosophila* embryonic gonad. Interestingly, these male-specific SGPs (msSGPs) express Sox100B, a homolog of Sox9, which has been implicated as a male-specific sex determination factor in a wide array of vertebrate species. Although similar to other SGPs in terms of cell-biological markers, msSGPs are specified independent of *abdominal-A*, *tinman*, and *zfh-1*. The sexually dimorphic behavior of msSGPs is controlled at the level of apoptosis, whereby female-specific *transformer* and *doublesex* function are required for msSGPs to undergo apoptosis. This cell death is dependent on caspases and genes in the H99 deficiency. These studies suggest that apoptosis of the msSGPs is one mechanism used in the somatic gonad to initiate sexually dimorphic development. Moreover, investigation into the role of msSGPs will further our knowledge of testis formation. Our results are also consistent with growing evidence that the regulation of sexual dimorphism may be an evolutionarily conserved process.

712. **Xite, X-Inactivation Intergenic Transcription Elements that Regulate the Probability of Choice.** Yuya Ogawa and Jeannie T. Lee. Howard Hughes Medical Institute, Massachusetts General Hospital, Harvard Medical School.

Allelic expression differences contribute to phenotypic variation. In X-chromosome inactivation (XCI), unfavorable XCI ratios promote X-linked disease penetrance in females. During XCI, one X is randomly silenced by Xist. X-chromosome choice is determined by asymmetric expression of Tsix whose antisense action represses Xist. Here, we discover a novel cis-element in the mouse X-inactivation center that regulates Tsix. Xite harbors intergenic transcription start sites and DNaseI hypersensitive sites with allelic differences. At the onset of XCI, deleting Xite down-regulates Tsix in cis and skews XCI ratios, suggesting that Xite promotes Tsix persistence on the active X. Truncating Xite RNA is inconsequential, indicating that Xite action does not require intact transcripts. We propose that allele-specific Xite action promotes Tsix asymmetry and generates X-chromosome inequality. Therefore, Xite is a candidate for the Xce, the classical modifier of XCI ratios.

713. **Evolution in Black and White? Pigmentation Pattern Diversity in *Drosophila*.** Sean B. Carroll. HHMI, University of Wisconsin-Madison.

Pigmentation patterns offer many attractive features as models of phenotypic evolution. Foremost among these is their relative simplicity (as compared with the three dimensional anatomy of body parts) which may be under the control of a modest number of genes. There is abundant diversity throughout the *Drosophila* genus in melanic pigmentation patterns, as well as significant variation within species. I will focus on three general questions concerning the genetic architecture and molecular mechanisms of pigment pattern evolution, including: 1) Which genes underlie phenotypic differences between species?; 2) What is the molecular basis of genetic differences that affect phenotypic diversity; and 3) Are similar phenotypic changes due to evolution at the same loci? Our findings in *Drosophila* offer potential insights into such classic evolutionary models as industrial melanism and mimicry.

714. **Evolutionary Genetics and Development of Stripe Loss in *Danio albolineatus*.** R. R. Roberts, J. Manuel, and D. M. Parichy. Institute for Cellular and Molecular Biology, University of Texas, Austin Texas 78712.

Vertebrate pigment cells are derived from neural crest cells and patterns formed by these cells are a tractable model for understanding the evolution of form. Relatives of the zebrafish *Danio rerio* exhibit a diverse array of pigment patterns including horizontal stripes, vertical bars, and spots. Here we examine the genetic and developmental bases for stripe loss, and the resulting uniform distribution of pigment cells in *D. albolineatus*. Inter-specific complementation testing and segregation analyses support a role for the receptor tyrosine kinase *Fms* in generating the pigment pattern difference between *D. rerio* and *D. albolineatus*. Cellular and molecular analyses further identify differences in pigment cell morphogenesis and differentiation underlying the different pigment patterns between species. These results suggest testable hypotheses for the mechanisms underlying pigment pattern diversification in danios more generally.

715. **Dogfish Hox Genes and the Evolution of Fin Development.** Renata Freitas and Martin J. Cohn. Department of Zoology, University of Florida, Gainesville, FL 36211.

In order to identify developmental mechanisms involved in the origin and diversification of fins, we have undertaken a comparative analysis of fin development. Sharks, the most primitive group of extant vertebrates with paired fins, occupy a key phylogenetic position as basal gnathostomes. As such, sharks provide a unique opportunity to study the mechanisms that govern development of primitive fin morphologies. To this end, we have cloned and characterized posterior *HoxA* and *HoxD* genes and analyzed their expression in the dogfish *Scyliorhinus canicula*. We find that early *Hox* gene expression in lateral plate mesoderm is associated with regionalization of the pectoral, pelvic and inter-fin levels. These patterns become dynamic and, as in tetrapod limbs, exhibit temporal, spatial and quantitative colinearity within the fin buds. Striking differences emerge during patterning of the paired fin and limb skeletons. The unpaired median fins develop within a contiguous median fin fold. We find that *Hox* genes are differentially expressed within this fold prior to its subdivision into dorsal, anal and caudal fins. Later, specific

patterns of expression are associated with each of the median fins. This is consistent with the idea that *Hox* genes are involved in determining the position and pattern of unpaired fins. Our results suggest that *Hox* genes may have played a role in subdivision of an evolutionarily ancient median fin fold.

716. **The Evolution of Key Bilaterian Traits: Insights into Axial Patterning and Mesoderm Formation from the Sea Anemone *Nematostella*, a Non-Bilaterian Animal.** John R. Finnerty,* Kevin Pang,† Pat Burton,* Mark Q. Martindale*. *Boston University, †University of Hawaii.

The most recent common ancestor of the Bilateria possessed bilateral symmetry, an anterior-posterior axis, and mesoderm. These features are regarded as key innovations, critical to the subsequent radiation of bilaterian bodyplans. The origins of these traits cannot be understood solely by studying extant bilaterians because these features were already well entrenched prior to the initial radiation of the Bilateria, some 545 million years ago. Outgroups that diverged prior to the origin of these traits may reveal the pre-bilaterian condition and how the bilaterian condition originated. We developed the sea anemone, *Nematostella* into a new developmental model system for the phylum Cnidaria, the major extant group of non-bilaterians. We cloned and tracked developmental expression of over 30 axial patterning, mesoderm and neural patterning genes and developed approaches for manipulating gene expression. In this talk, we will show expression data for five *Hox* genes as well as a *ParaHox* gene, even-skipped, *dpp*, *snail*, *twist*, *mef2*, *nk-2*, *otx*, *mox*, *GATA*, a muscle-specific *lim* gene, and *tropomyosin*. Our data prove pre-bilaterian origins for these genes and reveal aspects of the ancestral bilaterian condition. For example, genes associated with mesodermal differentiation in bilaterians are expressed in the bi-functional gastrodermis of *Nematostella*, yielding insights into the origin of mesendoderm. Furthermore *Hox* gene expression along the main body axis of *Nematostella* suggests that the primary body axis of animals evolved before the bilaterian-cnidarian split.

717. **Gli3 Antagonizes the Shh Response in the Developing Spinal Cord.** Néva P. Meyer and Henk Roelink. University of Washington.

A concentration gradient of *Shh* is thought to pattern the ventral neural tube, and in *shh*^{-/-} mice ventral cell patterning is absent. Based on in vitro and genetic studies, the zinc finger-containing transcription factors *Gli1*, 2 and 3 are mediators of the *Shh* intracellular response. The floorplate and adjacent ventral cell types are absent in *gli1*^{-/-}; *gli2*^{-/-} mice, but most ventral cell types that are absent in *shh*^{-/-} mice are restored in the *shh*^{-/-}; *gli3*^{-/-} double mutant. This is consistent with the predicted role of *Gli3* as a repressor of the *Shh* response. Likewise, *Shh* is thought to block *Gli3* repressor activity by preventing post-translational cleavage of *Gli3*. In order to test the role of the cleaved, repressor form of *Gli3* in the neural tube, a truncated version of *Gli3* (*Gli3R*^{*}) was designed to mimic a Pallister Hall allele. *Gli3R*^{*} acts as a constitutive repressor independent of *Shh* signaling. Misexpression of *Gli3R*^{*} in the chick neural tube caused a cell-autonomous, ventral expansion of class-I, dorsal progenitor proteins and a loss of class-II, ventral progenitor proteins consistent with expected activity as a repressor of the *Shh* response. Misexpression of *Gli3R*^{*} did not affect expression of dorsal progenitor proteins that are not directly *Shh*-responsive.

Activation of the BMP response is sufficient to maintain gli3 expression in neural plate explants, which might be a mechanism by which BMPs antagonize the Shh response. These experiments emphasize the role of Gli3 as a critical element in dorsoventral patterning of the spinal cord whose activity is independently regulated by Shh and BMP signaling.

718. Abstract #718 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

719. **Vax Homeobox Genes are Fundamental Regulators of Neural Retina Precursor Cells.** Stefano Bertuzzi,* Michela Curradi,*† Stina Mui,** Jin Woo Kim,** and Greg Lemke**. *Dulbecco Telethon Institute at CNR-ITB. Segrate (MI) Italy; †Newron Pharmaceuticals, Gerenzano (MI), Italy; and **The Salk Institute. La Jolla, California.

The vertebrate eye develops from an evagination of the neuroepithelium, called optic vesicle (OV). The ventral-proximal part of the

OV will give rise only to the glial cells of the optic nerve, while the distal part of it will give rise to the six different cell types (neurons and glia) present in the neural retina (NR). The precursor cells of these two adjacent territories, which develop from the same early embryonic neuroepithelium, have very different potentials for differentiation. In Vax1/Vax2 double knock-out (DKO) mice we observe a dramatic expansion of the retinal tissue at the expenses of the glial cells of the optic nerve, with a consequent generation of retinal tissue throughout the ventral forebrain. Using different approaches (ISH, IHC and cultures of optic vesicles) we demonstrate that this loss of boundary between eye and nerve in Vax DKO mice is due to the specific recruitment of ventral precursor cells from the optic stalk territory to become NR. Our data demonstrate that Vax genes are fundamental regulators for the territorialization of the eye field. In particular, we propose a model in which Vax genes repress Pax6, restricting it to the NR territory. In DKO mice, this inhibition is lacking allowing therefore the expression of Pax6 in the OS (normally a territory of Vax1) where it forces precursor cells toward NR differentiation, with the end result of the generation of two giant well differentiated and well laminated retinæ.