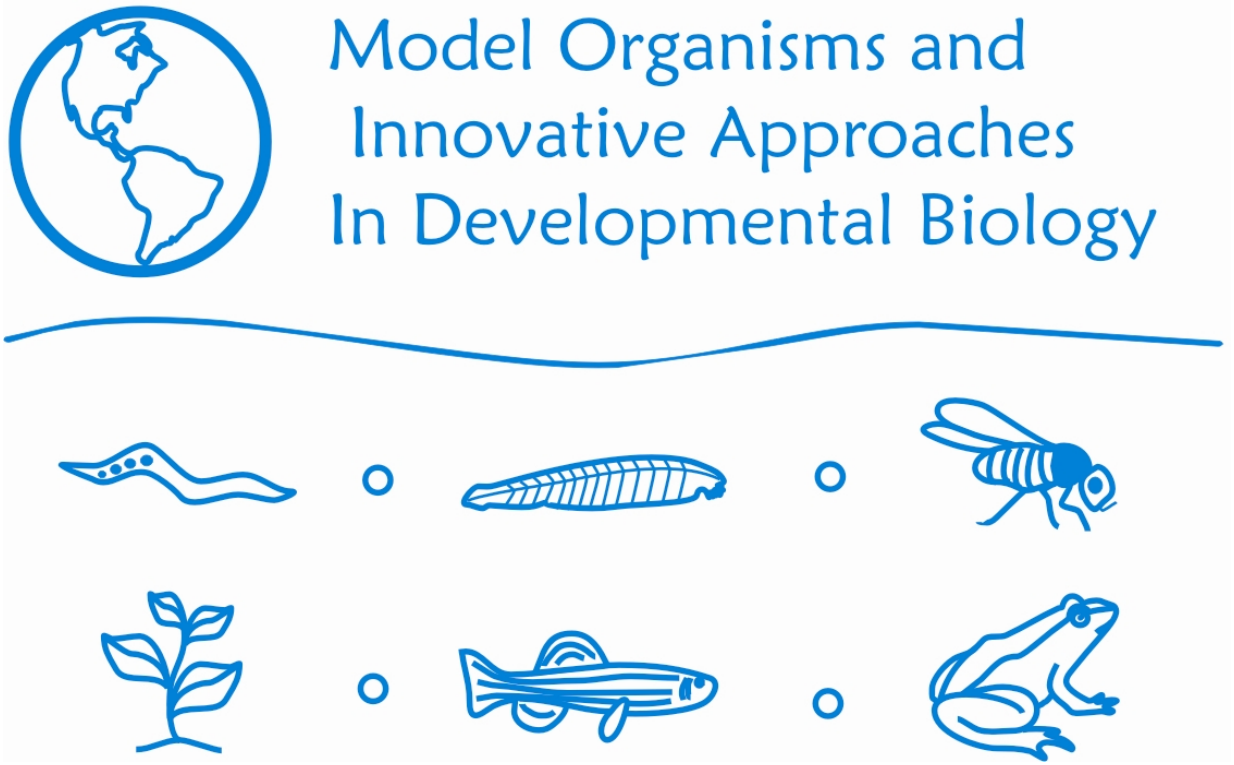


Development in Zebrafish, a Genetic Approach

Didier Stainier
University of California

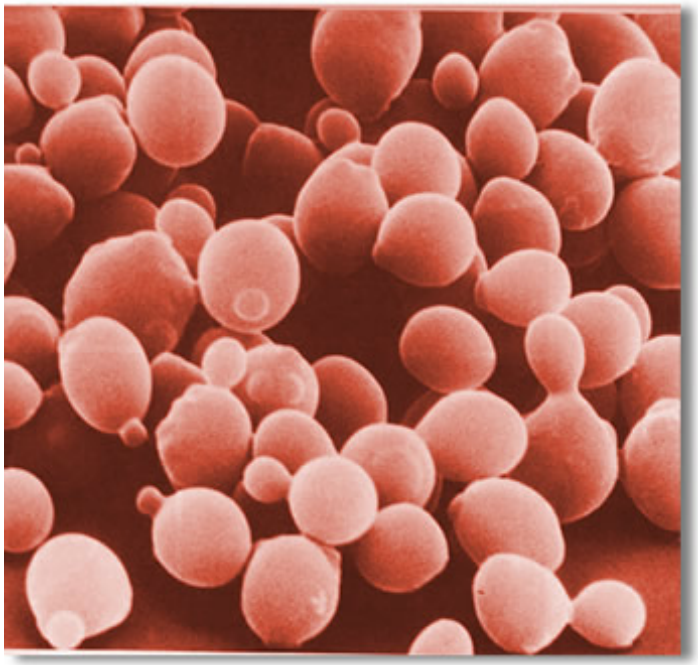
dstainier@biochem.ucsf.edu

Zebrafish genetics

Didier Stainier, UCSF Biochemistry

Juquehy, Brazil

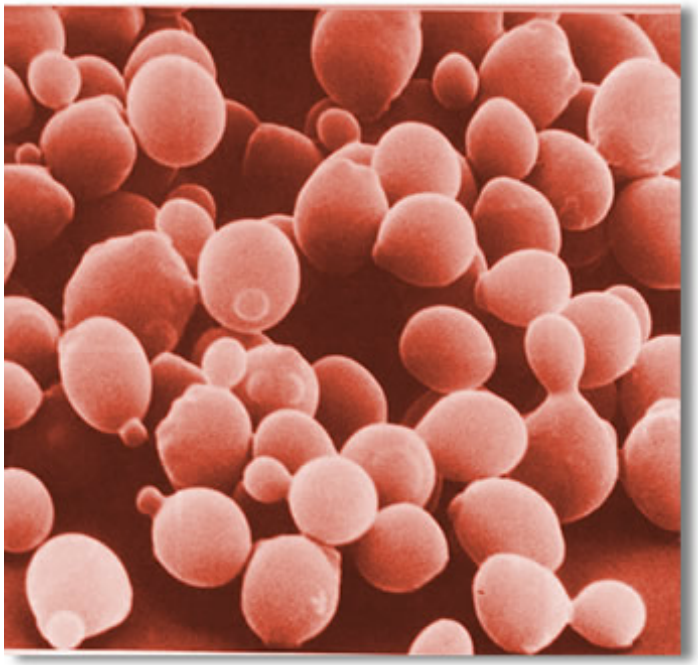
5/2/05



Saccharomyces cerevisiae

Caenorhabditis elegans

Drosophila melanogaster



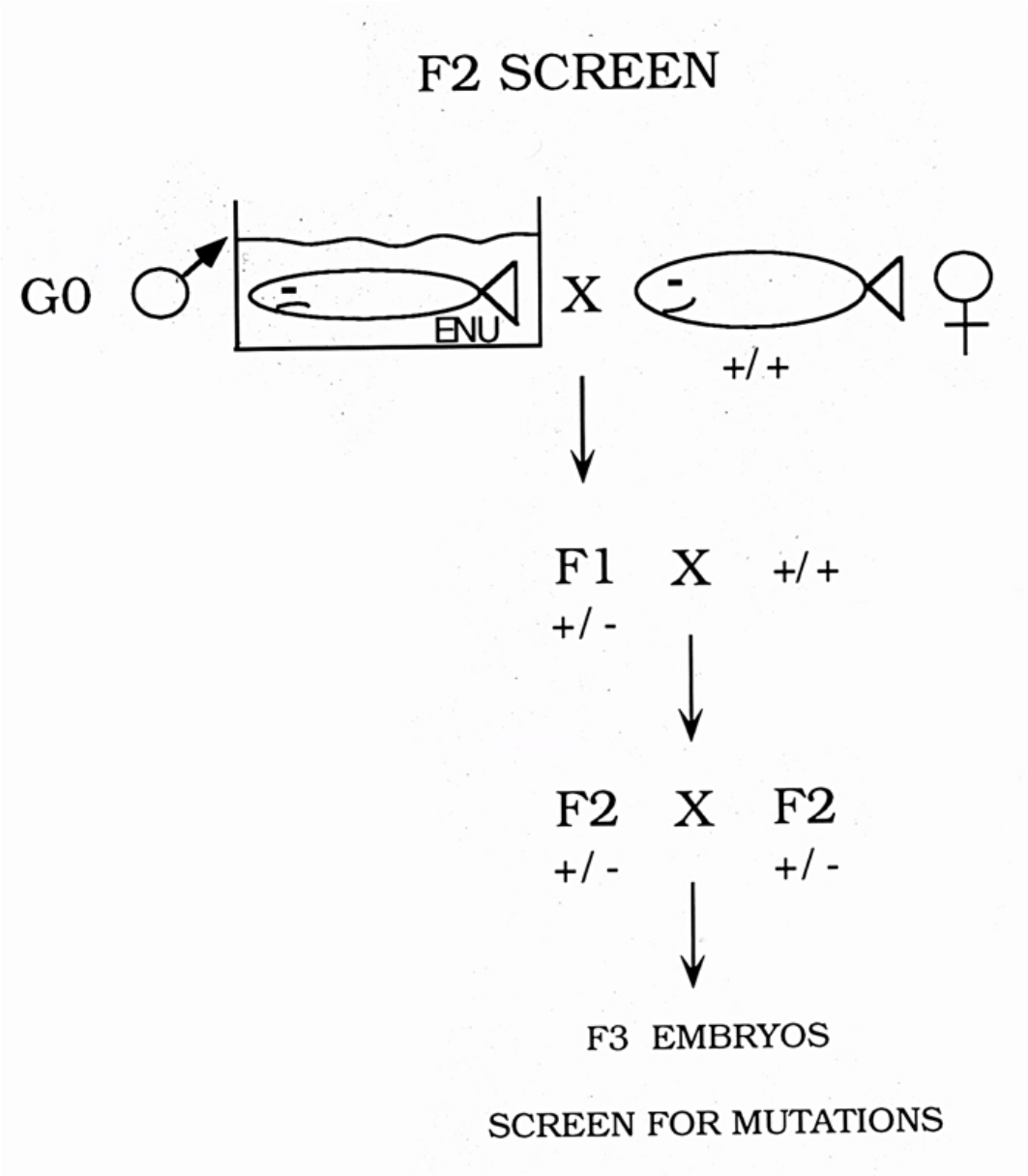
Saccharomyces cerevisiae

Caenorhabditis elegans

Drosophila melanogaster

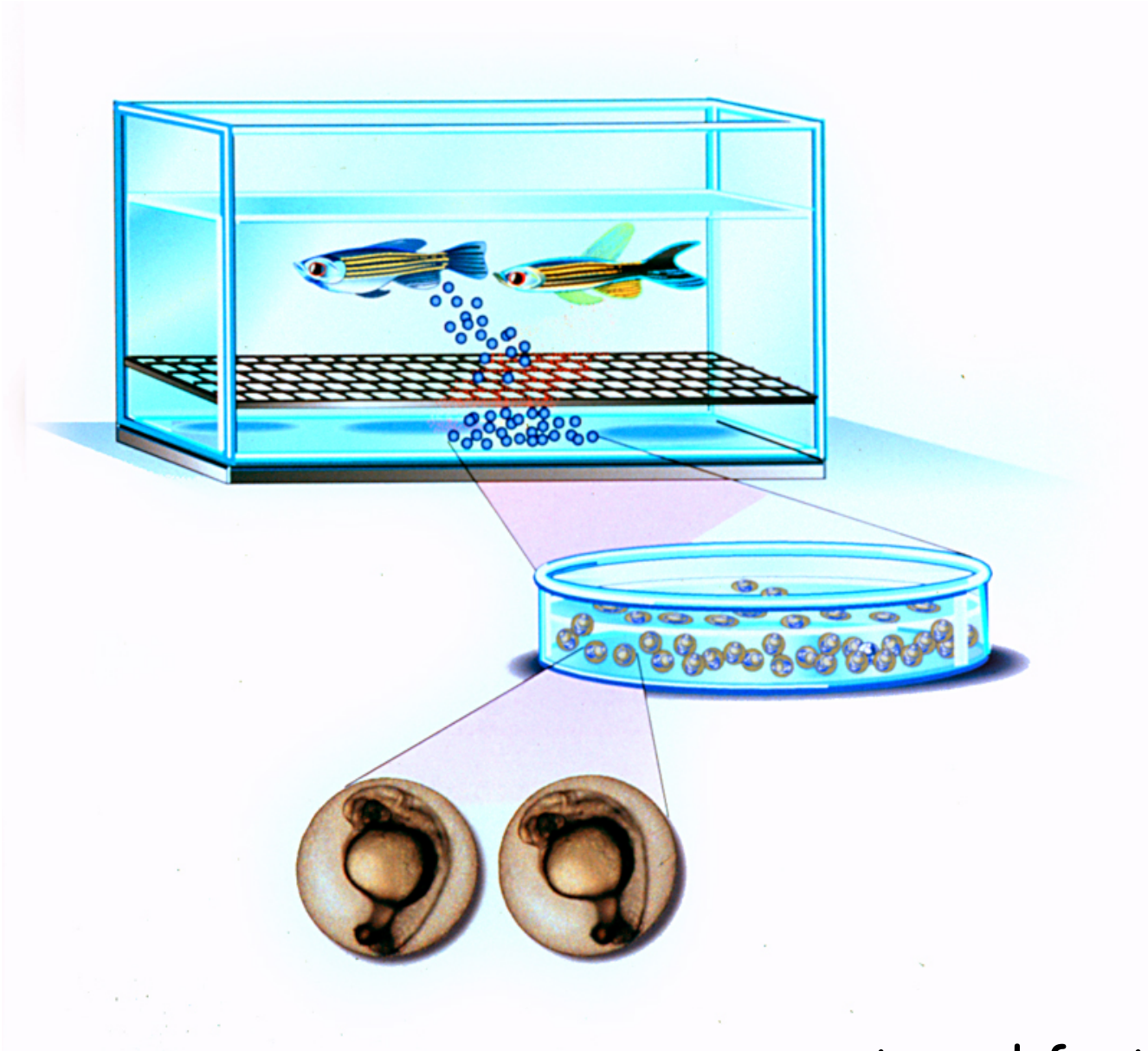
Danio rerio

GENETIC SCREENS

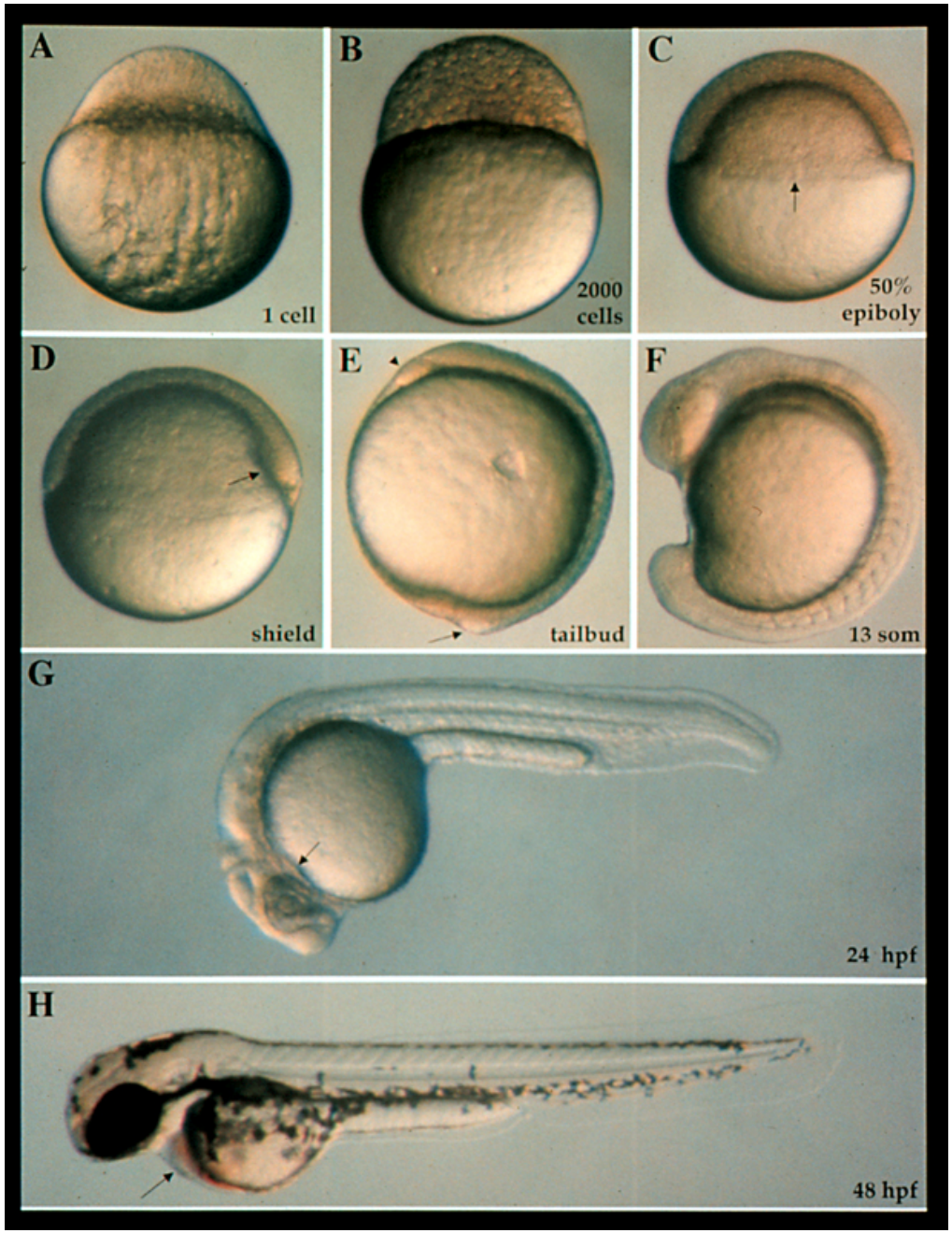


What can one screen for?

- developmental processes
- physiological processes
- behavior
- other



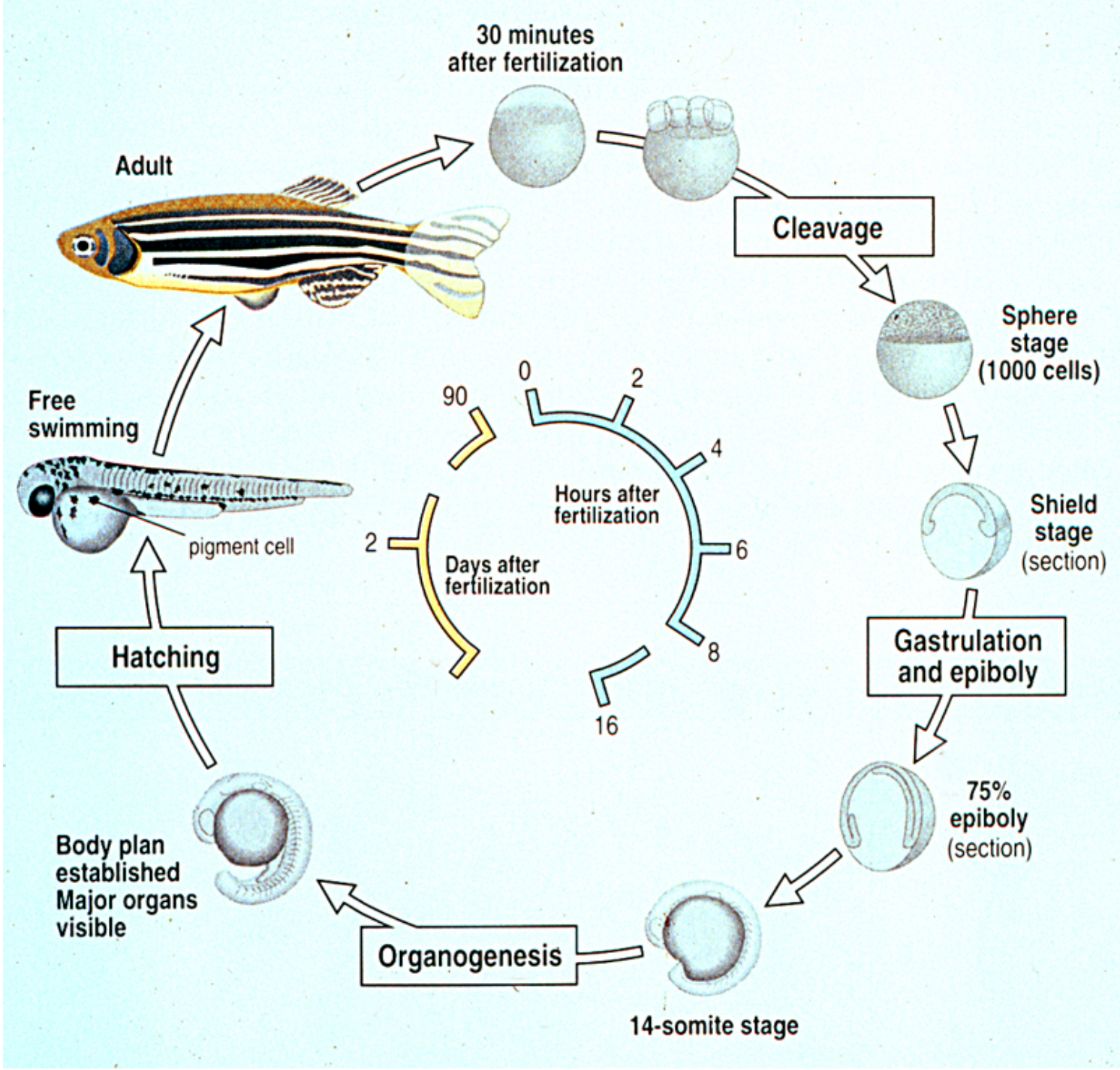
external fertilization

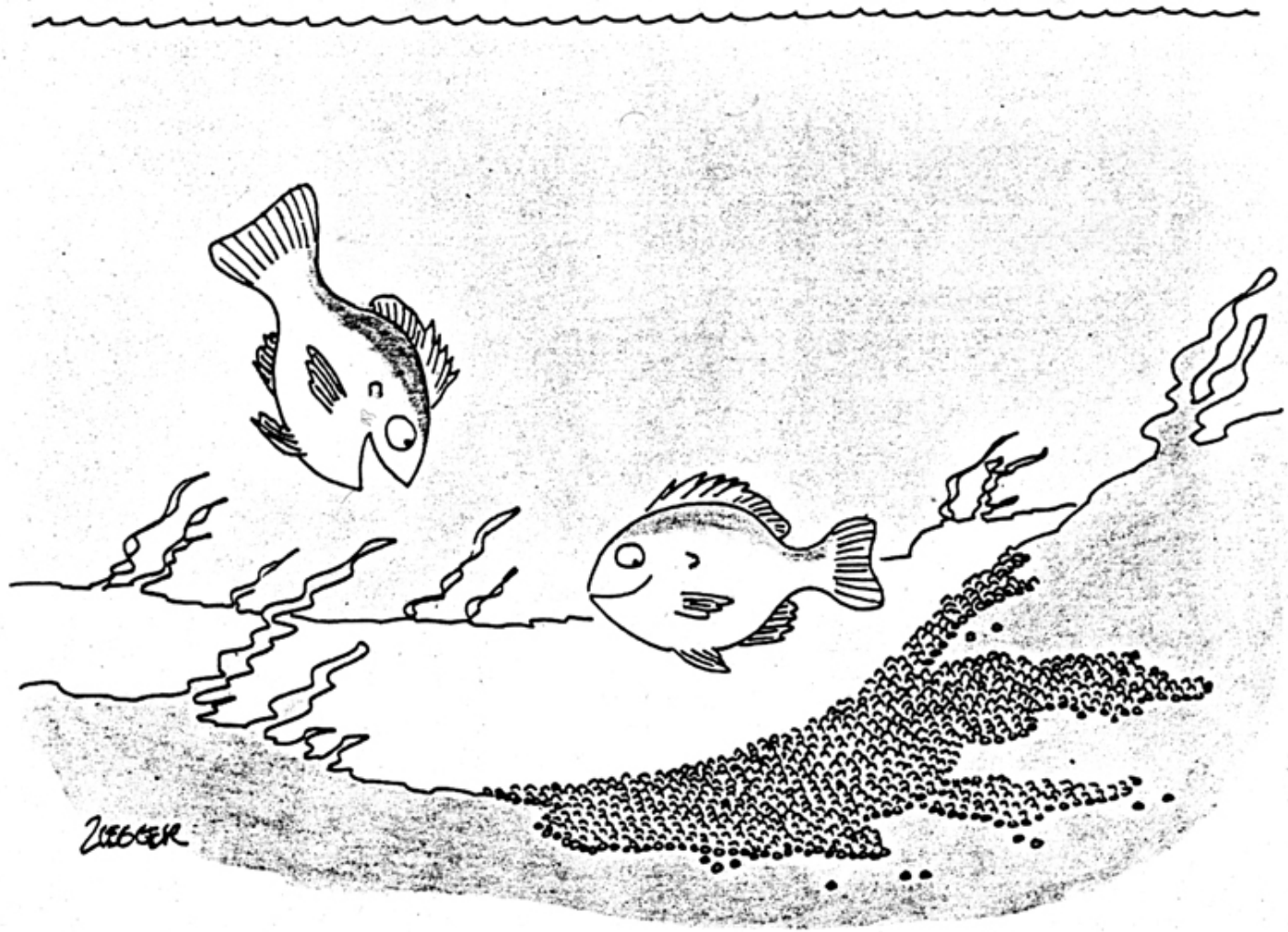


some key developmental stages

Zebrafish embryonic development

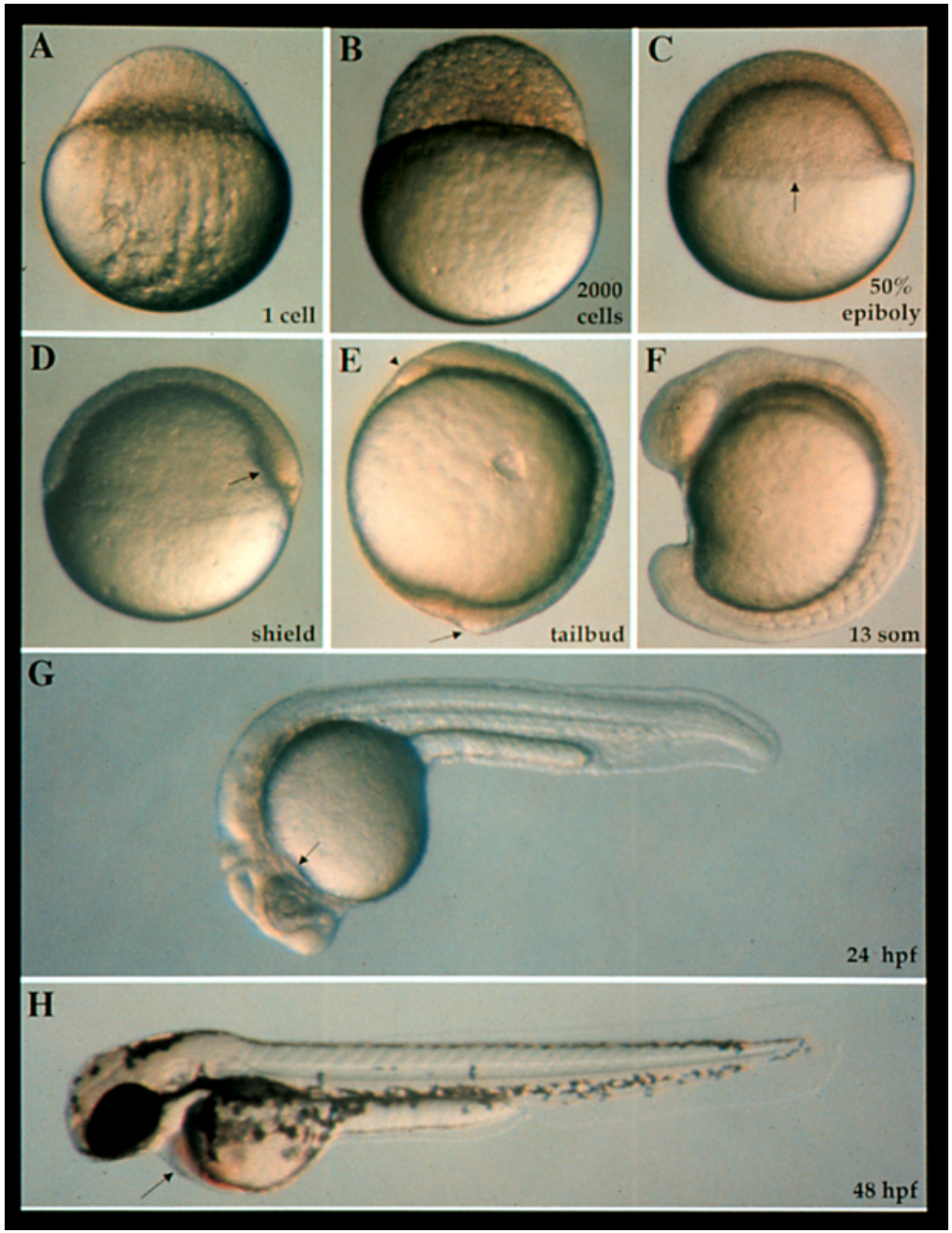
QuickTime™ and a
Animation decompressor
are needed to see this picture.


30 minutes
after fertilization
Cleavage
Sphere
stage
(1000 cells)
Shield
stage
(section)
Gastrulation
and epiboly
75%
epiboly
(section)
14-somite stage
Organogenesis
Body plan
established
Major organs
visible
Hatching
Free
swimming
pigment cell
Adult
0
2
4
6
8
16
Hours after
fertilization
90
2
Days after
fertilization



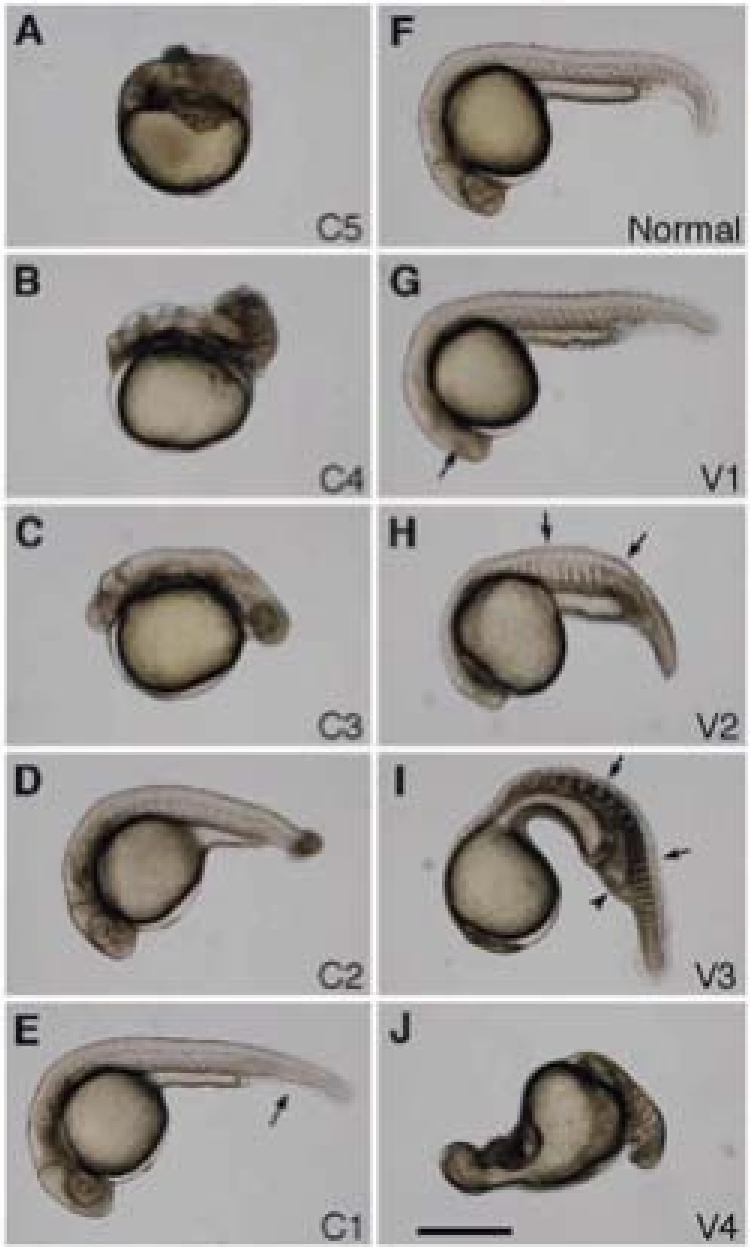
"*Oh, Maria—so many eggs!*"

large number of progeny



screening based on morphological traits

Zebrafish embryos showing abnormal DV patterning



V; ventralized

chordin mutants

C; dorsalized

bmp2 mutants
tolloid mutants

Cardiac valve formation

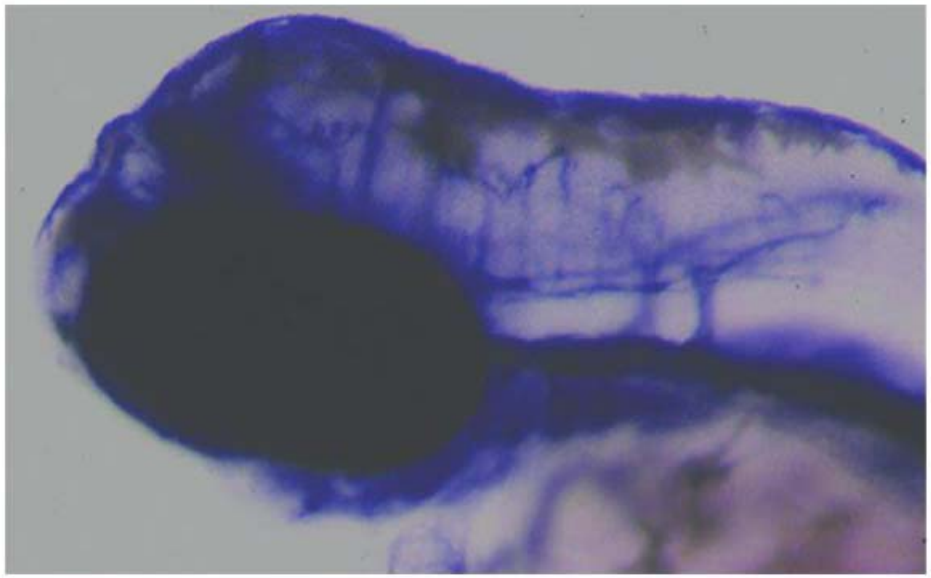
QuickTime™ and a
Video decompressor
are needed to see this picture.

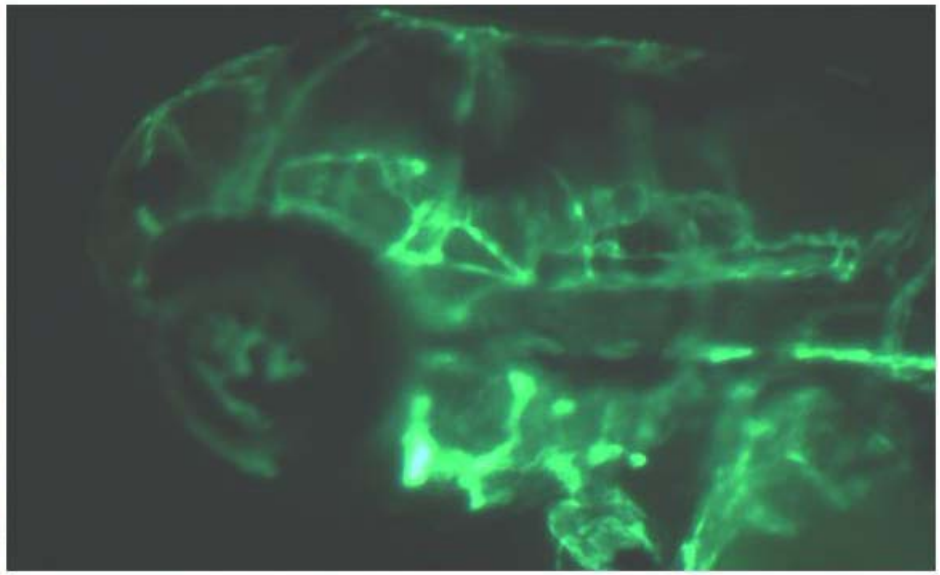
QuickTime™ and a
Video decompressor
are needed to see this picture.

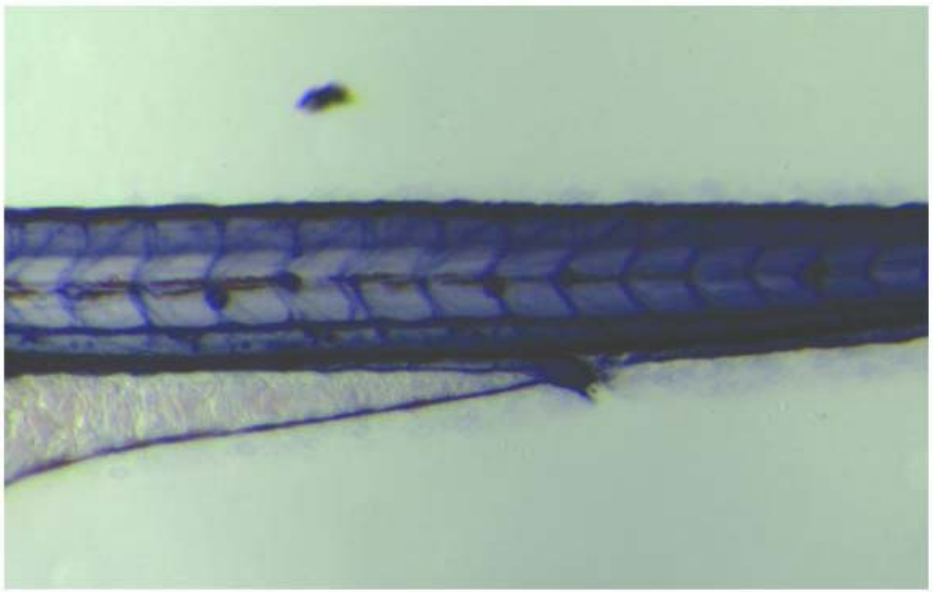

QuickTime™ and a
Cinepak decompressor
are needed to see this picture.

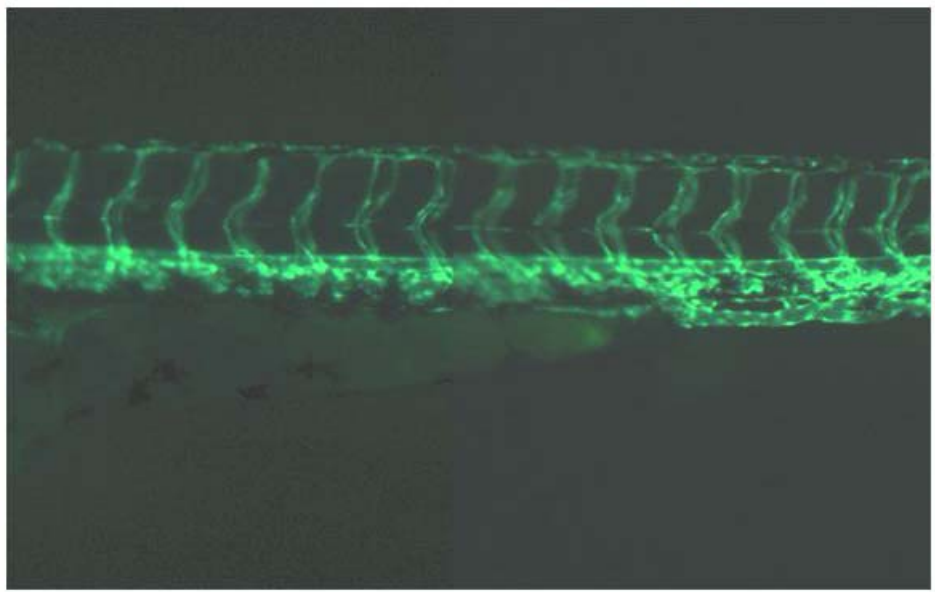
AP staining

flk1:GFP

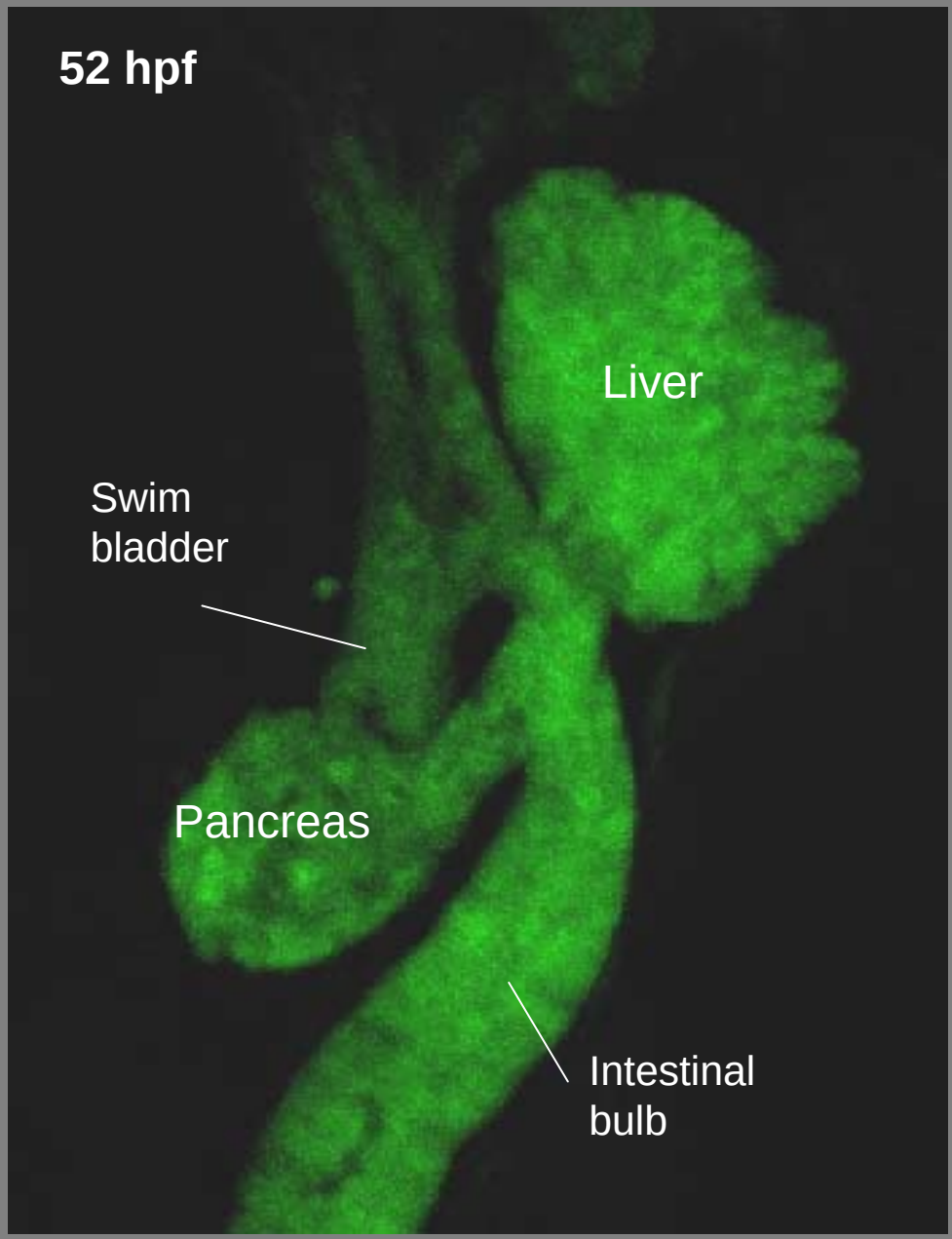








gutGFP line



Ventral View

Duc Dong

80 hpf

L

QuickTime™ and a
Motion JPEG A decompressor
are needed to see this picture.

P

lfabp-dsRed
elastase-GFP
insulin-dsRed

Ryan Anderson

forward genetic screens in zebrafish

- ENU

- insertional mutagenesis (retroviral vectors)

- large number of tanks (diploid screens)

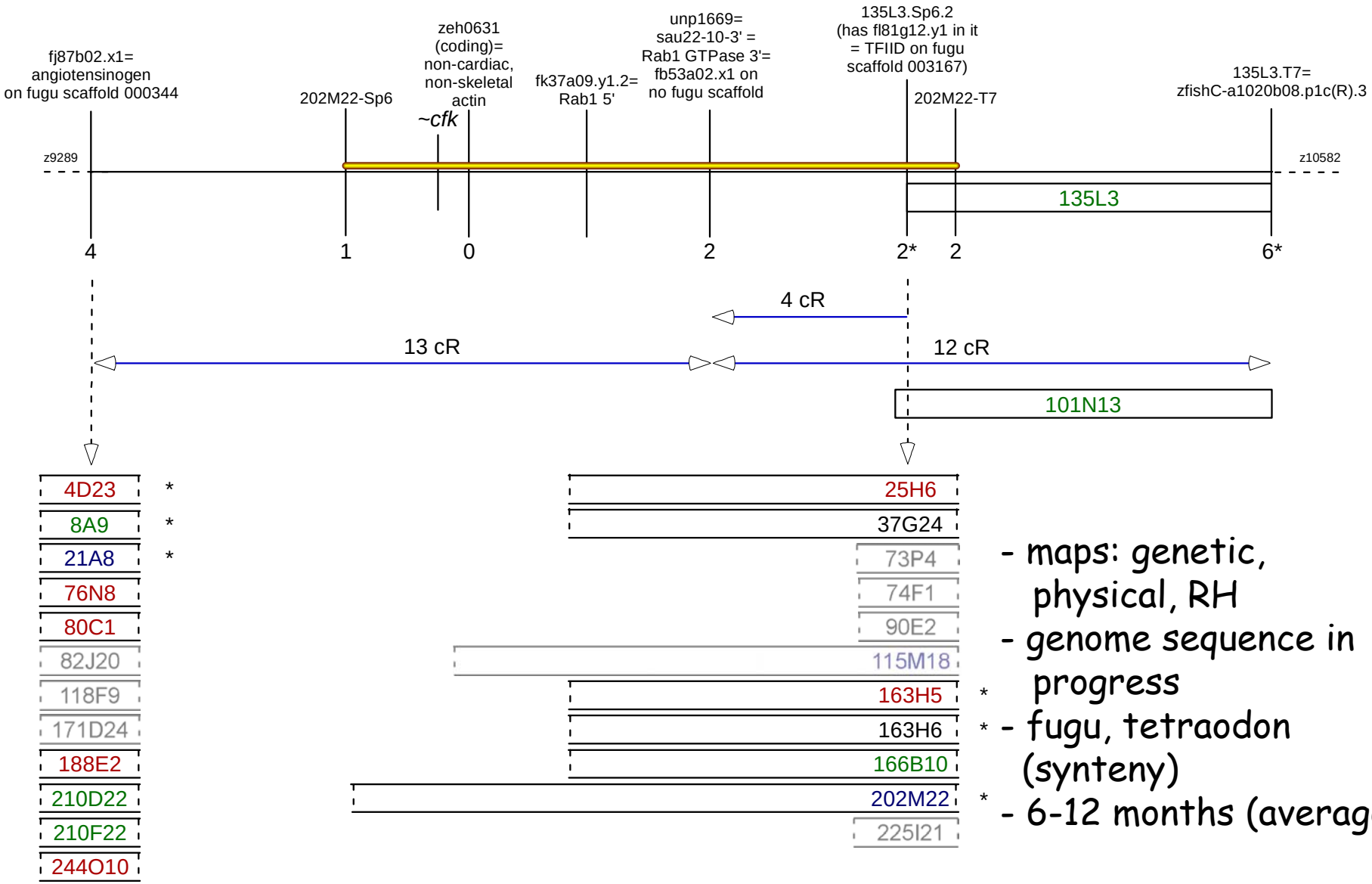
- 3-4 scientists

- 18-24 months

What can you do with your mutants?

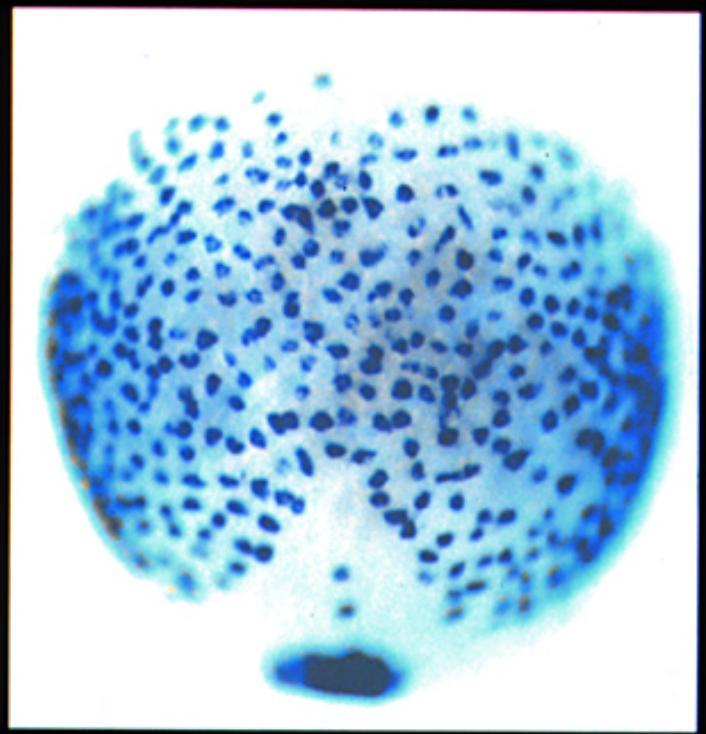
- clone the gene, look at its expression pattern
- analyze cell-autonomy
- gain-of-function experiments

Going from mutation to gene

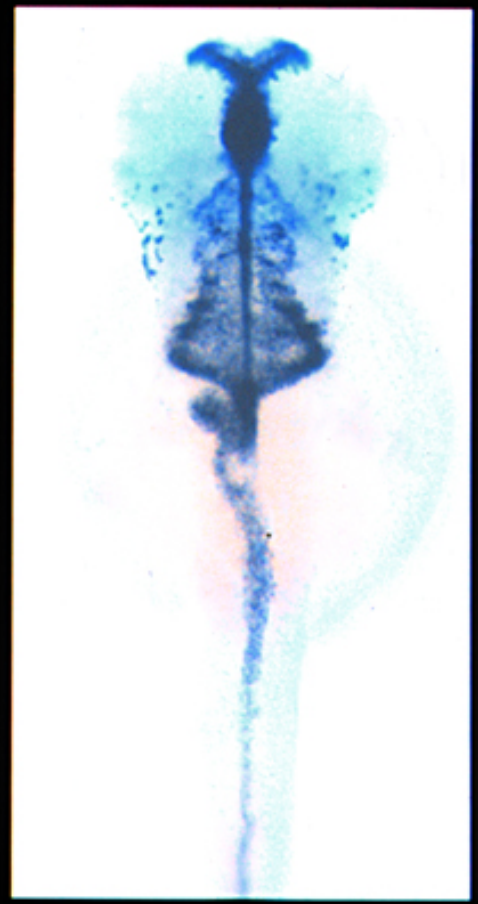


- maps: genetic, physical, RH
- genome sequence in progress
- fugu, tetraodon (synteny)
- 6-12 months (average)

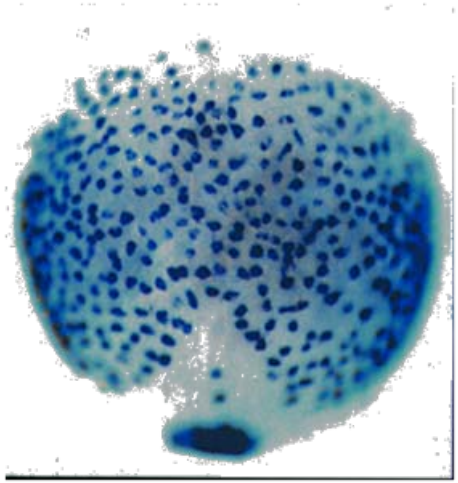
Endoderm Development in Zebrafish



Late gastrula
sox17



46 hours
fkd7



sox17 late gastrula

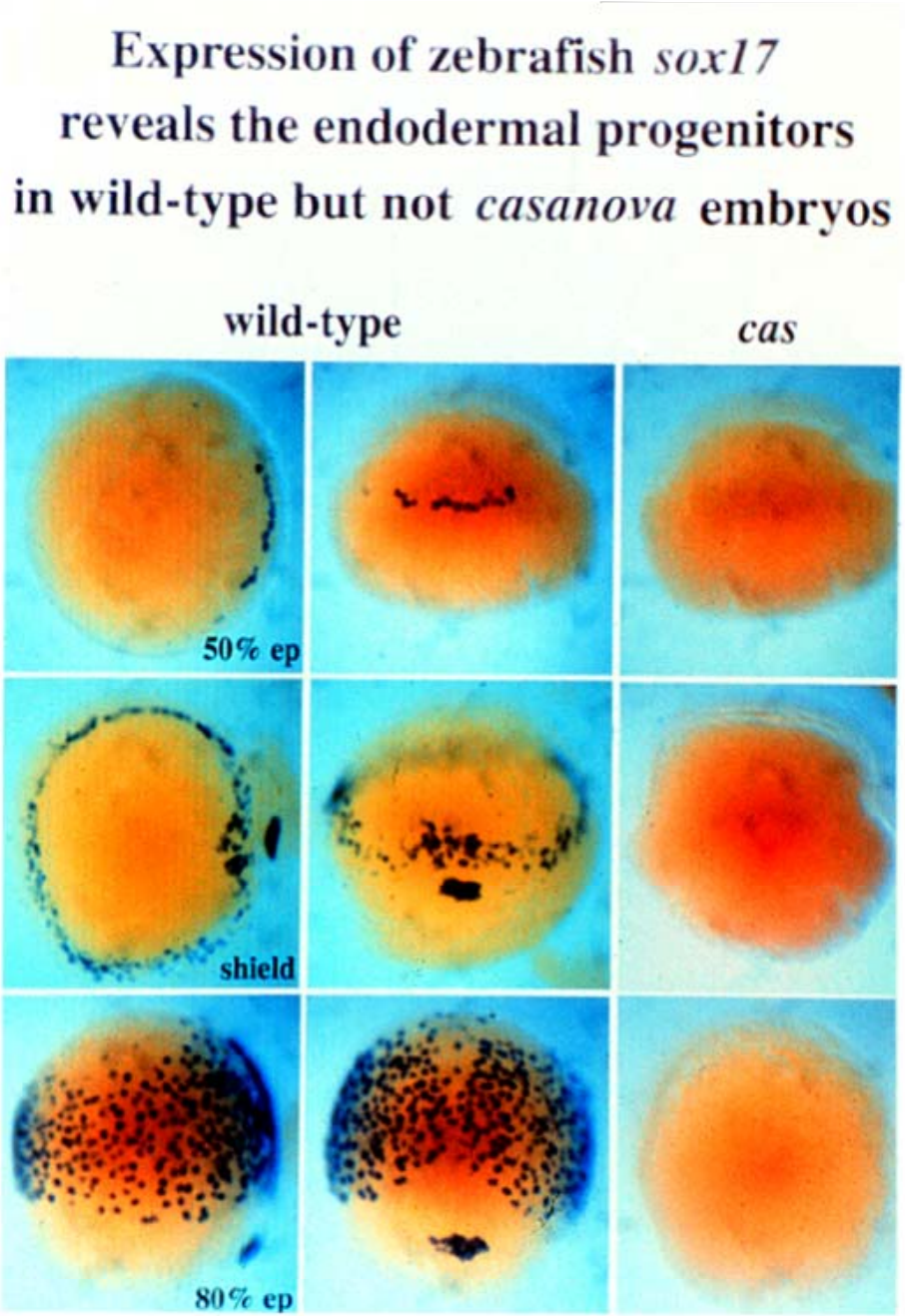
Mutations that affect early endoderm formation in zebrafish

- *one-eyed pinhead (oep)*
- *casanova (cas)*
- *bonnie and clyde (bon)*
- *faust (fau)*

all endodermal cells missing

90% of endodermal cells missing

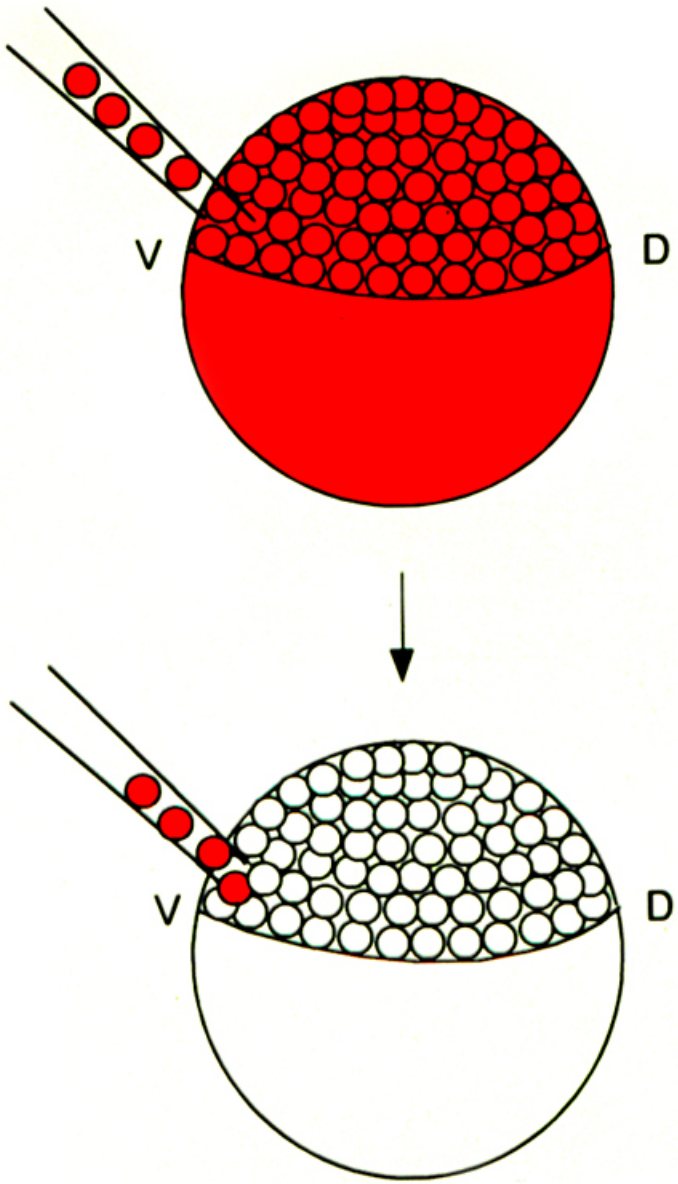
60% of endodermal cells missing



sox17 first implicated in endoderm formation in the frog *Xenopus laevis*

Alexander et al., 1999

Generating genetic mosaics in zebrafish

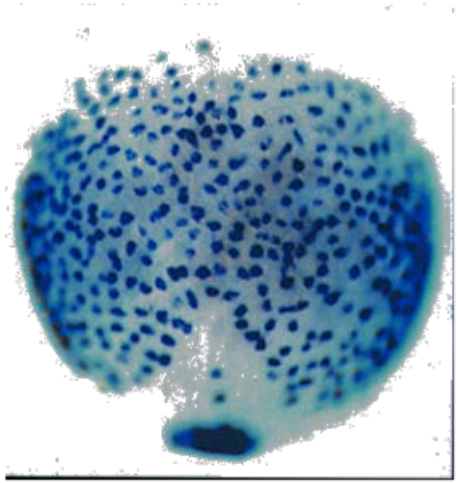


Results of the cell transplantation experiments

- *casanova* mutant cells never form endoderm (either in wild-type or mutant embryos)
- wild-type cells can form endoderm in *casanova* mutant embryos

The endoderm phenotype in *casanova* mutants is cell-autonomous, i.e. *casanova* functions within the endodermal lineage

Alexander et al., 1999

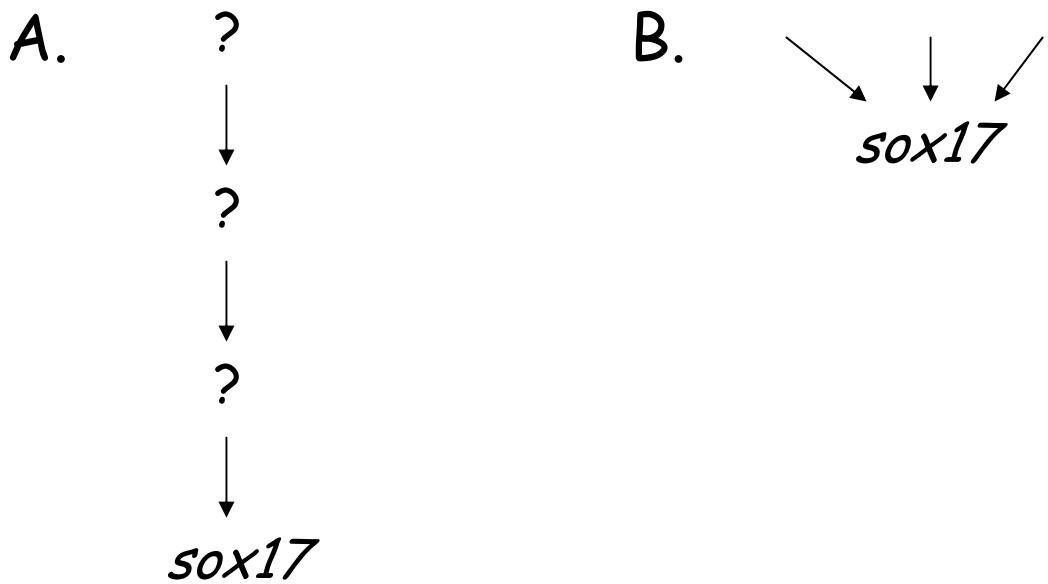


sox17 late gastrula

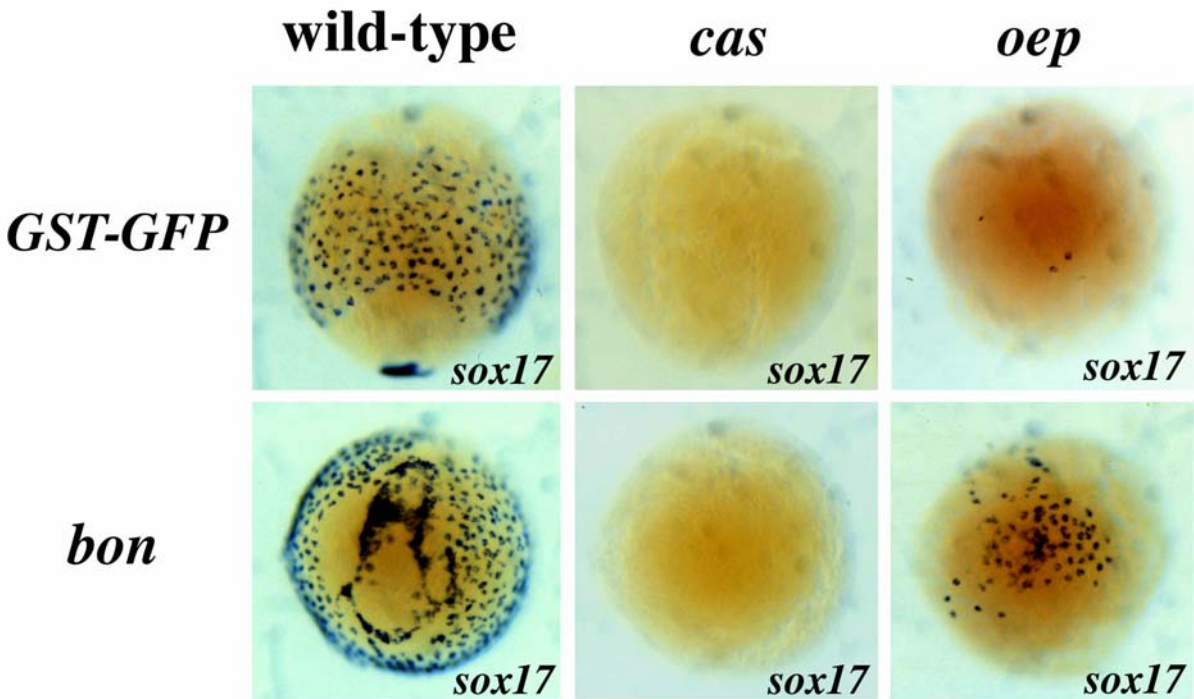
Proteins that affect early endoderm formation in zebrafish

- Oep (Nodal co-receptor. Schier; Whitman)
- Cas (Sox-related transcription factor)
- Bon (Mix-type HD transcription factor)
- Fau (Gata5: Zinc-finger transcription factor)

How are Oep,
Cas/Sox-related
Bon/Mix-type
Fau/Gata5
positioned relative to each other in the pathway leading to endoderm formation?

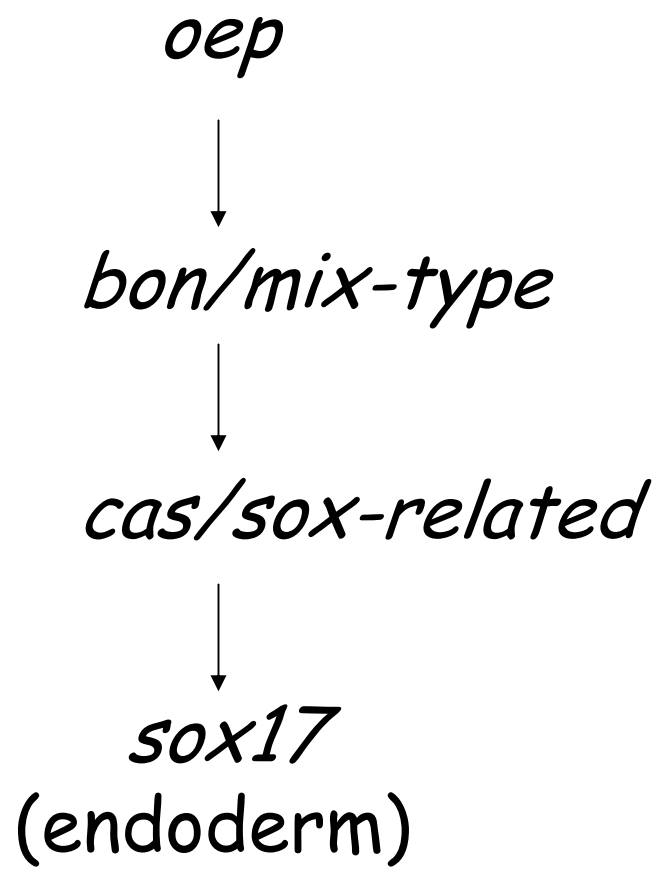


bon overexpression rescues endoderm formation in *oep* but not *cas* mutants

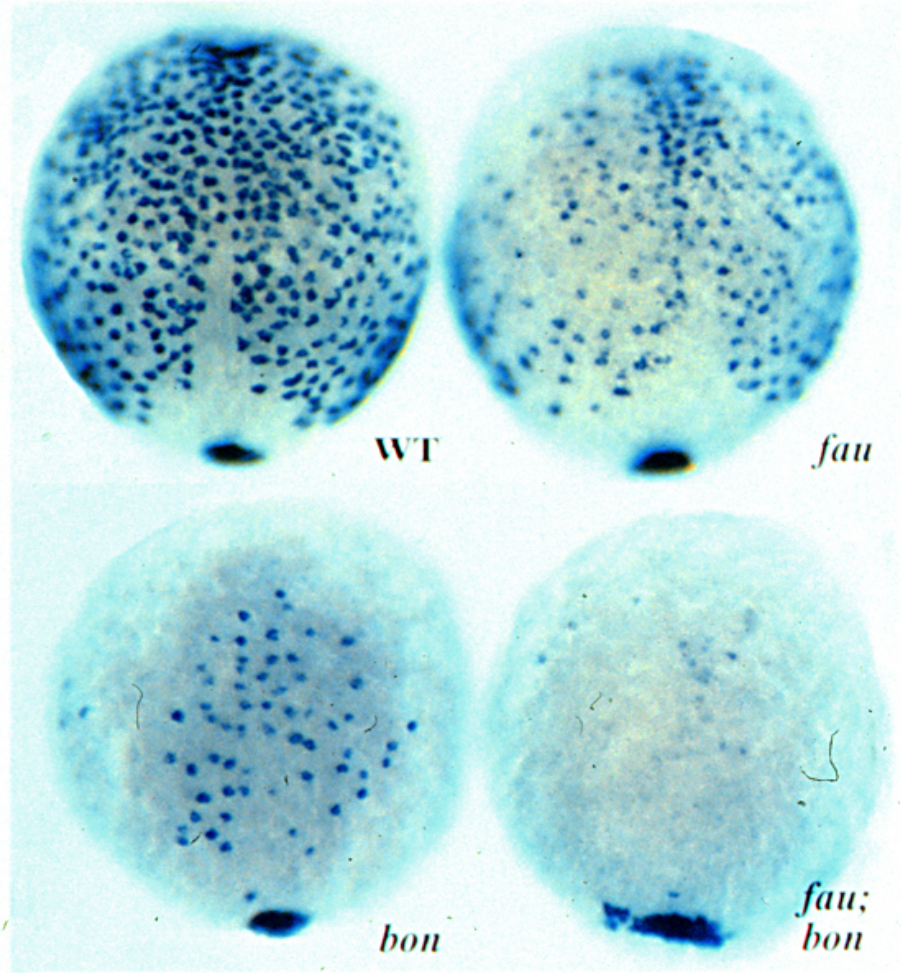


Alexander et al., 1999

Endoderm formation in zebrafish

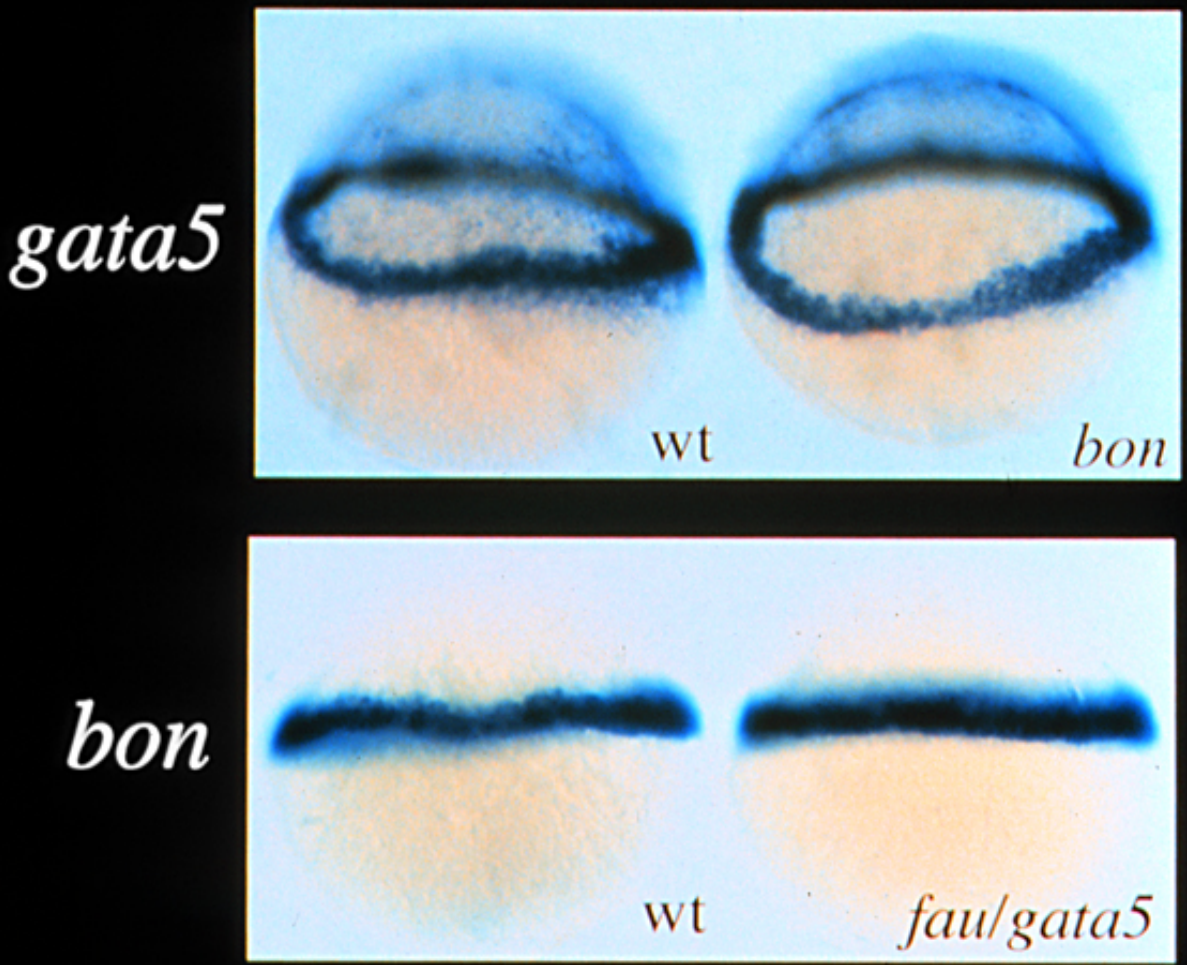


Endoderm formation is defective in *faust* and *bonnie and clyde* mutants

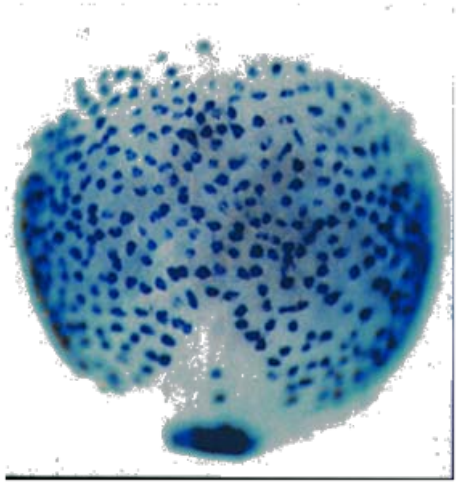


Reiter et al., 2001

Bon and Gata5 do not regulate each other

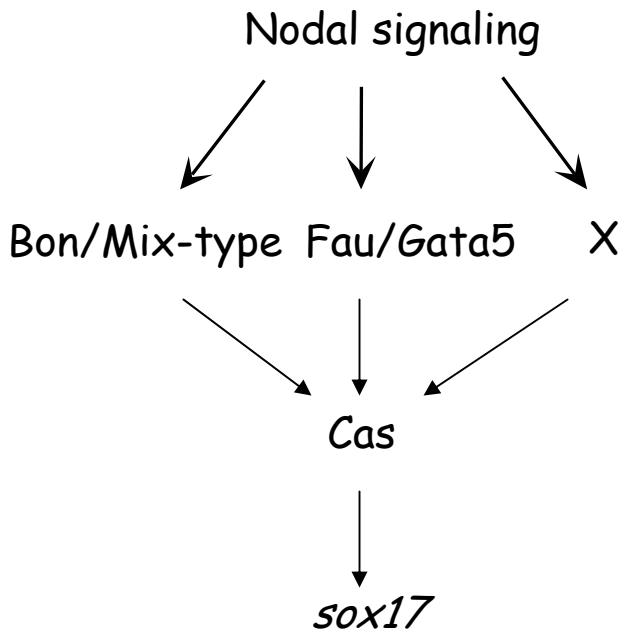


Reiter et al., 2001

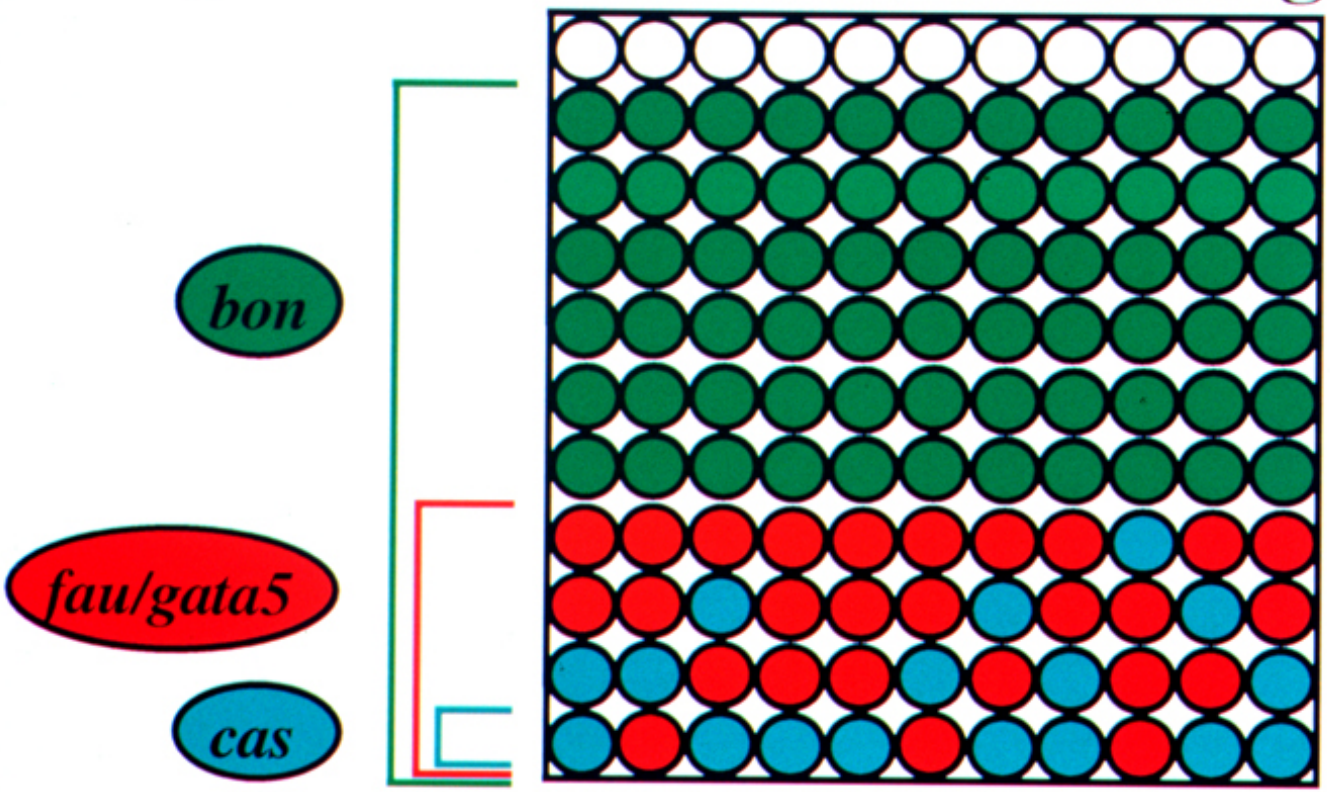


sox17 late gastrula

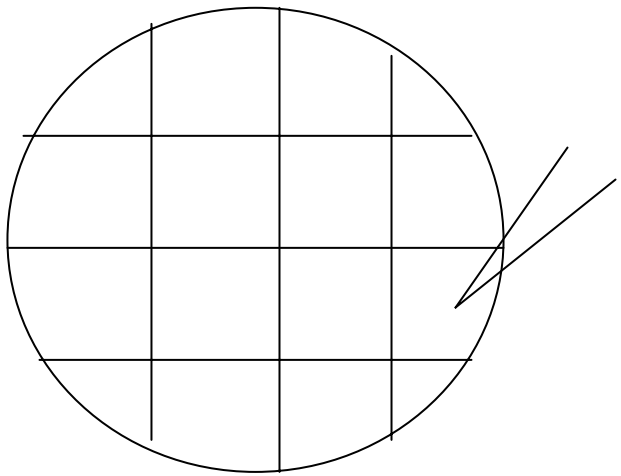
A possible model of early endoderm formation in zebrafish



Comparison of *bon*, *fau/gata5*, *cas* expression at late blastula stage



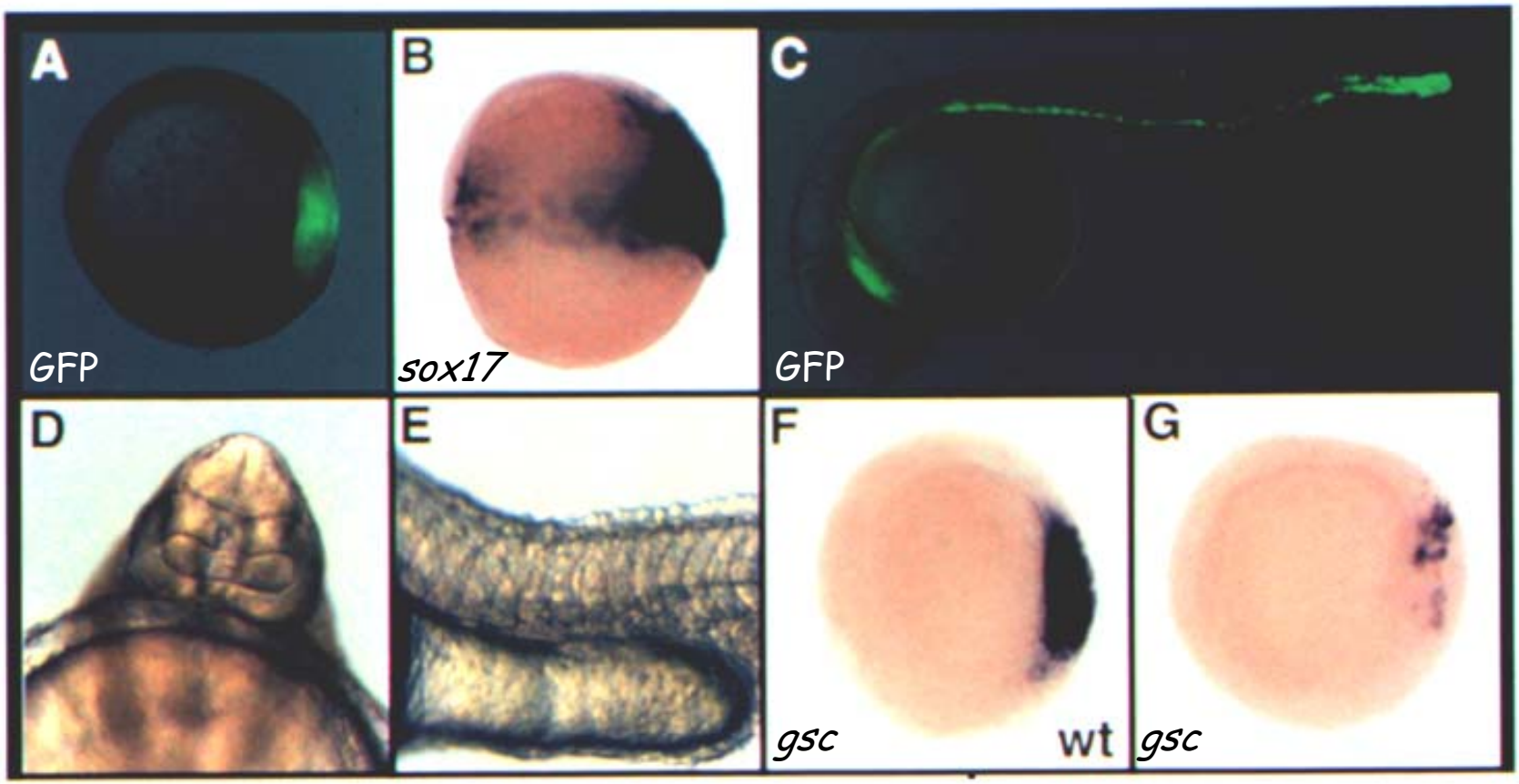
Analyzing the function of *cas* by gain-of-function (i.e. misexpression) experiments



cas mRNA and *GFP* mRNA

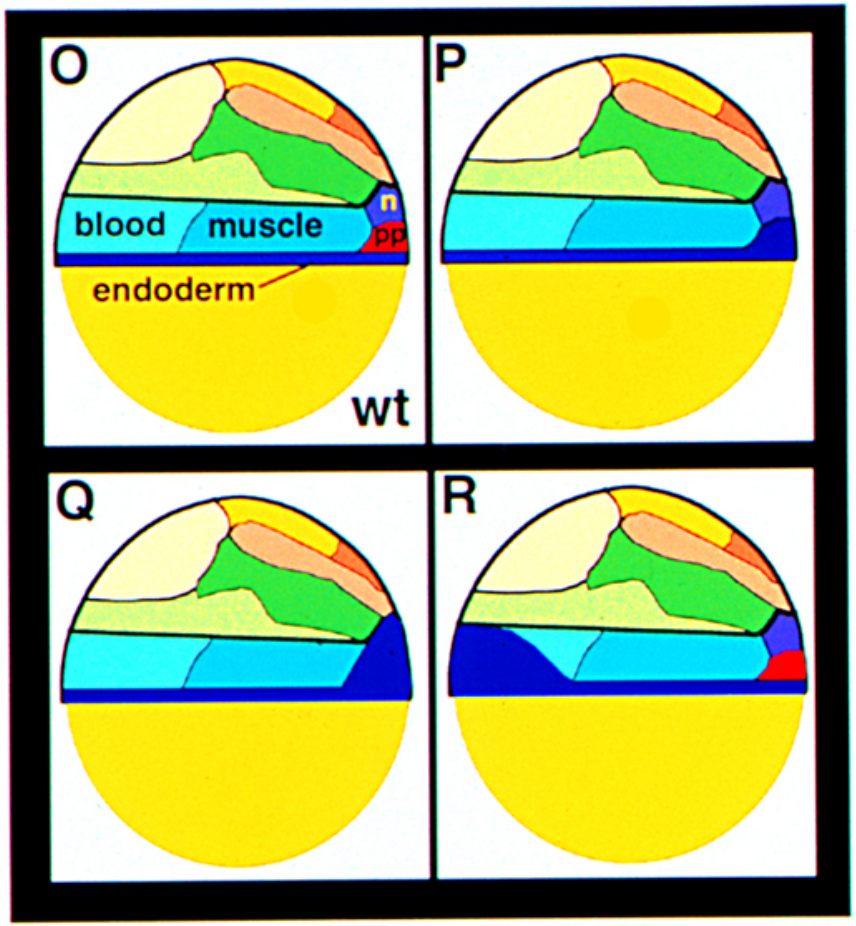
16-cell stage

Ectopic cas expression can transfate mesoderm into endoderm



Kikuchi et al., 2001

Transfating ability of *cas*



Kikuchi et al., 2001

What can you do with your mutants?

- clone the gene, look at its expression pattern
- analyze cell-autonomy
- gain-of-function experiments

Tools

forward genetics

reverse genetics; morpholinos (use with caution)
(use splice MOs whenever possible)

lineage analysis, cell transplantation

gain-of-function experiments:
- DNA, RNA injections
- spatial control (binary systems: Gal4-UAS (Fraser))
- temporal control (heat shock promoter)

other genetic tools:
- transposons, ires, cre-lox

Tools

what is missing?

homologous recombination

spatial and temporal control of gene knock-down

Developmental Biology

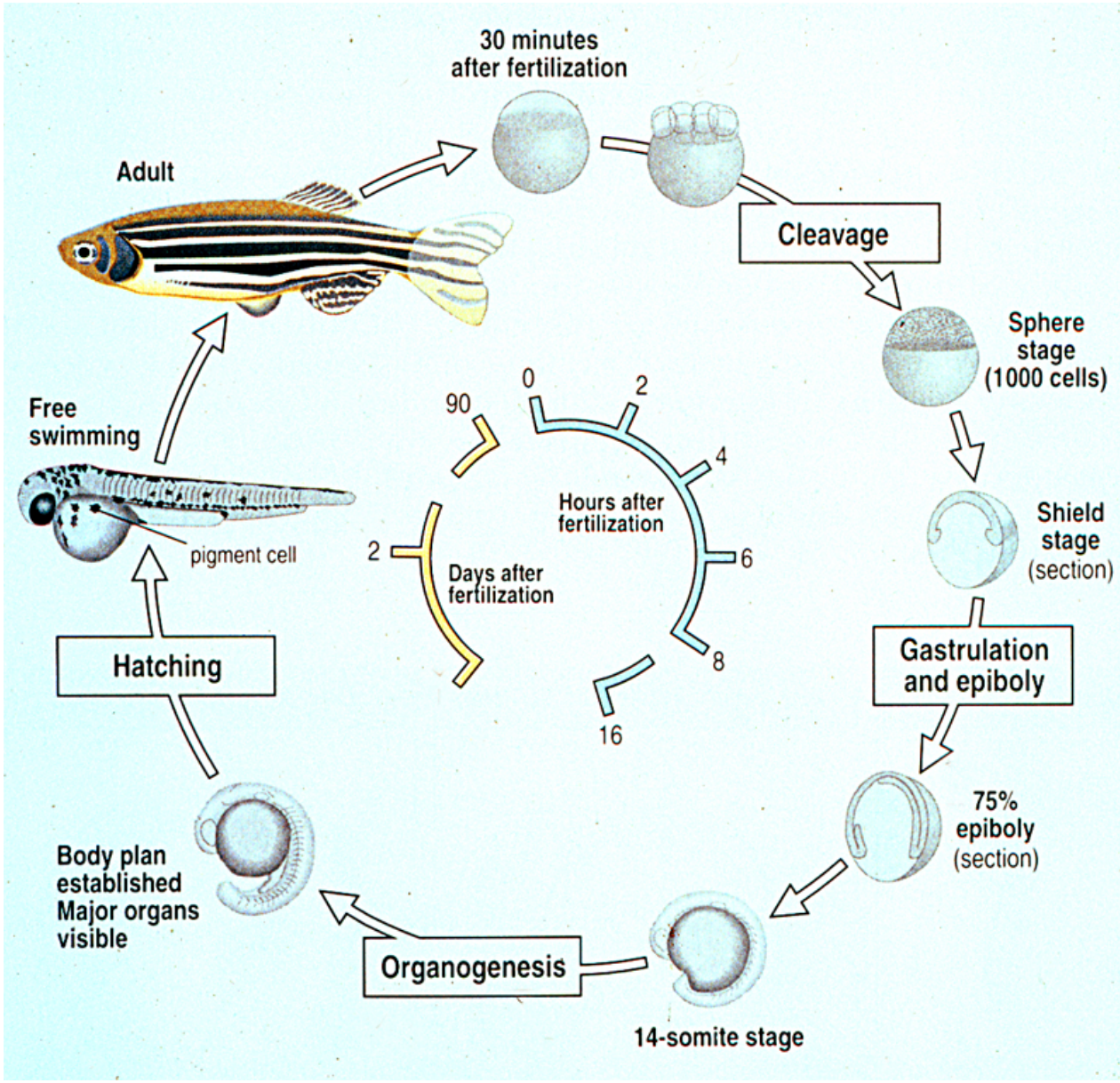
QUESTIONS

- cell differentiation
- morphogenesis

APPROACH

1) gene identification (forward genetics)
2) cell biological mechanisms
3) biochemical mechanisms

1. embryology
2. forward genetics
3. cell biology

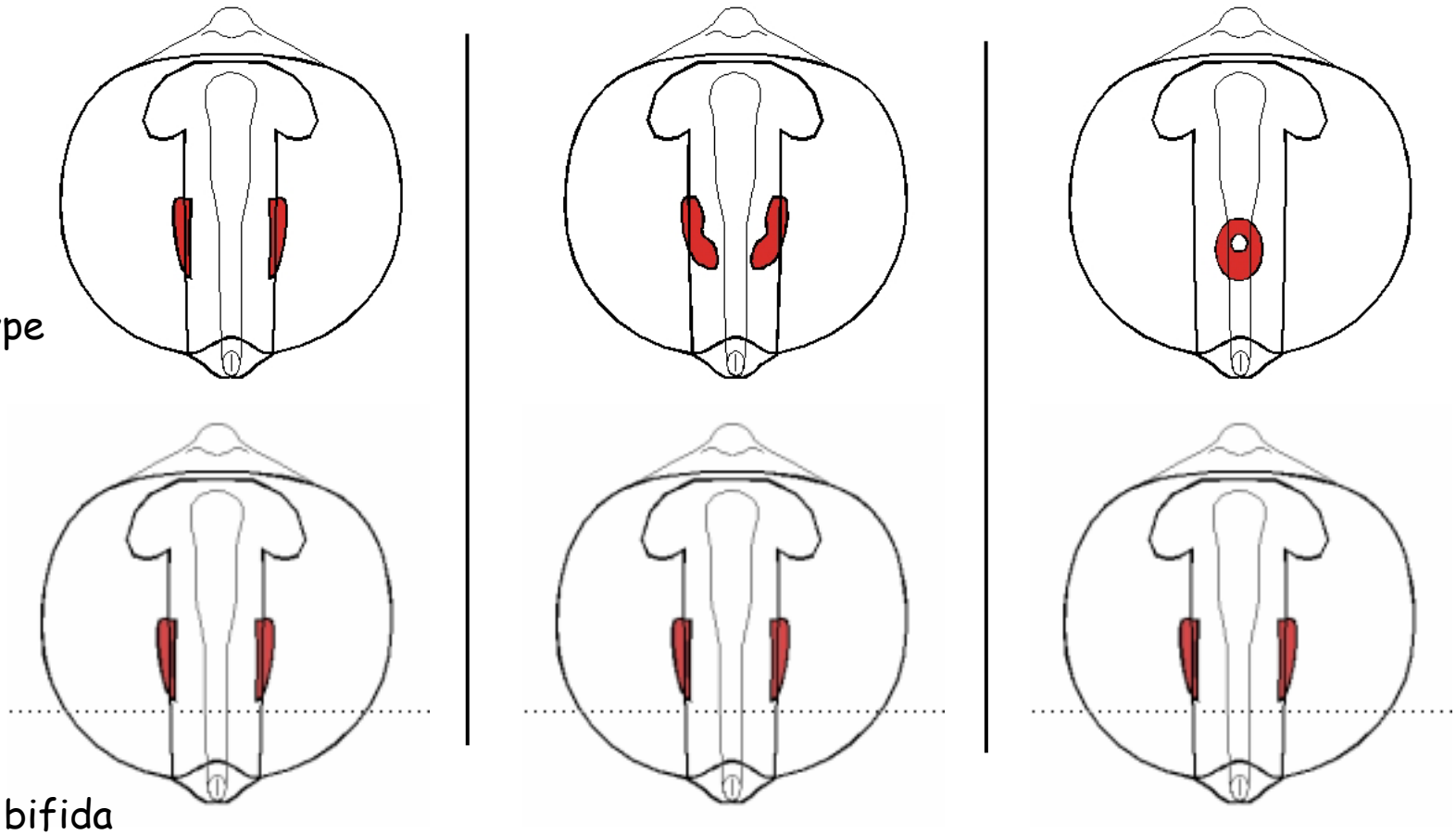


precardiac mesoderm morphogenesis in zebrafish

dorsal views

wild-type

cardia bifida mutants



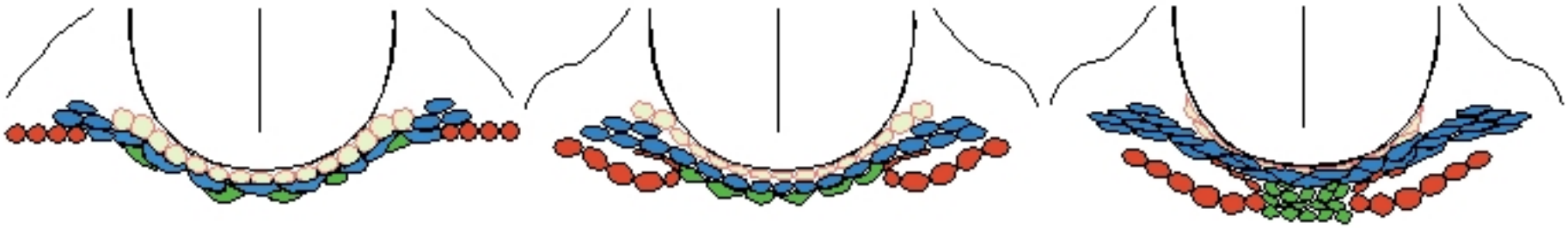
13 somite
15.5 hpf

17 somite
17.5 hpf

20 somite
19 hpf

precardiac mesoderm morphogenesis in zebrafish

transverse sections



13 somite
15.5 hpf

17 somite
17.5 hpf

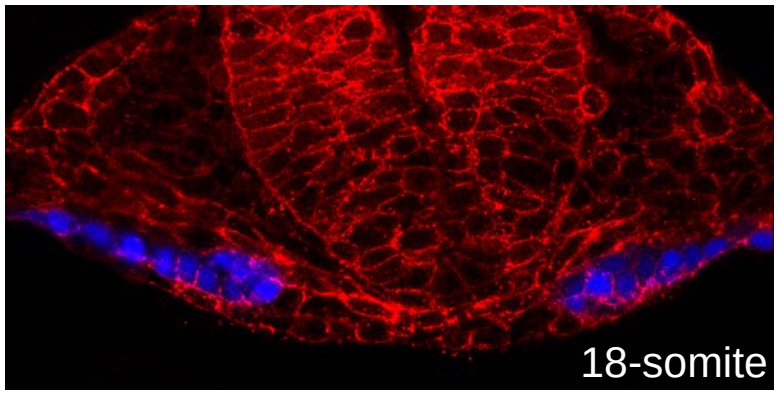
20 somite
19 hpf

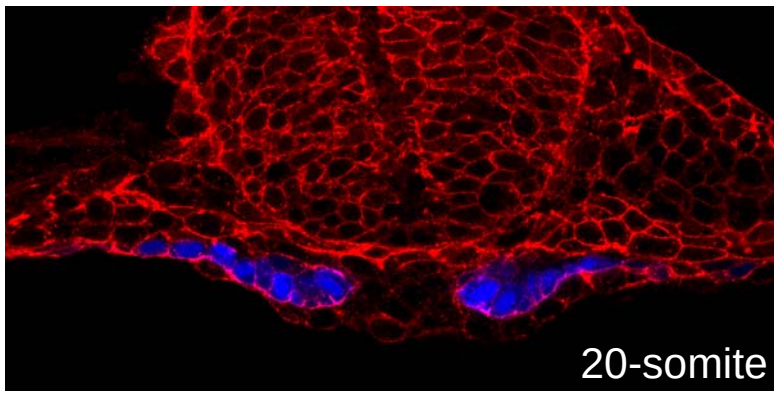
pre-myocardial cells

pre-endocardial cells

endoderm

Myocardial migration

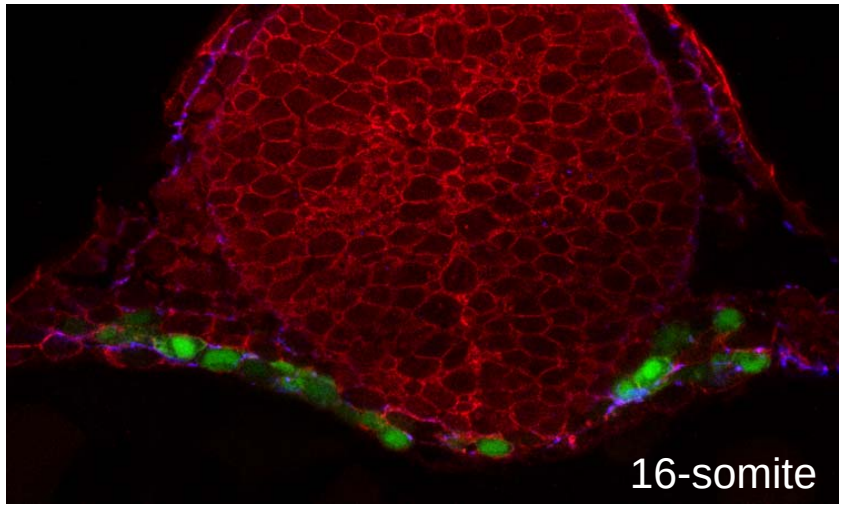


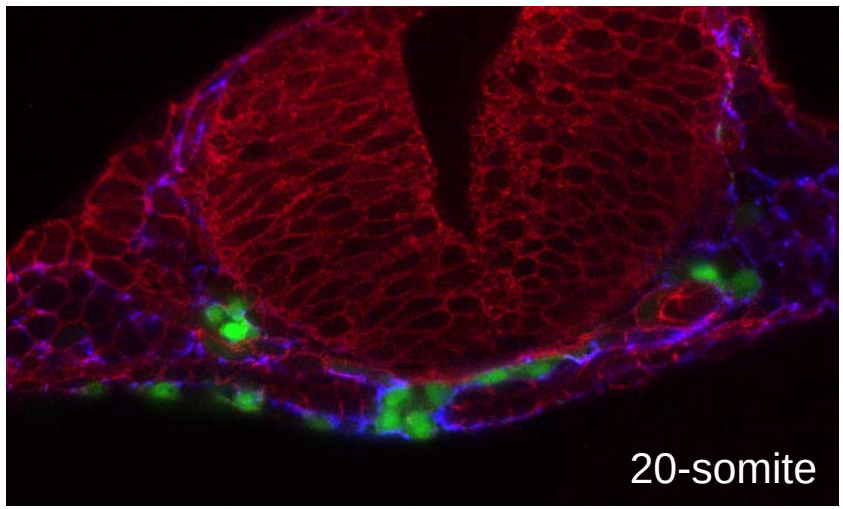


HJ Tsai

cmlc2-GFP

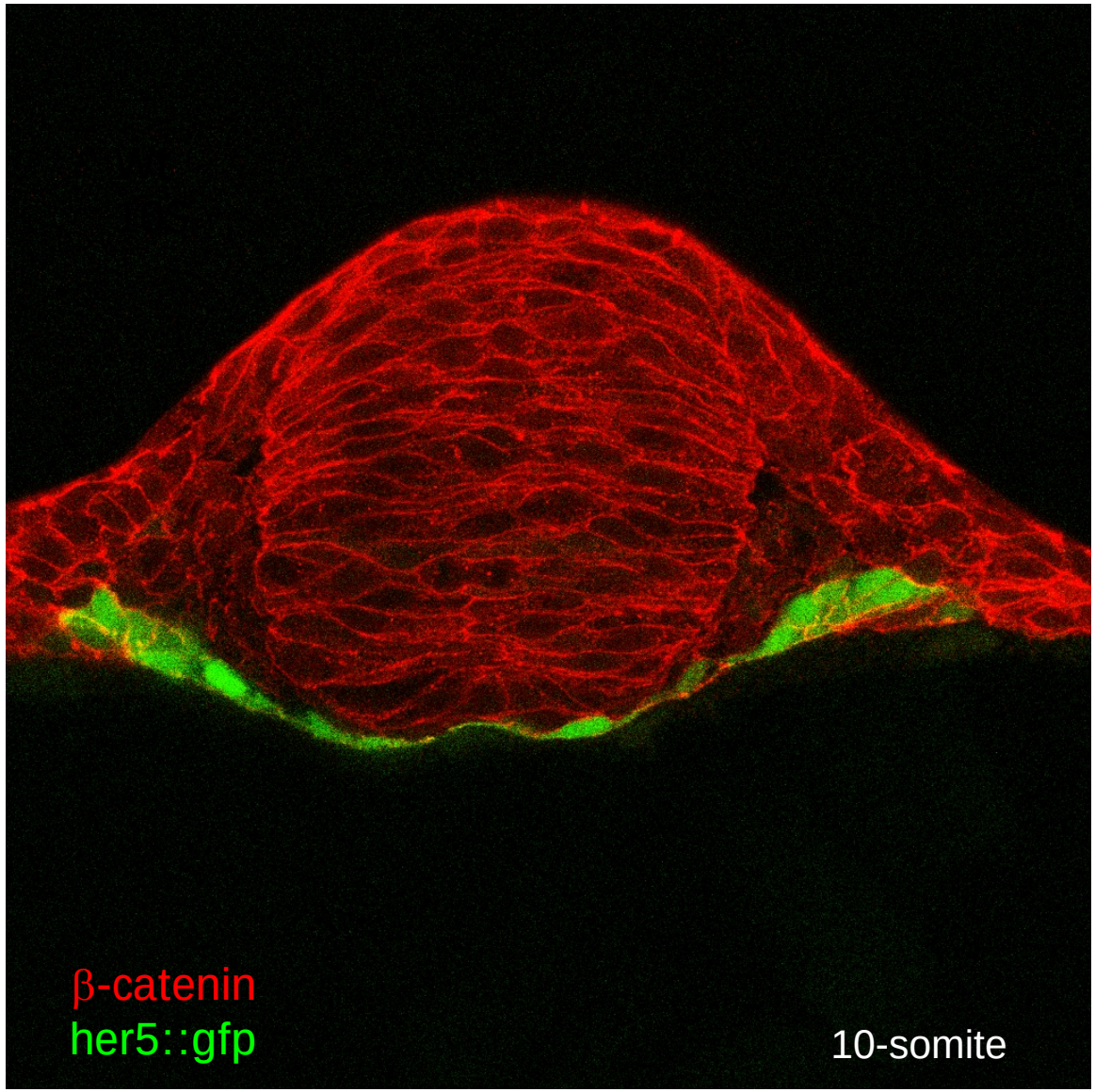
Endocardial migration





flk1-GFP

Endodermal GFP line



Nick Osborne

CARDIA BIFIDA MUTATIONS

1) AFFECTING MYOCARDIAL DIFFERENTIATION
   - *one-eyed pinhead* (*oep*) (CFC protein)
   - *faust* (*fau*) (Gata5)
   - *hands off* (*han*) (Dhand/Hand2)

2) AFFECTING HEART CELL MIGRATION
   A) VIA AFFECTING ENDODERM FORMATION
      - *one-eyed pinhead* (*oep*) (CFC protein)
      - *casanova* (*cas*) (Sox32)
      - *bonnie and clyde* (*bon*) (Mix-type HD)
      - *faust* (*fau*) (Gata5)

   B) VIA SOME OTHER MECHANISM
      - *natter* (*fibronectin*)
      - *miles apart* (*mil*) (S1P receptor)
      - *two-of-hearts* (*toh*)

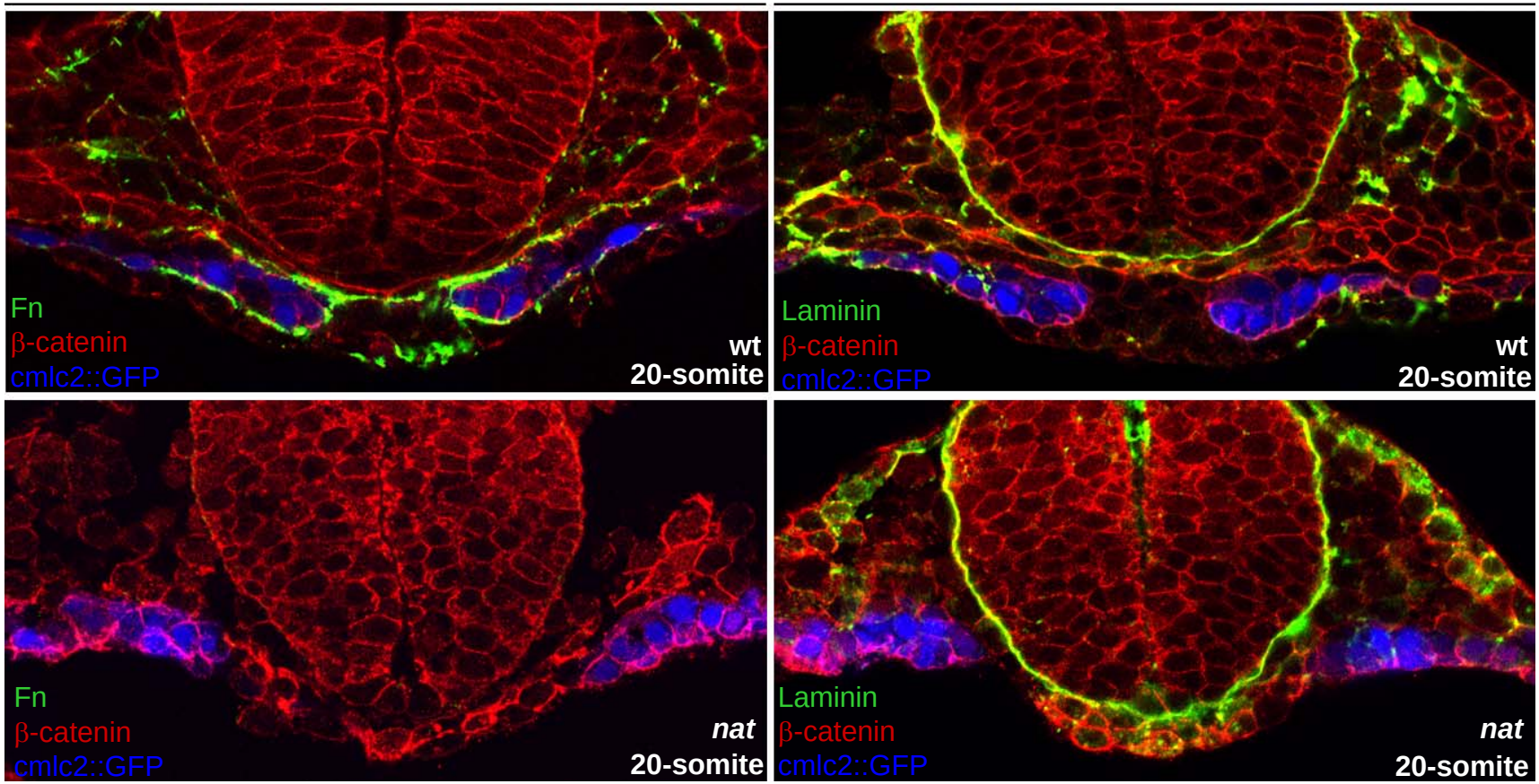
How does Fibronectin regulate myocardial migration?

- chick: Fn appears to be distributed in a gradient towards the midline (K. Linask)

- mouse: *Fn* mutants can show cardia bifida (R. Hynes)

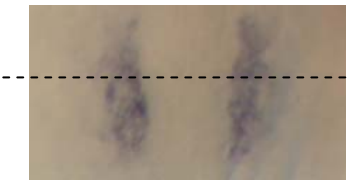
Model: haptotaxis (moving towards areas of greater adhesiveness)

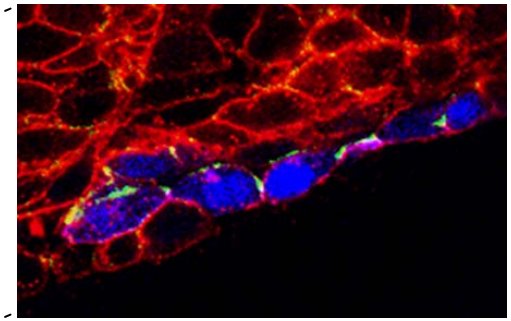
Lack of Fn deposition in *nat* -/- embryos

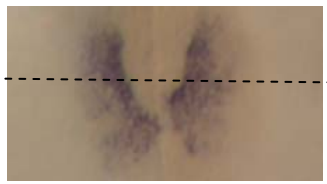


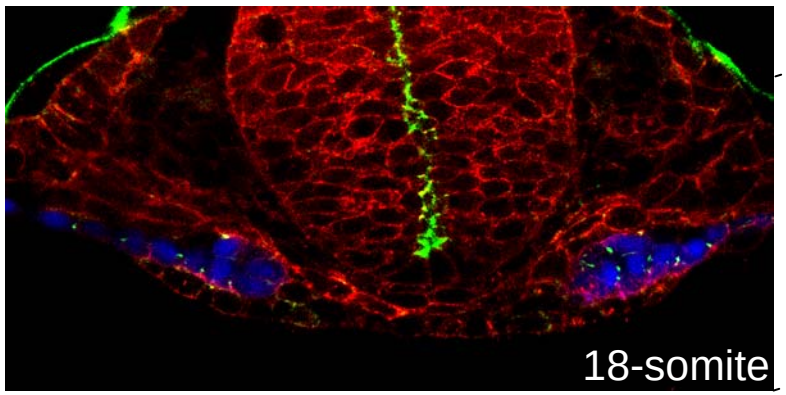
Le Trinh

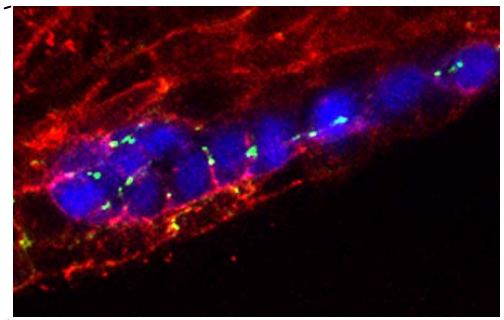
Myocardial precursors form maturing epithelia during the migration stages

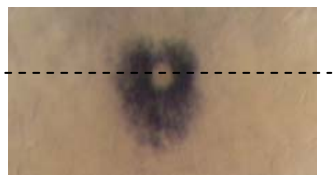


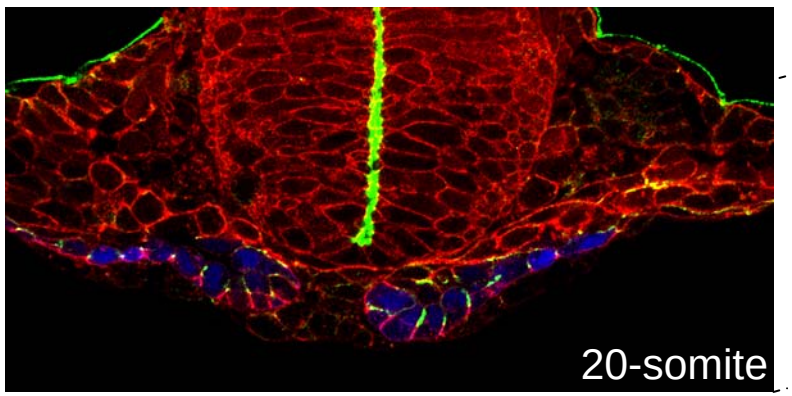


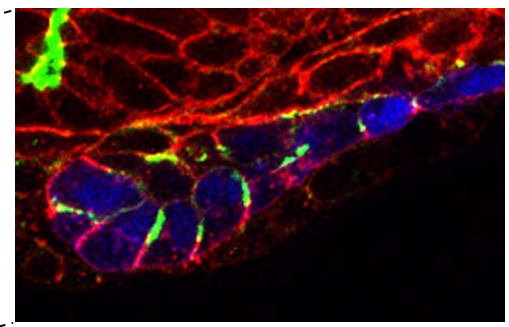






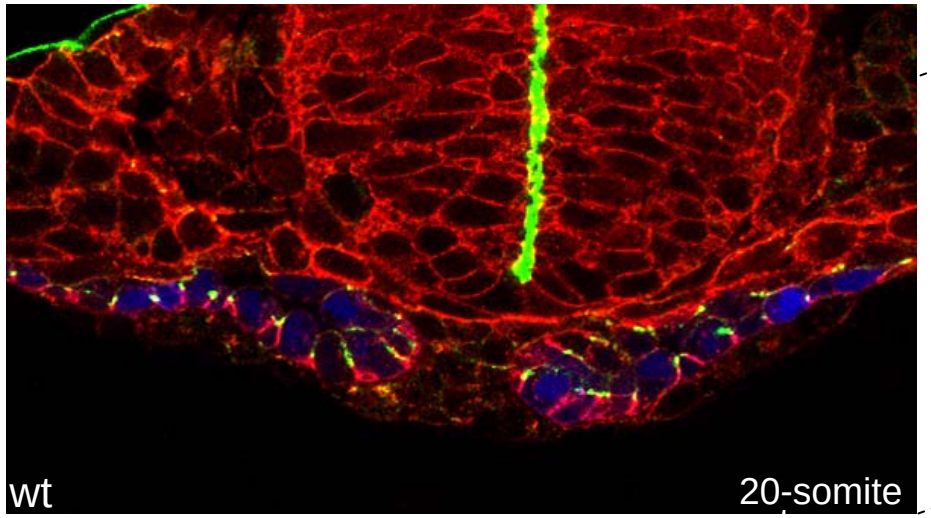


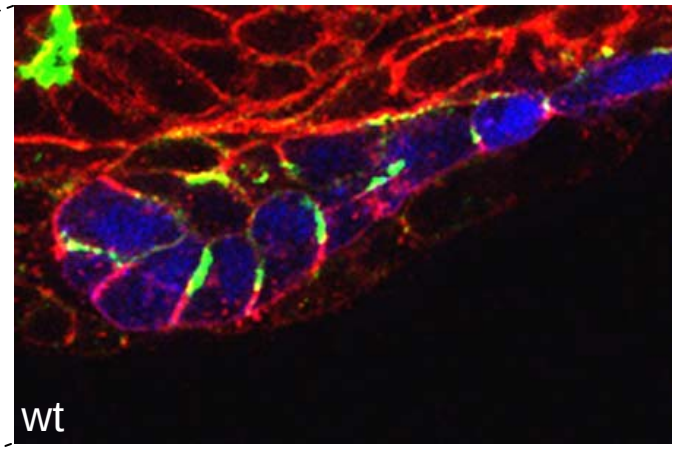


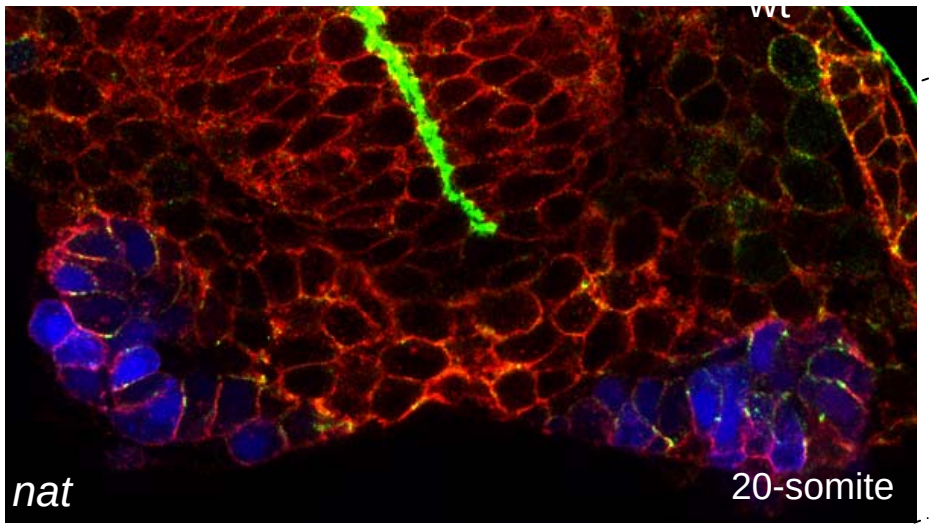


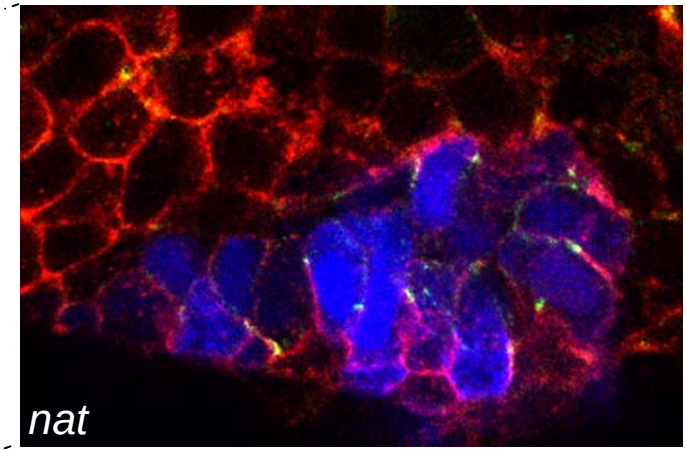
aPKCs
β-catenin
cmlc2::GFP

Fibronectin is required for proper junction formation in the myocardial precursors







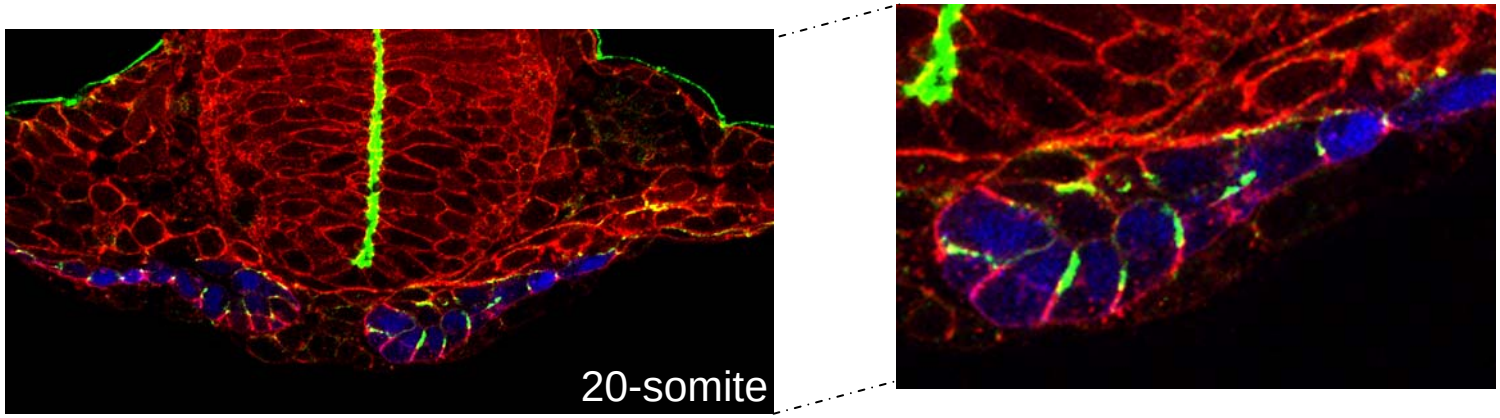


aPKCs
β-catenin
cmlc2::GFP

Le Trinh

Myocardial precursors form maturing epithelia during the migration stages

aPKCs
β-catenin
cmlc2::GFP



genetic requirements for myocardial polarization

- Fibronectin (Trinh and Stainier, *Dev Cell*, 2004)
- Dhand/Hand2 (Trinh, Yelon and Stainier, *Current Biology*, 2005)

cell biological mechanisms

cell polarity, cell shape, cell migration

intracellular trafficking, signaling, organelle biogenesis

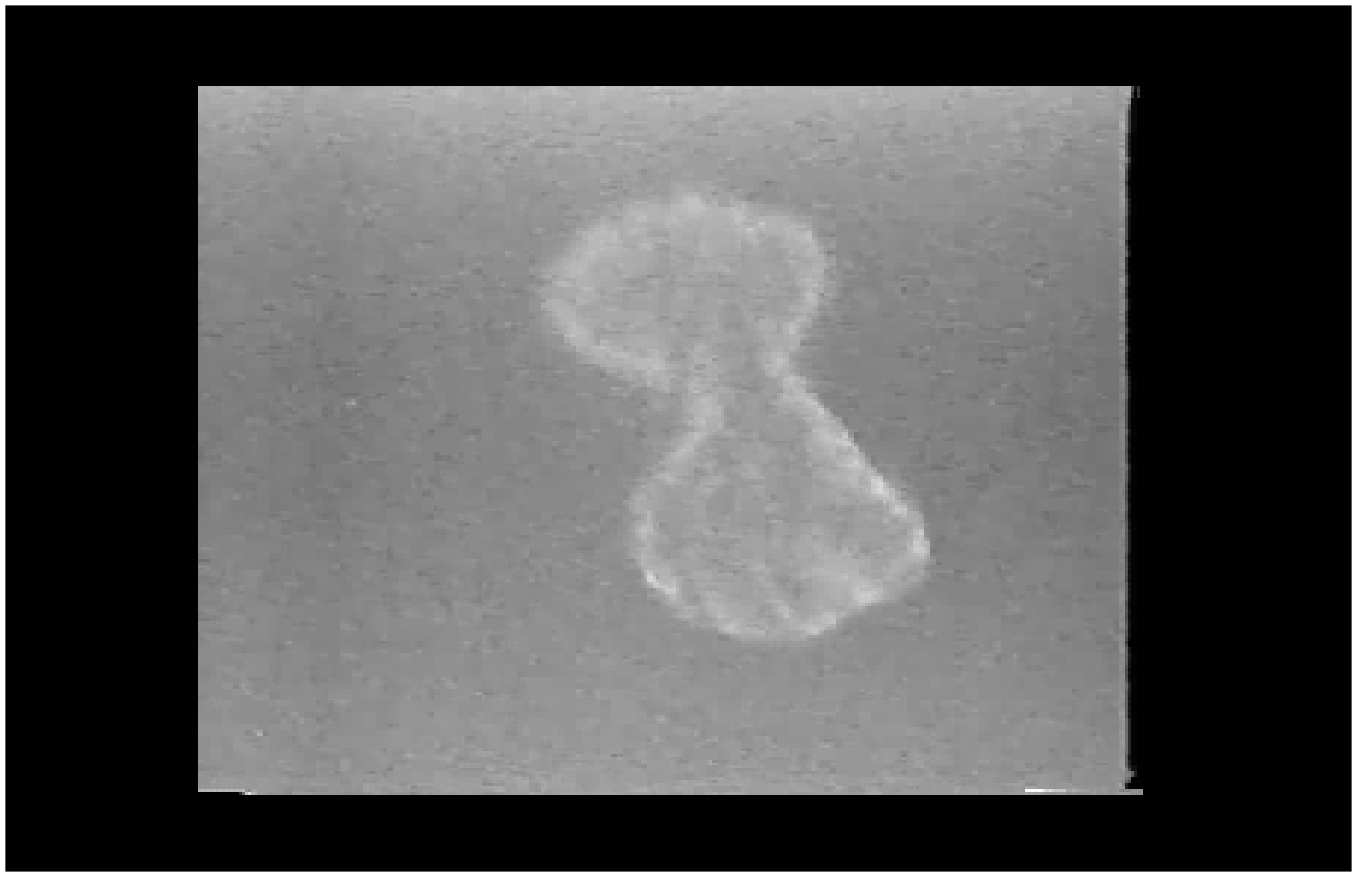
IMAGING

A novel transgenic line to visualize Ca^{++} flux

QuickTime™ and a
Animation decompressor
are needed to see this picture.

Neil Chi

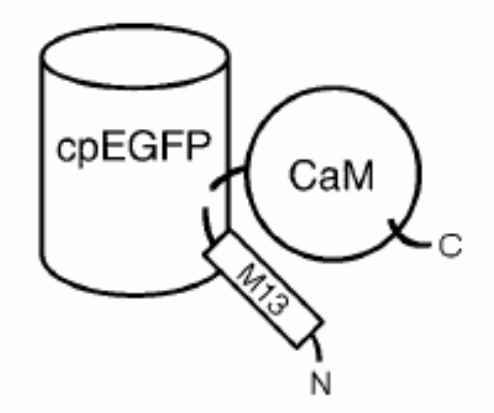
Calcium Green/Optical Mapping



Explanted 48 hpf hearts (wt and *sih* -/-) exposed to calcium green

Sehnert et al., 2001 *Nature Genetics*

gCAMP/Optical Mapping



Kd~190nM
F/F'~4

	F/F'
di-4ANNEPES	**1-2/100**
calcium green	**14**
FCIP FRET	**1-2/10**
gCAMP	**4**

generation of a transgenic line
(gCAMP expressed in myocardial cells) (Neil Chi)

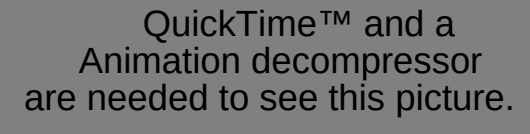
Nakai et al., 2001 NBT/Imoto Lab

G-CAMP/Optical Mapping

24 hpf

Raw
RT

QuickTime™ and a
Animation decompressor
are needed to see this picture.



Processed
1/3 RT

All embryos were treated with 2,3 BDM, an ATP myofibrillar inhibitor, in order to arrest cardiac contraction. Images were obtained with a CCD camera at a 20-30ms/frame capture rate. 40x objective used.

Neil Chi

G-CAMP/Optical Mapping

48 hpf

Raw
RT

QuickTime™ and a
Animation decompressor
are needed to see this picture.

QuickTime™ and a
Animation decompressor
are needed to see this picture.

Processed
1/3 RT

5 dpf

Raw
RT

QuickTime™ and a
Animation decompressor
are needed to see this picture.

QuickTime™ and a
Animation decompressor
are needed to see this picture.

Processed
1/3 RT

Neil Chi

connexin mutants display aberrant calcium waves

QuickTime™ and a
Animation decompressor
are needed to see this picture.

embryos were co-injected with *ctnt2* and *connexin* MOs

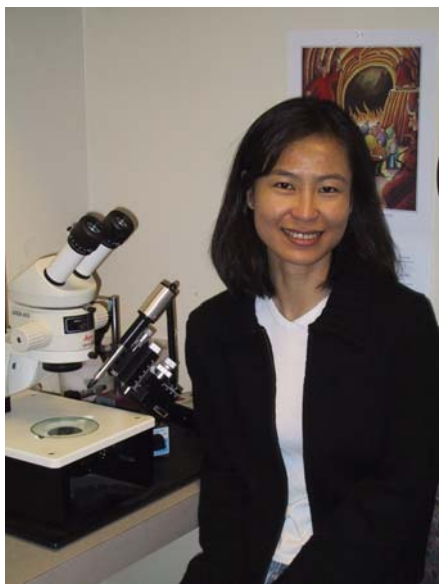
Neil Chi

ACKNOWLEDGEMENTS

Endoderm formation

*Jon Alexander
*Jeremy Reiter
*Yutaka Kikuchi, Ph.D.
Pia Aanstad, Ph.D.

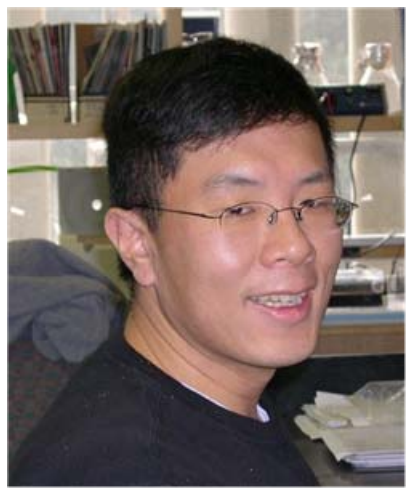
heart formation



Le Trinh
Fn, Hand2

Debbie Yelon
Skirball

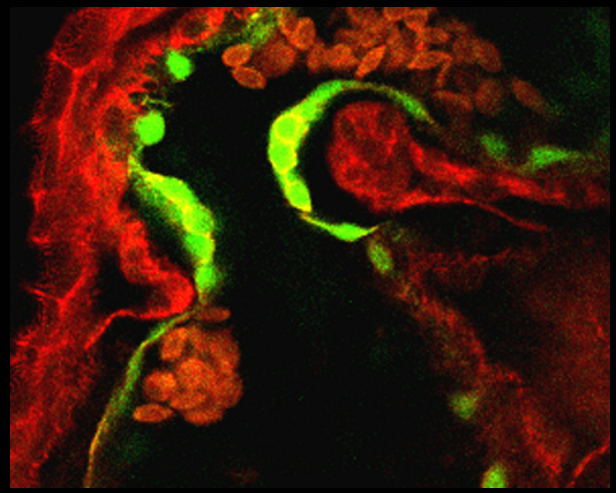
heart function



Neil Chi, M.D., Ph.D.
(cardiac function)

Robin Shaw, M.D., Ph.D.
Lily Jan

vasculogenesis



endocardial cushion formation

cardiac function