6 NEW INSIGHTS INTO VERTEBRATE ORIGINS

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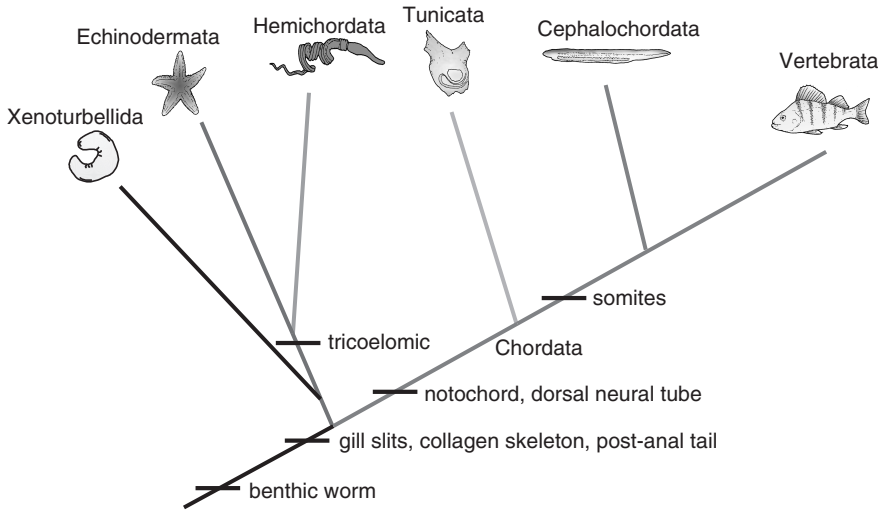
s0010 ## INTRODUCTION

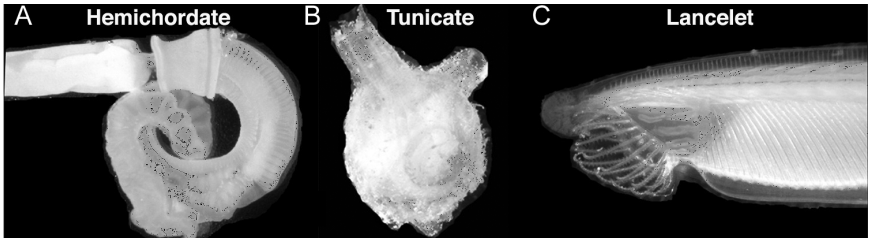
s0020 ### A. HISTORY OF HYPOTHESES OF CHORDATE ORIGINS

p0010 Vertebrates share several distinct morphological characters with three invertebrate groups: lancelets, tunicates, and hemichordates (Figure 6.1). Tunicates, lancelets, and vertebrates have traditionally been considered to be a monophyletic group—the chordates—that shares five morphologic features: a notochord, a dorsal neural tube, an endostyle, a muscular postanal tail, and pharyngeal gill slits. Hemichordates share some of these chordate features; the pharyngeal gill slits (Figure 6.2), an endostyle, and a postanal tail. Previously, hemichordates were thought to contain a notochord homolog (the stomochord) and a dorsal neural tube in the neck region, but recent evidence from developmental genetics has questioned these homologies. Developmental genetics and genomics have allowed for the reexamination of the question of chordate origins by comparing developmental gene expression in embryos of different phyla. This powerful approach has allowed new insights into the molecular mechanisms underlying morphologic changes. Genomics has allowed for investigations into the phylogenetic relationships of the chordates and their invertebrate relatives and for the comparison of the shared genetic pathways in related embryos. We review current research on this topic and show that our view of the chordate ancestor has changed during the past 10 years. For years, the chordate ancestor has been considered to be a filter-feeding, tunicate-like animal with a tiny chordate tadpole larva. However, recent evidence from my laboratory and others has shown that the chordate ancestor was more likely a benthic worm with a mouth and pharyngeal gill slits supported by cartilaginous gill bars (Cameron et al., 2000; Gerhart et al., 2005; Rychel et al., 2006). Further research in developmental genetics and genomics is likely to be fruitful

Principles of Developemental Genetics



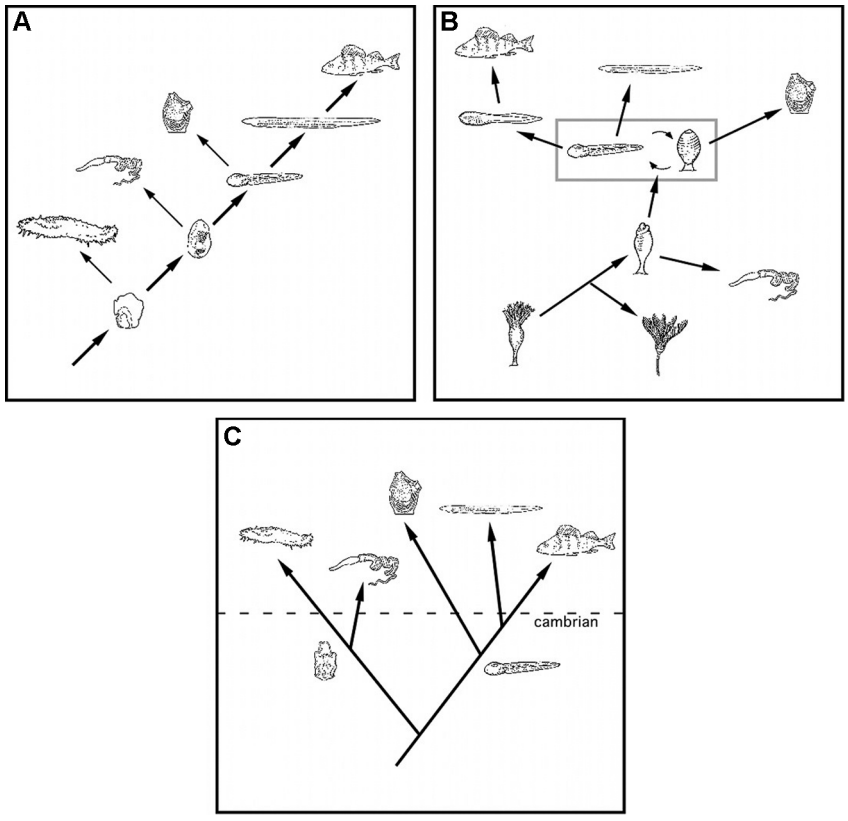
f0010 **FIGURE 6.1** Deuterostome phylogeny. There are five distinct adult body plans seen among the deuterostomes. Echinodermata and Hemichordata have distinctly different body plans but similar tricoelomic feeding larvae. Xenoturbellids are a newly described deuterostome phylum, and little is known about their development. The fourth group exhibiting a distinct adult body plan is the Tunicata, which is usually considered a subphylum within Chordata. The last groups are the Cephalochordata and the Vertebrata, which are considered Chordata subphyla with the tunicates. Morphologic characters that are shared between major groups are marked on the figure. Our evidence suggests that the chordate ancestor was a benthic worm that had gill slits, a collagenous skeleton, and a postanal tail. Somites are a developmental feature that unites Cephalochordata and the Vertebrata. Redrawn with modification from Rychel et al., 2006. Au16



f0020 **FIGURE 6.2** **Three distinct invertebrate body plans. A,** An adult enteropneust hemichordate, *Saccoglossus kowalevskii,* B, a tunicate molgulid ascidian, and C, a lancelet, *Branchistoma virginae*, showing the dramatic differences in their adult body plans. **A,** The mouth of the enteropneust hemichordate is hidden in the collar region, directly behind the anterior proboscis. **B,** The mouth of the tunicate is moved upward at metamorphosis and shown here as the siphon to the top left, whereas the anus empties into the buccal siphon, shown to the top right. **C,** The lancelet mouth has been modified for filter feeding, as shown by the cirri at the anterior, ventral side, to the left. All animals were collected and photographed at the Smithsonian Marine Station at Fort Pierce, Florida. Au17 Au18

for solving some of the remaining homologies between the hemichordates and the chordates.

p0020 Three basic hypotheses of chordate origins are shown in Figure 6.3 (Garstang, 1928; Romer, 1967; Jefferies, 1986; Jollie, 1973; Gee, 1996; Gerhart et al., 2005; Rychel et al., 2006). One early scenario of chordate origins, which is still quite popular, is the view of chordate origins that was first hypothesized by Garstang near the turn of the century (Figure 6.3, A; Garstang, 1928). This theory espouses the notion that the echinoderm and



f0030 **FIGURE 6.3** **Theories of chordate origins: which ones fit the available data? Several possible theories of chordate origins are depicted here. A,** Theory 1 was first proposed by Garstang, and it espouses the notion that the nonfeeding ascidian tadpole larva evolved from echinoderm-like and hemichordate-like larvae. However, developmental gene-expression patterns in the different larvae show that echinoderm larvae and hemichordate tornaria larvae are very similar but that both differ markedly from chordate expression patterns (Swalla, 2006). **B,** Theory 2 was popularized by Romer, and it depicts the deuterostomes as all evolving from a pterobranch hemichordate. Phylogenetic and fossil evidence suggest that this is an unlikely scenario, because chordates, hemichordates, and echinoderms all appear during the early Cambrian era. **C,** Theory 3 is a compilation of all available phylogenetic, fossil, and gene-expression data. Parts of this theory were put first put forth by Jollie, who considered Garstang's and Romer's theories to be extremely unlikely. The molecular evidence suggests that the chordate tadpole larva had an independent origin from an ancestor with a feeding dipleurulid larva. In this scenario, the chordate body plan would have evolved *de novo* in a direct-developing soft-bodied worm-like ancestor. The notochord would have evolved from the co-opting of genes used for other functions in the ancestor. Now the search is on to determine which of the genes were most important in the evolution of this novel structure.

hemichordate larvae "evolved" into the ascidian tadpole larvae and that the adults of echinoderms, hemichordates, and tunicates developed independently. However, as reviewed here, recent developmental data show that the features that hemichordates and chordates share are adult features rather than larval ones (Rychel et al., 2006; Swalla, 2006). These results would favor the evolution of chordates from a direct developing hemichordate (Gerhart et al., 2005; Rychel et al., 2006) rather than from hemichordate tornaria larvae. Further comments on Garstang's theory and genetic evidence against it are nicely summarized in Lacalli (2005).

p0030 Most textbooks still carry the scenario that all deuterostomes evolved from a colonial hemichordate: a pterobranch; this idea was first published by Romer in 1967 (Figure 6.3, B). This theory was popularized because the fossil record has an abundance of colonial hemichordates, called *graptolites,* and because lophophorates were thought related to deuterostomes (Romer, 1967; Gee, 1996). Molecular phylogenetics has shown that the lophophorates are part of a large group of animals called the *lophotrochozoa* (Halanych, 2004) and that the colonial pterobranchs are derived hemichordates (Cameron et al., 2000). Collectively, these new data bring into question the widely held view of deuterostome evolution that was popularized by Romer.

p0040 In 2000, we presented a new hypothesis based on the new molecular phylogenies and developmental gene-expression patterns (Figures 6.1 and 6.3, C; Cameron et al., 2000). In this scenario, the deuterostome ancestor is worm-like, and the larvae of hemichordates and echinoderms developed independent of ascidian tadpole larvae. During the ensuing years, developmental gene-expression data has continued to favor a worm-like deuterostome ancestor (Lowe et al., 2003; Gerhart et al., 2005; Rychel et al., 2006; Delsuc et al., 2006). Developmental genomics and genetics can provide key pieces of evidence for understanding chordate origins. Genomic information will soon be available for a single member of each of the deuterostome monophyletic groups: echinoderms (the purple sea urchin *Strongylocentrotus purpuratus*), hemichordates (an acorn worm, *Saccoglossus kowalevskii*), tunicates (a solitary ascidian *Ciona intestinalis* and the pelagic appendicularian *Oikopleura dioica*), cephalochordates (a lancelet *Branchiostoma floridae*), and many vertebrate species. Initial analyses suggest that deuterostomes share many developmental genetic pathways during early embryonic and larval development (Davidson and Erwin, 2006; Swalla, 2006). Developmental genetics can be highly informative by illuminating how these similar genetic pathways are expressed in different times and places to elaborate the final morphology of the larvae and the adults (Swalla, 2006). We next review the latest findings of molecular phylogenetics and genomic analyses, examine developmental gene expression in different deuterostome phyla, and discuss the origin of the vertebrates in the light of new data published during the past 15 years. Au1

s0030 B. Molecular Phylogenetics of Deuterostomes

p0050 Phylogenetic relationships within the deuterostomes are critical to understanding evolutionary changes that have occurred during chordate and vertebrate evolution (Figure 6.1; Zeng and Swalla, 2005). Deuterostome phylogenetic relationships have been reviewed extensively elsewhere (Halanych, 2004; Zeng and Swalla, 2005), so they will be briefly summarized here. Schaeffer (1987) examined morphologic and phylogenetic evidence and concluded that the deuterostomes (the group of animals that contains the vertebrates) were monophyletic. Later, in 1994, two papers examined deuterostome relationships using 18S rDNA for the first time. Wada and Satoh (1994) showed that deuterostomes were monophyletic, presented evidence that chaetognaths were not deuterostomes, and showed that echinoderms and hemichordates were sister groups (albeit with low bootstrap support). Turbeville and colleagues (1994) increased the deuterostome 18S rDNA data set and used the notochord as a morphologic marker to place ascidians as chordates. Later, Cameron and colleagues (2000) greatly increased the number of tunicates

and hemichordates in the deuterostome 18S rDNA database, and showed that echinoderm and hemichordates are sister groups with high bootstrap support with all methodologic analyses for phylogenetics reconstructions. Morphologic and molecular data since that time have continued to confirm the sister-group relationship of echinoderms and hemichordates (Halanych, 2004; Smith et al., 2004; Zeng and Swalla, 2005; Bourlat et al. 2006).

p0060 The tunicates, although monophyletic (Swalla et al., 2000), have been difficult to place within the deuterostomes with 18S and 28S combined ribosomal sequence analysis (Figure 6.1; Winchell et al., 2002). Recent genome phylogenies constructed with hundreds of genes have suggested that tunicates are more closely related to vertebrates than lancelets are (Blair and Hedges, 2005; Delsuc et al., 2006; Bourlat et al. 2006). Although the phylogenetic relationship of the tunicates to the vertebrates is still in question, it is clear that the developmental programs that are activated in ascidian embryos for specific tissues are quite similar to those seen in vertebrate development (Passamaneck and Di Gregorio, 2005; Swalla, 2004; 2006). Ascidians have a number of important transcription factors localized in the egg cytoplasm that are necessary for some tissue development, so they have been described as having mosaic development (Swalla, 2004; Nishida, 2005). In addition, ascidians have some unique features of tissue specification, such as cellulose production by the adult ectoderm, that are not found in vertebrates. These unique characteristics of ascidian development are thoroughly reviewed by Passamaneck and Di Gregorio (2005).

p0070 Specific, well-characterized genetic pathways are activated in embryos during embryonic development, and those genetic pathways are shared among the deuterostomes (Davidson and Erwin, 2006; Swalla, 2006). Examination of the timing and spatial expression of homologous genes during development can be informative in the understanding of which morphologic structures are homologous in animals with very different body plans (Gerhart et al., 2005; Rychel et al., 2006; Swalla, 2006). In the following sections, the expression of homologous genes in different deuterostome groups is discussed in the context of what these results tell us about the evolution of the vertebrates.

s0040 I. *HOX* GENE CLUSTER ORGANIZATION AND EXPRESSION IN DEUTEROSTOMES: ANTERIOR–POSTERIOR AXIS DEVELOPMENT

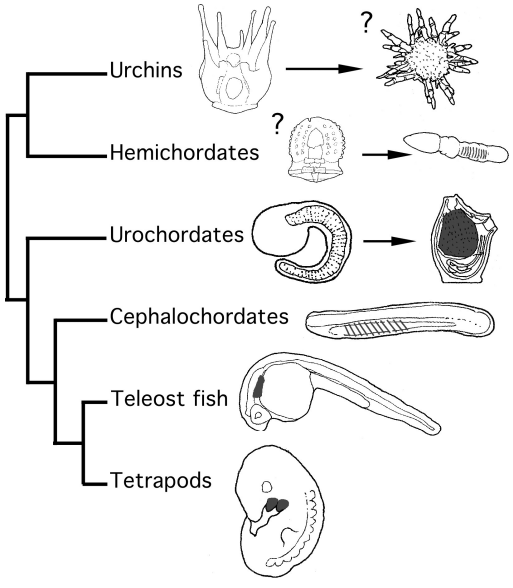
p0080 The *Hox* gene complex has shed light on both deuterostome relationships and the anterior–posterior homologies between body plans of the different phyla (Swalla, 2006). The *Hox* gene complex is a group of genes that are arranged from 3′ to 5′ colinearly on the chromosome and that are also expressed from anterior to posterior during embryonic development (see the chapter by Kenyon in this book). Invertebrate deuterostomes have a single *Hox* cluster, Au2 whereas vertebrates have multiple copies (Swalla, 2006, for review). The sea Au3 urchin *Hox* cluster has been mapped, and it has undergone an inversion so that the most posterior gene, *Hox* 11/13c, is next to *Hox* 3 (Cameron et al., 2006). Hemichordates and sea urchins share motifs in their three posterior *Hox* genes, called *Hox* 11/13a, *Hox* 11/13b, and *Hox* 11/13c, which suggests that the ancestors of these two phyla had posterior gene duplications independent of the chordate lineage (Peterson, 2004; Cameron et al., 2006; Aronowicz and Lowe, 2006).

p0090 *Hox* developmental gene expression provides evidence for anterior–posterior homologies between echinoderms, hemichordates, vertebrates, and lancelets (Lowe et al., 2003; Aronowicz and Lowe, 2006; Swalla, 2006). Developing hemichordates express their *Hox* genes in a colinear fashion from anterior to posterior, but, instead of expression only in the dorsal neural tube, expression is seen in the entire ectoderm of hemichordates (Lowe et al., 2003). These expression patterns have been interpreted as the hemichordate ectoderm having neural potential throughout the ectoderm, such as is seen in insects (Lowe et al., 2003). In echinoderms, colinear expression has been reported in the developing adult somatopleura (Cameron et al., 2006) and in the adult nerve ring (Morris and Byrne, 2005). By contrast, tunicates show widely differing expression patterns of *Hox* genes that depend on whether the gene is expressed during the larval or adult stage (Spagnuolo et al., 2003; Passamaneck and Di Gregorio, 2005; Swalla, 2006).

s0050 II. PHARYNGEAL GILLS AND CARTILAGE DEVELOPMENT

s0060 A. *Pax 1/9* Expression and *Hox* Expression in Deuterostome Gill Slits

p0100 Pharyngeal gill slits in hemichordates were originally used as a morphologic character uniting the hemichordate enteropneust worms with chordates (Figures 6.2 and 6.4; Romer, 1967; Schaeffer, 1987; Rychel et al., 2006).



f0040 **FIGURE 6.4** The expression of *Pax1/9* in the deuterostomes. Deuterostome phylogenetic relationships are shown from morphologic and molecular data summarized by Zeng and Swalla (2005). *Pax 1* and *Pax 9* are expressed in the developing and adult pharyngeal gill slits of all of the vertebrate gnathostomes (tetrapods and teleost fish). *Pax 1* and *Pax 9* expression patterns are shown in blue. *Pax 1/9*, a single gene that duplicated in vertebrates, is expressed in the lancelets (cephalochordates), tunicates (urochordates), and hemichordates. *Pax 1/9* has been reported to be present in sea urchins, but no expression has yet been published, so there is a question mark. Expression has not yet been reported in hemichordate tornaria larvae, so there is a question mark there as well.

Structures are considered to be homologous if they have similar morphology, similar function and similar developmental ontogenies (Rychel et al., 2006; Rychel and Swalla, 2007). The clear homology of pharyngeal gill slit structures is what causes the hemichordates to fall between the echinoderms and chordates by morphologic analysis (Figure 6.2, A; Schaeffer, 1987). The pharyngeal clefts and surrounding collagen skeleton of hemichordates, cephalochordates, and vertebrates are remarkably similar in form and function (Schaeffer, 1987; Rychel et al., 2006), but tunicates lack any cartilage skeleton in their pharyngeal structures (Figure 6.2). Developmental genetics allows for the comparison of morphologic structures at a new level: the level of genetic pathways expressed during the development of the structure. Recent work has shown that the pharyngeal slits in vertebrates, lancelets, and tunicates are all elaborated after the expression of specific *Pax* genes. The single gene called *Pax 1/9* in invertebrate deuterostomes has been duplicated in vertebrates to two genes: *Pax 1* and *Pax 9* (Neubüser et al., 1995; Holland and Holland, 1995; Ogasawara et al., 1999; Ogasawara et al., 2000a).

p0110 The expression of the paired box transcription factors *Pax 1* and *Pax 9* has been shown in the endodermal pharyngeal pouches during vertebrate development (Figure 6.4; Neubüser et al., 1995; Wallin et al., 1996; Peters et al., 1998; Ogasawara et al., 2000a). Furthermore, these transcription factors are necessary for the proper development of the pharyngeal pouches and the surrounding endodermal derivatives (e.g., the thymus) as seen by their absence in mice lacking either *Pax 1* or *Pax 9* (Wallin et al., 1996; Peters et al., 1998). There is a single homologue of these two vertebrate pharyngeal genes in lancelets (Holland and Holland, 1995), ascidians (Ogasawara et al., 1999), and hemichordates (Ogasawara et al., 1999), called *Pax 1/9*, which duplicated into the two separate copies (*Pax 1* and *Pax 9*) in vertebrates (Ogasawara et al., 1999). In both chordates and hemichordates, *Pax 1/9* is expressed in the endoderm of the pharynx and later in the pharyngeal slits (Figure 6.4). Notably, in ascidians, no expression was detected during embryogenesis. The first sign of *Pax 1/9* expression was in swimming tadpole larvae that were about to begin metamorphosis (Figure 6.4; Ogasawara et al., 1999). Likewise, expression in hemichordate adults was found to be highest in the gill endoderm (Figure 6.4; Ogasawara et al., 1999). These results suggest that the morphologic and functional similarity between the pharyngeal gill slits in hemichordates (Ogasawara et al., 1999), ascidians (Ogasawara et al., 1999), cephalochordates (Holland and Holland, 1995), and vertebrates (Neubüser et al., 1995; Wallin et al., 1996; Peters et al., 1998; Ogasawara et al., 2000) is a reflection of similar genetic programs activated in the pharyngeal endoderm at the time of differentiation by the *Pax 1/9* or *Pax 1* and *Pax 9* transcription factors. In the light of these results and the deuterostome phylogeny (Figure 6.1), the most parsimonious hypothesis is that the deuterostome ancestor had endodermally derived gill slits and that these were subsequently lost in the echinoderm lineage (Figure 6.3, C). The mitrate carpoids, fossils from the Devonian era, do appear to have both stereoms (similar to extant echinoderms) and gill slits (Jefferies, 1986; Gee, 1996, Smith et al., 2004). Therefore, early echinoderms may have had pharyngeal gills and then lost them (Smith et al., 2004; Rychel et al., 2006). Further examination of Cambrian echinoderms for evidence of pharyngeal gills will be informative, as will the cloning and characterization of the expression of echinoderm *Pax 1/9*. No expression data have been reported in echinoderms to date

(question mark in Figure 6.4), but it will be interesting to see if this gene has been co-opted for other functions in echinoderms. Au5

p0120 The pharyngeal gill slits share conserved transcription factors for their development as described previously and are localized in a similar manner along the anterior–posterior axis (Aronowicz and Lowe, 2006). For example, in vertebrates, *Hox 1* is first expressed at the level between the first and second pharyngeal pouch (Lowe et al., 2003). When *Hox* gene expression was examined in hemichordates, *Hox 1* was expressed between the first and second pharyngeal pouch, thereby suggesting that the location of the pharyngeal gills along the anterior–posterior axis is homologous between the hemichordates and the vertebrates (Lowe et al., 2003; Aronowicz and Lowe, 2006).

s0070 B. Pharyngeal Gill Cartilage in Hemichordates and Lancelets is Acellular

p0130 Therefore, the pharyngeal gill slits themselves appear to be homologous, but what about the cartilaginous gill bars that lie between the gill openings? The morphology and development of the gill bars in hemichordates is similar to lancelets (Schaeffer, 1987; Ruppert, 2005; Rychel et al., 2006). The bars appear as a thickening of the basal lamina between the pharyngeal endoderm, as was first reported by Hyman in 1959 and recently demonstrated by *in situ* hybridization (Rychel and Swalla, 2007). The cartilaginous bars of hemichordates stain with Alcian blue (Smith et al., 2003), but they are acellular (Rychel et al., 2006), whereas the gill bars of lamprey are made by neural crest cells and are cellular (Zhang et al., 2006). The development of gill bars in hemichordates and lancelets has been examined and it appears that their acellular cartilages are secreted by endoderm (Rychel and Swalla, 2007). This may have been the ancestral way of making cartilage in deuterostomes. Later in evolution, neural crest cells in vertebrates may have migrated into those areas and replaced the acellular cartilage with cellular cartilage. Therefore, the gill bar cartilage in hemichordate and lancelets appears to be homologous, but it is not clear whether these are homologous to any extant vertebrate cartilages. Au22 Au23

s0080 III. THE POSTANAL TAIL AND THE ENDOSTYLE OF HEMICHORDATES: GENE-EXPRESSION STUDIES

p0140 It is not clear how significant the postanal tail is as a defining chordate feature. Ascidians do not have an open gut as larva, so they do not have an anus, but both lancelets and vertebrates have a postanal tail (Gerhart et al., 2005). The vertebrate and lancelet posterior *Hox* genes are expressed in the tissues of the postanal tail. Phylogenetic analyses of hemichordate enteropneust worms shows that they fall into two separate monophyletic groups: those that have feeding larvae similar to echinoderms and those that are direct developers (Cameron et al., 2000). The direct-developing saccoglossids have a postanal tail and express the posterior *Hox* genes (Lowe et al., 2003), whereas the ptychoderids lack a postanal tail (Swalla, 2006). Instead, ptychoderid worms form an anus at the vegetal pole of the larvae that becomes the anus of the adult (Urata and Yamaguchi, 2004; Swalla, 2006). These results could be interpreted as evidence that the vertebrates evolved from a direct-developing hemichordate ancestor, because they are the only group of hemichordates that show a postanal tail. However, because the hemichordates would have

diverged from a chordate ancestor long before the Cambrian era (Blair and Hedges, 2006), there has been plenty of evolutionary time for the independent Au24 evolution of a postanal tail in both groups.

p0150 The endostyle present in lancelets and tunicates is thought to have homology to the vertebrate thyroid, so endostyle-specific genes have been isolated in an effort to examine this question with gene expression (Mazet, 2002; Ogasawara et al., 2000b; Sasaki et al., 2003). One of these genes is the homeobox gene thyroid transcription factor 1 *(TTF-1)*, which regulates thyroid peroxidase, the enzyme that iodinates thyroglobulin (Mazet, 2002; Ogasawara et al., 2000b; Sasaki et al., 2003). In lancelets, *TTF-1* is expressed throughout the six morphologic zones of the endostyle (Mazet, 2002), whereas, in tunicates, expression is limited to particular zones (Sasaki et al., 2003). Both the tunicate and lancelet endostyles also bind iodine, so their endostyles are considered to be homologous to the vertebrate thyroid gland (Sasaki et al., 2003; Ruppert, 2005). When the hemichordate *TTF-1* was cloned and gene expression was characterized, there was expression seen in the pharyngeal endoderm, stomochord, and hindgut (Takacs et al., 2002). The pharyngeal endoderm of hemichordates also binds iodine throughout, even in the regions that do not morphologically resemble an endostyle (Ruppert, 2005). These results could be interpreted to mean that the entire hemichordate pharynx fulfills endostyle function (Rychel et al., 2006) or that the hemichordate endostyle is not homologous to the tunicate and lancelet endostyle (Ruppert, 2005). Further developmental and functional studies will be necessary to distinguish between these two hypotheses.

s0090 IV. NO GENETIC EXPRESSION EVIDENCE FOR STOMOCHORD HOMOLOGY TO NOTOCHORD

p0160 Ultrastructural studies of the hemichordate stomochord suggested that this structure could be the homolog of the chordate notochord (Balser and Ruppert, 1990), so gene-expression studies of notochord-specific genes were expected to confirm this hypothesis. *Brachyury* is a T-box transcription factor that was first isolated during mesoderm formation in vertebrates (Wilkinson et al., 1990; Holland et al., 1995) and that is expressed exclusively in the ascidian notochord (Yasuo and Satoh, 1993; Swalla, 2006). When *Brachyury T* was cloned and described in echinoderms and hemichordates, it was expressed at the site of gastrulation at the vegetal pole, which later becomes the larval anus (Peterson et al., 1999; Swalla, 2006). These results suggest that the ancestral function of *Brachyury* as a transcription factor was in promoting the gastrulation and formation of the three germ layers and that the gene was later co-opted into notochord development (Swalla, 2006). Results from our laboratory have also shown that there is no collagen antibody staining in the stomochord, although we do see staining in the adult gill bars (Rychel et al., 2006). Unfortunately, these are all negative results, which collectively are evidence that the stomochord is not a notochord homolog; however, they do not conclusively prove it. Candidate gene-expression studies so far do not suggest any other hemichordate structure as a candidate for the notochord homolog (Gerhart et al., 2005).

s0100 V. EVOLUTION OF PLACODES AND NEURAL CREST IN CHORDATES

p0170 Neural crest has been widely touted as a vertebrate innovation that allowed for the development of complicated sensory structures in the anterior head and of the skull and pharyngeal bars (Gans and Northcutt, 1983; see chapter by Mayor in this book). Therefore, it has long been assumed that tunicates Au6 and lancelets would lack placodes. However, there is recent evidence from gene-expression studies that tunicates have well-developed sensory placodes and lateral placodes (Manni et al., 2004; Bassham and Postlewait, 2005; Mazet et al., 2005). The buccal cavity and palps at the anterior of tunicates express *Six 1/2, Six 3/6, Eya,* and *Pitx,* which suggests a homology to the hypophyseal and olfactory placodes of vertebrates (Manni et al., 2004; Bassham and Postlewait, 2005; Mazet et al., 2005). These results suggest that the common ancestors of vertebrates and tunicates had placodes and that their anterior ends had homologous structures. A rather startling result is that the excurrent buccal opening in tunicates early on expresses *Six 1/2, Six 4/5, Eya,* and *Fox 1,* which are vertebrate markers for otic placodes, lateral lines, and epibranchial placodes (Manni et al., 2004; Bassham and Postlewait, 2005; Mazet et al., 2005; see chapter by Moody in this book). As mentioned Au7 previously, tunicate larvae do not have an open gut, so they do not have an anus during larval life. After metamorphosis, the gut is emptied out of the excurrent buccal siphon (after the tail has retracted), and the mouth forms at the anterior of the larvae. This would suggest that the adult tunicate is defecating out of its ear, which is an odd symmetry twist for a chordate.

s0110 VI. CONCLUSIONS

p0180 In summary, developmental genomics and genetics have allowed new insights into the question of chordate origins (Cameron et al. 2000; Gerhart et al. 2005; Rychel et al. 2006; Rychel and Swalla, 2007). Genomics and gene- Au25 expression studies have been extremely informative in the understanding of the homology of various structures in invertebrate deuterostomes to vertebrates. Developmental gene-expression data allow one to analyze the genetic pathways that are deployed to make similar structures in genetically different organisms. Gene-expression data suggest that the anterior–posterior axes of hemichordates, lancelets, and vertebrates are very similar, except that the neural genes are expressed throughout the ectoderm of hemichordates (Lowe et al., 2003). Tunicates have lost some of the middle *Hox* genes, and they express some of their *Hox* genes as larvae and some as adults; only a few are expressed colinearly (Spagnuolo et al., 2003; Passamaneck and Di Gregorio, 2005). The gill slits of hemichordates, lancelets, and vertebrates are homologous, while the gill bars of lancelets and hemichordates are both acellular (Rychel et al. 2006) and secreted by the endoderm, suggesting they are homologous (Rychel and Swalla, 2007). In contrast, tunicates completely Au26 lack gill bars in their pharyngeal region. Tunicates have been shown to have both neural and nonneural placodes, which were thought to exist only in the vertebrates. It will be interesting to examine hemichordates for the existence of placodes by examining the gene expression of homologous genes. Although tunicates are clearly chordates, they have evolved some amazing

changes in body plan, and they are likely to have lost some structures evolutionarily at the time that the tunic evolved. Hemichordates have an anterior–posterior axis similar to that of chordates, but they lack a dorsal central nervous system. Our view of the chordate ancestor is that it was a benthic worm with gill slits and a mouth that was able to filter feed but also to ingest large particles. Further research on developmental gene expression in lancelets, tunicates, and hemichordates is likely to be fruitful for the better understanding of the evolution of vertebrates.

SUMMARY

- Hemichordates are a sister group to echinoderms but not to chordates. The relationship of tunicates to vertebrates is not yet clear.
- *Hox* genes are expressed in an anterior to posterior manner in hemichordates and chordates. Tunicates have lost the middle *Hox* genes and show rather different tissue-expression patterns. Echinoderms have a rearranged *Hox* cluster and show some colinearity of expression.
- Pharyngeal gill slits in hemichordates and chordates are homologous. Pharyngeal gill bars are similar in hemichordates and lancelets, but they differ from those of vertebrates in that they are acellular.
- On the basis of gene-expression studies, the postanal tail and endostyle in hemichordates and chordates are likely to be homologous.
- Chordates specify neural and nonneural ectoderm, whereas all ectoderm is neural in hemichordates. Possible chordate notochord and neural tube homologs in hemichordates have not yet been unambiguously identified.
- Tunicates contain sensory placodes, which suggests that they have some form of neural crest and that they form a secondary anus after metamorphosis, probably from the otic placode.

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GLOSSARY OF TERMS

Deuterostome
Literally means "second mouth" (*deutero:* two; *stome:* mouth). The blastopore is formed first during gastrulation, and the mouth is formed secondarily. This

mode of development applies to all deuterostomes. Echinodermata, Hemichordata, Xenoturbellida, and Chordata are considered deuterostome phyla.

Endostyle
An endodermal structure found in invertebrate chordates in the pharyngeal area. The endostyle secretes mucus to capture small particles and to increase the efficiency of filter feeding. In lancelets and tunicates, the endostyle also accumulates iodine, and it is considered homologous to the vertebrate thyroid gland.

Graptolites
These abundant fossils are believed to be colonial hemichordates or members of the hemichordate class Pterobranchia.

Hemichordates
This phylum includes enteropneust worms and colonial pterobranchs. Hemichordates are tripartite as adults, which means that they have three body regions. The most anterior is the proboscis (protostome), then the collar (mesosome) and the posterior trunk (metasome).

Lancelets
The common name for cephalochordates. These animals are frequently referred to by the taxonomically incorrect term *amphioxus*.

Notochord
The key chordate morphologic character is the notochord. The notochord forms a stiff rod that runs from anterior to posterior in chordates beneath the dorsal neural tube, and it is usually surrounded by a sheath of extracellular matrix. The gut is found just under the notochord in vertebrates and lancelets. In lancelets and appendicularians, the notochord persists in the adult, whereas in ascidians the notochord undergoes apoptosis at metamorphosis. In vertebrates, the notochord disappears as the vertebrae develop from somites.

Pharyngeal
The area of the digestive system that serves as a respiratory and feeding organ in hemichordates, tunicates, and lancelets. The vertebrate homolog is the pharynx, which develops into gills in aquatic vertebrates, but it is the area of the throat, including the thyroid gland and thymus, in amniotes (birds and mammals).

Pharyngeal gill bars
Cartilaginous elements made of extracellular matrix and located between the pharyngeal endoderm that give the pharynx of hemichordates, lancelets, and vertebrates structure. Pharyngeal gill bars are secreted from endoderm in hemichordates and lancelets, but they develop from neural crest cells in vertebrates.

Placodes
An area of an ectodermal thickening where cells can delaminate and eventually achieve a cell fate that is not epidermal. There are both neurogenic and nonneurogenic cranial placodes, which are associated with the nervous system in vertebrates. Placodes were thought to be found only in vertebrates, but they have recently been described in tunicates using both molecular markers and careful morphologic analyses.

Pterobranch

Class Pterobranchia is the group of colonial hemichordates or pterobranchs. Colonial hemichordates reproduce both sexually and asexually, and they have feeding tentacles to capture small particles for feeding. There are many fossil pterobranchs, called *graptolites,* but there are only two extant families: Rhabdopleuridae and Cephalodiscidae.

Stomochord

A projection of the endoderm that juts forward into the hemichordate proboscis, against which the hemichordate heart beats. The stomochord cells are vacuolated, and they make an extracellular sheath.

Tunicates

A monophyletic group of animals that includes ascidians, appendicularians, and thaliaceans. This group of animals is also sometimes called *urochordates,* but *tunicates* is the preferred term. There are more than 3000 species of tunicates.

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Biology of the protochordata: A collection of reviews published in the *Canadian Journal of Zoology*: Volume 83 #1. Available athttp://pubs.nrc-cnrc.gc.ca/cgi-bin/rp/rp2_tocs_e?cjz_cjz1–05_83.

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