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REVIEW

Man Is but a Worm: Chordate Origins

Federico D. Brown,[1,2] **Andrew Prendergast,**[1,3] **and Billie J. Swalla**[1,2]*

[1]Biology Department and Center for Developmental Biology, University of Washington, Seattle, Washington
[2]Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington
[3]Neurobiology and Behavior Graduate Program, University of Washington Medical School, Seattle, Washington

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Summary: The origin of chordates remains one of the major puzzles of zoology, even after more than a century of intense scientific inquiry, following Darwin's "Origin of Species". The chordates exhibit a unique body plan that evolved from a deuterostome ancestor some time before the Cambrian. Molecular data gathered from phylogenetics and developmental gene expression has changed our perception of the relationships within and between deuterostome phyla. Recent developmental gene expression data has shown that the chordates use similar gene families and networks to specify their anterior-posterior, dorsal-ventral and left-right body axes. The anterior-posterior axis is similarly established among deuterostomes and is determined by a related family of transcription factors, the *Hox* gene clusters and *Wnt* signaling pathways. In contrast, the dorsal-ventral axis is inverted in chordates, compared with other nonchordate invertebrates, while still determined by expression of *BMP* signaling pathway members and their antagonists. Finally, left-right asymmetries in diverse deuterostomes are determined by *nodal* signaling. These new data allow revised, testable hypotheses about our earliest ancestors. We present a new hypothesis for the origin of the chordates whereby the expansion of BMP during dorsal-ventral patterning allowed the evolution of noneural ectoderm and pharyngeal gill slits on the ventral side. We conclude that "Man is but a worm . . . ," that our chordate ancestors were worm-like deposit and/or filter feeders with pharyngeal slits, and an anterior tripartite unsegmented neurosensory region. genesis 46:605–613, 2008. © 2008 Wiley-Liss, Inc.

Key words: evolution and development; chordate evolution; tunicates; hemichordates; ascidians; deuterostomia; axis formation; BMP signaling

INTRODUCTION

The origin of the chordates has been a question of intense inquiry and speculation since at least as far back as Darwin's "Origin of Species" (Darwin, 1872). In 1866, Kowalevsky's startling discovery that tunicates possess tadpole larvae showed the importance of larvae in understanding animal evolutionary relationships (Fig. 1c, Kowalevsky, 1866) and led many to hypothesize that tunicates are the "ancestral chordates" (Berrill, 1955; Gee, 1996). Such thinking remains prevalent today, as tunicates, especially ascidians, are studied widely but frequently referred to as "ancestral chordates." However, many different theories of chordate origins have been put forth over the years (Berrill, 1955; Cameron *et al*., 2000; Garstang, 1928a, 1928b; Gee, 1996; Gerhart *et al*., 2005; Nielsen, 1999; Romer, 1967; Satoh, 2008; Swalla, 2007), and a new one is even suggested in this Genesis volume (Satoh, 2008). A few theories have been recently rejected with data from molecular phylogenetics and developmental gene expression, one of which is Garstang's larval theory for the origin of vertebrates (reviewed in Cameron *et al*., 2000; Gerhart *et al*., 2005; Lacalli, 2005; Satoh, 2008; Swalla and Smith, 2008).

Deuterostome phylogeny and the fossil record has recently been extensively reviewed elsewhere (Bourlat *et al*., 2006; Swalla and Smith, 2008) and will be discussed in this article only in the interpretation of recent developmental data concerning the possible homologies in hemichordates with specific chordate tissues. The evidence for echinoderms and hemichordates as sister groups in the clade termed Ambulacraria is very strong and generally accepted, whereas some conflict remains between genomic, ribosomal, and mitochondrial data in the relationships of the chordates (Bourlat *et al*., 2006; Delsuc *et al*., 2006; Swalla and Smith, 2008). Hypotheses discussed in this article are based on the understanding that hemichordates are the sister group to echinoderms, but echinoderms have highly derived adult body plans that share few homologies with chordates (Swalla, 2006). We have shown that hemichordates and lancelets share homologies that are not present in echinoderms or

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Current address for Federico D. Brown: Evolutionary Biology, Division 4, Max Planck Institute for Developmental Biology 72076 Tübingen, Germany.
*Correspondence to: Billie J. Swalla, Box 351800, Department of Biology, University of Washington, Seattle, WA 98195.
E-mail: bjswalla@u.washington.edu

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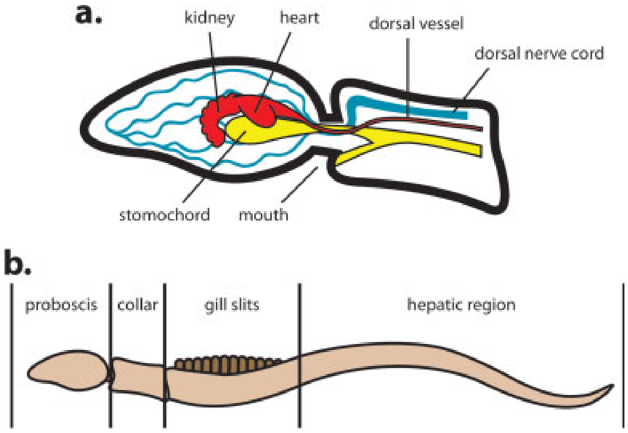

FIG. 1. Diagram of a ptychoderid hemichordate. (**a**) The anterior proboscis and collar region of a hemichordate enteropneust worm contains the anterior stomochord and heart/kidney complex. The dorsal nerve cord is found in the neck region and fans into a nerve net in the anterior proboscis. There is a nerve net just under the ectoderm throughout the hemichordate adult. (**b**) An adult ptychoderid hemichordate with the anterior proboscis, the middle collar region and the posterior abdomen that contains the gill slits and the hepatic sacs.

tunicates, such as unusual acellular pharyngeal cartilages (Rychel and Swalla, 2007). Consequently, morphological characters shared between hemichordates and chordates are likely to have been present in the deuterostome ancestor (Swalla and Smith, 2008). Here, we examine other structures and genetic control of body axes between hemichordates, lancelets, and vertebrates.

Chordates are considered to have four or five defining features: notochord, dorsal neural tube, pharyngeal gill slits, muscular post-anal tail, and an endostyle (Swalla, 2007). Hemichordates are the only deuterostome that shows a tripartite adult body plan, with a proboscis, a neck region, and a posterior abdominal region that has prominent gill slits (see Fig. 1). Developmental gene expression data and morphological analyses suggest that hemichordates share the pharyngeal gill slits and postanal tail with chordates, but homologies of the other features are currently being investigated (reviewed in Gerhart *et al.*, 2005; Satoh, 2008; Swalla, 2007). Hemichordates are the last major deuterostome phyla to have their complete genome sequenced, but a large number of ESTs are currently available for the direct developing hemichordate worm, *Saccoglossus kowalevskii* (Freeman *et al.*, 2008). In this article, we compare the anterior sensory organs, the dorsal neural tube, the A-P and D-V axes between ptychoderid hemichordates and lancelets, and suggest where studies will be most fruitful to evaluate further homologies.

Until the 1990s, most zoology or biology textbooks carried either Garstang's' (Garstang, 1928a,b) or Romer's (Romer, 1967) theory of deuterostome phylogeny and chordate evolution (Fig. 1, Swalla, 2007). Garstang realized the morphological similarities in echinoderm and hemichordate larvae and hypothesized that the ascidian chordate tadpole larvae evolved by the rolling of the ciliated bands of the ambulacraria larva into the dorsal neural tube (Fig. 2a). Although this was a favorite theory of many scientists for nearly a century, recent genetic and developmental gene expression data suggests that the feeding ambulacraria larvae evolved independently of the chordate tadpole larvae (Fig. 1c, Lacalli, 2005; Swalla, 2006). When gene expression is compared in developing hemichordate and echinoderm larvae, many similar expression patterns of transcription factors are seen, including brachyury, found in the blastopore (Peterson *et al.*, 1999a,b; Shoguchi *et al.*, 1999), *otx* and t-brain found in the apical tuft (Harada *et al.*, 2000; Tagawa *et al.*, 2000) and *BMP 2/4*, found expressed in the hydropore (Harada *et al.*, 2002). However, it is difficult to find homologies between ambulacraria larval expression patterns and the chordate expression patterns of these genes.

In contrast, Romer's theory of chordate evolution presupposed that that the lophophorates (bryozoans, brachiopods, and phoronids) were basal deuterostomes (Fig. 1b, Romer, 1967), a hypothesis disproven by molecular phylogenies in the late 1990s (Cameron *et al.*, 2000; Halanych *et al.*, 1995). First, the lophophorates were shown not to be deuterostomes, but actually part of a large group of metazoans now called the "lophotrochozoa" (Halanych, 2004; Halanych *et al.*, 1995). Then, the colonial pterobranch hemichordates were shown likely to be derived within enteropneust worms (Cameron *et al.*, 2000). Later analyses suggested that the brachiopods and phoronids are closely related, but bryozoans are distantly related, suggesting that feeding tentacles have evolved independently in many groups of filter-feeding marine invertebrates (Halanych, 2004).

In summary, phylogenetic and developmental data emerging since the mid-1990s have suggested that both Garstang's and Romer's theories of chordate origins are unlikely (Fig. 2, Swalla, 2007). In 2000, we first proposed a benthic worm with gill slits as the deuterostome ancestor, a theory that had not been suggested before, based on phylogenetic analyses of hemichordates and deuterostomes (Fig. 2, Cameron *et al.*, 2000; Swalla, 2001). Although hemichordate enteropneust worms had historically been identified for their similarities to chordates (Barrington, 1965; Bateson, 1886; Bullock, 1944), echinoderms or colonial pterobranch hemichordates were believed to be the basal deuterostomes (Garstang, 1928a,b; Gee, 1996; Romer, 1967; Swalla and Smith, 2008). However, morphological and molecular evidence continues to suggest that the basal hemichordates were similar to members of the Ptychoderidae, an enteropneust worm, with complex body plans and feeding larvae with ciliary bands (Cannon and Rychel *et al.* submitted). Therefore, the chordate ancestor would have evolved from the worm-like (vermiform) deuterostome ancestor by the acquisition of a neural tube and notochord (Swalla, 2007). In this arti-

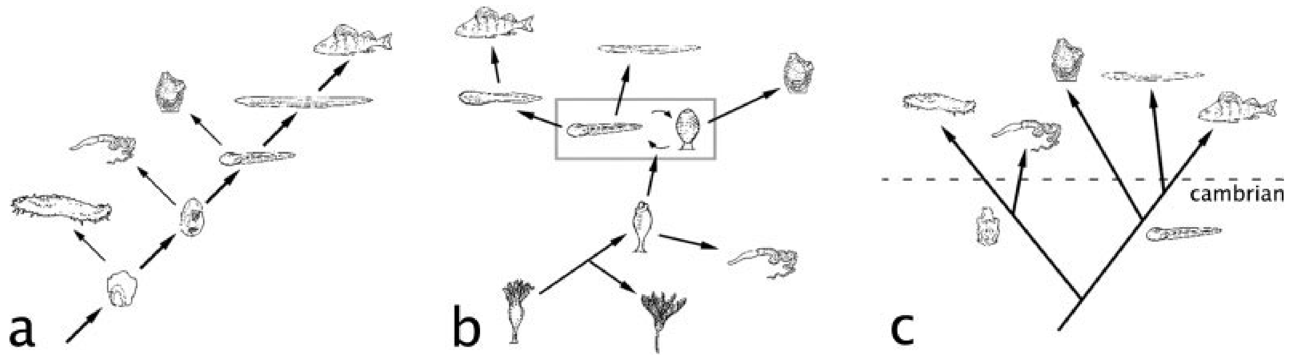

FIG. 2. Popular theories of Chordate Origins. (**a**) The hypothesis of the larval evolution of the chordates, first described by Garstang (1928a,b), is summarized. In this theory, echinoderm larvae are the ancestral deuterostome, then the hemichordate larvae evolved, and then chordate larvae evolved by the rolling up of the ciliary bands to form the neural tube. Most of the developmental gene expression data suggests that the tunicate tadpole larvae and the ambulacraria larvae evolved independently (Swalla, 2006), suggesting that this theory is incorrect. (**b**) Romer's theory of deuterostome origins (Romer, 1967). In this scenario, the ancestral deuterostome resembled a colonial pterobranch hemichordate, and then the various adult body plans evolved from this sessile animal. Hemichordate phylogenies and larval morphology suggest that enteropneust worms were the ancestral hemichordates, which cast doubt on this theory (Cameron *et al*., 2000, Swalla, 2006). (**c**) The theory that deuterostomes evolved from a worm-like animal with gill slits was first published in 2000 (Cameron *et al*., 2000; Swalla, 2001) and has been refined since then (Swalla, 2007).

cle, we continue to refine and extend our theory of chordate origins from an ancestral benthic worm with gill slits.

EVOLUTION OF EYESPOTS IN DEUTEROSTOME LARVAE

Echinoderms and hemichordates are united in a single monophyletic group, the ambulacraria (Swalla and Smith, 2008). This grouping reflects both molecular and morphological shared characters, especially the homologies of the ciliated feeding larvae, that is, the dipleurula larva (Hart, 1994; Nielsen, 1999; Strathmann and Bonar, 1976). In particular, a noteworthy shared anterior structure is the presence of a serotonergic nerve net in the anterior apical tuft of some echinoderm larvae (Byrne *et al*., 2007) and hemichordate tornaria larvae (Dautov and Nezlin, 1992; Nezlin and Yushin, 2004). Although echinoderm and hemichordate dipleurula larvae share many homologies, the larvae of ptychoderid hemichordates have two prominent eyespots in the tornaria larvae, which disappear as metamorphosis proceeds (see Fig. 3). These eyespots have been shown by morphological analyses to be rhabdomeric pigment cell cup photoreceptors (Brandenburger *et al*., 1973) that appear to have been lost by the echinoderm ancestors (Arendt and Wittbrodt, 2001). Lancelet and tunicate larvae contain both rhabdomeric and ciliary photoreceptor cells. In lancelets, these are retained in the adult (Arendt and Wittbrodt, 2001), whereas in ascidians, the larvae have a pigmented ocellus and otolith (Swalla, 2006) that undergo programmed cell death at metamorphosis. Therefore, we hypothesize that the deuterostome ancestor had a feeding larva with anterior sensory photoreceptor cells that were evolutionarily lost in adults of the ambulacraria. This hypothesis can be tested with future developmental and molecular work focused on the development and function of the eyespots in tornaria larvae and their subsequent disappearance during metamorphosis.

ANTERIOR-POSTERIOR AXES DETERMINATION IN DEUTEROSTOMES

For purposes of this review, we will focus on gene expression and axes determination in a lancelet,

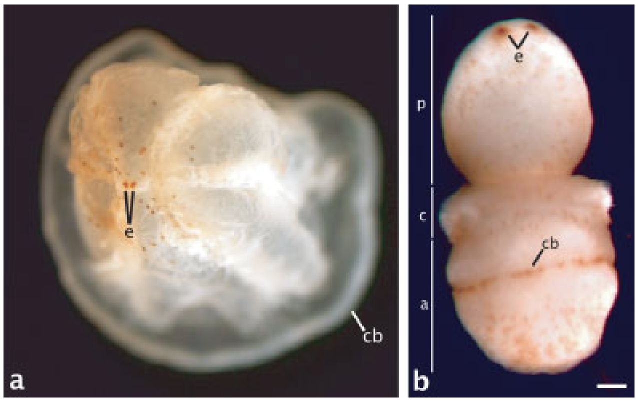

FIG. 3. Ptychoderid hemichordates contain pigmented eyespots in tornaria larvae and metamorphosing adults. (**a**) A ptychoderid tornaria larvae found in plankton tows near Bermuda, showing the eyepots (e) near the anterior apical tuft. The other brown spots are pigment spots, found in all ptychoderid hemichordate larvae. (**b**) A *Ptychodera flava* larva 1 week after metamorphosing from tornaria found in the plankton near Hawaii. Note at this stage that the larvae has elaborated the three body parts of a hemichordate adult—the anterior proboscis (p), the middle collar region (c), and the posterior abdomen (a), marked by the dark ciliated band (cb) that moves anterior to posterior as the animal develops after metamorphosis. There are two dark eyespots (e) visible at the anterior. This is the ventral side of the larvae, so the mouth would be found in the neck region. Scale bar is 100 μ.

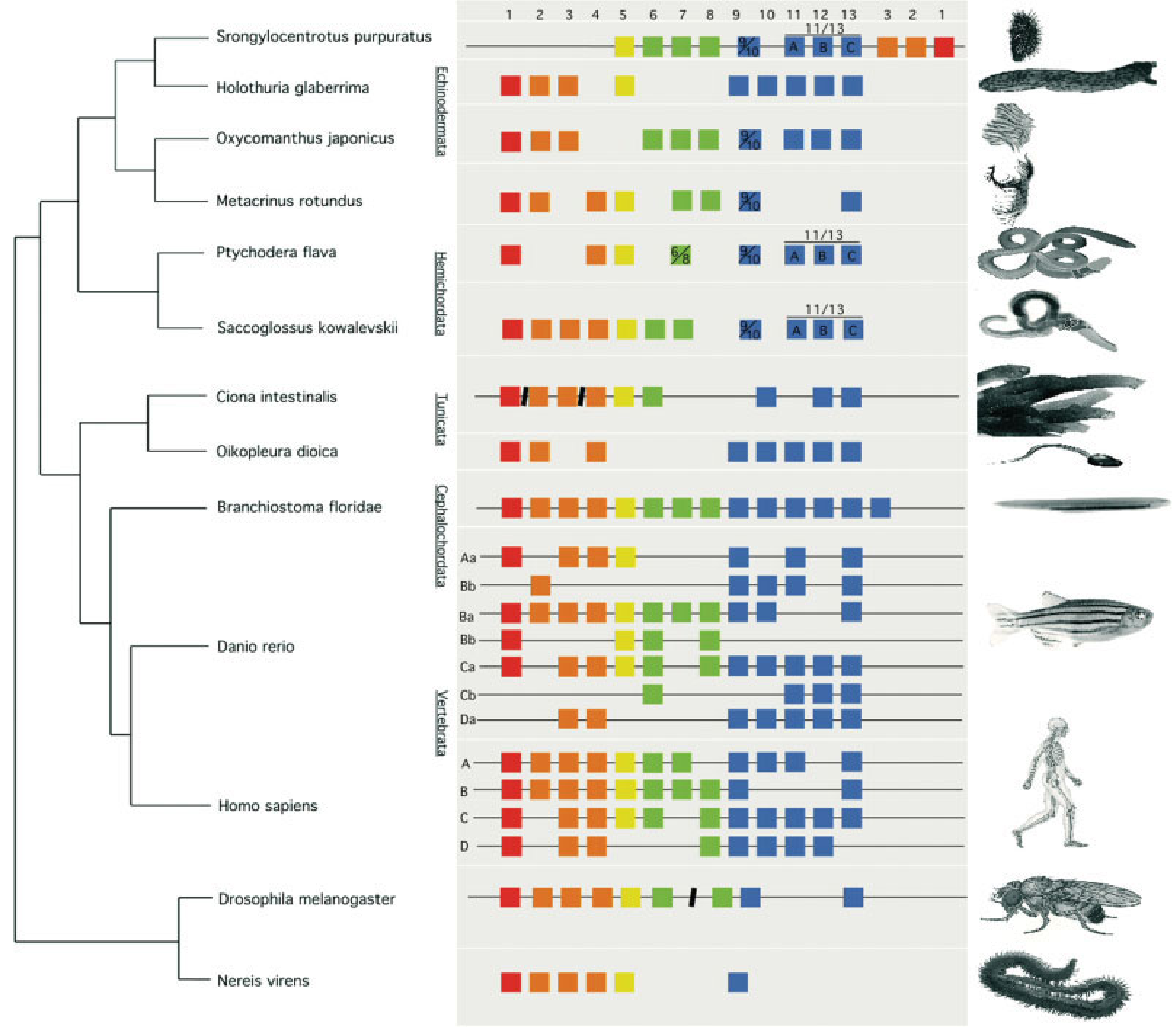

FIG. 4. Current knowledge of Deuterostome *Hox* clusters. An Ecdysozoa (fruit fly, *Drosophila melanogaster*) and a Lophotrochozoa (polychaete annelid, *Nereis virens*) are shown as outgroup protostome taxa. The most complete echinoderm and hemichordate Hox clusters are shown here with all available hemichordate data and major chordate clades. The two sequenced tunicates, *Oikopleura dioica* and *Ciona intestinalis* are shown with a recently sequenced cephalochordate, *Branchiostoma floridae* and a couple of representative vertebrates. Humans have four duplicate clusters, as do all vertebrates, except teleost fishes, (*Danio rerio*), that had a second gene duplication event, resulting in eight clusters. Lines through the boxes depict that the genome has been completed, and the organization of the cluster is known. Slashes indicate genomic breaks in the clusters. Note the unusual inversion seen in sea urchins (top row). References for figure updated from an earlier review in (Aronowicz and Lowe, 2006; Cameron *et al*., 2006; Méndez *et al*., 2000; Mito and Endo, 2000; Passamaneck and Di Gregario, 2005; Peterson, 2004; Swalla, 2006).

Branchiostoma floridae (amphioxus), which has recently been sequenced and shows remarkable synteny with the vertebrate genome, suggesting that the ancestral chordate had seventeen chromosomes (Putnam *et al*., 2008). The adult body plan in lancelets is also much more similar to vertebrates than the tunicate body plan, which has a U-shaped gut (Swalla, 2006), even though tunicates may be more closely related to vertebrates (Bourlat *et al*., 2006; Delsuc *et al*., 2006). In *B. floridae*, it has been shown that the anterior-posterior axis in the central nervous system is determined by a retinoic acid-Hox hierarchy, similar to vertebrates (Schubert *et al*., 2006). In addition, *Wnt* antagonists are expressed anteriorly, whereas *Wnt* signals are expressed posteriorly, suggesting that Wnt genes may also be involved in anterior-posterior patterning in lancelets (Yu *et al*., 2007).

Hox genes are a group of related genes encoding homeodomain transcription factors that evolved from repeated gene duplications that are involved in specifying the anterior–posterior axes in metazoans, but we

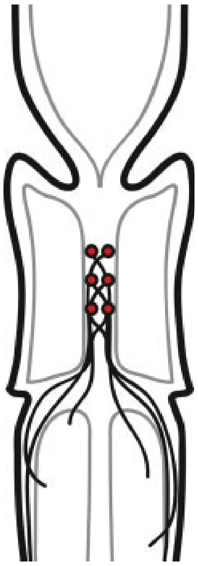

FIG. 5. Giant Cells in the collar nerve cord of a Balanoglossid enteropneust worm, redrawn from Bullock, 1944. The giant neuronal cells are found in worms of the family Ptychoderidae, especially balanoglossids and glossobalanids. These worms can vary in size from several centimeters long to over a meter. Depending on the species there would be less than a dozen or more than fifty of these giant neurons located in the collar nerve cord. These unipolar cells, first described by T.H. Bullock (Bullock, 1944), send their axons across the midline and posteriorly, into the dorsal cord of the trunk. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

focus here specifically on the deuterostomes (Duboule, 2007; Ferrier, 2007; Swalla, 2006). Hox clusters appear to have remained intact in the vertebrates that is, the genes are located in tandem on the chromosome in a 3′-5′ fashion and have cotemporal and colinear expression with the most 3′ genes expressed first and most anteriorly, then the middle, and finally the posterior 5′ genes (Duboule, 2007; Ferrier, 2007). Within the deuterostomes, independent posterior *Hox* gene duplications in the ambulacraria and the chordates likely occurred, as the ambulacraria show three combined *Hox 11/13a*, *11/13b*, and *11/13C*, while the chordates have *Hox 10*, *Hox 11*, *Hox 12*, and *Hox 13* (Peterson, 2004). In lancelets, lamprey and cartilaginous fish, there is a fifth posterior gene, *Hox 14* expressed in the most posterior tissues (Amemiya *et al*., 2008; Ferrier, 2007; Kuraku *et al*., 2008). However, the divergence of the posterior genes in the lancelets make it difficult to establish homology of those genes with the rest of the chordates (Amemiya *et al*., 2008).

Expression of Hox and a suite of other neural genes in the direct developing hemichordate, *Saccoglossus kowalevskii* is seen in an anterior to posterior manner similar to lancelets, although in the entire ectoderm, rather than in the neural tube (Aronowicz and Lowe, 2006; Lowe *et al*., 2003). Hemichordate worms have a nerve network just under the ectoderm and these neural gene expression patterns have been compared with the vertebrate expression patterns in the forebrain, midbrain, and hindbrain (Lowe *et al*., 2003). Remarkably, the expression of *Hox 1* is seen immediately after the formation of the first pharyngeal slit, suggesting that the pharyngeal slits are positioned similarly along the anterior–posterior axis in hemichordates, lancelets, and vertebrates (Aronowicz and Lowe, 2006; Lowe *et al*., 2003; Swalla, 2007). The anterior, nonsegmented, sensory proboscis has been shown to express similar neural genes as are expressed in the chordate forebrain (Lowe *et al*., 2003), yet there has been no characterization of the specific nerve types found in hemichordate proboscis nervous system. We suggest that such studies may be fruitful in understanding the origins and evolution of the vertebrate brain.

GIANT CELLS IN THE NERVOUS SYSTEMS OF HEMICHORDATES AND LANCELETS

In contrast to direct developing *Saccoglossus* species, *Ptychodera* genera within the Ptychoderidae family have a hollow nerve cord that runs the length of the neck region (Hyman, 1959; Willey, 1899). *Balanoglossus* or *Glossobalanus* genera show one or more hollow cavities partially represented throughout the entire neural tube (Bullock, 1944; Hyman, 1959). There are described giant neuron cells in balanoglossid and glossobalanus nerve cords that enervate into contralateral sides of the worm (Figs. 5–7, Bullock, 1965). Bullock noted that these cells were reminiscent of the giant cells also found in lancelets (Takeda *et al*., 2003). We have further characterized here the arrangement of the dorsal central nervous system in adult *Glossobalanus berkeleyii* (Willey, 1931) ptychoderid worms by using histochemical stains (see Fig. 6) and commercial antibodies to specific neurotransmitters, such as serotonin (see Fig. 7). The giant cells are all located in the central nervous system in the neck region (see Fig. 2), as found for other genera of enteropneust hemichordates, with the highest number of cells normally seen in the posterior neck region (Bullock, 1944).

When serial sections of an adult *G. berkeleyii* worm were stained with ponceau, we found at least two distinct cell types in the dorsal nervous system, as previously noted (Bullock, 1944; Hyman, 1959; Willey, 1899). The inner area has numerous nuclei and obvious giant cells, whereas the outer area is composed of axon tracts that label with α-tubulin (Figs. 6 and 7). Posteriorly, at the back of the collar region, the dorsal nerve lies dorsal to the proboscis skeleton (Figs. 6a-a′ and 7a-a‴) and many nerve nuclei are seen on the dorsal side by ponceau staining (Fig. 6a′) or by DAPI labeling (Fig. 7a). On the ventral side, numerous axon tracts (Fig. 6a′) label with α-tubulin (Fig. 7a′), but not serotonin (Fig. 7a″-a‴). However, in the middle and more anterior neck regions, very obvious giant cells can be observed (Fig. 6b-b′,c-c′). These giant cells label with α-tubulin (Fig. 7b′,c′) and serotonin antibody (Fig. 7b-b″,c-c″).

Giant cells have been proposed to be involved in hemichordate escape responses, the rapid contraction of the

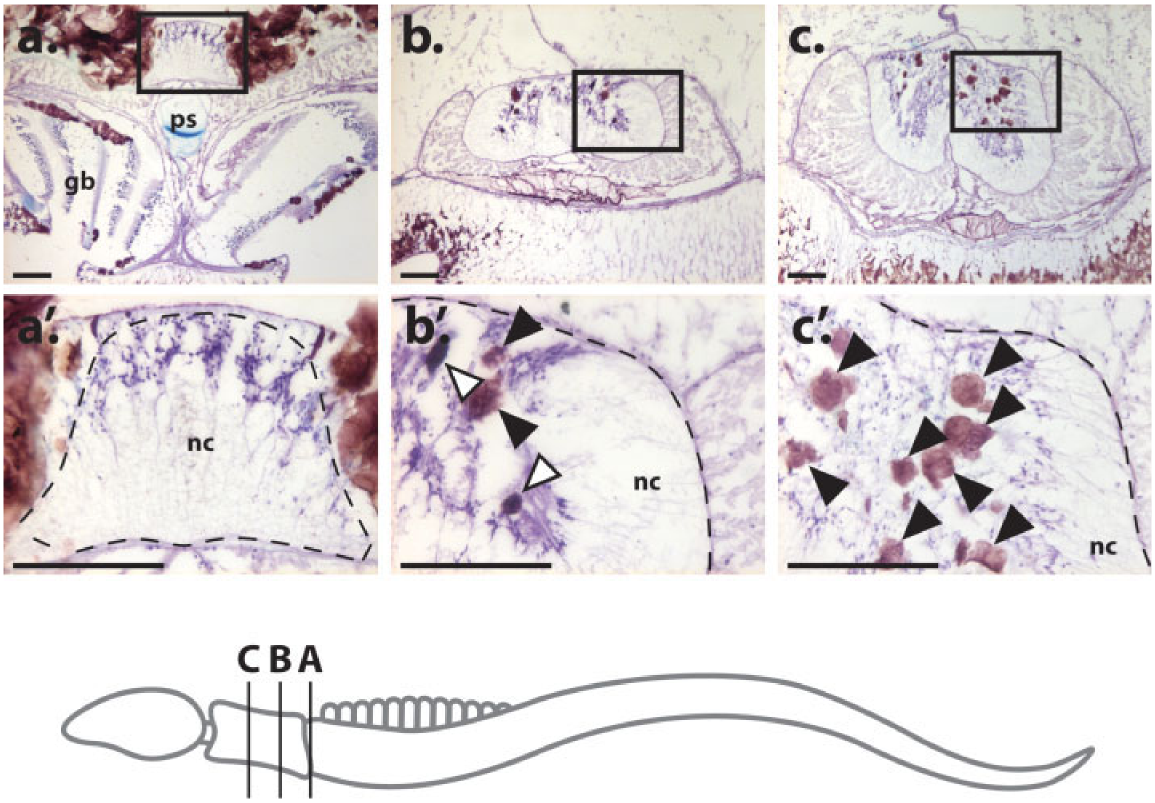

FIG. 6. Giant Neurons in the collar nerve cord of *Glossobalanus berkeleyi*. All micrographs are from cross sections taken through the collar region of the animal. All sections are stained with cresyl violet and Luxol Fast Blue. Schematic diagram illustrates the approximate position of each cross section relative to the whole animal. (**a**) Most posterior section through dorsal nerve cord. Box denotes image location in a′. (**a′**) Same section as a. No giant cells are apparent at this rostrocaudal level in the dorsal nerve cord (dNC). (**b**) A more anterior cross section through the dorsal nerve cord. Box denotes image location in b′. (**b′**) Same section as b. Cresyl violet staining suggests that the dorsal nerve cord is segregated into a more dorsomedial cell body layer and a more ventrolateral process layer. The cell layer of the dorsal nerve cord contains several large cells that are stained by cresyl violet, called giant neurons by Bullock (1965) marked with black arrowheads. Other neuronal nuclei are also visible, marked by open arrowheads. (**c**) Most anterior cross section through the dorsal nerve cord. Box denotes image location in c′. (**c′**) Same section as shown in c. Many giant neurons, seen as large cresyl violet positive cells and marked with black arrowheads, are present in the cell layer of the dorsal nerve cord at this rostrocaudal level. All scale bars are 100 μm.

posterior segments of the worm (Bullock, 1944, 1965). However, homology of these large cells to giant cells found in other invertebrates or chordates is still uncertain. Genomic resources, such as EST projects (Freeman *et al.*, 2008) and/or monoclonal antibodies, will be instrumental in isolating and characterizing specific genes expressed in these cells. This information may, in turn, be informative as far as the homology of these cells to large neuronal cells in nervous systems of other animal species.

DORSAL-VENTRAL AXIS INVERSION OR THE PLACEMENT OF THE GILL SLITS?

Recent investigations into the establishment of dorsal-ventral axis determination in hemichordates have shown that, like other invertebrates, BMP is expressed on the dorsal, rather than the ventral side, suggesting that chordates have an inverted D-V axis (summarized in Lowe *et al.*, 2006; Swalla and Smith, 2008). In addition, *nodal*, a gene involved in chordate left-right asymmetry, is expressed on the right side in chordates and the left side in sea urchins, further adding evidence to a body axis inversion in chordates (Swalla and Smith, 2008). This has led to the hypothesis that vertebrate evolution involved a rotation of the mouth of an enteropneust-like ancestor to the other side of the body on the same side as the gill slits (Christiaen *et al.*, 2007; Lacalli, 2006; Lowe *et al.*, 2006). However, following this scenario the neural tube would remain on the same side of the worm as the gill slits and the mouth or a “hot zone” of a neural organizing center must occur on the post oral dorsal side of the vertebrates (see details in Lacalli, 2006). Both hypotheses are very attractive, however the former assumes that the central nervous system of hemichordates and chordates have evolved independently, which is possible, but should be further investigated with modern molecular and cellular methods.

Here, we propose an alternative hypothesis to the rotation of the mouth that may have occurred during the

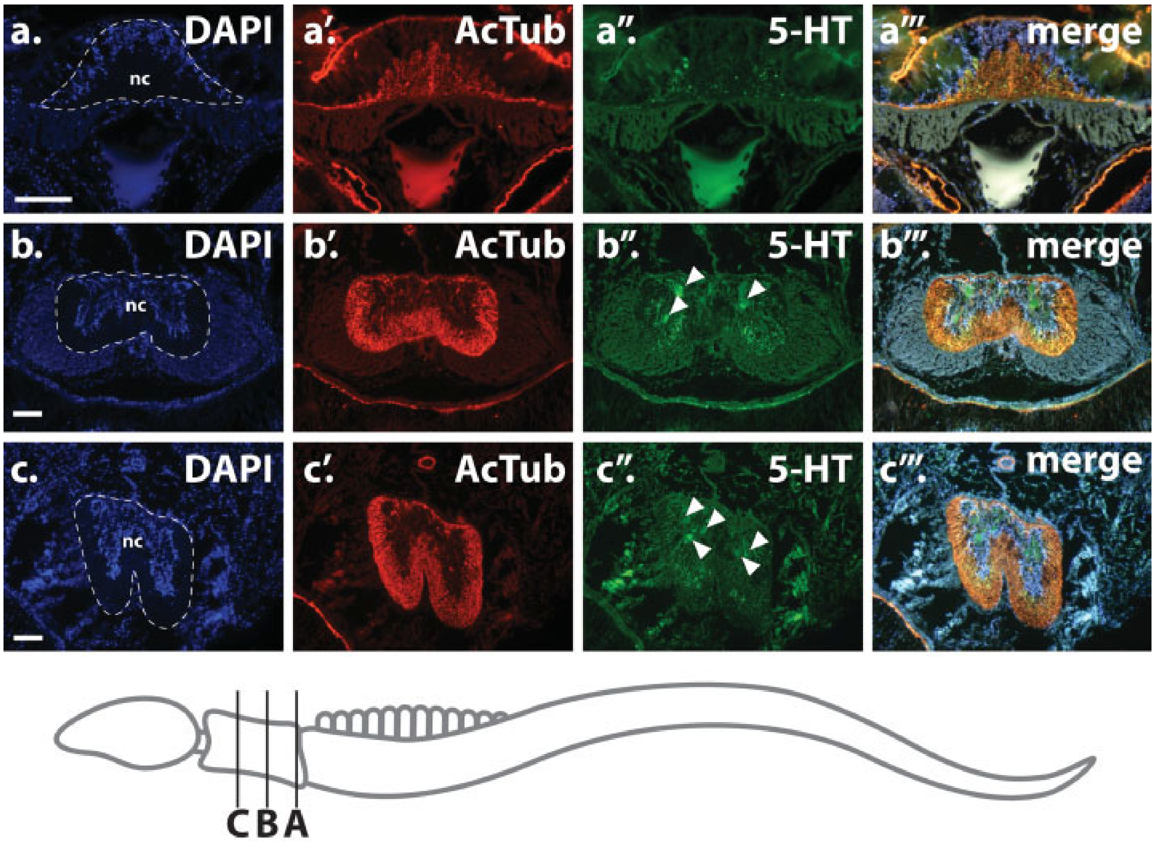

FIG. 7. Giant neurons in the collar nerve cord of *Glossobalanus berkeleyi* are serotonin positive. All micrographs are from cross sections taken through the collar region of the animal. Schematic illustrates the approximate position of each section relative to the whole animal. (**a-a**′, ″, ‴) Most posterior section through the dorsal nerve cord. (**a**) The nuclear counterstain DAPI shows that at this rostrocaudal level the dorsal nerve cord is largely free of nuclei. (a′) Staining with α-acetylated tubulin, a marker of neuronal processes, confirms that the nerve cord consists largely of projections at this rostrocaudal level. (a″) Staining with α-serotonin reveals sparse serotonergic projections. (a‴) Merged image of a-a″. (**b-b**′, ″, ‴) A more anterior cross section through dorsal nerve cord. (b) DAPI staining at this rostrocaudal level reveals organization of the dorsal nerve cord into a dorsomedial layer comprised of cell soma and a ventrolateral process layer. (**b**′) Acetylated tubulin staining confirms the layered organization seen in b. (**b**″) Large serotonin-positive cells are present in the cell soma layer of the dorsal nerve cord, marked by white arrowheads. (**b**‴) Merged image of b-b″. (**c-c**′, ″, ‴) Most anterior section through the dorsal nerve cord. (c) DAPI staining shows separation of cell soma and process layers in the dorsal nerve cord. (**c**′) Acetylated tubulin expression confirms the layered organization seen in c. (**c**″) Many, large serotonin-positive cells are also present at this more anterior position in the dorsal nerve cord, some which are marked with white arrowheads. (**c**‴) Merged image of c-c″. All scale bars are 100 μm.

origin of chordates. This hypothesis assumes that the dorsal neural tube in hemichordates is homologous to the dorsal neural tube in chordates, which still needs to be tested experimentally. We propose that *BMP* expression was first expanded on the dorsal side of a hemichordate or worm-like intermediate ancestor, facilitating the movement of the gill slits from the dorsal side of the worm to the ventral side of the worm, and inhibiting neural development, for example, diffuse nerve net system, in the ectoderm on the ventral side. At the same time, we propose that expression of *BMP* antagonists, such as chordin or noggin, were reduced to the future anterior region during gastrulation, rather than being expressed down the entire dorsal side of the animal. This led to the linking of anterior and dorsal axes, which is prevalent in chordate embryos, due to the anterior to posterior development.

We next examine homology within the deuterostomes of a structure that it established in an anterior to posterior manner, the pharyngeal slits. Hemichordates and lancelets contain pharyngeal bars or slits that are made of acellular cartilages with remarkably similar morphologies (Schaeffer, 1987). We have shown previously that these gill bars appear to be homologous to each other, but not to vertebrate cartilages (Rychel and Swalla, 2008). Therefore, we propose that the loss of the posterior coelom in a direct developing hemichordate or worm-like intermediate ancestor may have allowed extension of the neural tube down the length of the animal, accompanied by the evolution of somites and notochord as support structures. An increasingly complex set of structures evolving on the dorsal side of the animal may have resulted in a more ventral placement of the gill bars. Eventually, the gills would be displaced anteriorly as seen in present day chordates, as the posterior muscular tail became increasingly robust for swimming in the open ocean.

CONCLUSION

We believe that the weight of the current evidence argues strongly for a worm-like ancestor of the deuterostomes and the chordates, as previously suggested (Cameron *et al*., 2000; Swalla, 2001, 2007). This ancestor would have been solitary and sexually reproducing, not colonial, and fed through a mouth and/or filtering apparatus, similarly to enteropneust hemichordates. Such an ancestor may have specialized towards prey capture, as seen in vertebrates, and eventually developed sensory structures and jaws, losing the capacity to filter feed. In contrast, some lineages may have modified the mouth and/or pharyngeal structures by modifying the expression of key regulatory genes to enhance filter-feeding, as seen in the invertebrate chordates: lancelets and tunicates (Swalla, 2006).

FUTURE STUDIES AND NEW HYPOTHESES

Future studies need to be directed to understand the developmental origins and formation of the structures located in the anterior proboscis and neck region in enteropneust hemichordates, specifically the proboscis nerve net, stomochord, proboscis skeleton, and the heart/kidney complex in ptychoderid hemichordates following metamorphosis. These results can then be compared with the development of possible homologous tissues in the direct developing saccoglossid hemichordates, lancelets, and then to the vertebrates. Gene expression of tissue specific genes such as *tinman* could shed light on the possible homologies of the heart and/or the dorsal vessel to the chordate heart. Mesodermal and muscle specific gene expression, such as *snail* and the muscle determination factors, may allow insight to the origin of the proboscis muscle and the proboscis skeleton. Eventually, entire gene networks can be examined and the evolutionary changes that have occurred in them during the evolution of morphology elucidated. In conclusion, although much progress has been made in the past twenty years concerning homologies between chordates and the ambulacraria, there is much more to be investigated with modern genomics and developmental gene expression. We look forward to further understanding of the evolution and development of the unique chordate body plan within the deuterostomes.

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