

Molecular genetic insights into deuterostome evolution from the direct-developing hemichordate *Saccoglossus kowalevskii*

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Progress in developmental biology, phylogenomics and palaeontology over the past five years are all making major contributions to a long-enduring problem in comparative biology: the early origins of the deuterostome phyla. Recent advances in the developmental biology of hemichordates have given a unique insight into developmental similarities between this phylum and chordates. Transcriptional and signalling gene expression patterns between the two groups during the early development of the anteroposterior and dorsoventral axes reveal close similarities, despite large morphological disparity between the body plans. These genetic networks have been proposed to play conserved roles in patterning centralized nervous systems in metazoans, yet seem to play a conserved role in patterning the diffusely organized basiepithelial nerve net of the hemichordates. Developmental genetic data are providing a unique insight into early deuterostome evolution, revealing a complexity of genetic regulation previously attributed only to vertebrates. While these data allow for key insights into the development of early deuterostomes, their utility for reconstructing ancestral morphologies is less certain, and morphological, palaeontological and molecular datasets should all be considered carefully when speculating about ancestral deuterostome features.

Keywords: hemichordate; body plan; deuterostome; nervous system evolution

1. INTRODUCTION

The deuterostome phyla make up one of the two major bilaterian lineages (Hyman 1940; Brusca & Brusca 1990). The evolutionary history of this group has been the subject of debate in comparative biology for over a century (Gee 1996). The composition of true deuterostomes has been in a state of flux since the advent of molecular systematics, making attempts to reconstruct the early history of the group very difficult. However, the issue of which phyla belong within the deuterostomes is now largely resolved thanks to molecular phylogenetics (Turbeville *et al.* 1994; Halanych 1995; Bromham & Degnan 1999; Cameron *et al.* 2000; Boursat *et al.* 2003, 2006). The following four phyla make up the deuterostomes: chordates, hemichordates, echinoderms and xenoturbellids. Despite increased confidence in the relationships between the major deuterostome phyla, our understanding of early deuterostome evolution remains quite murky. There are two main factors that contribute to this uncertainty: a poor fossil record (Swalla & Smith 2008) and a large morphological disparity between the body plans of the four phyla. Both of these factors make reconstruction of ancestral features of early deuterostomes particularly challenging. This review will focus on the molecular genetic data from hemichordates that have facilitated more direct comparisons with the chordate body plan. First, I begin with a general introduction to the

deuterostome phyla and the challenges associated with reconstructing early deuterostome evolution. Second, I summarize the molecular genetic information, most recently generated from enteropneusts, involved in the anteroposterior and dorsoventral patterning of hemichordates. Finally, I will discuss what sort of insights can be gained from molecular genetic datasets and their usage for testing both general axial or organizational homologies and more traditional morphological homologies.

2. PROBLEMS RECONSTRUCTING ANCESTRAL DEUTEROSTOME CHARACTERS

One of the most significant barriers to understanding the evolution of early deuterostome evolution has been the difficulty of making direct comparisons between the adult body plans of the four deuterostome phyla: chordates; echinoderms; hemichordates; and xenoturbellids. There are few uncontested deuterostome synapomorphies and a poor early deuterostome fossil record, making attempts to reconstruct an ancestral deuterostome body plan very difficult. Echinoderms typify the difficulties of body plan comparison across the deuterostome phyla: the adult body is perhaps the most radical morphological departure of any of the bilaterian groups (Lowe & Wray 1997). Extant species have entirely lost ancestral bilateral symmetry, and have become pentaradially symmetric as adults, while maintaining a bilaterally symmetric larva. There are two novel mesodermally derived structures that are key components of their novel body plan: the calcitic endoskeleton and water vascular system. Their nervous

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system is largely diffuse and organized as a basiepithelial nerve net, with some evidence of integrative abilities in the radial nerves (Bullock & Horridge 1965). Even gross axial comparisons between extant echinoderms and other deuterostomes are problematic (Lowe & Wray 1997) and it is not clear whether valid comparisons can be made to the anteroposterior and dorsoventral axes of the bilaterian groups. However, early stem group fossil echinoderms give some clues to their early bilaterian origins. These fossils show evidence of gill slits (Dominguez *et al.* 2002) and possibly even a muscular stalk (Swalla & Smith 2008). There are a few molecular genetic studies that give some insights into these questions (Peterson *et al.* 2000; Morris & Byrne 2005; Hara *et al.* 2006), but many more data are required before any strong comparative conclusions can be drawn. The most recent addition to the deuterostomes, xenoturbellids (Bourlat *et al.* 2003, 2006) are morphologically rather unremarkable: they have a ventral mouth; a blind gut; and little in the way of external morphological features. They do share the general organizational features of the hemichordate nervous system (Pedersen & Pedersen 1986), but little else currently described in their anatomy could be referred to as a deuterostome synapomorphy. There are still very few published studies of their biology, though preliminary developmental studies suggest that embryos are brooded (Israelsson & Budd 2005). However, it is difficult to make any strong comparative conclusions based on current data from this group, and further study is needed, particularly in characterizing the development of this animal.

Hemichordates are perhaps the most promising of the non-chordate deuterostomes groups for addressing issues of both the early deuterostome evolution and the evolution of the chordate body plan (Cameron *et al.* 2000; Tagawa *et al.* 2001; Lowe *et al.* 2003). The phylum is divided into two classes: the enteropneust worms and the pterobranchs. Both groups possess a similar tripartite body organization, but are characterized by distinct feeding mechanisms. The pterobranchs use a lophophore, a ciliated extension from the mesosome, to filter feed (Halanych 1995), whereas enteropneusts use their highly muscular and ciliated proboscis (prosome) for direct particle ingestion and filter feeding (Cameron 2002). I will focus almost exclusively on the body plan of enteropneusts, as there are currently no published molecular data for pterobranchs. The most recent molecular phylogenies describe two main groups of enteropneusts: the Harrimaniidae on one lineage and the Ptychoderidae and Spengelidae on the other (Cameron *et al.* 2000). These two lineages have major life-history differences: harrimaniids are all direct developers, whereas the spengelids and ptychoderids are indirect developers with feeding larvae, which often spend many months in the plankton before metamorphosing into juveniles (Cameron *et al.* 2000). Phylogenetic relationships of the various hemichordate groups remain poorly resolved, and this area is in need of further research. Pterobranchs have classically been considered as basally branching hemichordates, based largely on the proposed homology of its lophophore with that in other lophophorate groups. However, reclassification of the

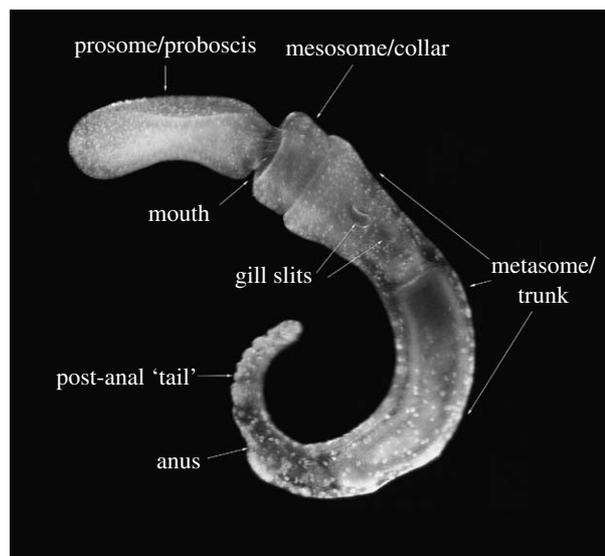


Figure 1. Adult body plan organization of enteropneusts. Light micrograph of a juvenile worm of the harrimaniid enteropneust *S. kowalevskii* at day 13 of development. All major body regions (prosome, mesosome and metasome) are well developed and several gill slits are perforated in the anterior metasome. The juvenile post-anal tail is still present, but is eventually lost in adult animals.

lophophorates as protostomes reveals that the structural similarities of lophophores are due to convergence rather than homology (Halanych *et al.* 1995). Further molecular phylogenetic studies have proposed that pterobranchs are perhaps nested within the enteropneusts (Cameron *et al.* 2000; Winchell *et al.* 2002), but this is weakly supported by current datasets. Clearly, this issue should be revisited with broader phylogenetic sampling.

Figure 1 outlines some of the main anatomical features of enteropneusts and shows a photomicrograph of a juvenile worm of the harrimaniid *Saccoglossus kowalevskii*: the tripartite body plan is divided into an anterior prosome or proboscis, a mesosome or collar and a metasome or trunk. The proboscis is muscular, ciliated and highly innervated with sensory neurons (Bullock 1945; Knight-Jones 1952), and its primary functions are digging and feeding. The mouth opens up on the ventral side and marks the boundary between the proboscis and the collar. In the most anterior region of the trunk, dorsolateral gill slits perforate the ectoderm. The gill slits can be very numerous and continue to be added as the animal grows (Bateson 1885; Hyman 1940). At the very far posterior end of the metasome, a ventral extension, sucker or tail, extends ventrally from the anus and is used for locomotion by the post-hatching juvenile worm. A post-anal extension is present only in the juvenile of the harrimaniids, but not in other enteropneust groups, and is lost in adult worms. Given the uncertainty over the relationships of the major groups within the hemichordates, the possible homology of this extension to the pterobranch stalk, and even more controversially, the chordate post-anal tail, remains unresolved (Cameron *et al.* 2000; Swalla & Smith 2008). The earliest descriptions of hemichordate anatomy by Bateson (1884, 1885, 1886) and Morgan (1891,

1894) resulted in various hypotheses of morphological homologies between hemichordates and chordates (Bateson 1886; Morgan 1891; Nübler-Jung & Arendt 1996). Most of these are now rather weakly supported by both morphological and molecular datasets (Peterson *et al.* 1999; Nieuwenhuys 2002; Lowe *et al.* 2003, 2006; Ruppert 2005). However, only gill slits, as primary ciliated pouches, may represent an ancestral feature of the deuterostomes (Ogasawara *et al.* 1999; Okai *et al.* 2000; Tagawa *et al.* 2001; Cameron 2002; Rychel *et al.* 2006; Rychel & Swalla 2007) and possibly the post-anal tail (Lowe *et al.* 2003). A much more in depth discussion of hemichordate and chordate potential morphological homologies is discussed in Ruppert (2005). Morphological homology aside, there has been little consensus over how to compare the body plans of hemichordates and chordates, even at a basic axial level.

3. THE POTENTIAL OF MOLECULAR GENETIC DATA FOR PROVIDING INSIGHTS INTO DEUTEROSTOME EVOLUTION

Although establishing robust morphological homologies between deuterostome groups is problematic, progress in developmental biology over the past 20 years, mainly from studies of arthropods and chordates, has allowed unprecedented axial comparisons between distantly related groups (Gerhart & Kirschner 1997; Carroll 2005). However, representation of the deuterostome adult body plans in broad metazoan comparative studies has been dominated by chordate developmental biology. There is an impressive literature on early embryonic and larval patterning in echinoderms, but only a handful of studies on the development of the adult body plan (Lowe & Wray 1997; Arenas-Mena *et al.* 2000; Ferkowicz & Raff 2001; Lowe *et al.* 2002; Sly *et al.* 2002; Morris & Byrne 2005). Recent studies in hemichordates have revealed a novel way to compare the adult body plans of chordates and hemichordates (Okai *et al.* 2000; Tagawa *et al.* 2000, 2001; Taguchi *et al.* 2002; Lowe *et al.* 2003, 2006). I will introduce these comparative datasets and their role in comparing anteroposterior and dorsoventral patterning of bilaterian groups. I will then review the current developmental genetic work from hemichordates and how this impacts our understanding of early deuterostome evolution.

Although it is now widely accepted that many of the developmental regulatory cascades controlling anteroposterior and dorsoventral axial patterning are probably homologous as regulatory modules (Gerhart & Kirschner 1997; Carroll 2005; Davidson 2006), the extent to which the comparative expression and functional studies involving regulatory genes are effective for testing hypotheses of morphological homology remains controversial (Arendt & Nübler-Jung 1996; De Robertis & Sasai 1996; Lowe *et al.* 2003, 2006; Lichtneckert & Reichert 2005; Denes *et al.* 2007). Most studies have focused on similarities in nervous system patterning along both dorsoventral and anteroposterior axes (Acampora *et al.* 2001; Reichert & Simeone 2001; Hirth *et al.* 2003;

Lichtneckert & Reichert 2005). Classical morphological comparisons have generally converged on the hypothesis that the central nervous systems of arthropods and chordates evolved independently and that early bilaterian nervous systems were generally quite simple (Holland 2003). More recent interpretations of the molecular genetic data based on model systems with the central nervous systems lead to quite different conclusions and propose a protostome–deuterostome ancestor with a complex, centralized nervous system with a regionalized brain. The homologous suites of genes involved in the patterning of the central nervous systems of model systems are very similarly deployed spatially during development (Arendt & Nübler-Jung 1994, 1996; Finkelstein & Boncinelli 1994; Sharman & Brand 1998; Hirth *et al.* 2003; Lichtneckert & Reichert 2005). Along the anteroposterior axis, the *hox* genes are involved in patterning the nerve cords of both arthropods and chordates. The boundary of the trunk and the rest of the anterior nervous system are marked by the homeobox gene *gbx* or *unplugged* (Hirth *et al.* 2003; Castro *et al.* 2006). *Gbx* is expressed at the boundary between *hox* genes and *otx* and marks a morphological transition in the organization of the nervous system. Other anteriorly localized homeobox genes such as *orthodenticle* (*otx*), *pax6*, *distalless* (*dlx*), *emx* and *retinal homeobox* (*rx*) play conserved roles in brain patterning and exhibit similar relative spatial localization during the central nervous system development (Lowe *et al.* 2003). In the dorsoventral axis, early patterning events of the ectoderm are similarly conservative between arthropods, vertebrates and annelids (Arendt & Nübler-Jung 1994, 1996; De Robertis & Sasai 1996; Holley & Ferguson 1997; Cornell & Ohlen 2000; Denes *et al.* 2007). The secreted factor *chordin/short gastrulation* is released dorsally in vertebrates and ventrally in arthropods, protecting the ectoderm from the neural-inhibiting effects of the *TgfB* ligand *Bmp*, which is expressed ventrally in vertebrates and dorsally in flies. The interaction of these secreted ligands results in the formation of the central nervous system on the dorsal side in vertebrates and the ventral side in arthropods and annelids. These data have revived the venerable dorsoventral axis inversion hypothesis by Dohrn (1875), which proposed that the dorsoventral organization of chordates is best explained by a complete body axis inversion in the lineage leading to chordates. Further similarities have been revealed in later dorsoventral patterning of the neurectoderm of all three groups, but most closely between the annelid *Platynereis dummerilii* and vertebrates (Denes *et al.* 2007). The similarities in relative expression domains and essential functions along both the dorsoventral and anteroposterior axes during central nervous system patterning have led to the proposal that a tripartite brain is ancestral for bilaterians, implying that the protostome/deuterostome ancestor and early deuterostomes were characterized by a complex, centralized nervous system. Within a purely phylogenetic framework, based on current molecular phylogenies, the outgroups to the bilaterians are both acoel flatworms and cnidarians, both of which are characterized by a nerve net (Holland 2003). Within the bilaterians, the basiepithelial nerve net is quite

common. Proposing a protostome/deuterostome ancestor with a complex central nervous system and brain implies that this organization has been lost multiple times in evolution of the bilaterian phyla.

Until recently, the vast majority of data generated in the developmental biology have been from terrestrial model systems that are characterized by central nervous systems. Recently, this bias has been redressed by a broad description of the genetic information involved in patterning the hemichordate enteropneust body plan and its diffuse basiepithelial nerve net (Lowe *et al.* 2003, 2006). The organization of the nerve net is based around a broad distribution of cell bodies throughout the ectoderm. Despite the general diffuse organization of the nervous system, there is a significant dorsoventral and anteroposterior polarity in its structure and organization, particularly in the dorsoventral dimension (Bullock 1945; Knight-Jones 1952). A mat of axons spreads out along the basement membrane, which is thickened in certain areas of the ectoderm, at the base of the proboscis, along the anterodorsal region of the body in the mesosome and in both the dorsal and ventral midlines of the metasome. In the proboscis ectoderm, there is a dense concentration of nerve cells that have been proposed to be primarily sensory (Bullock 1945; Knight-Jones 1952) and is particularly thick at the base of the proboscis (Brambell & Cole 1939).

Probably the most well-known aspect of hemichordate anatomy is the mid-dorsal region of the dorsal cord, or collar cord which is internalized into a hollow tube of epithelium in some species within the Ptychoderidae and in one species of the Spengelidae. However, in the harrimaniids, there is no contiguous hollow tube, but scattered blind lacunae (Bullock & Horridge 1965; Nieuwenhuys 2002; Ruppert 2005). This structure has been widely compared to the dorsal cord of chordates due not only to the superficial similarities of the hollow nerve cord but also to the collar cord forms, in some species, by a process that quite closely resembles chordate neurulation (Morgan 1891). However, the similarities have generally been over emphasized as it seems to be more of a conducting tract rather than processing centre (Ruppert 2005) as evidenced by both ultrastructural (Dilly *et al.* 1970) and physiological data (Pickens 1970; Cameron & Mackie 1996). Another striking feature of the dorsal cord is the presence of giant axons in some species. The cell bodies project their axons across the midline and continue posteriorly within the collar cord. It is not known where the axons finally project; Bullock (1945) proposed that they innervate the ventrolateral muscles of the trunk and suspected that their primary function is to elicit a rapid contraction of these muscles. However, several groups do not possess giant axons and yet are still able to elicit a rapid retreat, so the role of the giant axons remains uncertain (Pickens 1973).

In the metasome, the third body region, the most prominent features are the ventral and dorsal nerve cords, which are both thickenings of the nerve plexus. The dorsal cord is contiguous with the collar cord and projects down the entire length of the metasome. The ventral cord is comparatively much thicker, with more associated cell bodies, but both cords are largely

described as through axon tracts. However, at least one study describes the ventral cord as having some integrative function (Pickens 1970). It seems to play a role in rapid retreat of the animals following anterior stimulation (Knight-Jones 1952; Bullock & Horridge 1965; Pickens 1973).

4. ANTEROPOSTERIOR PATTERNING IN HEMICHORDATES

Molecular genetic patterning information in hemichordates has the potential to address two major areas of comparative interest. First, these data could facilitate another means to compare deuterostome body plans giving insights into early deuterostome evolution. Second, hemichordates are representative of the first basiepithelial nervous system to be characterized molecularly and allow insights into whether the complex networks of regulatory genes involved in patterning complex central nervous systems play similar roles in less complex, more diffusely organized, nervous systems. These questions are the focus of several papers over the past 10 years investigating the roles of body patterning genes in hemichordates. The first suite of papers focused on *Ptychodera flava*, an indirect-developing species with a ciliated feeding larva, and an extended planktonic larval period: most of these initial studies focused on the establishment of the larval body plan (Peterson *et al.* 1999; Harada *et al.* 2000, 2002; Okai *et al.* 2000; Tagawa *et al.* 2000, 2001, 2002). More recently, I have been involved in developing a direct-developing species, the harrimaniid enteropneust, *S. kowalevskii*, to investigate more directly the patterning of the adult rather than larval body plan of hemichordates. In the first of two papers on body patterning, we investigated anteroposterior patterning (Lowe *et al.* 2003) by examining the expression of orthologues of 22 transcription factors that have conserved roles in the patterning of the brain and spinal cord of vertebrates along the anteroposterior axis. At least 14 of these genes also play conserved roles in the patterning of the central nervous system of the fruit fly *Drosophila melanogaster*. The 22 transcription factors can be divided into three broad expression and functional domains during the development of the vertebrate brain and central nervous system: (i) expressed and involved in forebrain development, (ii) expressed during midbrain development, and (iii) expressed and functionally involved in the patterning of the hindbrain and spinal cord. Quite surprisingly a group of six transcription factors—*six3*, *brain factor 1 (bf-1)*, *distal less (dlx)*, *nk2-1 ventral anterior homeobox (vax)* and *retinal homeobox (rx)*—were all expressed in similar domains during the early development of the embryo and juvenile (figure 2). Their expression was restricted, for the most part, to the developing proboscis ectoderm, the most anterior region of ectoderm. Unlike vertebrates and panarthropods, this expression is not restricted to the dorsal or ventral side, but rather forms rings encircling the entire dorsoventral aspect of the animal reflecting the inherent diffuse organization of the basiepithelial nerve net. The second group of genes is expressed with the posterior limit of expression marking the

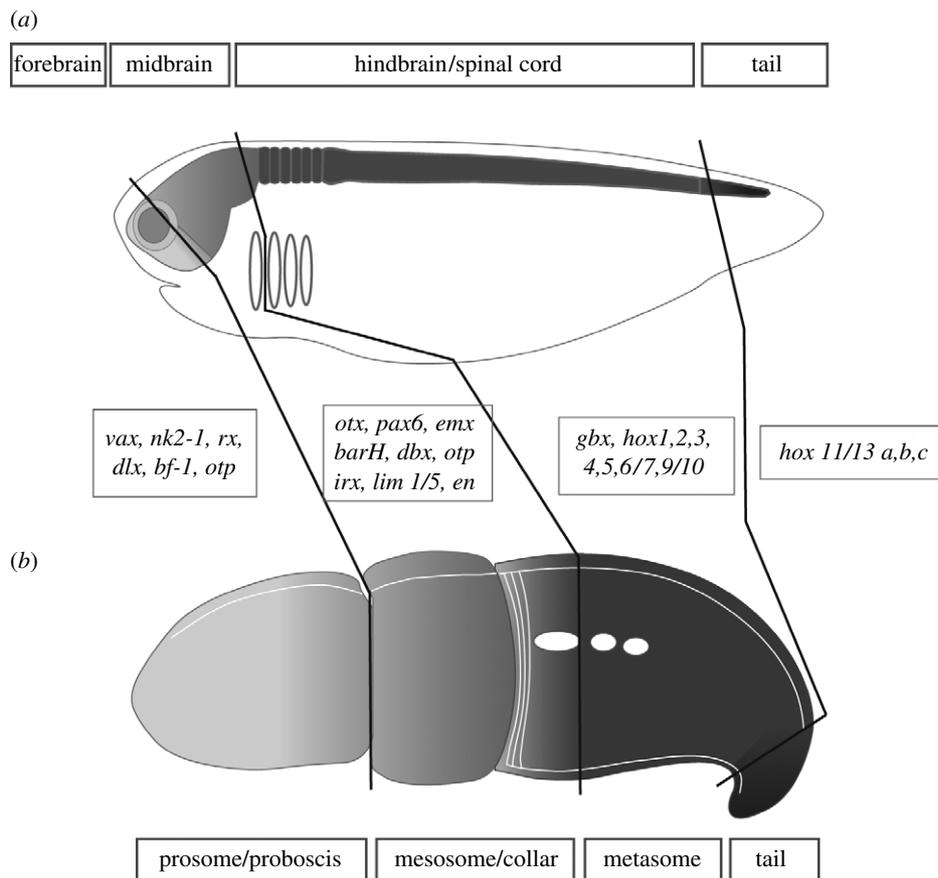


Figure 2. Summary of similarities between the enteropneust hemichordate *S. kowalevskii* and vertebrates in the ectodermal expression of conserved transcriptional developmental regulatory genes. Representation of (a) an idealized vertebrate and (b) a juvenile hemichordate. The various tones of grey represent similarities in gene expression between the two groups.

midbrain, and sometime hindbrain of vertebrates including: *pax6*, *tailless (tll)*, *barH*, *emx*, *orthopedia (otp)*, *dorsal brain homeobox (dbx)*, *lim1/5*, *iroquois (irx)*, *orthodenticle (otx)* and *engrailed (en)*. Similar to vertebrates, this group of genes is expressed in a more posterior position along the anteroposterior axis of the developing hemichordate embryo, in the posterior proboscis and anterior collar. Of these genes, *en* is particularly interesting as it forms a sharp single ring of expression in the ectoderm of the anterior metasome over the forming first gill slit. *En* is a critical gene in the formation of the vertebrate isthmus. This then makes a compelling case to investigate other genes involved in the formation of the midbrain/hindbrain division of the vertebrate brain and to investigate how much of this signalling regulatory cassette is conserved, as most studies of basal chordates have suggested that ectodermal signalling centres evolved in association with complex vertebrate neural anatomy (Canestro *et al.* 2005).

The last group of genes includes *gbx* and *hox* genes. In vertebrates, the regulatory interaction between *otx* and *gbx* is involved in positioning the isthmus along the A/P axis, with *gbx* expressed posterior to *otx*. In amphioxus, *gbx* is also expressed in a mutually exclusive domain to *otx* in the central nervous system, suggesting a conserved interaction between the two genes (Castro *et al.* 2006), but is absent from the genome in ascidians. In *S. kowalevskii*, we observe a departure from chordates in that *otx* and *gbx* expression overlap extensively at all stages of development examined, suggesting that they do not share the same mutual

antagonism as found in vertebrates. Posterior to *gbx*, *hox* genes are expressed in closely abutting domains, but roughly in order down the rest of the trunk in ectodermal rings. This work was further developed in a study of *hox* genes (Aronowicz & Lowe 2006) which cloned a further 6 *hox* genes to make a complement of 11 *hox* genes. There is probably a true orthologue of *hox8* still to be cloned from the genome, but it is not clear whether further posterior class member duplications are present or the current complement, shared with sea urchins, represents the ancestral complement of deuterostomes, or just the Ambulacraria. There are currently no data on hemichordate cluster organization. This information will be critical to determine the full *hox* complement, and the *hox* gene order along the chromosome, to check for collinearity with gene expression as has been described in other groups (Aronowicz & Lowe 2006). *Hox* expression domains follow predictable nested domains with the most anterior *hox* genes expressed in the most anterior regions of the ectoderm, and the more posterior members in the most posterior domains. At the stages that were examined, expression of many of the genes was tightly grouped, with little evidence of difference in the anterior limits. Expression has not been examined for all genes at late developmental stages when the trunk begins to elongate and become further regionalized. Perhaps the anterior expression limits of *hox* genes become more markedly differentiated in later stages. Posterior *hox* family members were examined at later stages when the ventral post-anal

tail was developing, and the expression of these posterior members was restricted to the post-anal tail in these juveniles, which is similar to the expression of their orthologues in the dorsal post-anal tail of vertebrates. These data would support the proposed homology of the chordate and enteropneust post-anal tails, although it is certainly also possible that independently evolved posterior extensions are likely to express posterior *hox* genes already expressed in the posterior ectoderm.

A summary of the data from the development of the A/P axis of the hemichordates is diagrammed in figure 2. These data clearly demonstrate similar relative expression of transcription factors with critical roles in anteroposterior patterning between chordates and hemichordates. Although most comparative studies and speculations on the nervous system of hemichordates have focused on the dorsal and ventral axon tracts as potential homologues of chordate central neural structures, the results from this study suggest, as was proposed by Bullock in 1945, that the appropriate comparison is with the entire net. The cords are probably local thickenings of the nerve plexus rather than integrative centres (Dilly *et al.* 1970). The conclusions one can draw from these data are more complicated, particularly, whether these data help to reconstruct early morphological evolution of deuterostomes. First, it is most parsimonious to conclude that the majority of the similarities in the expression of multiple genes, with such close topological similarities, is conservation of a transcriptional regulatory network between vertebrates and hemichordates, and at that level, this is a homologous feature. It is highly unlikely that all the similarities of gene expression along the A/P axes of both groups are a result of co-option of individual genes into convergently similar domains. However, the organizational difference in the nervous systems of both groups suggests that, despite this regulatory conservation, the evolutionary possibilities of the downstream morphologies have not been constrained. The nervous system, in particular, demonstrates this point effectively: the development of both the central nervous system of chordates and the basiepithelial nerve net of hemichordates is probably regulated by this conserved regulatory map (although it is important to note that this was not directly tested in Lowe *et al.* 2003). Clearly, the forebrain of vertebrates and the proboscis of hemichordates are not homologous structures. So this suite of genes is not a reliable marker of morphological homology between groups. By considering these data alone, we can speculate that the deuterostome ancestor, and also the protostome/deuterostome ancestor, may have been characterized by a completely diffuse or fully centralized nervous system, and all possible intermediates. Reconstructing ancestral morphologies from gene expression data can be problematic, even with such large expression datasets. These data give a unique insight into the A/P patterning of the deuterostome ancestor, revealing a degree of transcriptional complexity previously attributed to the complex nervous system of vertebrates.

Finally, the nervous system of hemichordates has been described as barely more complex than the

cnidarian nervous system (Bullock 1945; Bullock & Horridge 1965) and yet there is an exquisite level of transcriptional patterning in the ectoderm. This may suggest a level of neural diversity currently not recognized in this group. Perhaps the complexity of the basiepithelial net of the hemichordates has been underestimated and would greatly benefit from a contemporary approach to describing the neural diversity. Detailed physiological and molecular studies would be required to address this hypothesis.

5. DORSOVENTRAL PATTERNING

Hemichordates have a distinctive and marked dorsoventral axis. The mouth opens on the ventral side by convention, and the most obvious dorsal markers are the paired dorsolateral gill slits. The stomochord is an anterodorsal projection from the gut supporting the axial complex or heart and kidney complex. As previously discussed, the nerve net also exhibits marked dorsoventral polarity in the distribution of dorsal and ventral cords, and the presence of giant axons in the dorsal cords.

The TGF β -signalling ligand, Bmp, and one of its antagonists, chordin, are involved in establishing the dorsoventral developmental axis in arthropods and chordates. This molecular axis has recently been investigated in *S. kowalevskii* (Lowe *et al.* 2006). Hemichordates occupy a key position for investigating the evolution of this developmental pathway in dorsoventral patterning of the bilaterians (Nübler-Jung & Arendt 1996; Lowe *et al.* 2006). The most striking feature of Bmp and chordin between vertebrates and arthropods is that their relative expression is inverted dorsoventrally with respect to each other (Arendt & Nübler-Jung 1994, 1996; De Robertis & Sasai 1996). In hemichordates, *bmp2/4* is expressed along the dorsal midline throughout all stages of development, along with all the members of the Bmp synexpression group (Niehrs & Pollet 1999; Karaulanov *et al.* 2004). At early developmental stages, *chordin* is expressed ventrally and very broadly, on the opposite side to *bmp2/4*, almost up to the dorsal midline, but is increasingly restricted to the ventral side as development progresses. There are many genes that exhibit marked dorsoventrally restricted expression domains along either dorsal or ventral midlines in ectoderm (*tbx2/3*, *dlx*, *olig*, *netrin*, *pitx*, *poxN*, *lim3*, *admp*, *sim*), endoderm (*mnx*, *admp*, *sim*, *nk2.3/2.5*) and mesoderm (*mox/gax*). These data reveal a molecular dorsoventral asymmetry that perhaps underlies the morphological asymmetry along this axis. Although in hemichordates the expression of *chordin* and *bmp* in relation to the dorsoventral axis is similar to protostomes, the early developmental action of Bmp and chordin does not result in segregation of a central nervous system from the general ectoderm: there is no central nervous system, but a diffuse and broadly distributed nerve net. What is the early role of Bmp in an animal without a non-neural ectoderm? Over expression and knockdown analyses addressed this question, and two main conclusions were presented: first, over expression of Bmp did not result in the repression of neural fates; second, *bmp* plays a central and critical role in dorsoventral patterning (Lowe *et al.* 2006). In

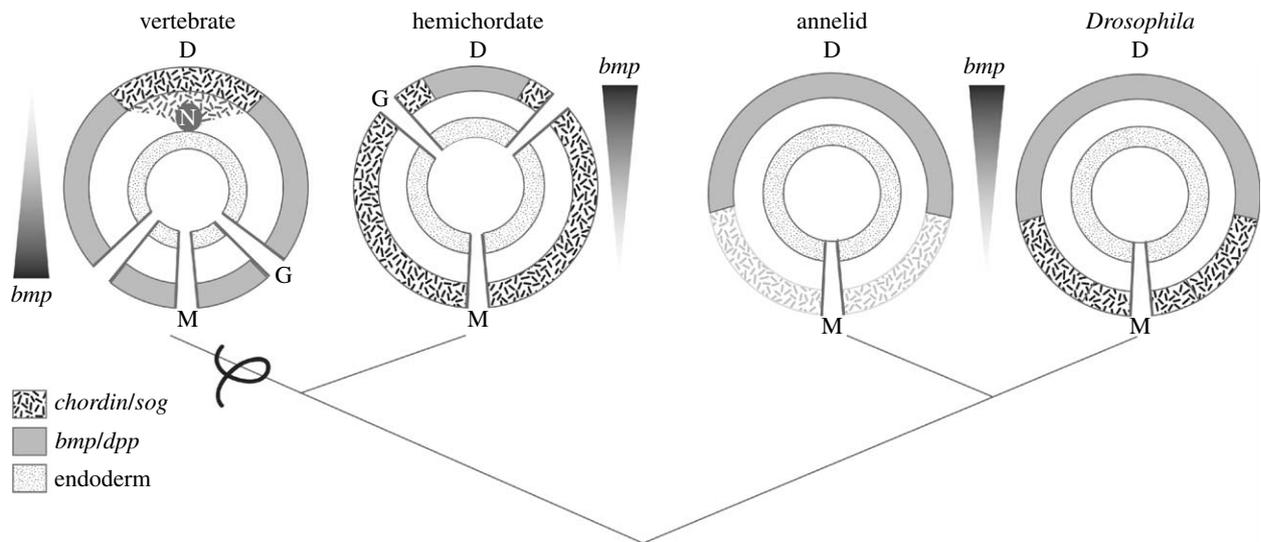


Figure 3. Expression of *bmp/chordin* in bilaterians. The diagrams represent idealized cross sections through embryos, with dorsal (D) oriented up and ventral down. In vertebrates, the source of *chordin* is largely from the notochord, marked as a grey circle and marked with 'N', dorsal to the gut. The mouth in all panels is represented by M, and in hemichordates and vertebrates, G represents the position of the gill slits. In annelids, the ventral, light grey colour represents the predicted domain of *chordin* expression, as this has yet to be published. The black symbol under the vertebrate model represents a potential dorsoventral axis inversion on the lineage leading to chordates.

embryos incubated with recombinant vertebrate Bmp4 protein, endogenous hemichordate *bmp2/4* expression was activated throughout the ectoderm, rather than localized along the dorsal midline in normal embryos. These treated embryos do not perforate a mouth, and with high levels of Bmp protein do not perforate gill slits. Additionally, in the endoderm, dorsolateral endodermal pouches, precursors to the gill slits, do not form, and the entire endoderm projects into the protocoel rather than a thin dorsal projection that would normally develop into the stomochord. Knockdown or diminished expression of *bmp2/4* by injection of short-interfering RNAs (siRNA) resulted in a complementary phenotype, particularly in relation to the mouth, which normally perforates on the ventral side. In injected embryos, the mouth develops circumferentially, and eventually results in the detachment of the entire prosome. The morphological interpretation of Bmp modulation experiments suggests that over expression of Bmp dorsalizes embryos, and knockdown of Bmp ventralizes embryos. This was confirmed by further molecular analysis: markers of the dorsal midline, in both the ectoderm and endoderm, expanded into circumferential rings in Bmp ligand-treated embryos, suggesting that in normal embryos, they are activated by Bmp signalling on the dorsal midline. Some of the same dorsal markers failed to activate expression following siRNA injection, adding further support for a role of Bmp in patterning dorsal cell fates. Further experimental evidence suggested that Bmp is involved in restricting the expression of ventrally expressed genes to the ventral midline, as Bmp ligand-treated embryos failed to express ventral markers, and these same markers expand to the dorsal side in siRNA-injected embryos.

The major differences between the hemichordates and vertebrates are summarized by two major criteria. First, in the disposition of the Bmp/*chordin* axis, which is inverted (figure 3): hemichordates more closely

resemble the protostomes with *chordin* expressed ventrally, and Bmp dorsally. Second, the mouth opens on the side of the embryo expressing *chordin* in hemichordates and other protostomes, but in the *bmp* domain in vertebrates. Functional experiments in hemichordates suggest that the Bmp/*chordin* axis is fundamental for the development of many components of the dorsoventral axis, and particularly important for the formation of the mouth. Lastly, although Bmp is directly involved in repressing neural fates in the developing epidermis of vertebrates, it plays no role in repressing neural cell fate in hemichordates as neural markers are not downregulated following Bmp4 treatment of embryos. This is also not surprising based on previous descriptions of the distribution of neural cell bodies, which are present along the dorsal midline where Bmp is normally expressed. How can the differences between hemichordates and vertebrates be explained, and do these data give any critical insights into early deuterostome evolution? One way to explain the data is partially to accept the basic model of inversion as proposed by Dohrn (1875). However, the modification to the model is that a hypothetical ancestor was not necessarily characterized by a central nervous system: the data from *S. kowalevskii* demonstrate that a dorsoventrally distributed Bmp/*chordin* axis, although fundamentally involved in dorsoventral patterning, is not always linked to the formation of a central nervous system. Therefore, issues of inversion and centralization can be uncoupled and considered separately. It is formally possible that inversion of an animal with a diffuse nervous system gave rise to the chordates, and centralization happened secondarily. Following inversion, the definitive chordate mouth must have either migrated from the dorsal side or a new mouth formed de novo. The new mouth of vertebrates seems to have a novel relationship to the Bmp/*chordin* axis as it forms in a region of Bmp expression, which in hemichordates inhibits the formation of the mouth.

6. CONCLUSIONS

The molecular genetic body patterning data presented in this review reveal some critical insights into the body plan of the deuterostome ancestor, and a unique way to compare the adult hemichordate body plan to that of chordates. The detailed similarities in the transcriptional and signalling networks are not likely to be a result of recruitment of individual genes into convergently similar expression topologies. These exquisite similarities are almost certainly a result of homology. However, what we can most confidently reconstruct is an ancestral gene network rather than ancestral morphologies. Most of the gene networks discussed have been used comparatively to investigate the nature of ancestral nervous systems, and yet hemichordates are a good example of how homologous gene regulatory networks can be deployed to regulate the development of nervous systems with fundamental differences in their organizational base. While gene networks are conserved over large evolutionary time scales, the broad range of morphologies that they regulate have not been constrained by the higher-level regulatory control. Tight regulatory conservation is the foundation of both the highly complex vertebrate central nervous system and the basiepithelial nerve net of the hemichordates. Although these genetic networks have potential for testing hypotheses of morphological homology, their reliability as informative characters is questionable given the range of neural morphologies regulated by this network. Caution should be used when reconstructing ancestral neuroanatomies based on these data. Broader sampling and incorporation of fossil datasets will all be required for a more rigorous assessment of ancestral features of early deuterostomes.

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