

# The deuterostome context of chordate origins

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**Our understanding of vertebrate origins is powerfully informed by comparative morphology, embryology and genomics of chordates, hemichordates and echinoderms, which together make up the deuterostome clade. Striking body–plan differences among these phyla have historically hindered the identification of ancestral morphological features, but recent progress in molecular genetics and embryology has revealed deep similarities in body–axis formation and organization across deuterostomes, at stages before morphological differences develop. These developmental genetic features, along with robust support of pharyngeal gill slits as a shared deuterostome character, provide the foundation for the emergence of chordates.**

The mystery of chordate origins has endured for more than 150 years. Shortly after Darwin's *On the Origin of Species*, acorn worms were discovered to have chordate-like pharyngeal gill slits<sup>1,2</sup> and to metamorphose from echinoderm-like larva<sup>3</sup>, thus linking the evolution of chordates, hemichordates and echinoderms. Modern phylogenetic analysis has confirmed the union of these three phyla in a single clade. This group, the deuterostomes, provides the phylogenetic framework for developing hypotheses about the origin of chordate features through comparative morphology, embryology and genomics.

The emergence of comparative molecular developmental biology over the past quarter of a century has revived interest in classic hypotheses of animal body–plan evolution<sup>4</sup>. The comparative approach focuses on identifying morphological, developmental and genetic traits that are shared across phyla by virtue of their inheritance from a common ancestor, and provides an understanding of how such ancestral traits can arise and be subsequently modified. Although many recent hypotheses on chordate and vertebrate origins on the basis of molecular data are motivated primarily by projections from the bilaterian ancestor<sup>4,5</sup>, a growing body of data from hemichordates, echinoderms and invertebrate chordates serves as the foundation for new hypotheses based on deuterostome ancestral characters<sup>6–14</sup>.

Despite the impressive morphological disparity among deuterostome phyla, we are making progress identifying conserved anatomical and molecular ancestral characters. Each phylum is a fascinating natural experiment in body–plan evolution, but their dazzling diversity presents a major challenge for reconstructing early deuterostome evolutionary history in morphological terms (Box 1)<sup>15</sup>. In this Review we highlight recent advances in deuterostome phylogenetics, developmental biology and genomics that have contributed to our understanding of the early evolution of deuterostomes and the subsequent origin of chordates.

## Deuterostome phylogeny

The first step in unravelling chordate origins is the establishment of a robust deuterostome phylogeny (Fig. 1). The chordates, uniting vertebrates, tunicates and cephalochordates, were first recognized by Haeckel<sup>16</sup>, partly based on shared developmental characteristics. A key insight came from Kowalevsky's<sup>17</sup> recognition that the tadpole larva of ascidians shared many characteristics with vertebrates, an observation that greatly

impressed Darwin<sup>18</sup>. Kowalevsky also recognized the vertebrate-like gill slits of the invertebrate acorn worms<sup>2</sup>. The link between chordates and acorn worms was emphasized by Bateson, who proposed further morphological affinities between them in the late 1800s, and named the acorn worms 'hemichordates'<sup>1</sup>. Around the same time, Metchnikoff recognized the similar larval forms of hemichordates and echinoderms, and united these two phyla into the 'Ambulacraria'<sup>3</sup> (Box 2).

The unity of chordates, hemichordates and echinoderms was inferred by Grobben<sup>19</sup> on the basis of three shared developmental features: 'deuterostomous' development (derivation of the mouth from a secondary opening rather than the blastopore), radial cleavage and enterocoely (the pouching out of mesoderm from the archenteron wall). Although he named this lineage the 'deuterostomes' (second mouth), we now recognize that these features are not unique to the chordate–hemichordate–echinoderm clade, and are found in several other phyla<sup>20</sup>, the result of either shared ancestry or convergence. This leads to the nomenclatural embarrassment that some phyla with deuterostomous development are not deuterostomes. Nevertheless the name has stuck, and by convention we refer to the chordate–hemichordate–echinoderm clade as the deuterostomes.

The advent of molecular phylogenomics has brought new methods to bear on the relationships between and within deuterostome phyla (Fig. 1). Ambulacraria, the surprising grouping of hemichordates and echinoderms, is strongly supported by molecular characters<sup>15,21–23</sup>, and is clearly the sister group of chordates. Within chordates, it is now widely recognized that the cephalochordate lineage (amphioxus) diverged before the split between tunicates and vertebrates<sup>21,24</sup>. This recent discovery overturned earlier thinking that tunicates diverged first, which had implied that the simple ascidian tadpole larva represents ancestral chordate features (Box 1).

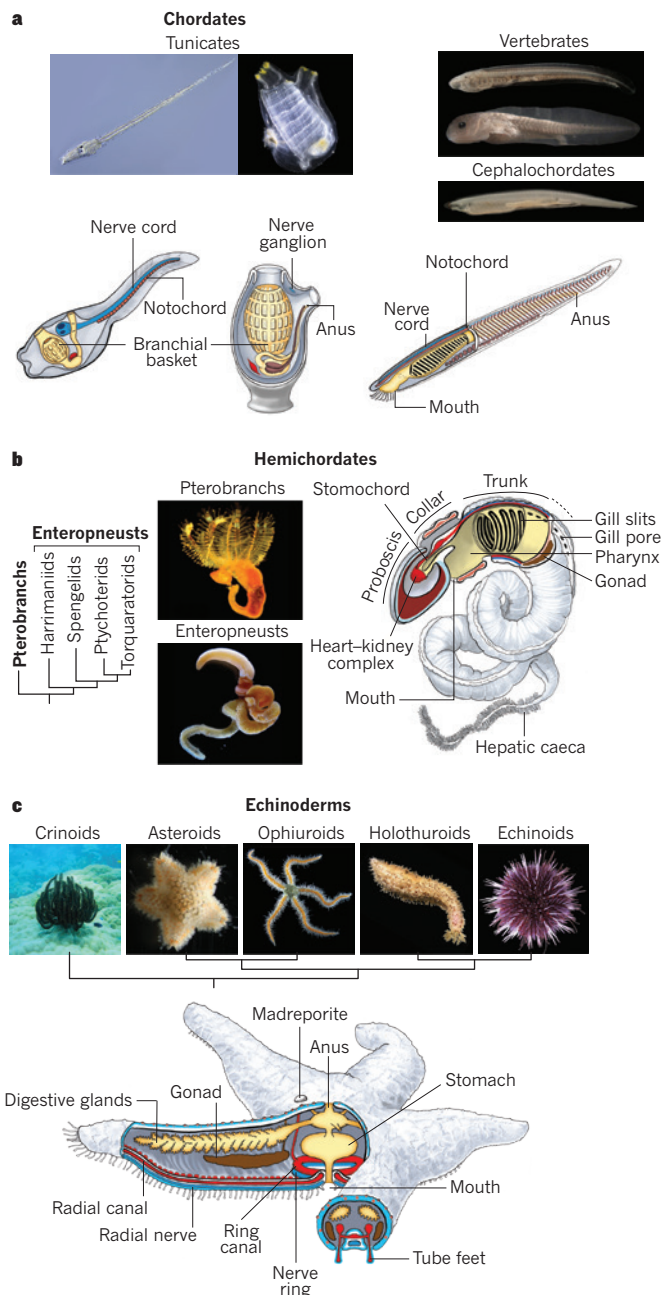
Although classic embryological criteria suggested that lophophorates (phoronids, brachiopods and bryozoans) and/or chaetognaths should also be grouped among the deuterostomes, molecular phylogenetics robustly supports their position in the protostomes<sup>22,25,26</sup>. Xenoturbellid worms are a more challenging case: these animals resemble acoelomorphs (acoel flatworms and nematodermatids) and have been grouped with them in a 'Xenacoelomorpha' clade<sup>27,28</sup>. Some molecular analyses also identify *Xenoturbella* and its relatives as ambulacrarians, and therefore deuterostomes<sup>27</sup>, whereas other studies find that acoelomorphs diverge from

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## BOX 1

## Deuterostome diversity

Unambiguous homologies between deuterostome phyla with morphologically disparate body plans are difficult to establish, leading to a wide range of often contradictory hypotheses about chordate origins<sup>1,6,29,96,100,107,108,114–116</sup>. We present basic descriptions of the adult body plans of the uncontested deuterostome phyla: chordates, hemichordates and echinoderms. All mesodermally derived structures are red, ectoderm are blue and endoderm are yellow. **a**, Chordates are set apart from other deuterostomes by a suite of features that enable swimming by paired muscles along a trunk that extends post-anally. These muscles exert forces on the notochord, a flexible rod that provides elastic recoil to power movement. Chordates also have a unique tubular central nervous system (CNS)<sup>29</sup>. Of the subphyla, vertebrates are distinguished from other chordates by the elaboration of the head region with an enlarged anterior CNS with paired sense organs, evident here in a lamprey ammocoete larva (top) and an axolotl tadpole (bottom). Tunicates (larvaceans, ascidians and thaliacians) are a diverse group of marine filter feeders that display a range of body plans and life-history strategies, including solitary, colonial, sessile and free-swimming forms<sup>117</sup>. They are represented here by ascidians. Chordate affinities are most evident in the larval form: an ascidian tadpole (left) has a tubular nerve cord, a notochord and a post-anal tail. These features regress at metamorphosis, leaving the branchial basket, a small nerve ganglion and the endostyle as the only chordate characters remaining in the adult (right). Cephalochordates, represented by amphioxus, are filter feeders that burrow in sand with their mouths open to the water column. Amphioxus shares much of its basic anatomy with vertebrates, including segmented musculature, and a vertebrate-like heart and circulatory system<sup>30,118</sup>. They have a modest CNS consisting of a neural tube with simplified vertebrate-type patterning along both the anteroposterior and dorsoventral axes<sup>8,118,119</sup>. **b**, Hemichordates are a clade of marine worms divided into two groups: enteropneusts and pterobranchs. Hemichordate phylogeny is based on Cannon *et al.*<sup>120</sup>. Pterobranchs, shown here by *Cephalodiscus*, are small largely colonial animals that live within the protection of a secreted fibrous tube and use a ciliated lophophore for filter feeding<sup>50,58</sup>. Enteropneusts, or acorn worms, are solitary, burrowing worms that feed using a combination of deposit and filter feeding<sup>52,121</sup>. The harrimaniid *Saccoglossus kowalevskii*, which has been used for many developmental studies<sup>12</sup>, is pictured (micrograph). Both groups of hemichordates are united by their tripartite body plan, which includes proboscis, collar and trunk (as shown in the illustration of a spengelid enteropneust). The proboscis is used for digging and feeding and contains the gut diverticulum called the stomochord that supports a heart–kidney complex<sup>56,60</sup>. The mouth opens ventrally into the pharynx within the collar region, and the anterior trunk is perforated by a series of dorsolateral gill slits<sup>58</sup>. **c**, Echinoderms have considerably modified the ancestral bilaterian body plan to become pentaradially symmetrical as adults, although their larvae are bilaterally symmetric (Box 2). Even basic axis comparisons with other deuterostomes are problematic, and the evolutionary origins of this phylum remain a mystery. All five extant classes of echinoderms: crinoids (sea lilies), asteroids (sea stars), ophiuroids (brittle stars), holothuroids (sea cucumbers) and echinoids (sea urchins) are characterized by a conserved body plan shown by a diagram of an asteroid with cutaways to show internal anatomy; the



mesodermally derived water vascular system, a hydraulic system that drives the distinctive tube feet used for feeding and locomotion; five radial nerves along each arm/ambulacrum linked by a nerve ring, and the mesodermally derived skeleton. Asteroids most clearly exhibit the basic components of the body plan. Phylogenetic relationships are based on refs 120, 122.

the bilaterian stem before the protostome–deuterostome split<sup>28</sup> (Fig. 1). We note, however, that even if xenoturbellids and/or acoelomorphs are deuterostomes, their simple body plans would represent secondary loss from a more complex deuterostome ancestor. The resolution of the phylogenetic placement of these taxa is therefore unlikely to provide substantial insight into vertebrate origins.

### Ancestral chordate characters

On the basis of shared features of living chordates we have gained a rather detailed view of the development, morphology and life history of the last common chordate ancestor. Most classic and modern reconstructions of ancestral chordates propose a filter feeder with a notochord, gill slits, endostyle, dorsal hollow nerve cord and post-anal tail<sup>29</sup>. The recent

revision of the chordate family tree has added to this list of ancestral chordate features. The basal position of cephalochordates among chordates suggests that similarities between amphioxus and vertebrates represent ancestral chordate features lost in tunicates<sup>21,24</sup>. Thus, in addition to the core features listed earlier, the Early Cambrian or Pre-Cambrian chordate ancestor probably possessed myomeres, a vertebrate-like circulatory system and a central nervous system (CNS)<sup>30</sup>. The life history of cephalochordates, and the fact that larval lampreys and adult hemichordates are burrowing filter feeders, further suggest that this ancestor was a solitary, endobenthic filter feeder that was capable of short swims.

The striking similarities between amphioxus- and vertebrate-developmental mechanisms allow a fairly comprehensive reconstruction of early development in primitive chordates. As in vertebrates and cephalochordates, the anteroposterior (AP) and dorsoventral (DV) axes of the ancestral chordate were probably determined during gastrula stages by organizing centres much like Spemann's organizer of vertebrates, secreting long-range patterning signals<sup>11</sup>. Opposing Nodal and BMP signalling gradients established the DV axis, with Chordin-mediated BMP inhibition in the dorsal ectoderm segregating the presumptive CNS from the epidermal (or general) ectoderm<sup>11,31</sup>. Along the AP axis, Wnt and retinoic acid signalling probably acted on Hox genes and other transcription-factor genes to establish the regional identities of AP domains of the body axis, including the boundary between the foregut and hindgut and the main subdivisions of the CNS<sup>8,32,33</sup>.

Comparisons between amphioxus and vertebrates suggest a deep ancestry of the major divisions of the CNS along the AP axis. Later in development, fine-scale patterning of the ancestral chordate CNS was also vertebrate-like, but simpler. Along the DV axis of the CNS, all chordates have a molecularly distinct dorsal domain that expresses *pax3/7*, *msx* and *zic* genes and generates sensory interneuron cells<sup>34</sup>, a ventral floor plate expressing hedgehog ligands<sup>35</sup>, and an intervening bilateral domain flanking the neural tube lumen and generating motor and visceral neurons. The expression domains of transcription factors and signalling molecules along the AP axis of the CNS are also mostly conserved across chordates, and presumably reflect expression domains of the chordate ancestor<sup>8</sup>. Precisely how this patterning was generated is less clear, as current data suggest that neither amphioxus nor tunicates have unambiguous, functionally validated homologues of two vertebrate CNS signalling centres, the isthmus organizer or the zona limitans intrathalamica (although these signalling mechanisms may have been present in a deuterostome ancestor, see later)<sup>8,36,37</sup>.

Segmented musculature of the ancestral chordate almost certainly developed from somites, and at least some formed by enterocoely<sup>35,38</sup>. In amphioxus, the anterior-most somites form by enterocoely, whereas posterior somites pinch off sequentially from the tail bud<sup>36,39</sup>. In vertebrates, a 'clock and wavefront' mechanism, involving oscillating Notch and Wnt

signalling and a posterior fibroblast growth factor (FGF)-signalling gradient divides the paraxial mesoderm into a series of somites<sup>40</sup>. Despite these mechanistic differences, amphioxus displays vertebrate-like segmental expression of Notch and Wnt signalling components in nascent somites, and requires FGF signalling for forming and maturing the anterior and posterior somites<sup>41,42</sup>. Thus, somitogenesis in all living chordates, and presumably their last common ancestor, involved iterated Notch–Delta and Wnt signalling, and FGFs.

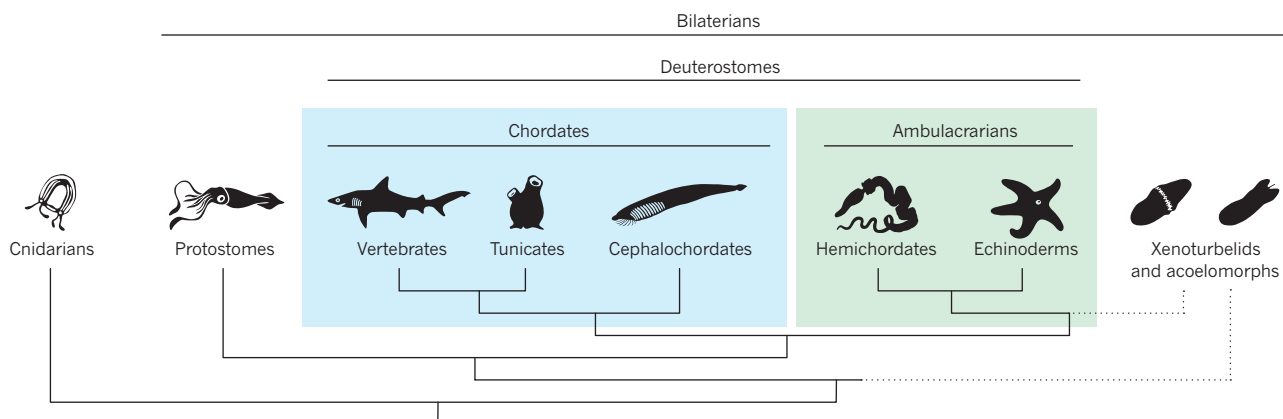
Despite differences in when and how the pharyngeal gill slits form in the three chordate clades, recent work reveals conserved aspects of their development, presumably inherited from the chordate common ancestor. In amphioxus and vertebrates, the pharyngeal endoderm is specified by attenuated retinoic acid signalling, and marked by conserved expression of several transcription factors including *pax1/9*, *six1/2*, *six4/5*, *six3/6*, *eya*, *foxC* and *foxL1* (refs 32, 43, 44). In addition, recent work has shown that the chordate ancestor probably had a collagen-based pharyngeal skeleton incorporating cellular<sup>45</sup> and acellular cartilage<sup>46,47</sup> derived from pharyngeal mesoderm. Whereas the pharyngeal walls develop pharyngeal pouches and gill slits, the floor develops endostyle specializations related to trapping food particles during filter feeding, as well as to hormonal and protective functions.

### The deuterostome roots of chordate characteristics

Work on hemichordates and echinoderms has informed our understanding of ancestral deuterostome features, with different taxa contributing complementary insights. Integrating insights from echinoderms is challenging owing to the divergent radial body plan of adults, although studies of echinoderm larval development have made essential contributions to our understanding of early deuterostome embryogenesis<sup>13,14,48,49</sup>. Pterobranch hemichordates are relatively understudied<sup>50</sup>. In this Review, therefore, we focus primarily on insights derived from the study of enteropneust hemichordates (acorn worms) as they relate to our understanding of early deuterostome evolution.

As first described by Kowalevsky<sup>2</sup>, the anterior gut of hemichordates is perforated in the dorsolateral region by a series of ciliated gill slits, now known to be supported by gill bars composed of an acellular collagen secreted by the endoderm (Fig. 2a, Box 1)<sup>46</sup>. Although there is no equivalent structure in extant echinoderms, fossils reveal compelling evidence that gill slits were present in stem echinoderms and subsequently lost<sup>51</sup>. On the basis of morphological and functional criteria, enteropneust gill slits closely resemble those of cephalochordates and are plausibly homologous<sup>1,46,52</sup>. In two species of enteropneust, studies of patterning genes with conserved roles in chordate gill-pouch development, namely *pax1/9*, *foxC*, *foxL1*, *eya*, *six1* and *foxI*, also strongly support homology<sup>53–55</sup>.

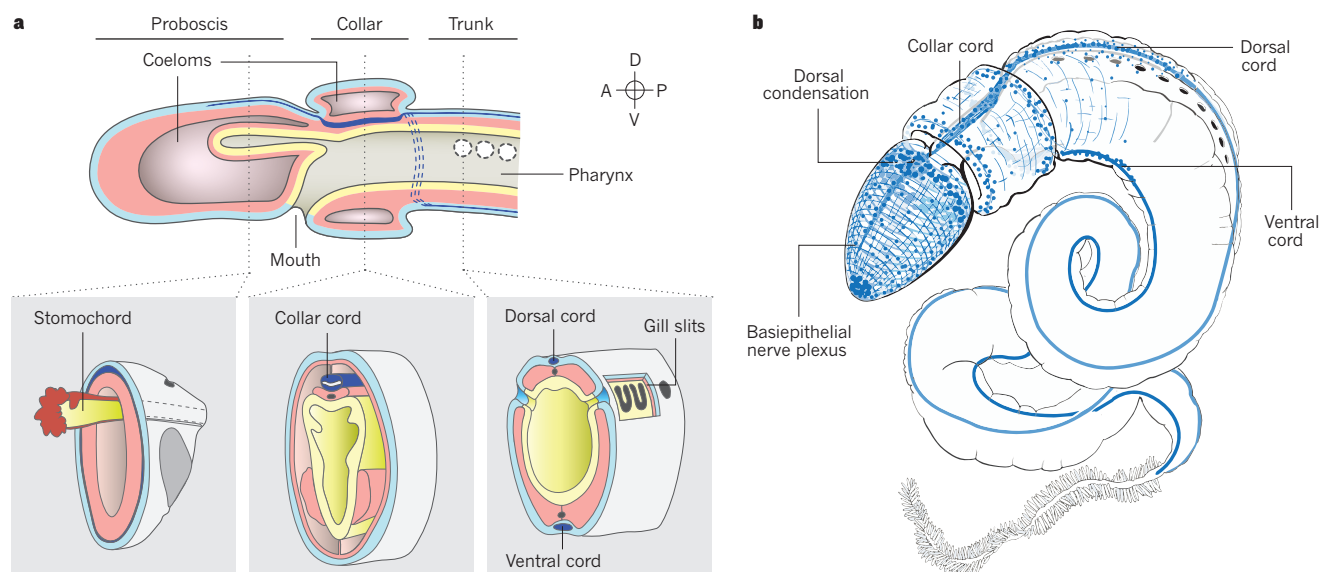
The stomochord in hemichordates has drawn much comparative interest as a notochord-like ancestral trait<sup>1,29,56,57</sup>. It is a diverticulum of



**Figure 1 | Deuterostome phylogeny.** A consensus cladogram of deuterostome groups based on recent phylogenomic data sets<sup>21,22,24,28,113</sup>. There are three major phyla of extant deuterostomes, which are grouped into two diverse clades: the ambulacrarian phyla (green), consisting of hemichordates

and echinoderms, and chordates (blue), consisting of the cephalochordate, tunicate and vertebrate lineages. Recent analyses have proposed either a grouping of xenoturbellid and acoelomorph flatworms as sister group to ambulacrarians<sup>27</sup>, or at the base of the bilaterians<sup>28</sup> (dashed lines).





**Figure 2 | Key anatomical features of the enteropneust body plan.**  
**a**, Longitudinal and transverse sections through an adult enteropneust hemichordate, highlighting morphological characters that have featured prominently in classic hypotheses of deuterostome evolution and chordate

the anterior gut that extends into the posterior proboscis supporting the heart–kidney complex on its dorsal surface (Fig. 2a, Box 1). Stomochord cells are vacuolated and surrounded by a sheath, similar in tissue organization to a notochord<sup>1,56,58</sup>. However, homology of these two structures is weakly supported by both morphological and molecular criteria<sup>59–61</sup>. In chordates the developing notochord is a key source of the secreted BMP antagonists Chordin, Noggin and Follistatin, and the ventralizing ligand Shh<sup>62</sup>. Of these genetic markers, only *hh* (the homologue of *Shh*) is expressed in the stomochord, but it is also observed in surrounding anterior endoderm<sup>57,63</sup>. Possible alternatives to notochord homology are suggested by the stomochord expression of genes such as *otx*, *dmbx*, *hex* and *foxE* that are expressed in prechordal endomesoderm of chordates, but not in the notochord. These markers suggest that the stomochord is an anterior endodermal structure with stronger affinities to the endostyle than the notochord<sup>61</sup>.

The hemichordate nervous system is characterized by two contrasting organizational features (Fig. 2b): a broad basiepithelial plexus, particularly prominent in proboscis ectoderm, and a pair of nerve cords. The ventral cord extends the length of the trunk and the dorsal cord runs from the base of the proboscis down the length of the animal and joins to the ventral cord by lateral nerve rings. Both cords are superficial condensations of the nerve plexus except in a short length that spans the collar, where the cord is internalized into a tube with a prominent lumen in some species, and is formed by a developmental process that resembles chordate neurulation<sup>59,64–67</sup>. Various authors have proposed both cords as possible homologues of the chordate dorsal cord<sup>57,59,68,69</sup>, however, the internalized collar cord has attracted the most attention<sup>6,57,59,69</sup>. Early reports suggested that the dorsal cord was simply a through conduction tract of axons<sup>70,71</sup>. Molecular studies, however, have shown condensations of cell bodies associated with this cord<sup>6,69</sup>, and a further study in *Balanoglossus simodensis* revealed *bmp2/4*, *pax3/7* and *msx* expression in the collar cord<sup>57</sup>, similar to that of the most lateral parts of the vertebrate neural plate and in other bilaterians during CNS development. Although these similarities are supportive of homology of the collar cord and chordate nerve cord, other neural molecular markers complicate this interpretation. In *Saccoglossus kowalevskii*, markers of medial rather than lateral neural plate are not expressed in the dorsal cord as predicted, but rather along the ventral midline associated with the ventral cord. In addition, several neural markers are not only expressed in the collar cord, but also throughout the length of the superficial cord in the trunk, suggesting a patterning role

origins. A, anterior; P, posterior; D, dorsal; V, ventral. **b**, The nervous system of an adult enteropneust showing both the broad basiepithelial plexus throughout the ectoderm and nerve chords along the dorsal and ventral midlines. The blue spots represent cell bodies and the lines represent neural processes.

throughout the dorsal midline<sup>72</sup>. When considering the general organization of the nervous system in enteropneusts, no simple homology statements can yet be made in relation to other nervous systems.

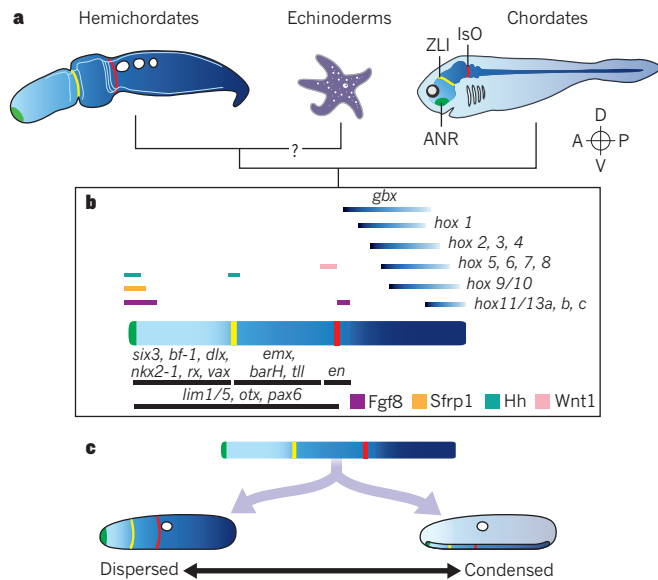
Although it seems likely that ancestral deuterostomes inherited some elements of nervous system centralization from the bilaterian common ancestor, a comprehensive characterization of key molecular markers is needed to test competing hypotheses of nervous system evolution further. It remains unclear whether the main features of the unusual enteropneust nervous system can be ascribed to the filter-feeding deuterostome ancestor, thereafter modified in the chordate line, or whether they are secondary derivatives of the hemichordate lineage.

### Axial patterning of deuterostome body plans

The discovery of conserved, pan-bilaterian mechanisms for the development of the animal–vegetal, AP and DV body axes has transformed our thinking about animal evolution<sup>4,5,73</sup>. This deep conservation initially surprised biologists because of the great morphological diversity of bilaterians, but made more sense when it was realized that the early axiation processes of the embryo are separate from the later processes of morphogenesis, organogenesis and cell differentiation. Conserved suites of genes are responsible for establishing basic regional differences of cells along all three axes of bilaterian embryos, reflecting an extensive genetic regulatory network spread across the developing embryo. The resulting map of conserved expression domains represents an ‘invisible anatomy’<sup>74</sup> that reveals clear relationships between disparate body plans, and provides a window into the organization of expression domains in the deuterostome ancestor. In this Review, we focus on the mechanisms by which these axes are formed in deuterostomes, and the patterns of transcription-factor and signalling-gene-expression domains produced along these axes.

### The animal–vegetal axis and formation of endomesoderm

One of the first developmental decisions in embryogenesis is the establishment of the animal–vegetal axis. This axis sets up the formation of the three germ layers: endoderm, mesoderm and ectoderm. Ectoderm derives from the animal pole, and endomesoderm from the vegetal pole, which later divides into endoderm and mesoderm. In all three major deuterostome phyla, the formation of endomesoderm is triggered by  $\beta$ -catenin protein, the intracellular effector of the canonical Wnt signalling pathway.  $\beta$ -Catenin is stabilized preferentially in the vegetal pole of early embryos and activates genes of the endomesodermal cellular program<sup>75,76</sup>.



**Figure 3 | A conserved molecular network for the deuterostome anteroposterior axis.** **a**, Schematic representation of the distribution of ectodermal expression domains of anteroposterior (AP) transcription factors (blue gradient) and ectodermal signalling centres (green, yellow and red) in relation to the body plans of deuterostome phyla. Chordate neuroectodermal signalling centres depicted are the anterior neural ridge (ANR), zona limitans intrathalamica (ZLI) and isthmic organizer (IsO). Broad conservation of expression domains between hemichordates and chordates allows for the reconstruction of an ancestral patterning network, which is shown without any explicit inference of ancestral morphologies (**b**). Insufficient data exist from echinoderms to infer to what extent they share this conserved AP patterning network during adult patterning, although much of the anterior network is conserved in larvae<sup>13,49</sup>. **b**, Domain map for the conserved transcription factors and signalling ligands in relation to the AP axis<sup>63,85,86</sup>. **c**, Current data allow for the reconstruction of a conserved molecular coordinate system for the AP axis of the last common deuterostome ancestor, but not for the reconstruction of discrete morphologies of that ancestor, because this AP patterning network is deployed in a variety of morphological contexts, as evidenced by comparative data from hemichordates (dispersed; AP expression domains encircling the body) and chordates (condensed; AP domains largely restricted to regions near the dorsal midline). A, anterior; P, posterior; D, dorsal; V, ventral.

In hemichordates and echinoderms, knockdown of the gene that encodes  $\beta$ -catenin protein results in the ‘animalization’ of the embryo — excess ectoderm and no endomesoderm. Conversely, stabilization of the protein throughout the embryo results in ‘vegetalization’ of the embryo — excess endomesoderm and no ectoderm. This mechanism has also been demonstrated in protostomes from work on nemertine embryos<sup>77</sup>, and  $\beta$ -catenin protein is also involved in endoderm formation in cnidarians<sup>78</sup>, suggesting a deep eumetazoan ancestry for this process<sup>73</sup>.

Later specification of mesoderm from the endomesoderm occurs by either of two generic mechanisms: autonomous specification by a cell’s inheritance of a sequestered cytoplasmic determinant, or induction by a signal from neighbouring tissue. In all deuterostomes except ascidians, mesoderm formation occurs by induction. In vertebrates, two main signalling pathways are involved in mesoderm specification: Nodal and FGF<sup>79</sup>. In amphioxus, FGF signalling specifies anterior mesoderm that forms by enterocoely<sup>42</sup>. Similarly, in the hemichordate *S. kowalevskii*, FGF signalling induces mesoderm and enterocoely, which raises the possibility of an ancestral role of FGF in deuterostome mesoderm formation<sup>80</sup>. As a classic deuterostome character, a mechanistic link of enterocoely to FGF signalling would support homology of this trait, at least within the deuterostomes. In echinoderms, however, the role of FGF has yet to be fully characterized, and there is some variation in inductive cues involved in mesoderm specification: Notch–Delta signalling is important in early mesoderm specification of echinoids, but not asteroids<sup>81</sup>. The differences

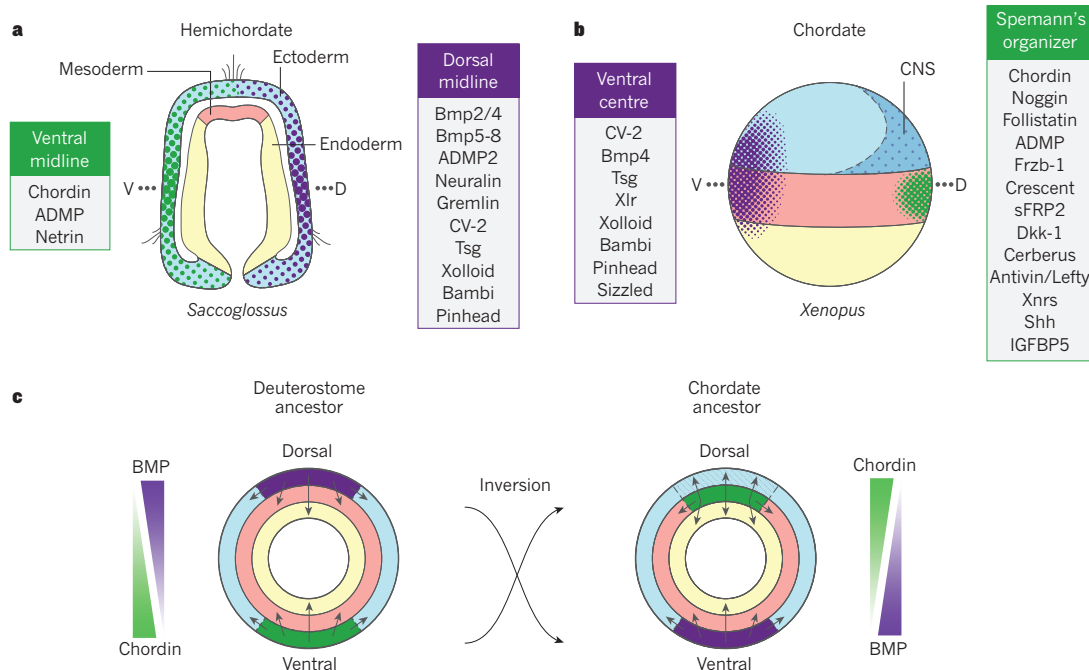
between deuterostomes in specifying endomesoderm and mesoderm preclude the definitive inference of the pathway of the deuterostome ancestor, except that  $\beta$ -catenin protein is required at the start, and various inductive signals are required later.

### Anteroposterior axis

Although deuterostome taxa show an impressive array of morphologies, organs and cell types along the AP axis, many of the early developmental steps of axis formation are highly conserved and probably date back to the bilaterian ancestor. Wnt signalling through  $\beta$ -catenin has emerged as the earliest conserved determinant of AP pattern in deuterostomes. (Note that this time and place of usage of  $\beta$ -catenin is separate from its role in endomesoderm formation discussed earlier.) In vertebrates, Wnt proteins act as posteriorizing signals in all three germ layers, but are most analysed in CNS patterning<sup>82,83</sup>. Whereas Wnts are produced posteriorly, Wnt antagonists are produced anteriorly from the mesoderm of Spemann’s organizer, and their interaction sets up a graded Wnt distribution prefiguring the eventual anatomical AP axis<sup>84</sup>. In both sea urchin larvae and the direct-developing *S. kowalevskii*, Wnt signalling is also important for establishing AP patterning<sup>48,63,75</sup>, suggesting that generating a Wnt signalling gradient (high posteriorly, low anteriorly) is a key step in AP-axis formation in all three phyla, for both adult and larval body plans. Different intensities of Wnt signalling along the graded distribution then activate distinct genes encoding different transcription factors and signalling ligands, producing a long-lasting AP map of gene expression domains that is collinear with the Wnt distribution. The ectodermal map is strikingly similar in the identity and relative expression of the constituent regulatory genes across bilaterians<sup>85,86</sup>.

This conserved AP map provides a novel basis for comparing body plans (Fig. 3a, b)<sup>74</sup>. In the most anterior regions, coexpression of genes such as *sfrp1/5*, *fgf8/17/18*, *foxG*, *retinal homeobox*, *dlx* and *nk2-1* define ectodermal territories that later form proboscis ectoderm in hemichordates and forebrain in vertebrates. Further posteriorly, expression domains of *emx*, *barH*, *dmbx* and *pax6* define the collar ectoderm of hemichordates and midbrain of vertebrates; still more posteriorly, domains of *gbx*, *engrailed*, *pax2/5/8* and the collinearly expressed Hox genes, regulate pharynx and trunk patterning of hemichordates and the hindbrain and spinal cord in vertebrates (Fig. 3a)<sup>63,85,86</sup>. Enteropneust Hox genes are organized as an intact cluster<sup>87</sup>, and in both cases the posterior group Hox genes are expressed in post-anal parts of the body axis, perhaps indicating domain-level homology of these deuterostomian posterior appendages. AP map similarities even extend to three signalling centres, producing the same signals and occupying equivalent map positions, that are important for vertebrate brain patterning and for hemichordate ectodermal development at the anterior tip, proboscis–collar boundary and collar–trunk boundary (Fig. 3a, b) (for an alternative perspective see ref. 88). In hemichordates, the conserved AP map of ectodermal expression domains covers both neural and epidermal tissue, and domains encircle the body. In chordates most comparative studies have focused on the role of this network in patterning the dorsal CNS, but more recent studies demonstrate that expression of many of the genes extend ventrally into sensory neurons and epidermis, suggesting a more general role in ectodermal patterning<sup>88</sup>.

The AP map of expression domains provides a positional criterion for evaluating morphological homologies between disparate body plans. Thus, the homology of chordate and hemichordate gill slits is supported by the observation that in both groups the first slit perforates the same region of the AP expression map, near the midbrain–hindbrain boundary in chordates and the collar–trunk boundary in enteropneusts. The map also provokes comparisons: if the hemichordate collar cord is homologous to the chordate dorsal nerve cord, it should express AP genes similar to those of the chordate midbrain. We can confidently reconstruct this AP patterning network in the ectoderm of the deuterostome ancestor, and as previously mentioned, much of the map probably dates back to the bilaterian ancestor. Indeed, more comparisons with protostomes are needed to illuminate which few domains are deuterostome-unique, for example,



**Figure 4 | Comparison of the dorsoventral patterning mechanisms of hemichordates and chordates.** **a**, BMP–Chordin signalling components expressed in the dorsal and ventral midline ectoderm (blue) in the late gastrula stage of *Saccoglossus kowalevskii*. **b**, BMP–Chordin signalling components expressed either on the ventral side or dorsally in Spemann's

organizer in the early gastrula of *Xenopus*. CNS, central nervous system. **c**, The inversion of dorsoventral (DV) signalling centres and the relocation of the Chordin source from the ectoderm (yellow) to mesoderm (red) were innovations in DV patterning at the base of the chordates (ancestral location shown by grey shading).

in pharyngeal ectoderm and endoderm.

The AP axial homology of chordates and hemichordates with echinoderms is far less clear<sup>89–93</sup>. During the development of the larvae of asteroids, echinoids and crinoids (Box 2), anterior regulatory genes are expressed throughout the anterior ectoderm<sup>49,94</sup>, whereas posterior markers such as Hox genes are entirely absent during early patterning. In both echinoids and crinoids, it is not until adult patterning begins in the late larva that Hox genes begin expression in a collinear pattern, not in ectoderm, but in posterior coelomic mesoderm<sup>90,95</sup>. Some anterior markers are expressed in the oral ectoderm and tube feet of pentaradial adults, but current data are too fragmentary to make valid comparisons of adult echinoderms with other deuterostome adults. Comprehensive characterization of the patterning of echinoderm adults is badly needed to unravel the evolution of this unusual body plan.

Although the AP map is conserved across deuterostomes (and in most aspects, across bilaterians) the differentiated morphologies built on it are probably not (Fig. 3c). The morphological outcomes of development differ in each phylum because the transcription factors and signals of the conserved map activate and repress different target genes<sup>63</sup>. These target genes, in turn, direct the final steps of organogenesis, morphogenesis and cell-type formation.

### The dorsoventral dimension

The DV axis evolved on the Pre-Cambrian stem leading to the bilaterian ancestor, and is intimately tied with the origin of bilateral symmetry. Its formation in early embryogenesis is analogous to AP axis formation. One midline of the embryo produces Bmp, and the opposite midline produces the Bmp antagonist Chordin<sup>96</sup>. Through complex interactions, this antagonism generates a graded distribution of Bmp across the embryo, a graded occupancy of Bmp receptors, and a corresponding graded distribution of activated Smad1/5 transcription factor in embryonic cells. This gradient of activated Smad1/5 stimulates and represses different genes encoding transcription factors and other signalling ligands, generating a long-lasting DV map of expression domains of these genes<sup>5,97</sup> (Fig. 4a, b).

The patterns of transcription-factor and signalling-ligand expression established along the DV direction generate the corresponding anatomical

axis by driving the expression of genes for the development of different tissues and cell types in different regions. Some of the definitive tissues and cell types are remarkably conserved among bilaterians, as demonstrated by the similarities between the DV development of protostomes such as the fruit fly and the annelid *Platynereis dumerilii* and vertebrates such as *Xenopus*, mice and zebrafish<sup>97,98</sup>. Domains from the Chordin side of the Bmp distribution activate axial (striated) muscle development in the mesoderm and nerve-cell development in the ectoderm, especially motor neurons and interneurons that assemble into the CNS, whereas domains from the Bmp side activate heart tube and coelom development from the mesoderm and epidermis and sensory-nerve-cell development from the ectoderm<sup>97</sup>. The Bmp distribution patterns all three germ layers.

Although deuterostomes as a group inherited the basic mechanism of DV axis formation from the bilateral ancestor, there are important differences among them that can inform hypotheses of chordate origins. It is immediately apparent that the Chordin and Bmp sides of the molecular DV axis have different anatomical names in deuterostomes and protostomes. In deuterostomes, the Bmp side is called 'dorsal' and the Chordin side is 'ventral', but in *Drosophila* and other protostomes the molecular and anatomical links are reversed. By zoological convention, sides are named according to the animal's orientation to the substratum and the location of the mouth. The difference was resolved by the proposal that the chordate ancestor underwent a dorsoventral inversion of the body relative to the substratum. This transition simultaneously inverted the Bmp–Chordin axis, the domain map, and axis of anatomical differentiations<sup>5,96</sup>. As a final refinement the mouth was relocated to the Bmp side, whereas most protostomes (for example, *Drosophila*), and invertebrate deuterostomes, form the mouth on the Chordin side. Although seeming modest as a novelty, body inversion must be considered when discussing innovations of the chordate line.

*S. kowalevskii* provides an excellent example of bilaterian DV axiation, probably conserved from the deuterostome ancestor<sup>72</sup>. (Indirect developing hemichordates and echinoderms also exhibit Bmp–Chordin-based DV patterning, modified for larval body plans, although we cannot cover these here<sup>9,99</sup>.) At gastrula stages, *bmp2/4* is strongly expressed on the dorsal ectodermal midline of *S. kowalevskii*, accompanied by genes for a large

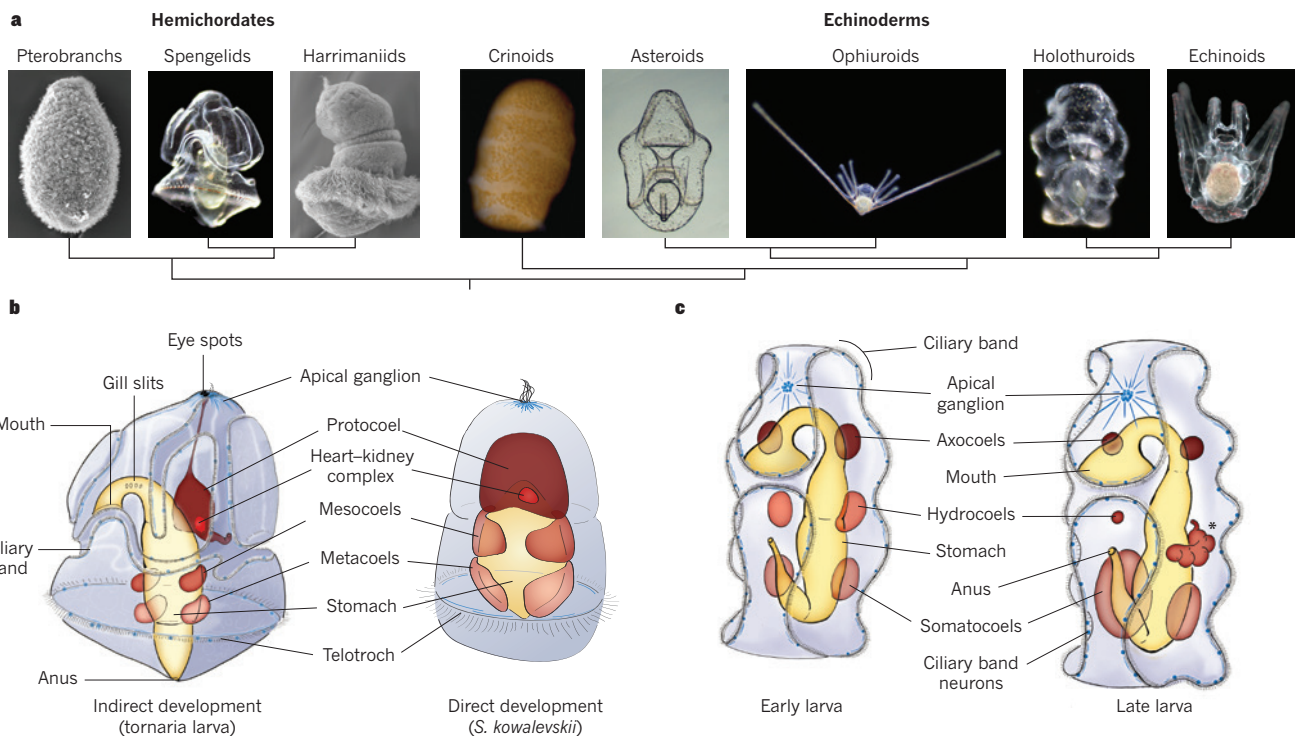


## BOX 2

## Deuterostome larval diversity

**a**, Hemichordates and echinoderms include lineages that are characterized by both direct-developers, forming the adult body plan from embryogenesis in a matter of days; and by indirect-developers, first forming planktonic feeding larvae that may swim and feed for months before metamorphosing to produce a distinct adult body plan. The harrimaniid enteropneusts and pterobranchs (reproduced from ref. 49) are examples of direct-developers. **b**, A model of a two-day-old embryo of *Saccoglossus kowalevskii* contrasts with the month-old late spengelid tornaria larva. **c**, The organization of the tornaria larval body plan is very similar to the organization of the echinoderm larva represented here by a holothuroid auricularian larva. However, echinoderms have a spectacular variety of larval forms from the ophiopluteus and echinopluteus with similar elaborate skeletons to the asteroid bipinnarian and holothuroid auricularian larvae with similar convoluted ciliary bands. Many researchers have focused on the morphological and developmental similarities between the diverse ambulacrarian larval types, suggesting the existence of an ancestral 'dipleurula' (small two-sided) larval form from which ambulacrarian larval diversity arose<sup>114</sup>. The dipleurula ectoderm is

characterized by a convoluted ciliary band used for swimming and feeding. In hemichordates, a robust additional posterior band of compound cilia, the telotroch, is purely locomotory (**a**, **b**). The nervous system is divided into two domains: an apical ganglion underlying the sensory ciliated apical organ, and neurons underlying the length of the ciliary bands (**b**, **c**). The dipleurula mesoderm is formed by enterocoely and organized into three compartments: anterior, middle and posterior. In echinoderms, the adult body plan is initiated by the left middle coelom, which expands and forms five lobes midway through larval development (**b**, asterisk in **c**). An influential theory of Garstang<sup>108</sup> further elaborated by a variety of authors (see Review by Holland *et al.* on page 450), proposed that the deuterostome ancestor also had a dipleurula larva, and that chordates evolved by paedomorphosis from such forms. A central tenet of this theory is that the dorsal central nervous system of chordates evolved through the dorsal migration and fusion of the lateral ciliary bands of the dipleurula larvae, and their underlying neurons<sup>114</sup>. More recently, this hypothesis has fallen out of favour on the basis of both phylogenetic and body-patterning data<sup>21,24,109</sup>.



set of signal modulating proteins and other Bmp-related proteins. Conversely, *chordin* and *admp* are strongly expressed on the opposite, ventral midline (Fig. 4a). Following the Bmp distribution gradient, transcription-factor genes are activated in a DV map that generally parallels the expression of orthologous genes in *Drosophila* and vertebrates. The DV domain map and subsequent differentiated structures of the overt anatomical axis depend entirely on the Bmp distribution, as shown by the development of dorsialized embryos in the presence of excess uniform Bmp2/4 protein, and of ventralized embryos when Bmp2/4 is eliminated<sup>72</sup>. Tissues, organs and cell types of the three germ layers are patterned by the Bmp–Chordin distribution, including the gill slits, the mouth and the two nerve cords (Fig. 2a). In embryos dorsialized by excessive Bmp, nerve cells still form in abundance. Although this might seem contrary to chordate neural

patterning in which Bmp initially represses neural development in the epidermis, it is not; the hemichordate dorsal nerve cord normally forms at the midline of high Bmp concentration, and the lateral parts of the chordate neural plate are themselves patterned by high Bmp concentrations. Overall, the hemichordate findings affirm general insights about bilaterian DV axis formation. In its body orientation, *S. kowalevskii* resembles protostomes: Bmp foretells the ventral side and Chordin the dorsal, leaving chordates as the single 'inverted' phylum (Fig. 4c). One of the key questions about chordate origins remains the evolution of the dorsal hollow nerve cord from the nervous system of a less centralized ancestor with little or no capacity for neurulation. In general, hypotheses imply that in the early embryo, the formation of neural ectoderm (prospective for motor neurons and interneurons) was increasingly repressed towards

one midline, and the neurulation process was induced along the edges of the narrowed neurectoderm territory. Thereafter Bmp exerted its neural patterning effects from the neural plate borders. This, of course, remains an area for future investigation<sup>6,68,100</sup>.

Chordates differ from hemichordates in that Chordin and other Bmp antagonists are produced mostly in midline mesoderm, and specifically in mesoderm of Spemann's organizer, a region formed in the late blastula embryo at a location of high Nodal signalling and low Bmp signalling<sup>101</sup>. Organizer cells are precursors of the notochord and head mesoderm. Notochord precursors undergo extreme convergent extension by cell intercalation, forcefully repacking a cube of cells into a rod one-cell wide and lengthening the embryonic midline. Simultaneously they secrete their dorsoventral patterning molecules, neuralizing nearly half the embryo's overlying ectoderm and initiating neurulation morphogenesis. In chordates, neurogenic ectoderm produces little or no Bmp antagonist, whereas in hemichordates it is the main source. Recently, a possible notochord homologue, the axochord, was described for the polychaete annelid *P. dumerilii*; it is a midline mesodermal structure of muscle cells contained in a strong sheath into which lateral muscles attach, but there is no evidence that it is a notochord-like signalling source<sup>102</sup>. Rather, the midline signalling source of this protostome is presumably nearby neural ectoderm that determines, among other things, where the axochord itself develops.

Taken together, these data suggest that hemichordates are like protostomes in their dorsoventral development, whereas chordates have considerably modified the ancestral patterning mode (Fig. 4c), by adding organizer mesoderm as the Chordin source, and acquiring a large-scale neuralization response in the ectoderm. These innovations in DV axisiation must be considered in any discussion of chordate origins.

### Gill slits are a deuterostome innovation

The single unambiguous anatomical homology that is a clear deuterostome synapomorphy is the pharyngeal gill-slit complex<sup>6,46,53,55,60,103</sup>. These perforations of pharyngeal endoderm and ectoderm, ringed by beating cilia, imply that the ancestor fed by ingesting food particles carried by water flow entering the mouth and exiting the slits. The complex, which can include more than a hundred (bilaterally symmetrical) gill-slit pairs, is a major developmental and morphological modification beyond the bilaterian ancestor's pharynx, although presumably elaborated from it. Although hemichordates do not have a well-defined pharyngeal endostyle like chordates, the pharynx as a whole, and even the proboscis, probably makes endostyle-like mucociliary contributions to food trapping and conveyance to the gut<sup>52,60</sup>. Some of these functions may be deuterostome synapomorphies. To coordinate the functions of gill-slit-mediated water propulsion, food intake, trapping and conveyance, the pharyngeal nervous system is likely to have become modified from that of the bilateral ancestor. Given that pharyngeal innovations may represent the signature morphological, developmental and genomic innovations of deuterostomes, their development and physiology should be characterized more comprehensively.

Among extant animals, the filter-feeding lifestyle correlates with simplified body plans — radialized dorsoventral dimensions, more dispersed nervous systems, less cephalization of sensory systems, and less motility by trunk and tail axial muscles — when compared with extant food-seeking or predatory arthropods, annelids and jawed vertebrates. Such simplifications are presumably anatomical or physiological adaptations that benefit gill-slit-mediated filter feeding, and it seems plausible that evolution along the deuterostome stem involved considerable morphological modifications relative to earliest bilaterian body plans. If true, it is nonetheless apparent from AP and DV domain maps that the deuterostome ancestor suffered no concomitant loss of body-plan complexity at the molecular genetic developmental level. Rather, it shows that bilaterian domain maps are remarkably stable and can support wide-ranging morphologies, organogenesis and cytodifferentiations. An example of such modification is the muscular proboscis of hemichordates. The proboscis is used to dig and to trap food, while containing most of the conserved basic patterning elements of the

vertebrate forebrain, here spread over a basiepithelial nerve plexus<sup>63,85</sup>. There would be no intrinsic reason for the deuterostome ancestor to preserve the morphology and differentiations of the bilaterian ancestor if it no longer lived that ancestor's lifestyle. Finally, there is some palaeontological support for a filter-feeding deuterostome ancestor. On the basis of molecular clock estimates, deuterostome phyla would have diverged in the Ediacaran period, well before the Cambrian explosion. The lack of an obvious fossil record, except for small Precambrian trace fossils and the enigmatic Ediacaran fossils, and evidence of abundant filterable food sources in the form of microbial mats and plankton, suggest that bilaterians of that time were probably small and simple filter feeders<sup>104–106</sup>.

This interpretation of the deuterostome ancestor has important consequences for the origin of chordates. Relative to that ancestor, the chordate stem lineage achieved major developmental and morphological innovations, including the evolution of a true notochord from the archenteron roof, centralizing many morphogenetic activities of the ancestral archenteron and taking over the signalling activities of the ancestral ectoderm for both AP and DV axial patterning (by producing Bmp and Wnt antagonists), to become the centrepiece of Spemann's organizer. Concomitantly, the innovations of neural induction (neuralization) and full-length neurulation of the ectoderm generated a hollow nerve cord along the entire body length (a length now defined by the elongating notochord), rather than just the short and late collar cord neurulation of hemichordates (although this limited neurulation shows that the ancestor possessed the basic morphogenetic process and components). At some point later, dorsoventral inversion of the chordate body took place, with mouth relocation out of the neural ectoderm<sup>107</sup>. All of this occurred on the chordate stem, perhaps after the elimination of an ambulacrarian-type larva, to open up uninterrupted embryonic development of the adult body plan (see ref. 100 for further elaboration of this hypothesis).

### Future directions

Insights into deuterostome evolution are emerging from research in developmental biology, phylogenomics, genomics and zoology. A particular focus has been the pharyngeal gill-slit complex, which is supported as an ancestral deuterostome feature by strong morphological and developmental data. The implication that the deuterostome ancestor was a filter feeder naturally draws attention to other integrated pharyngeal specializations, including endostyle-like food-trapping organs. Further study of these organs, especially in amphioxus and hemichordates, has the immediate potential to reveal clues about deuterostome and chordate origins.

One of the most important differences between hemichordates and chordates, revealed by comparative developmental studies, is the source of Bmp antagonists involved in establishing DV axial polarity in early development. These antagonists are expressed in the ectoderm of hemichordates and the mesoderm of the chordate organizer. Spemann's organizer is a key chordate developmental innovation defined by various secreted factors modulating Bmp, Nodal and Wnt signalling. A more comprehensive description of the roles of these signals and their antagonists in the patterning of hemichordate mesoderm and ectoderm will be required to devise and test hypotheses about the evolution of the chordate organizer.

Most developmental insights from hemichordates have so far come from studies of direct developing hemichordates, but a distinct larval life-history stage is probably an ancestral trait of Ambulacraria and perhaps of deuterostomes (Box 2). More comprehensive developmental studies in indirect-developing echinoderms and hemichordates, with distinct larval body plans, are needed to determine the importance of complex life cycles and the role of larvae in the early diversification of deuterostome body plans. Garstang's influential auricularian hypothesis derived the chordate body plan from an ancestral larval body plan<sup>108</sup>, but this hypothesis has recently lost support due to revisions in chordate phylogeny and close similarities between adult rather than larval body patterning<sup>109</sup>. Comparative data sets on larval patterning will be key for reconstructing ancestral developmental strategies of early



deuterostomes and testing hypotheses of larval homology.

Finally, advances in genomics have begun to shed light on the gene content and chromosomal organization of invertebrate deuterostomes, including the purple sea urchin (*Strongylocentrotus purpuratus*)<sup>110</sup>, the acorn worms *S. kowalevskii* and *Ptychodera flava*<sup>87,111</sup>, and the crown-of-thorns sea star (*Acanthaster planci*)<sup>112</sup>. Given the apparent conservation of not only the pan-deuterostome axial maps but also many of the downstream factors that control organogenesis, it will be exciting to explore the gene-regulatory elements that underlie this deep conservation through a combination of comparative genomics and experimental developmental biology, revealing features of the ancestral deuterostome down to the nucleotide level. ■

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