Scenarios for the making of vertebrates

Nicholas D. Holland[1], Linda Z. Holland[1] & Peter W. H. Holland[2]

Over the past 200 years, almost every invertebrate phylum has been proposed as a starting point for evolving vertebrates. Most of these scenarios are outdated, but several are still seriously considered. The short-range transition from ancestral invertebrate chordates (similar to amphioxus and tunicates) to vertebrates is well accepted. However, longer-range transitions leading up to the invertebrate chordates themselves are more controversial. Opinion is divided between the annelid and the enteropneust scenarios, predicting, respectively, a complex or a simple ancestor for bilaterian animals. Deciding between these ideas will be facilitated by further comparative studies of multicellular animals, including enigmatic taxa such as xenacoelomorphs.

Biologists have considered nearly every major taxon of animals as the key starting point for the evolution of vertebrates. We survey these ideas, many of which are no longer tenable in the light of subsequent advances in biology, and then concentrate on the few scenarios that are currently the subject of major research programmes. Lamarck was the first to propose an evolutionary conversion from an invertebrate to a vertebrate. In 1809, he depicted a phylogenetic tree, including an invertebrate-to-vertebrate transition in which molluscs gave rise to fishes[1]. During the next few decades, several others speculated on how body plans of invertebrates and vertebrates might be related; however, those biologists were generally in search of an underlying unity of organismal design. Evolution was not explicitly mentioned by key figures such as Geoffroy Saint-Hilaire[2], although one senses that he was on the verge of believing in it.

Aside from Lamarck's proposal, explicitly evolutionary schemes that derived vertebrates from invertebrates started appearing only after the publication of *On the Origin of Species* in 1859. In general, the scenarios were based on the morphology of developmental stages and adults of extant animals. Palaeontological evidence was considered less often[3–5], and molecular evidence was not widely considered until the 1980s with the advent of molecular phylogenetics and evolutionary developmental biology.

In Fig. 1, scenarios for the origin of vertebrates are arranged on a timeline extending from the publication of *On the Origin of Species* to the present. The references are broadly divided into those focused on larval type and those concerned with adults — a dichotomy reflecting two opposing views of life-history evolution. The first considers pelagic larvae as primal with benthic stages added later, and the second considers benthic stages as primitive with pelagic larvae interpolated later. Classification of the scenarios is not straightforward because relatively few proposed a linear ancestor–descendant relationship. More commonly, they were presented in the context of branching, sister-group relationships. For example, if enteropneusts were considered the sister group of the chordates (as in Fig. 2a), the ancestral node is often referred to as enteropneust-like. Finally, when a given scheme involves an evolutionary pathway through several major taxa to the vertebrates, the scenario is named for the invertebrate group receiving the most attention from the original author.

Scenarios currently the subject of active research

Contemporary research on the origin of vertebrates from invertebrates falls into two broad categories: the short-range transition from invertebrate chordates (amphioxus-like and tunicate-like ancestors) to vertebrates, and longer-range transitions from the base of bilaterally symmetrical animals or from the base of deuterostomes to vertebrates. Scenarios starting with invertebrate chordates are less controversial than the two long-range scenarios being actively studied: the annelid and the enteropneust theory.

Invertebrate chordate to vertebrate transition

The nearest relatives of the vertebrates are the invertebrate chordates, although it is still not settled whether chordate evolution should be considered from the viewpoint of larvae being primal[6] or larvae being interpolations[7,8]. Although invertebrate chordate scenarios ignore the deeper history of the vertebrate lineage, they still centre on events initiated more than 500 million years ago and involve remarkable evolutionary changes that are considered in companion reviews in this issue. Recently, the major chordate taxa were rearranged (Fig. 2b) on the basis of morphology and molecular phylogenetics, which have decisively shown that amphioxus is the sister group to tunicates and vertebrates[9,10]. The new arrangement implies that the tunicates have secondarily lost segmentation, coeloms and kidneys, but are vertebrate-like in features such as intercellular tight junctions, proto-neural crest, striated heart muscles, proto-placode derivatives and voluminous blood plasma with abundant circulating corpuscles.

The annelid theory

The first of the two long-range scenarios is the annelid theory. When initially published 140 years ago, it proposed a direct conversion of annelid worms into vertebrates[11,12]. Now, however, the starting point is often considered to be an annelid-like urbilaterian[13] (Fig. 2a, b). The annelid theory has its roots in arthropod biology, because these two groups were long considered to be very close relatives (Fig. 2a), and results for one were generally considered to be valid for the other.

In the original annelid scenario, Dohrn[11] started with a worm that inverted the body on the way to evolving into a vertebrate, thus positioning the old mouth on the top of the head and necessitating the formation of a new mouth on the ventral side of the body; thereafter, the old mouth disappeared, while the new one persisted (Fig. 3a–c). Several of Dohrn's colleagues modified his scenario in attempts to improve it[14–21], but the theory went into eclipse early in the twentieth century when the bilaterian animals were rearranged into two superphyla — the protostomes and the

[1]Marine Biology Research Division, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California 92093, USA. [2]Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK.
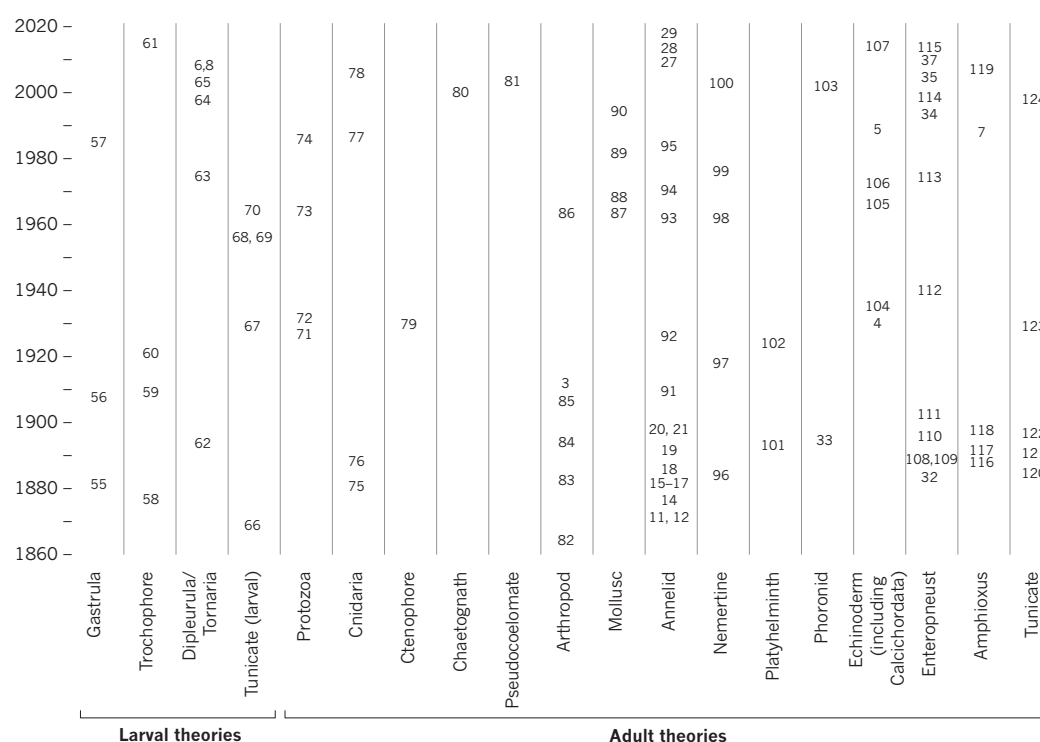
Figure 1 | Scenarios for the invertebrate-to-vertebrate transition. Each scenario is categorized according to the larval type or to the taxon of adult invertebrate proposed as ancestral to the vertebrates[3–8,11,12,14–21,27–29,32–35,37,55–124]. For prolix authors, only their most inclusive publications are given. Also omitted are references (typically textbooks) that repeat previous ideas without adding new information. References to problematic fossil ancestors of vertebrates are not included (except calcichordates, which are considered to be echinoderms here).

deuterostomes. The resulting relocation of annelids and arthropods at a considerable phylogenetic distance from the vertebrates (Fig. 2a) weakened the idea of a complex urbilaterian and shifted opinion towards a simple urbilaterian, which was imagined to be rather like an acoel flatworm that independently gave rise to annelids and vertebrates with their complex, but only superficially similar, body plans.

In the 1990s, advances in developmental genetics — again with arthropods leading the way — set the stage for the revival of the annelid theory. The fly *dpp* gene was found to be expressed dorsally and to have dorsalizing activity, whereas the homologous frog *bmp4* was expressed ventrally and found to have ventralizing activity[22]. Arendt and Nübler-Jung interpreted this pattern as support for homology between arthropod and vertebrate nerve cords and indicative of a dorsoventral inversion of the body during the invertebrate-to-vertebrate transition[23]. The proposed nerve-cord homology was strengthened by the discovery that the fly *sog* gene was expressed ventrally and had ventralizing activity, whereas the homologous frog *chordin* gene was expressed dorsally and had dorsalizing activity. In addition, *sog/chordin* and *dpp/bmp4* antagonized one another to establish a dorsoventral axis that was reversed between flies and frogs[24]. Additional support came from the finding that neural progenitor cells in the central nervous system (CNS) were organized in longitudinal bands each characterized by a distinctive suite of gene expression that was homologous between flies and vertebrates, and that gene expression in these bands was comparable mediolaterally in both organisms[25].

The developmental genetic comparison between arthropods and vertebrates[22–25], reinforced by details from neurochemistry and neural circuitry, favoured the revival of the inverted annelid theory. Direct comparisons between annelids and vertebrates also revealed commonalities in anterior–posterior regionalization by Hox genes[26], genetic specification of several kinds of nerve cells[27,28] and the formation of notochord-like structures[29]. As already mentioned, the revived annelid scenario posits the evolution of an already complex urbilaterian ancestor into a vertebrate. Such a transition would be most parsimonious if it proceeded through consistently complex intermediates. However, some features, such as segmentation and a clearly centralized nerve cord, are absent from several taxa associated with the presumed evolutionary lineage that leads to the vertebrates, possibly due to secondary losses. Such losses would have occurred in echinoderms, at least some hemichordates and

xenacoelomorphs, although the deuterostome nature of the last has not yet been firmly established[30,31]. Continuity between annelid-like ancestors and vertebrates could be strengthened if complex, segmented fossils of basal deuterostomes were known. Although several such fossils have been proposed as ancestral deuterostomes (vetulicolians and *Herpetogaster*), their taxonomic affinities remain highly controversial.

The enteropneust theory

The second long-range scenario of vertebrate origins currently under active study is the enteropneust theory. These marine worms (Fig. 3d), characterized by three body regions (proboscis, collar and trunk), belong to the Hemichordata, a phylum that also includes the minute pterobranchs (Fig. 3e), which comprise a flattened oral shield corresponding to the enteropneust proboscis, a collar extending into tentacle-fringed arms and a trunk. According to the original enteropneust theory[32], proposed by Bateson in 1886, the body axis of enteropneusts was not inverted relative to that of vertebrates. For him, the stomochord (Fig. 3d) corresponded to a vertebrate notochord, the collar cord (which he considered dorsal) corresponded to the vertebrate CNS, and the pharyngeal gill slits in both groups were homologous. Such an enteropneust was much like a vertebrate except that it lacked segmented musculature along the anterior–posterior axis. At the time, Bateson was uncertain about the deeper evolutionary source of the enteropneusts, although he tentatively suggested that they might have evolved from nemerteans or even tunicates. However, at the close of the nineteenth century, Masterman[33] proposed what seemed to be a firmer connection between enteropneusts and the rest of the animal kingdom through relatively complex precursors — pterobranchs (already mentioned) and the worm-like phoronids, which live mostly buried, but extend their tentacle crown into the sea water.

Through much of the twentieth century, Bateson's hypothesis, although not universally accepted, persisted. This inactivity ended in 1996, when Nübler-Jung and Arendt made a striking alteration[34]. They proposed that enteropneusts had an annelid-like CNS comprising three contiguous nerve tracts (the collar cord, the circumenteric nerve ring and the trunk ventral nerve cord), all recognizable by their giant nerve fibres. Such an enteropneust (Fig. 3f) complemented their earlier revival of the annelid theory[23] by approximating an intermediate stage in the conversion of a complex urbilaterian into a vertebrate. Because this CNS was oriented
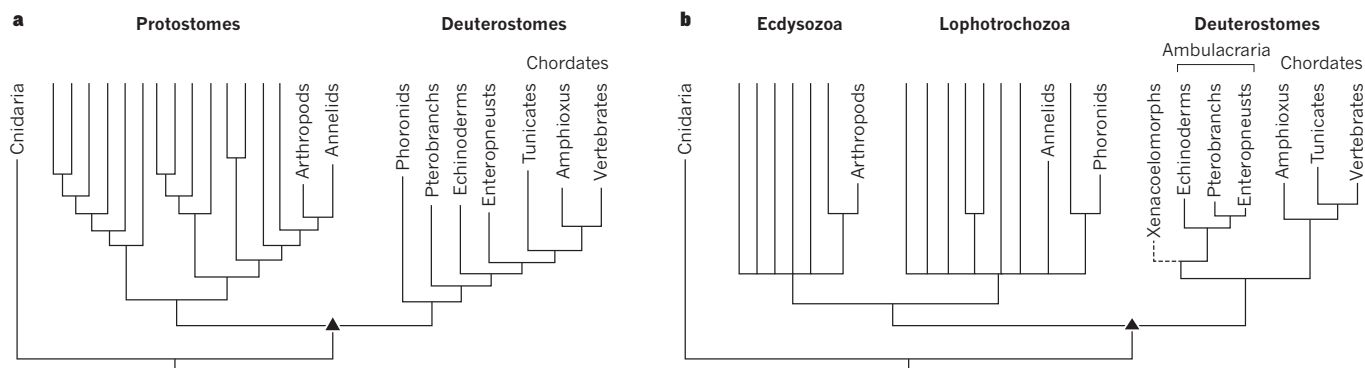
Figure 2 | Simplified trees of metazoan animal life. Taxa not mentioned in this Review are indicated by unlabelled branches (that are reduced in number and intended to be diagrammatic); the position of the Urbilateria is indicated by a triangle. **a**, Morphology-based tree[65]. **b**, Sequence-based tree[125]; the dashed line emphasizes the current uncertainty about the placement of the xenacoelomorphs.

as in annelids, the conversion into a vertebrate-like descendant (Fig. 3g) would require dorsoventral inversion, in contrast to Bateson's original scenario. While Nübler-Jung and Arendt were revising the enteropneust theory[34], molecular phylogenetics revealed that the relatively complex phoronids are neither deuterostomes nor their close relatives[30] (Fig. 2b). One interpretation of the new phylogeny was that the ancestors of the enteropneusts had relatively simple body plans — traceable back to an even simpler urbilaterian. The new phylogenetic arrangement triggered the definitive revival of the enteropneust theory that is still in progress.

The chief proponent of this newest revival of the enteropneust theory is Lowe, who gathered support for it with developmental genetic studies (see Review on page 456). He first considered a score of genes with homologues patterning the vertebrate CNS along its anterior–posterior axis[35]. Most of these enteropneust genes were expressed in the same anterior–posterior order as their homologues in the vertebrate CNS — but in annular bands of ectoderm and not in any tissue that might be interpreted as a CNS[35]. He concluded that the nervous system lacked any CNS component and consisted exclusively of an ectodermal nerve net. Although vertebrate homologues of many of the genes studied by Lowe help to establish borders separating neuronal populations in the vertebrate CNS[36], no corresponding neuroanatomical or neurophysiological discontinuities have yet been found in any enteropneust tissue. In Lowe's original scenario, the transition of enteropneust-like ancestors into vertebrates involved a loss of most of the ectodermal neurons, except along the midline of the body, where a CNS was elaborated. By similar, but independent paths, the dispersed nerve net of a structurally simple urbilaterian would have given rise to the complex CNS of annelids and arthropods.

Lowe subsequently studied the genes involved in establishing the dorsoventral axis of enteropneusts[37] and found that *BMP* and *chordin* were expressed, respectively, on the dorsal and ventral sides — if the body is assumed to be oriented similarly to annelids and arthropods. However, unlike the situation in amphioxus and vertebrates, upregulation experiments failed to alter neuron distribution, although some non-neural structures (the mouth, for example) were repositioned as expected. These results suggested that the *BMP–chordin* axis initially patterned exclusively non-neural structures and only later in evolution became linked to positioning neurons. This linkage to neural development was thought to have occurred independently in annelids, arthropods and vertebrates. Lowe[37] considered, but initially rejected, the converse possibility: that the relation between dorsoventral signalling and nervous-system development was ancient and was secondarily lost in the lineage leading to the enteropneusts.

More recent work challenges one point in the revived enteropneust scenario: that no CNS is present. First, Nomaksteinsky *et al.*[38] suggested that the proboscis plexus, collar cord, circumenteric nerves, and trunk dorsal and ventral cords have some properties of a CNS — cell bodies of neurons are present and extend their neurites into an adjacent neuropil — and that the epidermis outside the nerve cords includes only widely scattered nerve cells representing a sparse peripheral nervous system instead of a nerve net. In addition, Cunningham and Casey[39] found enteropneust neuronal

marker genes expressed along both the dorsal and ventral cord of the trunk, which they too suggested might be parts of a CNS. Neither study could resolve the dorsoventral orientation of the enteropneust body. In an attempt to answer this question, the left–right asymmetry of *Nodal* gene expression was compared during development of several deuterostomes. Right-sided expression in echinoderms and enteropneusts contrasted with left-sided expression in vertebrates, indicating that the dorsoventral axis of vertebrates is indeed inverted relative to that of echinoderms and enteropneusts[40,41]. As a caveat, however, although *Nodal* is involved in establishing the left–right axes of echinoderms and vertebrates, it evidently has no comparable functional role in enteropneusts[42].

To complicate matters further, Miyamoto and Wada[43] found that the endoderm of the enteropneust stomochord and the roof of the buccal cavity are sources of Hedgehog signals that evidently induce and pattern the collar nerve cord. This parallels Hedgehog signalling from the notochord to the nascent neural tube during vertebrate development. Their data could be interpreted to mean that dorsoventral inversion did not take place during the enteropneust-to-vertebrate transition, that the stomochord is homologous to a notochord, and that the collar cord corresponds to at least part of the vertebrate CNS. These conclusions are close to those reached by Bateson in his original scenario[32], although Miyamoto and Wada acknowledge that co-option of gene networks cannot be ruled out. These disagreements about the enteropneust nervous system seem likely to be resolved by additional neuroanatomical studies. However, that would still leave the nature of the urbilaterian unsettled, which will be considered in the next section.

Progress, problems and prospects

At the end of an argumentative symposium on the origin of vertebrates a century ago[44], one participant summed up progress with the mischievous words: "When we return home and our friends gleefully enquire, 'What then has been decided as to the Origin of Vertebrates?', so far we seem to have no reply ready, except that the disputants agreed on one single point, namely, that their opponents were all in the wrong." Although prospects for solving the riddle of vertebrate origins at that time did not look good, there has been progress. In particular, we now know where vertebrates fit in the animal phylogenetic tree. This knowledge helps to refine the remaining questions. To start with, we can consider an evolutionary tree as including a nested series of ancestors, each defining a different node of the tree, progressively deeper in time. As we climb down the tree, back in time from the living vertebrates, we encounter each ancestral node in turn. As we proceed, we should not be asking what did the ancestor of vertebrates look like? But instead what did each successive ancestor of the vertebrates look like? This logic can be applied to the node-based ancestors, but we should remember that there must have been an unbroken, genealogically connected series of ancestors between each node that are all but invisible to comparative biology based on living taxa.

Logically, the most recent node-based ancestor of all living vertebrates was itself a vertebrate, and possessed characters shared by lampreys,

hagfish and jawed vertebrates. This animal, living more than half a billion years ago, had a well-developed head and brain, complex cranial sense organs, segmented musculature and a vertebral column (recently shown to be present but secondarily reduced in hagfish[45]), but no jaws or paired fins. The ancestor also probably shared the genome duplications that set vertebrates apart from other deuterostomes. The subsequent course of evolution in the vertebrates is considered in several companion Reviews in this Insight; however, here we are concerned with looking the other way — towards the invertebrate roots of the vertebrates.

The two closest lineages to the vertebrates are the tunicates and the cephalochordates (such as amphioxus). Like vertebrates, both are chordates. The chordate ancestor had segmented muscle blocks, a notochord and a dorsal CNS. It also probably gathered food particles on secretions produced by a glandular endostyle located in an expanded, perforated pharynx. Controversy remains over what the head region of this long-extinct ancestor looked like, because the anterior region of tunicates (or their larvae) is so different from that of amphioxus. Did this ancestor have mesodermal somites (segments) in its anterior region, like a modern amphioxus, or was the anterior unsegmented as it is in tunicate larvae? This may sound like a minor issue, but it is important to resolve if we wish to understand how our own head and brain arose in evolution. This old debate remains unsettled, and more work is needed to compare gene expression and cellular fates in the cranial regions of each chordate group as well as between the cranial and somitic mesoderm of vertebrates.

At the next node-based ancestor, the basal deuterostome, the rival claims of the annelid and enteropneust theories first begin competing for our attention. The chordates are the sister group to the Ambulacraria[46], a clade comprising enteropneusts, pterobranchs and echinoderms (Fig. 2b).

Somewhere in the mix may also be the acoels and nemertodermatids (tiny animals with an inconspicuous nervous system) and possibly the larger, but similarly simple, xenoturbellids (here, we will accept the unification of these three groups as xenocoelomorphs[31,47]). The placement of the xenacoelomorphs in the evolutionary tree is also debated; for example, molecular phylogenies that place them as sisters to Ambulacraria — plus or minus the chordates — do not sit easily with other features such as their simple Hox gene cluster[47]. To understand the importance of xenocoelomorphs, we need to consider the common ancestor of Ambulacraria and Chordata. This animal in our series of vertebrate ancestors possessed pharyngeal slits (homologous in enteropneusts and chordates[48]), but what else? Did it have a brain and a CNS, for example? Chordates have a dorsal centralized nerve cord, whereas at least echinoderms have a dispersed nervous system that may be relatively condensed in some regions and not generally considered a CNS, although there is an element of subjectivity in deciding what constitutes a CNS. The putative CNS nature of enteropneust nerve cords[38] has been noted earlier. A similar debate surrounds xenacoelomorphs: xenoturbellids are not considered to have a brain, whereas acoels and nemertodermatids have small anterior aggregations of neural tissue that some have considered to be brain-like[47]. If xenacoelomorphs are basal in the deuterostomes, one might envisage the common ancestor of chordates and ambulacrarians to be enteropneust-like in lacking a clear CNS and a 'brain', although secondary simplification might have occurred[31].

Does this mean that the enteropneust theory wins over the annelid theory? Unfortunately, things are not simple. First, centralized nerve cords are widely distributed (although far from the rule) among bilaterian animals. Thus, concluding that the urbilaterian (and in turn the later ambulacrarian and chordate common ancestor) possessed a CNS would not be
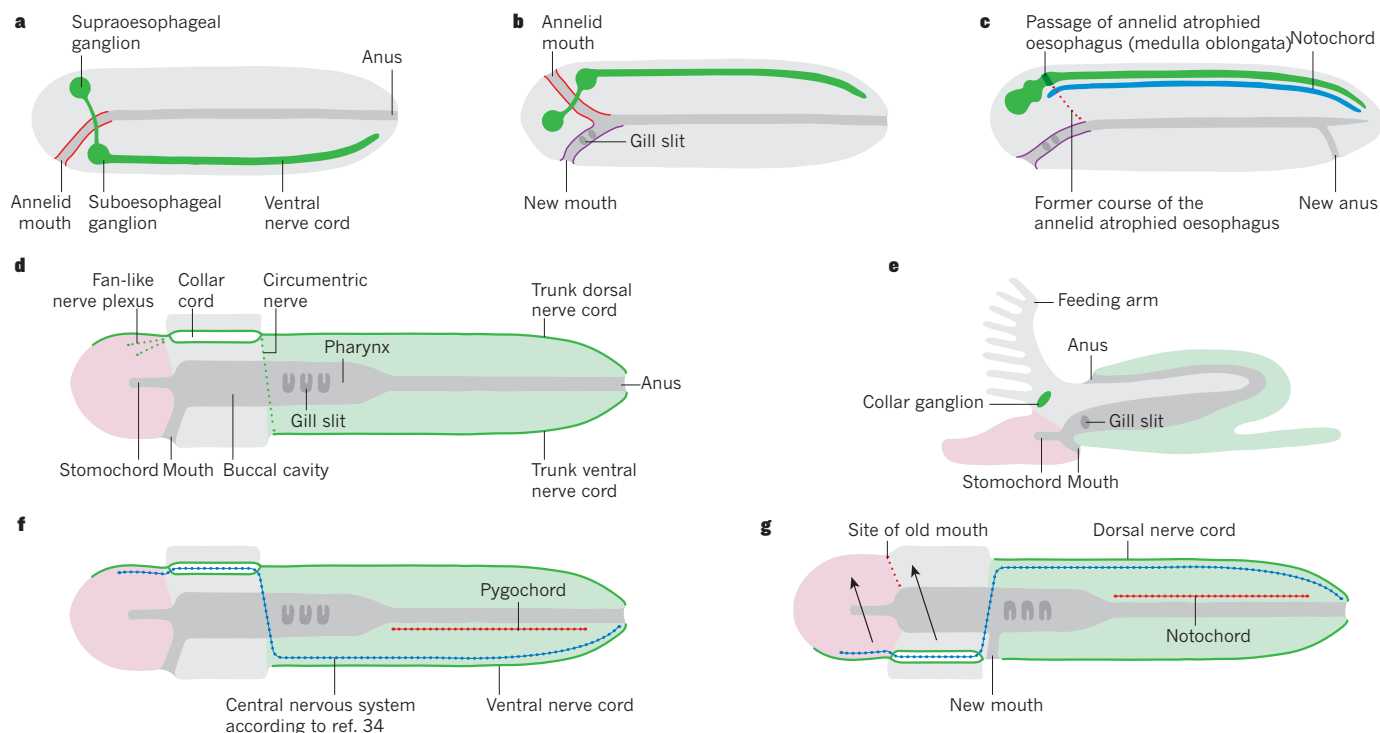

Figure 3 | Annelid and enteropneust theories. a, An annelid with a central nervous system (CNS; green) comprising supraoesophageal and suboesophageal ganglia, circumoesophageal connectives and ventral nerve cord. **b**, Dorsoventral inversion[11] produces a new foregut (purple) penetrated by gill slits. **c**, Annelid-to-vertebrate transition. The new foregut persists, but the old one atrophies, permitting union of the supra- and suboesophageal ganglia into a vertebrate-like brain. A notochord (blue) originates from connective tissue surrounding the nerve cord, and a new anus opens. **d**, Enteropneust according to Bateson[32], showing proboscis (pink), collar (grey) and trunk (light green). The ventral mouth opens into a buccal cavity, giving off a small diverticulum (the stomochord) anteriorly and connecting with the pharynx posteriorly. Gill slits penetrate either side of the pharynx,

and the post-pharyngeal gut ends posteriorly at the anus. **e**, A pterobranch hemichordate (*Rhabdopleura*), comprising a cephalic shield (pink), collar with feeding arms (grey) and trunk (light green). **f**, Enteropneust as conceived by Nübler-Jung and Arendt[34] with the blue line showing the extent of the CNS. The red line indicates the pygochord. **g**, Proposed inversion during enteropneust-to-vertebrate transition[34]. The pygochord becomes the notochord; the trunk ventral nerve cord becomes the dorsal nerve cord; a dorsal shift of the proboscis plexus and collar cord (arrows) supplies anterior brain regions; and a new mouth forms, while the old one disappears. The transition proposed by Nübler-Jung and Arendt (shown here between **f** and **g**) has now been supplanted by the more current scenario of Lowe[35,37], which is covered in detail by the Review on page 456.

unreasonable. This would imply secondary reduction in basal deuterostomes. A key issue is whether the deuterostome ancestor was segmented along the body axis[49]. This question is inextricably linked to the question of whether the urbilaterian was also segmented. Several distantly related invertebrates are segmented along the body axis, including arthropods and annelids. If their segmentation is homologous with that of chordates, then, as has been suggested[50], the ancestors of both deuterostomes and protostomes were segmented, and enteropneusts lost their segments.

However, the segmentation issue is still vigorously debated. Molecular similarities in the control of segmentation between arthropods and some annelids are striking[6,51], and there are commonalities in gene expression between mesodermal segmentation in these two phyla and in chordates[52]. Even so, deciding whether the similarities in segmentation are due to inheritance from a common ancestor or to independent co-option of parts of the same molecular machinery[53] is not straightforward. New modes of segmentation (in the broad sense[49]), such as hindbrain rhombomeres of vertebrates and reiterated pharyngeal slits of deuterostomes, can arise in evolution. Molecular and cellular studies of segmental patterning mechanisms across the animal kingdom and the nature of cycling gene networks are needed to tackle this issue. At present, therefore, we suggest that the common ancestor of ambulacrarians and chordates probably mixed the enteropneust character of pharyngeal slits and the annelid and chordate character of a centralized nerve cord. We cannot say with certainty that this ancestor was segmented along the body axis. Finally, some salient chordate characters seem to be novelties without precedents in either annelids or enteropneusts. For example, the organization of vertebrate muscle blocks working together with a notochord for active undulatory swimming[7]; this arrangement differs distinctively from the disposition of circular and longitudinal muscles in most other bilaterian animals.

If we now consider the next deepest node-based ancestor of the vertebrates, the urbilaterian ancestor to all bilateral animals, we can use the same logic as earlier, and many of the same data, to approach the reconstruction of the body plan. To pick up on just the three key morphological features discussed earlier — pharyngeal slits, a central nerve cord and segments — we deduce that it lacked pharyngeal slits and might have possessed a central nerve cord. However, there is too much uncertainty to decide whether it had segments along the body axis. It is reasonable to assume that the urbilaterian was unlike any animal alive today, but shared characters both with modern annelids and with modern enteropneusts. To turn this around, each of these two living groups seem to retain some of the characters from their, and our, distant ancestor.

We conclude, therefore, that the annelid and enteropneust scenarios are both partly correct. Some of the early proponents of the vast range of scenarios for the origin of vertebrates (Fig. 1) viewed living animals as proxies for long extinct ancestors. In reality, more progress has been made by comparing living animals with one another to deduce the combinations of morphological characters present in ancestors, a task that requires critical evaluation of homology, incorporating developmental, cellular and molecular approaches in an ever-widening range of animal taxa. Ultimately, a wealth of reliable and detailed information over a wide spectrum of taxa will be needed to sort out relationships among the animal phyla and their component characters[53,54]. This Review began with an appreciation of the older ideas in the field, and some of these will continue to guide us as we move ahead with technological advances and new discoveries in biology and palaeontology to gain insights into the origin of the vertebrates and our own distant history. ∎

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