To celebrate the golden jubilee of *On the Origin of Species*, in 1909, the Linnean Society of London held a special meeting on a hot biological topic of the day — the origin of the vertebrates. Such was the lack of consensus that one commentator, the zoologist T. R. R. Stebbing, wrote that “the disputants agreed on one single point, namely, that their opponents were all in the wrong.”

The problem is easily stated — vertebrates have so many special features, from large brains to complex physiologies to unique tissues such as enamel and bone — that their evolution from invertebrates is obscure. The question had intrigued Aristotle, and foiled minds as keen as those of William Bateson and Thomas Hunt Morgan, who, by way of finding a more rewarding problem, went off to discover genetics instead.

The same tools that Bateson and Hunt Morgan helped to create have now returned to address the old problem. Although our understanding is far from complete, it is much better than it was even 20 years ago, and is summarized in this collection of reviews.

Nicholas Holland and colleagues set out how the varied theories advanced to explain vertebrate origins, before Lowe *et al.* show how they fit in to the deuterostomes, a larger branch of the animal kingdom. Diogo *et al.* add new perspectives to a central question of vertebrate origins, namely, the origin of the head. Marianne Bronner and colleagues then look at the embryonic tissue known as neural crest, another uniquely vertebrate feature. Philippe Janvier surveys the wealth of newly found, and often curious, fossil evidence, and Martin Brazeau and Matt Friedman chart the evolution of jawed vertebrates from jawless forms. If Stebbing was able to peruse this collection, I hope he would agree that we have come a long way.

Henry Gee
*Senior Editor*
Scenarios for the making of vertebrates

Nicholas D. Holland¹, Linda Z. Holland¹ & Peter W. H. Holland²

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 Most of these scenarios are outdated, but several are still seriously considered.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. During the next few decades, several others speculated on how body plans of invertebrates and vertebrates might be related; however, these 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, 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, 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.

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 or larvae being intercalations. 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. 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, 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. Now, however, the starting point is often considered to be an annelid-like unir bilateral. The annelid theory has its roots in arthropod biology, because these two groups were long considered to be very close relatives, and results for one were generally considered to be valid for the other.

In the original annelid scenario, Dohrn 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. Several of Dohrn’s colleagues modified his scenario in attempts to improve it, but the theory went into eclipse early in the twentieth century when the bilaterian animals were reared into two superphyla — the protostomes and the
The developmental genetic comparison between arthropods and deuterostomes. The resulting relocation of annelids and arthropods at a considerable phylogenetic distance from the vertebrates (Fig. 2a) weakened the idea of a complex urbilateral and shifted opinion towards a simple urbilateral, 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\(^\text{22-25}\). Arendt and Nübler-Jung interpreted this pattern as support for homology between arthropod and vertebrate nerve cords and indicative of a dorsovenal inversion of the body during the invertebrate-to-vertebrate transition\(^\text{25}\). 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 dorsalvenal axis that was reversed between flies and frogs\(^\text{22-25}\). 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\(^\text{22-25}\).

The developmental genetic comparison between arthropods and vertebrates\(^\text{22-25}\), 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\(^\text{25}\), genetic specification of several kinds of nerve cells\(^\text{22,23}\) and the formation of notochord-like structures\(^\text{25}\). As already mentioned, the revised annelid scenario posits the evolution of an already complex urbilateral 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\(^\text{18-31}\). 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 (vetulicilians 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 (already mentioned) and the worm-like phoronids, 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\(^\text{22-25}\), 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 eunicates. However, at the close of the nineteenth century, Masterman\(^\text{33}\) 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 to the enteropneust proboscis, a collar extending into tentacle-fringed arms and a trunk. According to the original enteropneust theory\(^\text{22-25}\), 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 eunicates. However, at the close of the nineteenth century, Masterman\(^\text{33}\) 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\(^\text{22-25}\). 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\(^\text{22-25}\) by approximating an intermediate stage in the conversion of a complex urbilateral into a vertebrate. Because this CNS was oriented

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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\textsuperscript{38,39}, molecular phylogenetics revealed that the relatively complex phoronids are neither deuterostomes nor their close relatives\textsuperscript{40} (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\textsuperscript{41}. 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\textsuperscript{42}. 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\textsuperscript{39}, 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\textsuperscript{43} 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\textsuperscript{44} 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.\textsuperscript{32} suggested that the proboscis plexus, collar cord, circumcerebral 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\textsuperscript{30} 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\textsuperscript{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\textsuperscript{42}.

To complicate matters further, Miyamoto and Wada\textsuperscript{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\textsuperscript{2}, 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\textsuperscript{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 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 hagfishes\(^{46}\)), 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 mesoderms 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\(^{41,47}\)). The placement of the xenocoelomorphs in the evolutionary tree is also debated; for example, molecular phylogenies that place them as sisters to Ambulacaria — 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 Ambulacaria 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\(^{49}\) has been noted earlier. A similar debate surrounds xenocoelomorphs: 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 xenocoelomorphs 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 unbilaterian (and in turn the later ambulacrarian and chordate common ancestor) possessed a CNS would not be
in the latter part of the twentieth century.


This influential scenario did much to focus the attention of vertebrate biologists back on questions about the evolutionary origin of their group.


This is the original annelid theory.


This publication launched the current revival of the annelid theory.


This application of developmental genetic data to classic questions of animal phylogeny attracted much attention to the young field of evolutionary developmental biology.


This article is the original enteropneust theory.


This publication launched the current revival of the enteropneust theory.


57. Deaster-Jones, L. & Dray, N. Feathers ran high about vertebrate-origin scenarios, for instance, J. W. van Wijhe after his pioneering study in molecular phylogenetics indicated that the greater part of the animal kingdom is divisible into three major super-phyletic groups. Proc. Natl. Acad. Sci. USA 109, 10626–10633 (2012).

Acknowledgements We are grateful to R. Jenner and T. Lollaci for their criticisms during preparation of our manuscript. We dedicate this Review to the memory of Yoshiki Sasai, whose elucidation of the genetic control of dorsoventral patterning will endure as a landmark in the field of evolutionary developmental biology.

Author Information Reprints and permissions information is available at http://www.nature.com/reprints. The authors declare no competing interests. Readers are welcome to comment on the online version of this paper at go.nature.com/y7dnfd. Correspondence should be addressed to N.D.H. (nholland@ucsd.edu).
The deuterostome context of chordate origins

Christopher J. Lowe1, D. Nathaniel Clarke2, Daniel M. Medeiros3, Daniel S. Rokhsar1,4,5 & John Gerhart1

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 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 slitss and to metamorphose from echinoderm-like larva5, 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 evolution1. 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 ancestor16, a growing body of data from hemichordates, echinoderms and invertebrate chordates serves as the foundation for new hypotheses based on deuterostome ancestral characters17–19.

Despite the impressive morphological disparity among deuterostome phyla, we are making progress identifying conserved anatomical and molecular ancestral characteristics. 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)20. 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 Haeckel17, partly based on shared developmental characteristics. A key insight came from Kowalevsky’s21,22 recognition that the tadpole larva of ascidians shared many characteristics with vertebrates, an observation that greatly impressed Darwin19. Kowalevsky also recognized the vertebrate-like gill slits of the invertebrate acorn worms2. 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’23. Around the same time, Metchnikoff recognized the similar larval forms of hemichordates and echinoderms, and united these two phyla into the ‘Ambulacraria’2 (Box 2).

The unity of chordates, hemichordates and echinoderms was inferred by Grobben1 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 phyla24, 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 characters25–27, 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 vertebrates21,22,28. 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 bryozoa) and/or chaetognaths should also be grouped among the deuterostomes, molecular phylogenetics robustly supports their position in the protostomes29–31. Xenoturbellid worms are a more challenging case: these animals resemble acoelomorphs (acoel flatworms and nematodermatids) and have been grouped with them in a ‘Xenacoelomorpha’ clade32–34. Some molecular analyses also identify *Xenoturbella* and its relatives as ambulacrarians, and therefore deuterostomes35, whereas other studies find that acoelomorphs diverge from...
**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. 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)\textsuperscript{29}. 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 (larvae, ascidians and thalacians) 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\textsuperscript{17}. 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\textsuperscript{36,118}. b. Hemichordates are a clade of marine worms divided into two groups: enteropneusts and pterobranchs. Hemichordate phylogeny is based on Cannon \textit{et al}..\textsuperscript{120} Pterobranchs, shown here by \textit{Cephalodiscus}, are small largely colonial animals that live within the protection of a secreted fibrous tube and use a ciliated lophophore for filter feeding\textsuperscript{5,18}. Enteropneusts, or acorn worms, are solitary, burrowing worms that feed using a combination of deposit and filter feeding\textsuperscript{22,121}. The Harrimaniid \textit{Saccoglossus kowaleskii}, which has been used for many developmental studies\textsuperscript{12}, 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 feeding and contains the gut diverticulum called the stomochord that supports a heart–kidney complex\textsuperscript{29,60}. The mouth opens ventrally into the pharynx within the collar region, and the anterior trunk is perforated by a series of dorso lateral gill slits\textsuperscript{56}. 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.

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**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\textsuperscript{29}. 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. 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). 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. 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. 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.

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, a ventral floor plate expressing hedgehog ligands, 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. 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 isthmic organizer or the zona limitans intrathalamica (although these signalling mechanisms may have been present in a deuterostome ancestor, see later).

Segmented musculature of the ancestral chordate almost certainly developed from somites, and at least some formed by enterocoely. In amphioxus, the anterior-most somites form by enterocoely, whereas posterior somites pinch off sequentially from the tail bud. 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. 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. 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).

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.

As first described by Kowalevsky, 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). Although there is no equivalent structure in extant echinoderms, fossils reveal compelling evidence that gill slits were present in stem echinoderms and subsequently lost. On the basis of morphological and functional criteria, enteropneust hemichordates (acorn worms) as they relate to our understanding of early deuterostome evolution.

The stomochord in hemichordates has drawn much comparative interest as a notochord-like ancestral trait. It is a diverticulum of...
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. However, homology of these two structures is weakly supported by both morphological and molecular criteria. In chordates the developing notochord is a key source of the secreted BMP antagonists Chordin, Noggin and Follistatin, and the ventralizing ligand Shh. Of these genetic markers, only hh (the homologue of Shh) is expressed in the stomochord, but it is also observed in surrounding anterior endoderm. Possible alternatives to notochord homology are suggested by the stomochord expression of genes such as otx, dmtn, hox and foxE that are expressed in prechordal endomesoderms of chordates, but not in the notochord. These markers suggest that the stomochord is an anterior endodermal structure with stronger affinities to the endoderm than the notochord.

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. Various authors have proposed both cords as possible homologues of the chordate dorsal cord, however, the internalized collar cord has attracted the most attention. Early reports suggested that the dorsal cord was simply a through conduction tract of axons through the collar cord has attracted the most attention. In chordates the developing notochord is a key source of the secreted BMP antagonists Chordin, Noggin and Follistatin, and the ventralizing ligand Shh. Of these genetic markers, only hh (the homologue of Shh) is expressed in the stomochord, but it is also observed in surrounding anterior endoderm. Possible alternatives to notochord homology are suggested by the stomochord expression of genes such as otx, dmtn, hox and foxE that are expressed in prechordal endomesoderms of chordates, but not in the notochord. These markers suggest that the stomochord is an anterior endodermal structure with stronger affinities to the endoderm than the notochord.

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 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 cords along the dorsal and ventral midlines. The blue spots represent cell bodies and the lines represent neural processes.

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. 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 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 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 endomesoderms

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 β-catenin protein, the intracellular effector of the canonical Wnt signalling pathway. β-Catenin is stabilized preferentially in the vegetal pole of early embryos and activates genes of the endomesodermal cellular program.
In hemichordates and echinoderms, knockdown of the gene that encodes β-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 endoderm and no mesoderm. This mechanism has also been demonstrated in protochordates from work on nemertine embryos77, and β-catenin protein is also involved in endoderm formation in cnidarians78, suggesting a deep eumetazoan ancestry for this process79.

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 FGF87. In amphioxus, FGF signalling specifies anterior mesoderm that forms by enterocoely92. 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 formation93. 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 asteroids94. The differences between deuterostomes in specifying endomesoderm and mesoderm preclude the definitive inference of the pathway of the deuterostome ancestor, except that β-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 β-catenin has emerged as the earliest conserved determinant of AP pattern in deuterostomes. (Note that this time and place of usage of β-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 patterning25,26. 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 axis95. In both sea urchin larvae and the direct-developing S. kowalevskii, Wnt signalling is also important for establishing AP patterning96,97, 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 bilaterians95,86.

This conserved AP map provides a novel basis for comparing body plans (Fig. 3a, b)96. In the most anterior regions, expression of genes such as *srfp1*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 *enx*, *barH*, *dmxb* 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)93,85,86. Enteroneust Hox genes are organized as an intact cluster88, 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 also extend to three signalling centres, producing the same signals and occupying equivalent map positions, that are important for vertebrate brain patterning and for hemichordate cephalization 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 patterning89.

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 enteroneusts. 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.
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. 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 (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. 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. 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 differentiation. 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. (Indirect developing hemichordates and echinoderms also exhibit Bmp–Chordin-based DV patterning, modified for larval body plans, although we cannot cover these here.) At gastrula stages, bmp2/4 is strongly expressed on the dorsal ectodermal midline of S. kowalevskii, accompanied by genes for a large number of transcription factors and signalling molecules (Fig. 4a, b). A comparison of the dorsoventral patterning mechanisms of hemichordates and chordates is shown in Figure 4. The patterns of transcription-factor and signalling-ligand expression established along the DV direction are conserved across deuterostomes because the transcription factors and signals of the Bmp–Chordin axis, the domain map, and axis of anatomical differentiation are conserved across deuterostomes (and in most protostomes) despite extensive innovations in DV patterning at the base of the chordates (ancestral location shown by grey shading).
Late larva

Echinoids

Spengelids in abundance. Although this might seem contrary to chordate neural (Fig. 2a). In embryos dorsalized by excessive Bmp, nerve cells still form distribution, including the gill slits, the mouth and the two nerve cords and cell types of the three germ layers are patterned by the Bmp–Chordin (37x36)

and of ventralized embryos when Bmp2/4 is eliminated of dorsalized embryos in the presence of excess uniform Bmp2/4 protein, depend entirely on the Bmp distribution, as shown by the development map and subsequent differentiated structures of the overt anatomical axis (21,24,109) are examples of direct-developers. A model of a two-day-old embryo of Saccoglossus kowalevskii contrasts with the month-old late spengelid tornaria larva. 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 echinozoeus 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 ambulacranian larval types, suggesting the existence of an ancestral ‘dipleurula’ (small two-sided) larval form from which ambulacranian larval diversity arose114. 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 Garstang108 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 neurons114. More recently, this hypothesis has fallen out of favour on the basis of both phylogenetic and body-patterning data31,24,109.

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 dorsalized embryos in the presence of excess uniform Bmp2/4 protein, and of ventralized embryos when Bmp2/4 is eliminated35. 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 dorsalized 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 proboscophores: 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
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. 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, neutralizing 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. 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 neutralization response in the ectoderm. These innovations in DV axiation 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. 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. 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 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

vertebrate forebrain, here spread over a basiepipithelial nerve plexus. There would be no intrinsic reason for the deuterostome ancestor to preserve the morphology and differentiation of the bilaterian ancestor if it no longer lived that ancestor’s lifestyle. Finally, there is some paleontological 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.

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. 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 Ambulacaria 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, but this hypothesis has recently lost support due to revisions in chordate phylogeny and close similarities between adult rather than larval body patterning. 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)\textsuperscript{110}, the acorn worms S. kowalevskii and Psychodura fluvi\textsuperscript{9},\textsuperscript{111} and the crown-of-thorns sea star (Acanthaster planci)\textsuperscript{112}. 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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31. This report shows extensive patterning similarities between the development of chordate and enteropneust gills, further supporting morphological homology.
36. This manuscript demonstrates similar mediolateral patterning mechanisms between the hemichordate collar cord and chordate dorsal cord.
This paper presents evidence that ectodermal signalling centres thought to have been uniquely associated with the evolution of vertebrate brains are present in hemichordates as part of a conserved ancient deuterostome patterning network.


This paper shows clear molecular evidence for the presence of cell bodies in the dorsal nerve cord of enteropneusts and proposes the deep ancestry of a CNS in the deuterostomes.


This manuscript demonstrates the role of BMP signalling in the formation of the hindbrain of the deuterostome adult body plan.


This paper provides evidence for a conserved transcriptional gene regulatory network between hemichordates and chordates despite large organizational differences in their basic body plans.


A new heart for a new head in vertebrate cardiopharyngeal evolution

Rui Diogo1, Robert G. Kelly2, Lionel Christiaen3, Michael Levine3, Janine M. Ziermann1, Julia L. Molnar1, Drew M. Noden2 & Eldad Tzahor4

It has been more than 30 years since the publication of the new heart hypothesis, which proposed that the vertebrate heart is an evolutionary novelty resulting from the emergence of neural crest and cranial placodes. Neural crest generates the skull and associated connective tissues, whereas placodes produce sensory organs. However, neither crest nor placodes produce head muscles, which are a crucial component of the complex vertebrate head. We discuss emerging evidence for a surprising link between the evolution of head muscles and chambered hearts — both systems arise from a common pool of mesoderm progenitor cells within the cardiopharyngeal field of vertebrate embryos. We consider the origin of this field in non-vertebrate chordates and its evolution in vertebrates.

In their influential 1983 paper, Gans and Northcutt1 proposed that early vertebrates evolved from invertebrates principally through innovations in the head. These include the muscularization of the ventrolateral mesoderm, or hypomere, to form branchiomioccur muscles and the emergence of two novel ectodermal structures: the neurogenic placodes and the neural crest. Neural crest cells produce most of the cartilage, bone, dentine and other connective tissues of the vertebrate head, whereas the placodes give rise to the sensory neurons that are essential for the formation of vertebrates’ complex sensory systems2–4. The new head hypothesis proposed that these evolutionary innovations were associated with a shift from passive filter-feeding to active predation. Increased sensory capabilities and a muscularized pharynx arguably permitted more efficient prey detection and capture, as well as higher rates of respiratory gas exchange, which accompany the predatory lifestyle. This major behavioural and ecological transition also coincided with the emergence of a chambered heart, which presumably allowed for the increased growth and metabolism that was demanded by active predation. However, the new head hypothesis was primarily concerned with derivatives of neural crest and placodes, which are better represented in the fossil record than soft tissues such as muscles5,6. In this Review, we provide an up-to-date multidisciplinary discussion of the origin and evolution of vertebrate head muscles, taking into account surprising new evidence for shared developmental origins of several head muscles and the heart, and the ancient (pre-vertebrate) origin of this association.

The emerging concept of the cardiopharyngeal field

The cardiopharyngeal field (CPF) is a developmental domain that gives rise to the heart and branchiomioccur muscles (Box 1 and Figs 1, 2). The amniote heart is made up of cardiomyocytes derived from two adjacent progenitor cell populations in the early embryo7. Early differentiating cardiac progenitor cells of the first heart field (FHF) give rise to the linear heart tube and later form the left ventricle and parts of the atria8,9. Subsequently, second-heart-field (SHF) progenitors, located in pharyngeal mesoderm, produce cardiac muscle tissue (myocardium) of the outflow tract, right ventricle and parts of the atria10–12 (Fig. 2). The SHF can be divided into anterior and posterior progenitor cell populations that contribute to the arterial and venous poles of the heart, respectively7,9. Cells from pharyngeal mesoderm can form either cardiac or skeletal muscles, depending on signals from adjacent pharyngeal endoderm, surface ectoderm and neural crest cells9,13–16. The latter have important roles in regulating the development of the CPF — they are required for the deployment of SHF-derived cells to the heart’s arterial pole, and neural-crest-derived mesenchyme patterns branchiomioccur muscle formation and gives rise to associated fascia and tendons17–19. A suite of regulatory factors integrates the intercellular signals that coordinate the formation of cardiac and branchiomioccur muscles from a common pool of mesodermal progenitor cells. Within the CPF there is considerable overlap in the expression of genes that encode cardiomioccur regulatory factors (for example, Isl1 (also known as Islet1) and Nkx2-5) and those that specify head muscles (for example, Tbx1, Tcf21 (also known as capsulin), Mscl (also known as MyoR) and Pitx2)13,15,20. Importantly, many of the intercellular signalling pathways and transcription factors that control branchiomioccur myogenesis upstream of the Myod family of myogenic determination factors differ fundamentally from those operating in the trunk11,21. Here we focus on Isl1, Nkx2-5 and Tbx1. The LIM-homeodomain protein Isl1 is required in a broad subset of cardiovascular progenitor cells in mouse embryos22 and it is expressed in pharyngeal mesoderm, including the pharyngeal arches and SHF. Isl1 progenitor cells substantially contribute to the heart and branchiomioccur muscles, but not to hypobranchial (for example, tongue) or extraocular (eye) muscles13,23,24. Expression and functional studies indicate that Isl1 delays differentiation of branchiomioccur muscles13,24. Isl1 thus marks a subset of CPF cells and plays an important part in the development of distinct cardiovascular and skeletal muscle progenitors24. The cardiac transcription factor Nkx2-5 regulates proliferation in the SHF and acts with Isl1 to modulate SHF progenitor-specific gene expression25–27. Tbx1 is required within the CPF for both heart and head muscle development, and is the major candidate gene for the congenital condition DiGeorge syndrome (or 22q11.2 deletion syndrome), which is characterized by a spectrum of cardiovascular defects and craniofacial anomalies. Like Isl1, Tbx1 has a crucial and conserved role in extending the heart’s arterial pole by promoting proliferation and delaying differentiation of SHF cells28–30. Tbx1 is also required for activation of branchiomioccur myogenesis and may directly regulate the myogenic determination gene MyoD22–34.

1Department of Anatomy, Howard University College of Medicine, Washington DC 20059, USA. 2Aix Marseille Université, Centre National de la Recherche Scientifique, Institut de Biologie du Développement de Marseille UMR 7288, 13288 Marseille, France. 3Center for Developmental Genetics, Department of Biology, New York University, New York 10003, USA. 4Department of Molecular and Cell Biology, University of California at Berkeley, California 94720, USA. 5Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA. 6Department of Biological Regulation, Weizmann Institute of Science, Rehovot 76100, Israel.

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Branchiomeric muscles. Muscles formed from progenitor cells found in the pharyngeal arches. In vertebrates, they comprise the mandibular (first arch muscles, such as jaw muscles), hyoid (second arch muscles, such as the facial expression muscles of mammals) and branchial (from more posterior arches, including muscles of the larynx and pharynx, and the cucullaris-derived neck muscles trapezius and sternocleidomastoideus, in amniotes) muscles.

Pharyngeal (or branchial) arches. Bilateral swellings on either side of the pharynx comprising outer (ectodermal) and inner (endodermal) epithelia, neural-crest-derived mesenchyme and a mesodermal core.

First heart field. Population of early differentiating cardiac progenitor cells that arise in anterior lateral mesoderm and give rise to the linear heart tube and, later, to the left ventricle and parts of the atria.

Second heart field. Population of late differentiating cardiac progenitors that contribute to the developing heart after the linear heart tube stage to give rise to myocardium of the right ventricle and outflow tract, and to inflow tract myocardium, including parts of the atria.

Cardiopharyngeal field. Includes anterior lateral mesoderm of the first heart field plus contiguous pharyngeal mesoderm that gives rise to second-heart-field-derived regions of the heart and branchiomeric muscles.

Cardiopharyngeal ontogenetic motif. Lineage-specific progression through cardiopharyngeal progenitor cell identities, with conserved clonal relationships between first heart, second heart and pharyngeal muscle precursors characterized by specific gene expression and regulatory activities.

Pharyngeal mesoderm. Cranial mesoderm associated with the forming foregut or pharynx that populates pharyngeal arches and contributes to second-heart-field-derived regions of the heart and branchiomeric muscles.

Origins and diversity of cardiopharyngeal structures

The heads of mammals, including humans, contain more than 60 muscles, which control eye movements and allow food uptake, respiration, and facial and vocal communication. Strikingly, the human head includes at least six different groups of muscles with distinct developmental origins and evolutionary histories (Fig. 1). Full recognition and detailed knowledge of this heterogeneity has enormous basic science and clinical implications because long accepted anatomy concepts, mainly based on adult function and physiology (for example, skeletal compared with cardiac muscles) do not correspond to the true developmental and evolutionary origins of body structures. Even the conventional classification of head muscle groups based on topographical relations masks the true heterogeneity of muscle origins and progenitor fates (for example, molecular profiling of early determinative signalling molecules and transcription factors reveals almost as much heterogeneity within each group — such as, branchial, extracranial and tongue — as between them)

Comparative anatomical studies identified homologues of many amniote branchiomeric muscles in gnathostome (jawed) fish such as sharks, suggesting that they have ancient origins. (Fig. 3). Cyclostomes (hagfish and lampreys) lack some of these muscles (for example, the cullarisis group), but like some chordichthyans (Selachii and Holocophali) they possess an additional, seventh group of head muscles: epibranchial muscles, which are derived from anterior somites. Thus, extraoral, branchiomeric, and both hypobranchial and epibranchial somite-derived muscles were integral parts of the heterogenous head musculature of early vertebrates (Fig. 3). Moreover, lamprey embryos express homologues of IsL1, Nkx2-5 and Tbx1 in seemingly overlapping anterior and ventral mesodermal domains, comparable with the patterns of their homologues in the amniote CPF. Interestingly, the emergence of heterogenous head-muscle groups at the base of vertebrates coincided with the emergence of chambered hearts (Fig. 3). This intriguing correlation suggests that the two innovations are linked by their common developmental origin in the CPF.

Studies indicate that specific branchiomeric muscles were crucial for evolutionary innovations among vertebrates, such as the emergence of the tetrapod neck. The amniote neck muscles trapezius and sternocleidomastoideus (Fig. 1) derive from the cullarisis, a muscle that probably appeared in early gnathostomes and was found in fossil placoderms. Among extant gnathostomes, some of the anatomical and developmental characteristics of the cullarisis are shared with branchiomeric and somite-derived limb, epibranchial and hypobranchial muscles. Most available data, however, indicate that the cullarisis is a branchiomeric muscle derived from the posterior-most pharyngeal arches, as suggested by Edgeworth. Like other branchiomeric muscles, in most gnathostomes the cullarisis is attached to neural-cres-derived tendinous and skeletal elements. Furthermore, Tbx1 is active in core branchiomeric muscles (for example, the first and second arch muscles) and in the cullarisis-derived trapezius, whereas Pax3 is required in the somites for limb, diaphragm, tongue, infrayroid and trunk-muscle formation, but not for trapezius formation. These findings may also support Gegenbaur's hypothesis that the pectoral appendage, to which the cullarisis and its derivatives usually attach, probably originated as an integral part of the head. Thus, the evolutionary history of the cullarisis-related muscles illustrates the roles that branchiomeric muscles had in fostering anatomical and functional innovations during vertebrate evolution. Future studies are needed to investigate whether the emergence of the cullarisis at the base of gnathostomes coincided with cardiovascular innovations and, if so, whether this muscle also shares a common origin with a specific heart region (Fig. 1).

A urochordate cardiopharyngeal ontogenetic motif

Recent phylogenetic studies place the urochordates — not the cephalochordates (for example, amphioxus) — as the sister group of the
vertebrates. On the basis of these results, urochordates provide important insights for our understanding of the origin of vertebrates’ evolutionary innovations, particularly from a developmental perspective. For instance, the new hypothesis proposed that the emergence of branchiomeric muscles occurred during the transitions that led to the origin of vertebrates, and was associated with a shift from ‘passive’ filtration to more active feeding modes and the emergence of crest- and placode-derived sensory organs. However, recent studies have identified neural-crest-like cells, placodes and a CPF in tadpole-like larvae of the ascidian Ciona intestinalis, a model urochordate.

**Figure 1 | The striking heterogeneity of the human head and heart musculature.** The head includes at least six different muscle groups, all arising from the cardiopharyngeal field and being branchiomeric, except the hypobranchial and perhaps the extraocular muscles. On the left side of the body (right part of figure) the facial expression muscles have been removed to show the masticatory muscles. The six groups are: first/mandibular arch muscles, including cells clonally related to the right ventricle; left second/hyoid arch muscles related to myocardium at the base of the pulmonary trunk; right second/hyoid arch muscles, related to myocardium at the base of the aorta; muscles of the most posterior pharyngeal arches, including muscles of the pharynx and larynx and the cucullaris-derived neck muscles trapezius and sternocleidomastoides; extraocular muscles, which are often not considered to be branchiomeric, but according to classic embryological studies and recent retrospective clonal analyses in mice contain cells related to those of the branchiomeric mandibular muscles; and hypobranchial muscles, including tongue and infrahyoid muscles that derive from somites and migrate into the head and neck.

Ascidians and other urochordates possess a surprisingly sophisticated hearts, including localized pacemakers that drive a regular, rhythmic beat. The ascidian heart is derived from two Mesp+ cells in early embryos. These produce four trunk ventral cells, which express homologues of Nkx2-5, Gata4, 5 and 6 and Hand, and migrate towards the pharyngeal endoderm. They subsequently divide asymmetrically to produce medial heart precursors and secondary trunk ventral cells that divide again to produce second heart precursors and atrial siphone muscles, which migrate towards the atrial siphone placode. Thus, trunk ventral cells are multipotent cardiopharyngeal progenitors that produce bona fide heart and pharyngeal muscles, following a clonal pattern evocative of that seen in mice (Fig. 2). Gene-expression profiling data are also consistent with the idea that the trunk ventral cells are homologous to the vertebrate cardiopharyngeal progenitors: trunk ventral cells express Nkx4, the homologue of Nkx2-5, and secondary trunk ventral cells also express Tbx1/10, which is active in vertebrate pharyngeal mesoderm. Furthermore, the regulatory network governing interactions among the cardiopharyngeal specification genes seems to be highly conserved in ascidians and vertebrates. For example, cross-repressive interactions between Tbx1/10 and Nk4/Nkx2-5 delineate atrial siphone muscles and heart, respectively. Is1 is also expressed in the CPF, although there are differences from the precise expression profile seen in vertebrates, where Is1 is thought to delay muscle differentiation. It is nonetheless striking that all of the identified molecular determinants of the vertebrate SHF are expressed in ascidian trunk ventral cells.

There are additional parallels between the CPFs of ascidians and vertebrates in the regulatory circuitry underlying the differentiation of specialized muscles (Fig. 2). COE/Ebf downstream of Tbx1/10 and upstream of both Mtf/MyoD and Notch signalling to promote either early muscle differentiation or maintain undifferentiated precursors that produce most later atrial siphone and longitudinal muscles. Atrial siphone muscle precursors also associate with the Dlx atrial siphone placodes to form a ring of cells underlying the rosette-shaped placode in C. intestinalis swimming larvae. These events parallel the migration of vertebrate branchiormeric muscle precursors into pharyngeal arches, their association with Dlx cranial neural crest cells, and the maintenance and growth of a pool of undifferentiated progenitor cells. It is noteworthy that the ascidian FHF and SHF are each initially composed of four cells that independently arise from one of four multipotent cardiopharyngeal progenitors following a sequence of conserved regulatory interactions onto a stereotyped clonal pattern, producing FHF precursors and more closely related SHF and pharyngeal muscle precursors. We refer to this clonal sequence of cell divisions, gene expression and cell-fate choices as a cardiopharyngeal ontogenetic motif.

**Chordate origins of branchiomeric muscles**

Studies using cephalochordate axial skeleton further probed the early chordate origins of branchiomeric-like pharyngeal muscles. In the cephalochordate amphioxus, the larval mouth and unpaired primary gill develop five groups of orobranchial muscles. This musculature is anatomically reminiscent of the vertebrate branchiomeric muscles, and disappears through apoptosis during metamorphosis to give way to adult oral, velar and pterygial muscles. These are even more similar to vertebrate adult branchiomeric muscles. The oral and velar muscles, in particular, share anatomical similarities with the oral and velar muscles of lampreys and hagfish, although the pterygial muscles have a branchiomeric-like innervation pattern. Gans recognized this latter point and noted that this could mean that the branchiomeric muscles evolved before the last common ancestor (LCA) of vertebrates, as suggested by earlier authors, but contrary to the original new head hypothesis. Vestigial muscles appear transiently with secondary gill formation in amphioxus, providing additional evidence that bilateral muscular gills and a segmental pattern of branchiomeric muscles were already present in the LCA of extant chordates.

Molecular studies suggest that the amphioxus homologues of Tbx1, Nkx2-5 and Is1 are expressed in overlapping mesodermal domains in the pharyngeal region. This domain includes cells that also express the
vertebrate cardiac markers *Hand* and *Tbx20* (refs 59, 104) and is thought to produce the branchial artery, a possible — but controversial — homologue of the heart with diffuse contractility**. These observations raise the possibility that the LCA of extant chordates had a CPF. However, contrary to urochordates and vertebrates, cephalochordates have a rather diffuse heart-like vasculature and their branchial muscles seem to develop independently of *Ebf* and *Mrf* homologues**. Amphioxus *Mrf* homologues seem to be expressed exclusively in somites, overlapping with the Pax3/7 homologue 106,108, but also with the *Tbx1* homologue 102, suggesting the presence of distinct *Tbx1*, Pax3/7, *Mrf* somitic and *Tbx1*, Pax3/7, *Mrf* pharyngeal mesodermal domains in ancient chordates.

Branchiomeric-like muscles, such as the cephalochordate oral, velar and pterygial muscles (Fig. 4), thus probably predate the origin of a CPF as defined in urochordates and vertebrates (Fig. 3). Comparative anatomical studies suggest that the pterygial and orovelar muscles of adult amphioxus probably correspond to the atrial and oral siphon muscles of urochordates, respectively (Fig. 4). Remarkably, the ascidian oral siphon muscles (Fig. 4), which control mouth movements in post-metamorphic animals, do not derive from cardiopharyngeal progenitors 103,109,110, indicating the presence of distinct *Tbx1*, Pax3/7, *Mrf* somitic and *Tbx1*, Pax3/7, *Mrf* pharyngeal mesodermal domains in ancient chordates.

This is in contrast with the anterior oral muscles controlling mouth movements and in particular jaw opening (first (mandibular) arch muscles) in gastronomists, which are CPF derivatives (Fig. 2). Comparative studies of basal chordates, including that of the fossil *Haikouella*, suggested that their pharyngeal arch series started with the second (hyoid) arch and that only during early vertebrate evolution did parts of the anterior mesoderm become incorporated into the pharyngeal series by forming a new, *Hox*-independent first arch 111,112. Therefore, it is possible that the incorporation of the more anterior (first) arch in this series during vertebrate evolution was accompanied by integration of the associated oral and velar muscles into the CPF. This evolutionary scenario implies that the amphioxus orovelo muscles and urochordate oral siphon muscles may be homologous to the cyclostome orovelo muscles and gnathostome mandibular muscles, which could potentially explain why these muscles are derived from the CPF only in vertebrates.

**Bilaterian roots of the cardiopharyngeal network**

We have argued that the presence of a CPF, with dual cardiac and skeletal myogenic capacity, is probably a synapomorphy of olfactores (a derived feature shared by urochordates and vertebrates; Figs 2, 3). This argument raises the question: do the developmental, cellular and/or molecular units that form the CPF network of olfactores have even deeper evolutionary origins? Ambulacraria (echinoderms and hemichordates) is the sister group of chordates (Fig. 3). Hemichordates possess well-defined serial gill slits and a heart–kidney complex located in the anterior-most body part (prosobranch 115). Saccoglossus kowalevskii, an enteropneust hemichordate, is not expressed among non-deuterostome animals, nematodes lack a heart and a circulatory system, but possess pharyngeal muscles that contract rhythmically, exhibit electrical activity similar to mammalian cardiomyocytes, and require *ceh-22*, the homologue of Nkx2-5 (refs 9, 21, 22, 41, 42, 115). Flies lack anatomical structures that are comparable with the chordate pharyngeal apparatus, but the *Drosophila* homologues of *Tbx1*,
Nkx2-5, Isl, Ebf and Mrf/MyoD variably contribute to visceral, larval and adult skeletal and/or heart muscle specification. The diversity of myogenic networks driving muscle identity and differentiation in flies is reminiscent of the heterogeneity of myogenic origins and programs operating in the vertebrate head. Furthermore, visceral and dorsal larval muscles in Drosophila develop from mesoderm in proximity to the dorsal vessel or fly heart. It is therefore conceivable that many features of the CPF gene regulatory network predate the advent of chordates and, moreover, that this regulatory circuitry preceded the emergence of the well-studied myogenic hierarchies controlling vertebrate somitic muscle development.

Evolvable cardiopharyngeal units

Here, we summarize our arguments for the origins and diversification of the CPF (Fig. 3). Filter-feeding early chordates, endowed with serial gill slits inherited from deuterostome ancestors, already had gill-associated branchiomeric, or at least branchiomeric-like, muscles (Fig. 4).

A well-defined CPF then probably appeared in the ollactores. Ancestral vertebrates uncoupled myogenic specification and differentiation, thus increasing the population of cardiopharyngeal progenitors. This facilitated the emergence of cardiac chambers by progressive addition of progenitor cells to the growing heart tube during development. It also allowed for the expansion and diversification of branchiomeric muscles, contributing to increased muscularization of the pharyngeal apparatus that was essential for the transition to a predatory lifestyle. The latter was made possible by ollactores’ ancestral association between branchiomeric muscles and Dlx’ ectoderm cells. Elaboration of this interaction permitted coevolution of the branchiomeric musculature with the newly formed neural crest-derived craniofacial skeleton, linking the novel neural-crest-derived skeletal patterns with distinct branchiomeric muscles.

We propose that the heart and atrial siphon muscle gene network seen in the urochordate C. intestinalis illustrates the basic ontogenetic motif underlying the specification of the vertebrate CPF, and suggest three ways in which this blueprint was modified to produce the vast diversity of cardiopharyngeal patterns in vertebrates: the ontogenetic motif could be deployed in multiple independent embryonic progenitors; any given progenitor could self-renew, thus being transiently amplified, before generating distinct heart, in contrast with branchiomeric, muscle precursors and any given cell could migrate and/or be passively displaced and resume cardiopharyngeal development in different locations on receipt of appropriate signals. In contrast to their ascidian counterparts, vertebrate Tbx1 and Isl1 cardiac progenitors remain in an elusive niche in which they self-renew to produce SHF-derived heart precursors. During pharyngeal morphogenesis, these emerge sequentially to produce right ventricular and outflow tract cardiomyocytes. Conceivably, multiple independent cardiopharyngeal lineages developing in series may contribute to divergent cardiac and branchiomeric myogenic cell fates along the anterior–posterior pharyngeal mesoderm of vertebrates. This hypothesis is consistent with the observation that subsets of cardiac and branchiomeric muscles are more closely related to each other than to other heart and head muscles (Fig. 1). Future experiments will determine whether anteroposterior patterning of the CPF precedes segmentation of the pharyngeal region during arch morphogenesis.

General remarks and future directions

The CPF is a new paradigm to be reckoned with, and should take centre stage along with neural crest and cranial placodes when considering the origin of the vertebrate head. Importantly, novel insights from comparative, phylogenomic and developmental genetics studies have uncovered the deep evolutionary origins of the CPF, branchiomeric muscles, placodes and neural crest cells. Like vertebrates, urochordates have a CPF that gives rise to the SHF, SHF and branchiomeric muscles; moreover, apart from their neural-crest-like cells and placodes, at least some pelagic urochordates have highly developed brains. Data obtained after Gans and Northcutt’s new head hypothesis thus call into question the clear distinction between vertebrates and other animals, and show that the ‘new’ head arose instead by elaboration and modification of existing tissues, cell populations and gene networks through evolutionary ‘tinkering’. This revelation supports the proposal that the conventional view of vertebrates evolving from brainless ascidian-like filter-feeders through a progressive increase in complexity and emergence of several de novo structures, with no evolutionary losses or reversions, is an oversimplification. These data also emphasize the heterogeneity and complex developmental and evolutionary history of vertebrate heads and hearts, blurring the interface between head and trunk, extracranial and branchiomeric, and skeletal and somitomeric evolution).

Figure 3 | Some of the synapomorphies of the Chordata and its subgroups, according to our own data and review of the literature. a, Somites and branchiomeric muscles. b, Placodes, neural–crest-like cells and cardiopharyngeal field (CPF) (although within invertebrates, conclusive evidence for these features was only reported in urochordates, some of these features may have been already present in the last common ancestor of extant chordates) giving rise to first- and second-heart-field-derived parts of the heart and to branchiomeric muscles (possibly not all of them, that is, inclusion of oral/velar muscles into CPF might have occurred during vertebrate evolution). c, Skull, cardiac chambers, and differentiation of epibranchial and hypobranchial somitic muscles. d, Jaws and differentiation between hyapxial and epaxial somitic musculature; paired appendages and fin muscles; origin of the branchiomeric muscle cucullaris. e, Loss of epibranchial muscles; cucullaris divided into levatores arcuum branchialium (going to pharyngeal arches) and protractor pectoralis (going to pectoral girdle), an exaptation that later allowed the emergence of the tetrapod neck. f, Within sarcopterygians, the protractor pectoralis gave rise to the amniote neck muscles trapezius and sternocleidomastoideus.
cardiac myogenesis. Adult postcraniar structures, including the heart and part of the neck musculature, include cells derived from the CPF (Fig. 1); reciprocally, cephalic structures such as the tongue and infrathyroid muscles arise from somitic primordia located in the trunk. The discovery of the CPF therefore provides a more complete, and complex, view of the origin and early evolution of the vertebrate head.

However, many questions remain. For example, how is the multipotency of branchiomiwic and cardiac myocyte progenitor cells encoded in the CPF, and is there a defined molecular common niche in which these multipotent progenitor cells arise? How, and during what stages, are progenitor cell populations that give rise to different regions of the heart and head muscles specified in pharyngeal mesoderm? Recognition of the CPF also sets the stage for future discoveries in human medicine (Fig. 1). An important question is why many myopathies preferentially affect a specific subset of muscles, and whether these aetiologies are linked to the disparate embryonic histories of these muscles. As already noted, the clinical features of DiGeorge syndrome — one of the most common human congenital syndromes — include cardiovascular and craniofacial birth defects, highlighting the frequent link between these defects owing to their anatomical proximity during early embryogenesis and overlapping progenitor populations.

Therefore, the studies and data discussed here open promising new directions for biomedical research and the advancement of public health. For instance, future meta-analyses may reveal pathological relationships between specific branchiomeric muscles and regional congenital heart defects. The field of evolutionary developmental biology has progressed remarkably over the three decades since the new head hypothesis was published. With the recent revolutionarry discoveries and more exciting work already begun, the field is poised to move ahead anew.

Note added in proof: A paper has been published while the current Review was in press reporting the identification of a third group of bilateral common heart and skeletal muscle progenitor cells within the murine CPM. Using retrospective lineage analysis, cucullaris-derived neck muscles, the trapezius and sternocleidomastoid, were shown to be clonally related to myocardium at the venous pole of the heart, derived from the posterior SHF. These findings reinforce the hypothesis of a branchiomiwic origin of these neck muscles (F. Lescoart et al. Clonal analysis reveals a common origin between nonsonite-derived neck muscles and heart myocardium. Proc. Natl Acad. Sci. USA 112, 1446–1451; 2015).

This article demonstrates, using fate-mapping and experimental manipulation in the avian embryo, that cranial mesoderm gives rise both to head muscles and outflow tract myocardium.


This article demonstrates the diversity of lineages constituting craniofacial skeletal muscles and their associated satellite cells using a series of Cre lines to genetically trace trunk and cranial myogenic progenitor cells, leading to an isI1-lineage-based definition of CPF-derived cranial muscles.


This paper reports the genetic identification of Tbx1 as a regulator of branchiomyogenesis in mice, supporting the existence of distinct upstream regulatory hierarchies controlling head and trunk myogenesis.


This retrospective clonal analysis provides evidence for the existence of common progenitor cells in the mouse embryo that give rise to myocardiocyte of the right ventricle and first-arch-derived muscles, and to the arterial pole of the heart and second-arch-derived muscles.


38. Diogo, R. & Abdala, V. Muscles of Vertebrates: Comparative Anatomy, Evolution, Homologies and Development (CRC, 2010). This volume provides an overview on the comparative anatomy, evolution and homologies of the head and limb muscles in all major extant vertebrate groups with special focus on the developmental and evolutionary history of the muscles of Homo sapiens.


This 80-year-old publication continues to be the most complete compendium on the anatomical development of the head muscles of vertebrates.


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Evolution of vertebrates as viewed from the crest

Stephen A. Green1, Marcos Simoes-Costa1 & Marianne E. Bronner1

The origin of vertebrates was accompanied by the advent of a novel cell type: the neural crest. Emerging from the central nervous system, these cells migrate to diverse locations and differentiate into numerous derivatives. By coupling morphological and gene regulatory information from vertebrates and other chordates, we describe how addition of the neural–crest–specification program may have enabled cells at the neural plate border to acquire multipotency and migratory ability. Analysis of the topology of the neural crest gene regulatory network can serve as a useful template for understanding vertebrate evolution, including elaboration of neural crest derivatives.

The vertebrate body plan emerged in concert with extensive changes to anterior chordate morphology, including assembly of a craniofacial skeleton, expansion of the anterior neuroepithelium into a brain, reorganization of the pharynx and appearance of novel sensory systems1–3. The genesis of this vertebrate 'new head'2 has been fundamentally linked to the emergence of two cell types, neural crest cells and ectodermal placodal cells. The neural crest is a transient vertebrate cell type, characterized by its site of origin within the central nervous system (CNS), multipotency, and its ability to migrate and differentiate into numerous derivatives, as diverse as cartilage, bone, melanocytes, peripheral neurons and glia1. Together with ectodermal placodes that give rise to the sense organs of the head (see refs 5, 6 for discussion of placode evolution), neural crest cells have contributed to the remarkable array of novel anatomies that make vertebrates unique.

Neural crest cells are unlike any other cell type, and the advent of this progenitor cell population affected chordate evolution in an unprecedented manner. Although cells with subsets of neural crest characteristics are present in invertebrate chordates, only vertebrates have a bona fide neural crest that gives rise to structural elements of the head, glia, pigment cells and neurons. Imbued with broad developmental potential and extensive migratory ability, neural crest cells have gained developmental roles at nearly all axial levels and extensively interact with many other tissues. For these reasons, the neural crest is often referred to as the fourth germ layer7, associated with the emergence and elaboration of the vertebrate body plan1,8–9.

In this Review, we examine the morphological and genetic features that distinguish vertebrates from other chordates, focusing on cells and tissues derived from the neural crest. We place special emphasis on contributions that resulted in the assembly of the vertebrate head, which has played a crucial part in establishment and diversification of vertebrates. We discuss the gene regulatory network (GRN) underlying the formation of the early neural crest cells that are common to all vertebrates. We then use this network, together with morphological criteria, to discuss how neural crest cells may have emerged from the putative homologues that are present in invertebrate chordates, highlighting how addition of the neural–crest–specification program may have enabled cells at the CNS border to acquire multipotency and migratory ability. In this context, we examine how studies of neural crest GRNs may clarify patterns of morphological evolution within vertebrates, including expansion of neural crest derivatives during diversification of vertebrate taxa.

Taken together, the data paint a picture of the neural crest as a mal- leable population that has continued to imbue the vertebrate body with novel features.

Neural–crest–related innovations in early vertebrates

Emergence of the vertebrate lineage was accompanied by acquisition of the neural crest and its novel derivatives. All vertebrates have neural crest cells that arise from the dorsal portion of the CNS, exhibit multipotency by contributing to diverse derivatives, undergo an epithelial-to-mesenchymal transition (EMT), and have extensive migratory ability. 'Premigratory' neural crest cells initially reside in or adjacent to the dorsal neural tube, the newly formed CNS, of all vertebrates10. These cells undergo EMT to exit the CNS and migrate to numerous sites throughout the body, where they eventually contribute to their characteristic derivatives1 and progenitor cells.

Cell-lineage analyses have shown that many individual neural crest precursors can contribute to multiple cell types in vivo11–13 and in vitro14,15, and are thus ‘multipotent’ stem or progenitor cells.

Comparisons between the two major groups of living vertebrates, the jawed vertebrates (gnathostomes) and their sister group the cyclostomes (agnathans)16, identify many shared, derived traits likely to have been present in the neural crest of early vertebrates17–20. These include pigment cells, cellular pharyngeal cartilage and specialized pharyngeal musculature, an enteric nervous system, chromaffin cells, and perhaps cardiac valves17–21. Recent work has identified a new neural crest derivative, pillar cells22, that support vertebrate gill epithelia (Box 1). Because neural crest cells interact with many other tissues, they have a broad impact by modifying neuroepithelial patterning, craniofacial patterning, and cranial musculoskeletal development (Box 2).

Many early vertebrate innovations are unique to jawed vertebrates and absent in cyclostomes. Some of these traits are likely to have arisen in stem gnathostomes, the early fishes that are ancestral to the jawed vertebrates. One of these innovations is the appearance of jaws, through modifications of anterior pharyngeal arches. Other major gnathostome innovations include odontoblasts that produce dentine (Box 1), paravertebral sympathetic chain ganglia23 (Box 3) and exoskeletal armour. Although exoskeletal armour might have arisen from neural crest at cranial levels, it is likely that trunk armour instead arose from mesoderm (Box 4).

One central question in the early evolution of neural crest is the extent to which neural crest cell types are evolutionary novelties, rather than cell types (and regulatory programs) co-opted from other tissues. There are clearly some novel neural–crest–derived cell types, including
pillar cells and odontoblasts, but many neural crest cell types are similar to cells in related chordates\textsuperscript{24,25}. These cell types might either be homologous, representing a cell lineage that was co-opted and incorporated into the neural crest, or they might have arisen by convergent evolution. One example of co-option is the origin of pharyngeal cellular cartilage, probably accomplished by reuse of a program governing cellular cartilage formation in the oral region of invertebrate chordates\textsuperscript{26}. Assessment of co-option or novelty can be aided by evaluation of GRNs that govern their formation.

**A neural crest GRN is conserved across vertebrates**

From a gene regulatory perspective, the body plan of all metazoans is encoded in the genome. During embryonic development, this code emerges as a complex GRN formed by transcription factors and cis-regulatory elements that co-operate with non-coding RNAs and epigenetic factors to pattern the body and drive development of individual elements and cell types\textsuperscript{7}. According to this framework, the body-plan modifications observed during evolution are a direct consequence of changes in the developmental regulatory program\textsuperscript{8,10,19,43}. Extensive work in amniotes, frogs, teleosts and cyclostomes has revealed remarkable similarities in the overall structure of the neural crest GRN, demonstrating that it is virtually the same from amniotes to cyclostomes (Fig. 1b)\textsuperscript{10,15,23}. Some important species-specific differences exist, but they are likely to reflect the continuous restructuring of the GRN in individual clades. Nevertheless, expression patterns and epistatic interactions between FoxD3, SoxE, Snai1/2 and Pax3/7 transcription factors point to a very conserved module of neural crest specification\textsuperscript{28}. The overall conservation of the neural crest GRN correlates with conservation

The architecture of the neural crest GRN is thought to underlie the features observed in this cell population, such as multipotency and migratory capability. Functional experiments suggest that the neural crest GRN is comprised of distinct hierarchical levels\textsuperscript{36–39}. First, signalling events (GRN signalling module) initiate the specification process, by inducing co-expression of transcription factors that comprise the 'neural-plate-border module'. This in turn leads to specification of bona fide neural crest cells (neural-crest-specification module), their migration from the CNS to diverse sites (neural-crest-migration module), and finally to diversification into different derivatives through the deployment of distinct differentiation gene batteries\textsuperscript{36–39} (Fig. 1b). Each level of the neural crest GRN corresponds to a regulatory state that not only defines cell identity and behaviour at a given time point, but also drives transition to the next module of the network\textsuperscript{36}. From an evolutionary perspective, assessing conservation of different levels of the neural crest GRN helps to identify the origin of each subcircuit and reconstruct the evolutionary history of neural crest cells\textsuperscript{23–28}. As a result, the neural crest GRN provides a useful platform for understanding the molecular underpinnings of vertebrate evolution and how these cells may have participated in modifying vertebrate embryonic development. Neural-crest-GRN studies have indeed provided important clues regarding the establishment of the vertebrate lineage and its diversification\textsuperscript{23–42}.

Figure 1 | Gene regulatory interactions controlling vertebrate neural crest formation and the tunicate a9.49 cell lineage. a. Different stages in neural crest formation. Neural crest cells are defined by their origin at the neural plate border, epithelial to mesenchymal transition, migratory capacity and multipotency. b. A neural crest gene regulatory network (GRN) endows this cell population with its unique features. This GRN is composed of different modules arranged hierarchically, which control each step of neural crest development\textsuperscript{36}. The neural-crest-specification module seems to be missing from the neural plate border of invertebrate chordates. c. Regulatory circuit of a tunicate neural-crest (NC)-like pigmented cell precursor. Adapted from refs 38, 39 and based on the results from ref. 49.
Neural crest derivatives and the vertebrate pharynx

Changes in pharyngeal patterning are central to the evolution and diversification of vertebrate groups. Vertebrate pharyngeal arches have a similar general structure, characterized as a bilaterally symmetric series of endodermal evaginations that, with ectoderm, enclose a region of neural crest cells surrounding paraxial mesoderm. Neural crest cells and paraxial mesoderm give rise to pharyngeal skeletal elements and musculature, respectively.

Some aspects of vertebrate pharyngeal patterning are integrated within or modified from features common to many deuterostomes. Pharyngeal segmentation is a trait of ancestral deuterostomes, and unambiguous pharyngeal arch homologues with similar genetic controls are present in hemichordates, cephalochordates and adult urochordates, despite being secondarily lost in echinoderms. Pharyngeal mesoderm also has a broad phylogenetic distribution, being present throughout chordates. Neural-crest-derived cellular cartilage of vertebrates, rather than being a novelty of vertebrates, instead seems to have been co-opted from cellular cartilage homologous to that present within the oral cirri of cephalochordates.

Although some vertebrate pharyngeal patterning stems from ancestral conditions, many novel elements arise from vertebrate neural crest cells. Modification of early neural crest development was important for generating the diversity of pharyngeal structures observed throughout vertebrates. For example, in vertebrate gills, epithelial surfaces are supported by novel neural-crest-derived cells, pillar cells, which are ancestrally shared throughout vertebrates. In addition, in the transition from agnathans to gnathostomes, modifications to the anterior-most pharyngeal arch cartilages and neural-crest-modified musculature resulted in the formation of the jaws, as well as the formation of neck muscles.

Another vertebrate novelty associated with the pharynx and its integuments are odontodes: dental elements composed of mineral material and associated cells. In living jawed vertebrates, their formation is mediated by conserved gene regulatory subcircuits, identified by coexpression of transcription factors, including runx2 and eda/edar, among others, and require the inductive influence of neural-crest-derived mesenchyme. Fossil evidence suggests that odontodes emerged during the evolution of stem gnathostomes, in external dermal armour, consistent with the ‘outside-in’ model, which suggests that odontodes emerged first as structural elements associated with external integument, and were later incorporated into the oral cavity and pharynx. Mineralized dental elements found in conodont fossils are considered non-homologous to gnathostome teeth. Both groups of living cyclostomes, lampreys and hagfish, have keratinized dental elements, but these are morphologically distinct from gnathostome teeth and are probably not homologous. Continued analysis of cyclostome dental elements might clarify whether neural crest cells played a part in their ontogeny.

Do invertebrate chordates have neural crest cells?

Deciphering how the neural crest arose as a cell type is crucial for furthering our understanding of vertebrate evolution. Tackling this problem requires deeper knowledge of deuterostome embryonic development in multiple species, with particular attention to neural-crest-like cell types in other chordates. Recent studies have described intriguing embryonic cell populations in ascidians that have some, but not all, neural crest characteristics. For example, the trunk lateral cells in the colonial tunicate *Ecteinascidia turbinata* are derived from the A7.6 lineage, which originates in the vicinity of the neural tube, undergoes migration and gives rise to pigmented cell types. Similarly, in *Ciona intestinalis*, results show that the a9.49 cell lineage originates from the neural plate border and gives rise to the pigmented sensory cells of the otolith and the ocellus. These cells normally translocate only a few cell diameters, whereas misexpression of Twist in this lineage results in acquisition of mesenchymal morphology and long-range migration. In cephalochordates, there have been many proposed homologues of neural crest (see ref. 50 for a discussion), including a bipotential neuroepithelial precursor to pigment cells of the ocellus. Further assessment of this homology will require additional analyses of amphioxus ocellus development. Cephalochordates also have an ependymal cell in the neural tube that expresses *Snail*, a homologue of *Snai1* and a neural-crest-specifier gene in vertebrates, but this cell seems to be non-migratory.

The neural crest GRN is particularly useful for understanding assessment of GRN conservation outside of vertebrates. The available molecular data obtained from embryonic cell types in tunicates and cephalochordates suggest that gene regulatory interactions that specify the neural plate border (neural-plate-border module) are deeply conserved throughout chordates (Fig. 1c), and data from annelids suggest that this genetic program might be shared with protostomes, originating in stem bilaterians. Similarly, the terminal differentiation programs (differentiation gene batteries) that drive the neural crest to assume definitive fates are
conserved, as exemplified by control of pigment-cell differentiation. This is expected because most of the differentiation batteries are thought to be ancient subcircuits that were co-opted by different cell types\textsuperscript{27}. Although they are integral parts of the neural crest GRN, these neural-plate-border and differentiation subcircuits do not fully define neural crest identity in vertebrates. Proximally in the program, the neural plate border contains other cell types (neural tube and placode) in addition to neural crest, and is important for the delimitation of the neural plate. Distally, other deuterostomes have some differentiated cell types that in vertebrates can arise from neural crest: melanocytes, ectomesenchyme, autonomic neurons and glia. It has been proposed that during early vertebrate evolution, the neural-crest-specification module may have been assembled within the neural-plate-border cell lineage, interposed between the neural plate border and the distal differentiation modules of the network, to endow these cells with a full ‘neural crest’ phenotype.

Importantly, neural crest identity in all vertebrates is intrinsically linked to the neural-crest-specification kernel of the GRN, which endows these cells with its defining features such as multipotency, the ability to undergo EMT and migratory capacity\textsuperscript{40}. Important genes in the specification subcircuit include SoxE, FoxD and Snai1/2, homologues of which are present in the genomes of invertebrate chordates\textsuperscript{51,55}. For example, the amphioxus genome has all the transcription factors identified in the neural-crest-specifier module of the vertebrate neural crest GRN. However, only \textit{AmphiSnail} is expressed in the putative neural crest domain\textsuperscript{56}. Therefore, a key question is whether the neural-crest-like cells from tunicates possess this particular subcircuit. Molecular analyses suggest that tunicates and amphioxus have the neural-plate-border subcircuit\textsuperscript{44}, and thus invertebrate neural-crest-like cells may be homologous to neural-plate-border cells of vertebrates. However, although some neural-plate-specifier genes are expressed in these cells (for example, \textit{FoxD}\textsuperscript{49}) other crucial transcription-factor genes, notably SoxE genes, seem to be absent. In ascidians, it is not yet clear whether epistatic interactions between the transcription factors expressed in putative neural crest cells are similar to those observed in the vertebrate neural crest GRN (Fig. 1c). This, together with the fact that cells of the a9.49 lineage have not yet been shown to be multipotent, or to have extensive migratory capabilities, makes it more difficult to determine whether they are true neural crest homologues. Further gene-regulatory studies will be necessary to establish the relationship between these cells and the vertebrate neural crest.

As a cautionary note, there is inherent danger in assigning evolutionary relationships among cell types on the basis of molecular similarity alone, because transcription factors are reused throughout development, and are neither lineage- nor cell-type-specific. For instance, many bona fide neural crest transcription factors are expressed at the neural plate border, in later differentiation programs and in other lineages. Thus, one cannot attribute homology or lineage relationships on the basis of a few molecular markers. A more inclusive argument that includes morphological and behavioural information, expression data and, ideally, cis-regulatory studies\textsuperscript{57} perhaps provides the most reliable means to establish conservation of developmental mechanisms and ascribe homology between cell populations.

**Gene regulatory changes behind neural crest emergence**

Radical changes of body plan, such as those that took place in early vertebrate evolution, require substantial rearrangements in the structure of developmental GRNs\textsuperscript{27}. The emergence of the neural crest was dependent on the assembly of a specification subcircuit that allowed this cell population not only to exhibit its stereotypical behaviour, but also to drive multiple differentiation programs, resulting in its multipotent state. Understanding how a novel, complex specification subcircuit emerged during chordate evolution is a daunting task. However, observation of the neural crest GRN can provide important clues about vertebrate evolution and suggest likely scenarios for the creation of a novel cell type.

Given the deep conservation of the neural-plate-border-specification program\textsuperscript{28}, it seems reasonable to assume that this circuit was crucial for assembly of the vertebrate neural crest GRN. Because all of the neural-crest-specifier genes are present in the genomes of invertebrate chordates\textsuperscript{58,59}, it is likely that they were added to the GRN by deployment or co-option of transcription factors that were originally part of other developmental GRNs, such as the neural-plate-border subcircuit, mesodermal programs and terminal differentiation modules. According to this view, changes in their cis-regulatory apparatus placed the neural-crest-specifier genes downstream of the neural-plate-border program and signalling systems. Such cis-regulatory changes might have facilitated redeployment of neural-plate-border (\textit{Pax}3/7 and \textit{TEA}P2) and stem-cell genes (\textit{FoxD3}) in the specification module. For example, an amphioxus \textit{FoxD} enhancer that recapitulates endogenous amphioxus \textit{FoxD} expression provides the most reliable means to establish conservation of developmen-
A peripheral nervous system, including the sympathetic chain ganglia, is a common feature of all jawed vertebrates. Sympathetic ganglion cells are responsible for regulating homeostatic functions of peripheral organs. They arise from neural crest cells that migrate ventrally from the trunk neural tube to positions adjacent to the dorsal aorta, and form under the control of a gene regulatory circuit including Phox2, Hand2 and Ascl1. These genes collaborate to promote the construction of a sympathetic neural phenotype, including production of noradrenaline. In bony fishes and tetrapods, sympathetic ganglia are connected along the anteroposterior axis through chains, but in extant chordichyans (sharks, rays and skates) ganglia are largely separate. Cyclostomes do not seem to have a comparably organized sympathetic system, but very rare ganglion-like cells of unknown function have been identified\textsuperscript{[21]}. In general, autonomic function in cyclostomes seems to be controlled directly by spinal neurons of the central nervous system\textsuperscript{[21]}, which is similar to the peripheral organization of amphioxus, and thus is likely to represent a primitive condition for chordates. Taken together, these data suggest that sympathetic ganglia probably evolved in stem gnathostomes, and were further elaborated in stem osteichthyes.


d in somites and notochord\textsuperscript{[60]} was able to drive similar expression when electroporated into chick embryos\textsuperscript{[31]}. However, this enhancer failed to drive expression in the neural crest, suggesting that the novel neural crest expression domains rely on distinct gene regulatory processes that are absent in amphioxus\textsuperscript{[31]}. Similarly, co-option of EMT driver genes such as Snai2 (ref. 30) and Sip1 (ref. 47) may have allowed the neural crest to leave the neural plate border domain. This was probably accompanied by co-option of mesenchymal gene circuits that allowed these cells to exhibit migratory behaviour.

A key feature of the neural crest is its ability to form numerous derivatives (multipotency). Mechanistically, this implies that neural crest cells are capable of deploying a variety of differentiation gene batteries depending on signalling interactions during migration and once at their final sites. Neural-crest-specifier genes from the SoxE family play a crucial part in activating differentiation programs that lead to multiple derivatives, as diverse as neurons, Schwann cells, pigment cells and cartilage\textsuperscript{[48]}. Thus, a likely scenario was that a variety of differentiation gene batteries were placed downstream of the neural-crest-specification module by gain of function cis-regulatory changes, which placed differentiation driver genes (for example, Mitf, Ascl1 or Phox2b) under the control of neural-crest-specifier genes. Again, examples of redeployment of such ancient differentiation gene batteries by different cell types have been described in different contexts, and are thought to be a common feature in GRN evolution\textsuperscript{[77,84]}. Indeed, a recent study\textsuperscript{[26]} suggests that cis-regulatory changes in ancestral pro-chondrocytic genes allowed for their activation in the neural crest by factors such as SoxE and Timp2, allowing for the establishment of the vertebrate head skeleton. Thus, it is possible that the emergence of the neural-crest-specifier module served as a platform for the redeployment of multiple, pre-existing genetic subcircuits that endowed the neural crest with its defining features.

Although cis-regulatory changes were probably the most important events in the emergence of the neural-crest-specification module, it is also likely that changes in protein sequence had an important role therein. Neural crest cells employ a large repertoire of adhesion molecules, receptors and signalling molecules, and gene diversification and neofunctionalization might have enabled acquisition of the complex cell behaviours exhibited by the neural crest. Furthermore, recent data suggest that neofunctionalization of neural-crest-specifier genes such as FoxD3 was important for the emergence of this cell type\textsuperscript{[42]}, perhaps by mediating new protein–protein interactions and allowing for the assembly of novel, vertebrate-specific transcriptional complexes.

A role for gene duplications in early neural crest evolution

The extensive changes in gene regulation required for the evolution of the neural crest as a cell type might have been facilitated by large-scale genome duplications that took place early in the vertebrate lineage. It has long been suspected that rare, large-scale genomic rearrangements and genome-wide duplications in stem vertebrates had a key role in elaborating the vertebrate body plan\textsuperscript{[54,63–65]} and increasing vertebrate complexity\textsuperscript{[66,67]}. The presence of multiple homologous Hox clusters and conserved syntenic paralogy regions among jawed vertebrate chromosomes are usually taken to support the contention that there were two rounds of genome duplication during early vertebrate evolution\textsuperscript{[66]}. Recent analysis of the genome of the sea lamprey (Petromyzon marinus) suggested that ancestors of the lamprey (and hagfish) diverged from vertebrates after these two rounds of duplication\textsuperscript{[66–70]}, but this is still controversial, and an alternative model suggests that there was only a single round of duplication in stem vertebrates, followed by lineage-specific segmental duplications in jawed vertebrates and cyclostomes\textsuperscript{[71]}. Regardless of the precise number and timing of genome duplications, vertebrates have certainly undergone additional gene duplications relative to invertebrates, and these increases in gene number may have facilitated the evolution of vertebrate regulatory and anatomic complexity\textsuperscript{[63]}, potentially affecting the formation of the many novel cell types in vertebrates.

A full assessment of the extent to which gene and genome duplications have affected early vertebrate evolution remains incomplete, and is somewhat controversial\textsuperscript{[72]}. One way to approach this question is to determine whether the timing of the acquisition of particular traits compares with the inferred timing of gene duplications. Many traits were thought to arise in the vertebrate stem: these include key innovations such as the addition of neural-crest-derived pharyngeal cartilages, modification of cranial muscles, the development of segmented and Hox-patterned hindbrain\textsuperscript{[77]}, and perhaps the beginnings of peripheral nervous organization (Fig. 2). These distinct vertebrate characters are rooted in invertebrate chordates, but seem to have been fundamentally transformed by the innovation of neural crest cells and their interactions with other cell types. Thus, the timing of the acquisition of these traits correlates nicely with inferred instances of genome duplication, although one cannot distinguish cause from effect.

Ultimately, the fundamental question is how genomic duplications affected the organization of developmental GRNs. As has been discussed\textsuperscript{[65]}, such duplications may cause important shifts in gene regulatory mechanisms during vertebrate evolution. Indeed, it is possible that large-scale genome duplications may have facilitated extensive changes in the cis-regulatory apparatus controlling the transcription of neural crest genes\textsuperscript{[79]}, leading to their co-option and assembly into the neural-crest-specification module. Such events might have enabled the deployment of genes, such as those that encode SoxE transcription factors, in the neural-crest-specification module, potentially affecting vertebrate complexity.

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Evolution of crest populations along the rostrocaudal axis

Neural crest cells arising from different levels of the neural axis are endowed with distinct developmental potentials and behaviour. For example, the cranial neural crest of gnathostomes gives rise to ectomesenchymal derivatives (for example, the bone and cartilage of the face) in addition to melanocytes, glia and a subset of cranial sensory neurons. By contrast, the trunk neural crest is not able to contribute to cartilage or bone in vivo. Rather, these cells form melanocytes, dorsal root and sympathetic ganglia and chromaffin cells. Although the gene regulatory interactions underlying these differences remain unknown, they probably reflect disparities in the mechanisms of specification observed among neural crest subpopulations.

Classic heterotopic grafting experiments in the chick demonstrate that the trunk neural crest has restricted developmental potential compared with the cranial population (reviewed in ref. 4). Cranial neural crest cells transplanted to the trunk can not only give rise to all trunk neural crest derivatives, but also form ectopic cartilage nodules that are characteristic of their site of origin8,9,27. By contrast, trunk neural crest transplanted to the head fail to contribute to facial bone and cartilage, although they can form sensory neurons and glia28. These results indicate that there are cellular autonomous differences between neural crest subpopulations established during specification. This is consistent with cis-regulatory analysis of neural-crust-specifier genes, which shows that expression of both FoxD3 and Sox10 in the neural crest is controlled by separate enhancers in the head compared with the trunk33,34. Furthermore, activity of these enhancers depends on axial-specific inputs, suggesting that specification of the cranial and trunk neural crest cells relies on different genetic programs33,38.

The potential of the trunk neural crest has important implications for vertebrate evolution. For instance, it has been suggested that the neural crest played a central part in gnathostome evolution by giving rise to the exoskeleton of early vertebrates such as ostracoderms (armoured fishes)41. According to this scenario, at some point during vertebrate evolution the trunk neural crest was endowed with ectomesenchymal potential, which was subsequently lost in extant vertebrates. This hypothesis is based mainly on the fact that the skeletal plates that form the exoskeleton in armoured fishes were composed of dentine, a bona fide neural crest derivative29,30. Furthermore, studies in different model organisms suggest that the trunk neural crest exhibits at least some ectomesenchymal potential. For example, fate-map studies in zebrafish and frogs using vital dyes indicate that trunk neural crest contributes to the mesenchyme of the fins35,36. Finally, in vitro clonal analysis of avian trunk neural crest cells has shown that some clones exhibit gene expression that is characteristic of cartilage and bone37, suggesting that these cells might possess a latent ectomesenchymal potential, which can be unlocked by environmental signals37. These studies suggest that the trunk neural crest might have some residual capacity to form ectomesenchyme, consistent with the hypothesis that the trunk neural crest gave rise to the exoskeleton of basal gnathostomes.

Recently, however, this view has been challenged by a number of studies that employ genetic fate mapping and cell-transplantation analysis to define neural crest contributions in teleost fishes (Box 4). These data show that mesenchyme-derived structures formerly attributed to the trunk neural crest lineage, such as the fin osteoblast, fin mesenchyme and mineral-forming cells of the scales, are in fact of mesodermal origin84–87. Taken together, these studies suggest that the trunk neural crest of teleosts has the same developmental restrictions observed in amniotes, calling into question the neural crest origin of the exoskeleton in armoured fishes. Although further studies in other model organisms are necessary for a pan-vertebrate view of trunk neural crest potential, these results indicate that trunk neural crest has been devoid of skeletogenic potential throughout its evolutionary history. These findings suggest that alternative hypotheses for the evolution of the neural crest subpopulations require consideration.

A second scenario is that the cranial neural crest was endowed with gene regulatory mechanisms that are absent from the trunk and may have been ‘added on’ early in vertebrate evolution. So far, a few developmentally important cranial-specific regulators have been identified. In gnathostomes, for example, Ets1 (ref. 88) and Id1 (ref. 89) are enriched in cranial crest cells and are crucial neural crest-specifier genes for this subpopulation, but their expression is absent from the trunk. This raises the intriguing possibility that the genetic circuits underlying ectomesenchymal potential were added to an ancestral, trunk-like neural crest GRN. According to this view, the ectomesenchymal machinery was either co-opted from the mesoderm26 or assembled de novo in the cranial region. This scenario implies that trunk neural crest cells have a simpler GRN topology than cranial neural crest, an experimentally tractable hypothesis that can be addressed by comparative studies. This view is consistent with the large number of transcriptional regulators that are shared among all neural crest populations, consistent with a common origin.

However, a complication is that transcription of genes such as Sox10 and FoxD3 is activated uniformly along the entire neural axis, but by distinct enhancers with differential inputs in the trunk compared with cranial regions33,34. A third scenario is that neural crest subpopulations may have segregated early in vertebrate evolution and possess different GRN topology. Consistent with enhancer analysis, this hypothesis suggests that many ancestral neural crest GRN connections have been rewired during evolution and that these changes in topology resulted in two populations that have multiple differences in potential and behaviour, despite sharing a similar genetic toolbox. This scenario implies that the trunk and cranial neural crest GRNs have substantial differences, and predicts that pan-neural crest genes are generally controlled by distinct, axial-specific enhancers. Importantly, the hypotheses already discussed can be tested by in-depth analysis of the genetic pathways controlling neural crest formation at different axial levels. In particular, elucidating the circuits controlling ectomesenchymal differentiation of the neural crest will have a great effect on how we interpret the evolution of this cell population. Furthermore, additional neural crest subpopulations exist, including vagal and sacral subtypes, which have distinct migratory pathways and contribute to different derivatives. A more inclusive gene regulatory view of these subpopulations might clarify how the developmental potential of the neural crest...
Adult neural crest stem cells and post-embryonic growth

Many fossils suggest that the body size of the earliest vertebrates was, like many living invertebrates, quite small. Only later did vertebrates begin to attain larger sizes, presumably through a process that involved extending the duration of post-embryonic growth. Extended growth requires coordinated development of many cell types, possibly including the establishment of stem-cell niches that govern the growth and regeneration of novel tissues.

Until recently, there was little indication of how adult neural crest cell populations were maintained. Recent evidence suggests that amniotes have adult neural crest stem-cell populations that maintain multipotency into adulthood, presumably through a process that involved extending the duration of post-embryonic growth. Extended growth requires coordinated development of many cell types, possibly including the establishment of stem-cell niches that govern the growth and regeneration of novel tissues.

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Fig. 2 | Schematic cladogram of chordate features associated with neural crest cells or their derivatives. Labels at top indicate names of monophyletic groupings below. The timing of duplications is indicated in blue, whereas character changes are indicated by the bullet points. The order of character changes within a stem group is arbitrary. Adapted from ref. 97. CNS, central nervous system; NC, neural crest.

is established at the regulatory level, and have an impact on our views of the evolution of the vertebrate body plan.

Expansion of neural crest cell types

Development of the neural crest sets vertebrates apart from invertebrate chordates. Formation of this novel cell type was probably facilitated by the addition of a new and uniquely vertebrate ‘specification’ kernel to the GRN, which in turn conferred multipotency and migratory ability to cells at the neural plate border. During the course of vertebrate evolution, even more derivatives have emerged under the umbrella of the neural crest (for example, additional elements to the peripheral nervous system, elaboration of the jaw or formation of the middle ear). Consolidation of key neural crest specifier genes such as FoxD3, SoxE and TFAP2 in the neural-crest-specification module of its GRN may have facilitated evolution of this cell type, by allowing co-option of additional differentiation batteries under the control of neural crest regulators. Arguably, this has made the neural crest one of the most rapidly changing cell types in the vertebrate embryo and has perhaps contributed to the maintenance of neural crest stem cells in adults.

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This crucial paper identifies cellular cartilage in a cephalochordate, lending support to the contention that neural-crest-derived cartilage was co-opted from other tissues rather than constructed de novo.

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Facts and fancies about early fossil chordates and vertebrates

Philippe Janvier

The interrelationships between major living vertebrate, and even chordate, groups are now reasonably well resolved thanks to a large amount of generally congruent data derived from molecular sequences, anatomy and physiology. But fossils provide unexpected combinations of characters that help us to understand how the anatomy of modern groups was progressively shaped over millions of years. The dawn of vertebrates is documented by fossils that are preserved as either soft–tissue imprints, or minute skeletal fragments, and it is sometimes difficult for palaeontologists to tell which of them are reliable vertebrate remains and which merely reflect our idea of an ancestral vertebrate.

Vertebrates are a very small group among animals, but they show, along with arthropods and possibly echinoderms, a large number of ‘fossilizable’ complex characters that can be analysed to reconstruct their relationships; however, most of their anatomically informative fossil record appeared relatively late, about 470 million years ago (Ma). During the past 20 years or so, the fossil record of Palaeozoic era, 535–250 million year (Myr) old, jawless vertebrates has been enriched by the discovery of spectacular soft-bodied fossils preserved as imprints in famous fossil sites such as Chengjiang (535 Myr old) in China1 and the Burgess Shale in Canada2 (510 Myr old), but also in other, younger sites that yield exceptional preservation of soft tissues (referred to as ‘Kon
ervat-Lagerstätte’). These fossils, long considered to be trivial by palaeoanatomists, have gained a new dimension thanks to investigation and imaging techniques that allow the actual nature of the preserved tissues to be identified, as well as a better understanding of the processes involved in decay and fossilization, thereby avoiding their overinterpretation3–6.

Palaeontologists have been extensively tracing the earliest evidence for typical vertebrate hard tissues, such as bone, calcified cartilage, dentine (the ‘ivory’ of our teeth) or enamel, generally in the form of bone fragments, isolated scales or denticles made up of bioapatite (calcium phosphate) and found scattered in early Palaeozoic sediments7–9. This search for vertebrate ‘microremains’ or ‘ichthyoliths’ (often the only available vertebrate remains in the early Palaeozoic) yielded a large diversity of skeletal elements that could be compared with those of previously known, younger, complete fossils that belong to the major vertebrate groups, and provided evidence for the antiquity of most classic vertebrate hard tissues at least since the Lower or Middle Ordovician (about 477 Ma). However, this research also yielded some skeletal elements that, although suggesting the shape of scales or teeth, do not show all the characteristics of hitherto recognized vertebrate hard tissues. Such cases are frequent among Ordovician to Silurian (480–420 Myr old) microremains, which are dismissed by some, but regarded as possible vertebrates by others. The vertebrate fossil record is documented by an abundance of articulated specimens from periods since the late Silurian (about 430 Ma), but is either poorly represented or very puzzling in earlier periods. However, late Silurian (430 Myr old) articulated vertebrates still turn up (in Scotland, Canada and China10–13), and hint at exciting issues in deeper vertebrate history.

This may give the reader the impression that the early history (before the late Silurian) of vertebrate evolution is documented by fossils that look rather like squashed slugs and crushed lobster carapaces, although sometimes articulated. Uninformative data indeed, but, practically, it is all we can offer, except for extremely rare three-dimensionally preserved jawless vertebrates, such as the Ordovician astraspid and arandaspids11–13, which document the first occurrence of an extensive exoskeleton (or dermal skeleton, the superficial skeleton of vertebrates) with site-specific bones and a lateral-line system (the superficial sense organ of fishes).

Living vertebrates fall into two major clades, the cyclostomes (hagfishes and lampreys) and the gnathostomes (jawed vertebrates). Only the latter produce bone and dentine. Therefore, current vertebrate phylogenies that include fossils suggest that all the Palaeozoic jawless vertebrates that display at least an exoskeleton are more closely related to gnathostomes than to cyclostomes, and are thus ‘stem gnathostomes’, although lacking jaws14. These jawless stem gnathostomes that possess a calculated skeleton are informally referred to as ‘ostracoderms’ for historical reasons, but form

Figure 1 | Interrelationships of the major extant deuterostome clades. Distribution of the major tissues potentially preserved in fossil deuterostomes: no calcified hard tissue except for occasional calcified cartilage in vertebrates (blue), calcitic skeleton (green) and bone, dentine, enamel or enameloid (red).

1Muséum National d’Histoire Naturelle, UMR7207 du CNRS (Sorbonne Universités, UPMC), 8 rue Buffon, 75231 Paris, France.

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whose body shows indications of a series of myomeres (muscle blocks) distinguished from the metamery (repeated parts) of arthropods or annelids. The body musculature and gill apparatus has different developmental causes (the axial support of chordates, and precursor of the vertebral column), a deuterostome groups (Fig. 1). Most of these fossils have been referred to as either stem deuterostomes or stem protostomes (adapted from refs 31, 33). Vetulicolans31–33 (Vetulicol, Xiaozoon, Didazooon and Pomatrum; Fig. 2c) from Chengjiang and the somewhat similar Bannfia (Fig. 2d) from the Burgess Shale display a bipartite structure, with a balloon-shaped, cuticle-covered head laterally pierced by five presumed gill openings, and a flattened segmented tail19, Bannfia, however, seems devoid of gill openings and displays mid-gut diverticulae that rather suggest a protostome anatomy32. Again, the vetulicolan’s gill openings might suggest a stem deuterostome, but the purported presence of an endostyle (a gland unique to chordates) suggests stem chordate affinity32, Cathaymyrus (Fig. 2f), from Chengjiang, was described as “Pikaia-like”34. It has a worm-shaped body with a long series of myomers, and a distinct row of closely set pharyngeal slits that resemble those of cephalochordates. Other presumed chordates from Chengjiang are the debated tunicates Cheungkongella32 and Shankouclava35 (Fig. 2f). As a whole, all these presumed chordates from the Cambrian, mostly preserved as soft-tissue imprints, only provide tenuous information about their possible phylegetic relationships. And, despite their often spectacular preservation, there is a risk of overinterpreting their anatomy in the light of widely different living organisms. A notable example of this problem is Ainiktozoon (a much younger fossil from the Silurian (430 Ma) of Scotland), which has been interpreted both as a possible chordate because of its segmented body27 and as a thylacolephalan — a peculiar extant arthropod group46.

The myllokunmingiids (for example, Myllokunmingia and Haikouichthys; Fig. 2g)46–48 from Chengjiang and the similar Metaspriggina41 (Fig. 2h) from the Burgess Shale look more familiar to vertebrate specialists, as they are clearly fish-like. Despite their similarities, Metaspriggina provides better information about the arrangement of gill bars and eye structure. Although only a small number of characters can actually be observed on this kind of material, character analyses have resolved myllokunmingiids as paraphyletic, with Myllokunmingia as a stem vertebrate, and Haikouichthys as a stem lamprey39. More recent analyses suggest that all myllokunmingiids, and probably Metaspriggina, are stem vertebrates, but appear in a basal polytomy in the vertebrate tree, more crownward than Pikaia, but less so than any crown-group vertebrate (the last common ancestor to living vertebrates and all their fossil relatives)42. By combining myllokunmingiids and Metaspriggina data, a better reconstruction of the most likely Cambrian vertebrates is possible — a jawless ‘fish’ with a pair of large, anterodorsally facing camera eyes, a small median olfactory organ, 5–7 pairs of Gill arches, a stomach, a series of chevron-shaped myomeres and a median fin web (Fig. 2g, h), thereby remotely resembling old hypothetical reconstructions of ancestral vertebrates42 (Box 1).

The soft-bodied fossil record of the vertebrates is not limited to the Cambrian, and after the Cambrian ‘squashed slug’ episode comes the saga of the conodonts. Conodonts are minute tooth- or comb-like elements, or denticles, that are made up of bioapatite (like vertebrate teeth) and occur in marine sediments from the Cambrian to the Late Triassic (about 530–200 Ma). Depending on their internal structure, conodonts fall into three groups: protoconodonts, paraconodonts and eucodonts, the latter being the only monophyletic one49. For more than a century, conodonts have received diverse, sometimes fanciful interpretations, until the 1983 publication of the first conodont-bearing animal, from the Carboniferous...
(330 Ma): a conodont assemblage located in the mouth of an eel-shaped animal preserved as a soft-tissue imprint\(^5\). Other specimens have since turned up\(^\text{55}\), but so far all known articulated conodont-bearing animals are euconodonts. Anatomically, a euconodont-bearing animal has a small head with large paired eyes, a mouth or pharynx containing a large number of denticles, an elongated eel-shaped body with chevron-shaped myomeres, and a small caudal fin supported by possibly cartilaginous rods (Fig. 2i)\(^{46,47}\). Superficially, this agrees with vertebrate morphology, although the absence of more typical vertebrate structures, such as gill arches, remains puzzling. The most contentious question was whether euconodont denticles were homologous with vertebrate teeth and odontodes (skin denticles; Fig. 3a), a scenario that was advocated by some\(^58\), but rejected by others\(^59\). This controversy was finally resolved with the demonstration, by means of high-resolution microtomographic techniques, that euconodont denticle structure and growth techniques that are often associated with that of vertebrate odontodes\(^60\). Nevertheless, there remains a chordate- or vertebrate-like aspect to the euconodont body imprints, which does not preclude their position as either stem vertebrates or stem cyclostomes (Fig. 4). During the past 15 years, euconodonts were almost constantly considered in phylogenetic analyses of early vertebrates, and their position as basal-most stem gnathostomes was essentially supported by the presence of the phosphatic denticles\(^50\), which were then assumed to be homologues of gnathostome hard tissues, but lacking in all cyclostomes. However, an old hypothesis that euconodonts might be allied to cyclostomes, and more specifically hagfishes, periodically reappears in the literature\(^51-53\). For example, the enigmatic Carboniferous protoconodont-like soft-bodied fossil Conopiscius\(^6\) shows, like euconodont-bearing animals, a series of chevron-shaped myomeres, but a single pair of hollow, weakly mineralized denticles\(^52\). It has been suggested that conodont denticles were partly or entirely capped with a keratinous tissue\(^51,52\), which would remain in living cyclostomes. This hypothesis has now been dismissed\(^55\). The controversy about the homology of the para- and euconodont elements now seems to be settled, and all that soft-tissue data can currently suggest is that euconodonts might be either stem vertebrates, stem cyclostomes (Fig. 4) or, less likely, stem lampreys or stem hagfishes.

Other possible soft-bodied fossil chordates occur here and there, notably in Silurian to Carboniferous rocks, and some are more readily recognized as vertebrates, because they superficially resemble living hagfishes or lampreys. However, the risk of being misled by wishful thinking when making such comparisons is much the same as with odd Cambrian fossils. The lampreys came as a surprise when first discovered in Carboniferous 300–Myr-old rocks, because of their striking overall resemblance to modern forms. Mayomyzon\(^15\) (Fig. 2j), preserved as an imprint from the Mazon Creek Lagerstätte in Illinois, looks somewhat like a radiograph of a small modern lamprey. The image shows the outline of the body, the gill pouches and the characteristic cartilages of the ‘tongue’ apparatus. Other fossil lampreys turned up in the Carboniferous\(^46\) and the Late Devonian (around 360 Ma)\(^47\). The latter, Priscomyzon (Fig. 2k), shows annular cartilage that supports the characteristic oral funnel. The two presumed fossil hagfishes, both coeval with Mayomyzon, are more questionable. Myxinekeli\(^19\) (Fig. 2l) has cartilage imprints and tentacles that do resemble those of hagfishes, but Myxineidus\(^19\) was referred to as a hagfish based only on the impression of two V-shaped rows of keratinous teeth that resemble those of living hagfishes. The Mazon Creek Lagerstätte has also yielded peculiar presumed soft-bodied jawless vertebrates, Pipiscius and Gellichthys\(^54\). The former has a lamprey-like oral funnel, and the latter shows possible impressions of sharp, non-mineralized teeth that resemble those of hagfishes\(^57\). Yet this interpretation remains controversial\(^59\).

Another peculiar Palaeozoic soft-bodied vertebrate is Jamoytius (Fig. 2m)\(^45\), from the Silurian (about 438 Ma) of Scotland, which was first regarded as an ‘ancestral chordate’. New investigations show that the series of W-shaped imprints on the trunk of Jamoytius are not merely soft-tissue imprints of myomeres, but weakly mineralized scales\(^45\). With its median nostril and about ten gill openings, Jamoytius is otherwise suggestive of a lamprey and is often regarded as closely related to the younger, Devonian euphaneropids (Euphanerops, Corniovichthys, Achanarella; Fig. 2n), whose morphology is now best known from well-preserved 380-Myr-old Euphanerops material from the Late Devonian Miguasha Lagerstätte in Canada. Young individuals of Euphanerops are preserved as soft-tissue stains, but large individuals also show peculiar spongy calcifications of various elements of the endoskeleton (the internal, cartilaginous or bony skeleton of vertebrates), notably the fin radials, gill bars, vertebral elements, and elements that resemble the ‘tongue’ and annular cartilages of lampreys\(^4\). The most peculiar feature of Euphanerops is the large number (about 30 pairs) of gill bars that form its lamprey-like gill basket and extend back to the anal region. This is confirmed by a three dimensionally preserved specimen that shows impressions of the gill filaments\(^62\). Besides this feature, the overall appearance of Euphanerops resembles that of an anaspids, a group of Silurian–Devonian ‘ostracoderms’ that were long thought to be ancestral to lampreys, but are now regarded as being among the basal-most stem gnathostomes\(^60,61,63\). Like anaspids, Euphanerops displays a long, posteroventrally slanting tail and a large anal fin, suggested to be paired — a unique case among vertebrates\(^46\). However, this requires confirmation, as does the elongate, paired ventrolateral fins that seem to have extended ventrally to the gill basket\(^61\). Whatever their relationships to Jamoytius, euphaneropids did not possess mineralized scales, but do have some endoskeletal characters uniquely shared with lampreys\(^55\).

Finally, Palaeospondylus, from the Middle Devonian (390 Ma) of Scotland is still the most enigmatic early vertebrate, although it is known by hundreds of specimens. It is not preserved as a mere imprint, but clearly displays a vertebral column, a caudal fin with radials and fin supports, possible paired appendages, and its skull consists of several peculiar skeletal elements that cannot be clearly homologized with classic components of the vertebrate skull, be it a cyclostome or a gnathostome\(^60,66,67\). All of its skeletal elements are exclusively made up of a spongy calcified matter, which resembles that of the calcified endoskeleton of Euphanerops\(^61\), and

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**BOX 1**

**Fossils and ‘ancestors’**

When the first description of the myllokunmingiids was published\(^69\), early vertebrate palaeontologists were struck by the resemblance between these Lower Cambrian soft-bodied fossils from Chengjiang and various imaginary reconstructions of an ancestral vertebrate published during the twentieth century. For example, myllokunmingiids surprisingly resemble this imaginative reconstruction of an ‘ancestral cephalochordate’ (amphioxus) (see Figure) published at a time when some zoologists considered the absence of a complex head in living cephalochordates could be secondary. This reconstruction is a curious mix of a rather vertebrate-like, and even a ‘ostracoderm-like’ head, and some cephalochordate characters. It was thus intended to suggest that the overall morphology of the common ancestor to cephalochordates and vertebrates was rather vertebrate-like. Do such reconstructions of an entirely hypothetical ‘ancestor’, essentially based on inferences from extant and some fossil vertebrates, influence the way we interpret some zoologists considered the absence of a complex head in living cephalochordates could be secondary. This reconstruction is a curious mix of a rather vertebrate-like, and even a ‘ostracoderm-like’ head, and some cephalochordate characters. It was thus intended to suggest that the overall morphology of the common ancestor to cephalochordates and vertebrates was rather vertebrate-like. Do such reconstructions of an entirely hypothetical ‘ancestor’, essentially based on inferences from extant and some fossil vertebrates, influence the way we interpret such fossils lead us to search for such old and supposedly prophetic reconstructions to justify intuitions? Although palaeontologists try to take a cold look at characters, it is probable that such reconstructions, based on the tree of life in vogue at a given time, unconsciously affect the way researchers look at certain fossils and favour wishful thinking when in search of ancestors. This was probably also the case for the interpretation of Pikaia. Image adapted with permission from ref. 42.
is therefore interpreted as calcified cartilage. Its resemblance to embryonic cartilage of extant osteichthyans (bony jawed vertebrates) has even led to the suggestion that *Palaeospondylus* might be a peculiar bony fish that failed to develop bone. The anatomy of *Palaeospondylus* has been described, and this ‘fish’ has been tentatively referred to as practically all major fossil and extant vertebrate groups: hagfishes, lampreys, ‘placoderms’ (extinct armoured jawed fish), chondrichthyans (sharks, rays and chimaeras), teleosts, lungfish larvae and amphibian tadpoles. All these interpretations are either dismissed or still debated. However, data on hagfish skeletal development seem to enhance the superficial resemblance, already alluded to by some early authors, between the arrangement of certain elements of the *Palaeospondylus* skull and that of the cranial cartilages of late hagfish embryos. In addition, developmental data suggest that the absence of vertebral elements in hagfishes is probably secondary, and the vertebral column of *Palaeospondylus* may thus not preclude close relationships to hagfishes. Yet, no unambiguous character seems to be uniquely shared by hagfishes and *Palaeospondylus*.

These presumed soft-bodied chordates and vertebrates that were mostly devoid of hard tissue, except for occasional calcified cartilage, are generally collapsed and preserved as traces of variously transformed soft tissue. Their reconstruction in 3D is often difficult, even by means of sophisticated techniques, and their descriptions are characteristically cautious. Should we simply forget about them? Do they provide us with any useful information? Or are they merely material support to our imagination, which is in turn guided by current views about the interrelationships of living animal groups? The art of reconstruction for palaeontologists is usually to put flesh on bones, but it is difficult when there is only decayed flesh and no bone! However, it is worth trying.

**Hard–tissue data**

Early vertebrate hard tissues are reputedly easier to identify. Their structure can be studied in detail by means of material or virtual (microtomographic) sections, eventually in 3D, and classic scanning electron microscopy techniques. Their characteristics can then be compared with those of living or more recent and well-known species. Nevertheless, palaeontologists are confronted with many of the same problems as for soft-tissue preservations when dealing with the earliest presumed vertebrate skeletal remains. The first clues to vertebrate hard tissues are that they are made of bioapatite; the tissues often show an ornamentation of tubercles (odontodones), or ridges, with a structure that resembles that of our teeth; they have dentine that contains thin canals for cell processes; eventually enamel (enameloid) is present; and there is a pulp cavity (Fig. 3a). Other useful characters may be the surface ultra-sculpture, the small spaces that hosed bone cells, and the grooves or canals that hosed lateral-line sense organs. The exoskeleton of the earliest, articulated and duly recognized vertebrates, such as arandaspids or astraspids (Fig. 3b, c), show at least some of these characters. However, younger vertebrates known from complete specimens, such as the Silurian and Devonian anaspids or galeaspids, lack dentine, and many of the Cambrian to Silurian ‘microremains’, referred to as vertebrates owing to the aspect of their ornamentation or their scale-like shape, lack some of these characteristic tissues. Instead, they show other hard tissues that no longer exist, such as lamelline (acellular dentine). Therefore, the earliest evidence for possible vertebrate hard-tissue remains are barely less puzzling than the Cambrian soft-bodied animals.

The first controversy about these problematic skeletal fragments arose with the discovery of *Anatolespis* from the Lower Ordovician and Upper Cambrian. *Anatolespis* is represented by minute phosphatic fragments ornamented with elongate tubercles (Fig. 3d), which vaguely resemble the exoskeletal ornamentation of certain Silurian–Devonian ostracoderm, notably heterostraci (Fig. 4). Therefore, *Anatolespis* was first regarded as a possible heterostracan; this was immediately contested by some, whereas others considered it plausible. At around the same time, *Anatolespis* was tentatively referred to an arthropod, but again this raised debate. Later studies of the tissue structure of these fragments using new techniques showed that the tubercles of *Anatolespis* were in fact hollowed by a pulp cavity capped by a somewhat dentine-like tissue, and connected by a lamellar tissue, which was perforated by thin vertical canals (Fig. 3e). Nevertheless, these new data failed to convince the sceptics. *Anatolespis* may remain an enigma — as long as no articulated individual turns up. Other alleged Late Cambrian vertebrate bone fragments have been described from Australia and superficially resemble the exoskeletal bone ornamentation of the Ordovician arandaspid *Porophoraspis*; however, they are also strikingly similar to some Palaeozoic arthropod carapaces. In sum, apart from the euconodonts, whose possible vertebrate affinities essentially rest on soft-tissue characters, there is no undisputed evidence for Cambrian vertebrates that possess a mineralized skeleton. By contrast, the following Ordovician period not only yields articulated vertebrates covered with extensive mineralized armour and scales, but also numerous isolated bone fragments and scales. Most of these microremains, such as

![Figure 3 | Late Cambrian, Ordovician and early Silurian vertebrate exoskeletons. a, Ideal vertical section through a typical, ornamented vertebrate exoskeleton showing a tubercle (odontode) attached to a bony base (not to scale). b, c, The most complete articulated Ordovician vertebrates, *Astraspis* (b) and *Sacambambaspis* (c) (adapted from refs 86, 93). d, e, Exoskeleton fragment of the debated vertebrate *Anatolespis* (d) and vertical section of the possible odontodes (e) (adapted from ref. 77). f–I. Major types of isolated vertebrate scales retrieved from Upper Ordovician and Lower Silurian rocks: a thelodont (f), an ‘acanthodian’ (g), the possible chondrichthyan *Mongolepis* as an external view (h) and vertical section (i), and the vertebrates of uncertain affinities *Tesakioviaspis* (j), *Apedolepis* (k) and *Areyongalepis* (l). (f–I) adapted from ref. 8 and k, l from ref. 82). Scale bars are 1 cm (b, c), 0.5 mm (f–I) and 50 μm (e).](https://www.nature.com/articles/486146a)
Mayomyzon
Devonian
Pituriaspids
Permian
†
†
†
Porophoraspis
sity of the scales and other microremains retrieved from coeval Ordovi
classical Ordovician fishes looked like big armoured tadpoles. However, the diver
thetic platelets, suggesting a filtering function, as in the younger het
ings is unclear
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However, they provide no information about internal anatomy, apart from
series of numerous gill openings, and a scale-covered body and tail
Vertebrates that have an extensive exoskeleton with a large head shield
composed of either large plates or polygonal platelets, a posteriorly slant
sub-classical Palaeozoic jawless vertebrate groups, which are known from complete skeletons.
The three articulated Ordovician vertebrates, Astraspis (Fig. 3b), Aran
daspis and Sacabambaspis (Fig. 3c)\(^\text{11–13}\), and the bone assemblages of Erip
tichius\(^\text{36}\) and Ritchieichthys\(^\text{34}\) show the overall morphology of the earliest vertebrates that have an extensive exoskeleton with a large head shield composed of either large plates or polygonal platelets, a posteriorly slanting series of numerous gill openings, and a scale-covered body and tail\(^\text{34,36}\). However, they provide no information about internal anatomy, apart from uninformative fragments of calcified cartilage in Erip
tichius\(^\text{36}\), and faint internal impressions of the gill pouches in Astraspis and Sacabambaspis.\(^\text{34}\) Orbits indicate the presence of eyes, and paired dorsal openings in aran
daspids are interpreted as pineal foramina, but the position of nasal open
ing is unclear\(^\text{34}\). The lower lip of arandaspids is covered with a series of minute platelets, suggesting a filtering function, as in the younger heterostracans\(^\text{22}\). These articulated fossils may give the impression that all Ordovician fishes looked like big armoured tadpoles. However, the diver
sity of the scales and other microremains retrieved from coeval Ordovi
cian rocks suggests that different morphologies may have existed already.\(^\text{34}\) Porophoraspis is regarded as an arandaspid, but some relatively large plates referred to as this genus are difficult to reconcile with the head-skeleton morphology of either Arandaspis or Sacabambaspis\(^\text{34}\). Among the isolated scales retrieved from Ordovician and Early Silurian rocks, some clearly belong to thelodonts (a group of ‘ostracoderms’; Figs 3f, 4) and ‘acanthodians’ (Fig. 3g; presumed stem chondrichthyan). Both of these were known later by complete specimens, whereas others, such as Mongolepis (Fig. 3h, i), Teslepis, Sodolepis and Tesakoviaspis (Fig. 3j),\(^\text{34}\) all presumed chondrichthyanans (shark relatives), and still-unnamed forms\(^\text{34}\) may have belonged to vertebrates that had an entirely micromeric (composed of minute scales) exoskeleton like that of sharks. Their body structure will remain unknown unless articulated material is discovered in some still
elusive Lagerstätt. Although some of these scales are, by default, referred to as chondrichthyanans, they are in fact vertebrates in limbo.

After the Middle Ordovician, no articulated vertebrate turns up until the mid-Silurian (around 433 Ma), apart from the Late Ordovician euconodont Promissum\(^\text{45}\). Then, relatively complete representatives of the six major Silurian–Devonian ‘ostracoderm’ groups (anaspids, heterostracans, thelodonts, galeaspids, pituriaspids and osteostracans; Fig. 4) occur, and, shortly after (about 430 Ma) the earliest complete jawed vertebrates, notably ‘placoderms’\(^\text{30}\), ‘acanthodians’ and osteichthyanans (bony fishes)\(^\text{34}\). Such articulated or well-preserved material is generally the key to suggesting a systematic position for some of the microremains from the Ordovician and early Silurian, and tracing back the distribution of these major groups through time (Fig. 4). Moreover, the number of anatomical characters that this material now offers us allows for better supported reconstructions of the interrelationships of these groups.

The phylogenetic trees of fossil and living vertebrates generally agree on the position of the ‘ostracoderms’ as a series of jawless stem gnathostomes,
with galeaspid, osteostracans (and possibly pituriaspids) as successive sister groups of the jawed vertebrates.\textsuperscript{5,57,60,68} (Fig. 4). This is partly because galeaspid and osteostracans have an extensively calcified or ossified endoskeleton, which preserves the cavities and canals that housed the brain, sensory capsules, nerves and blood vessels, including the pectoral girdles and fins in osteostracans, thereby providing a wealth of anatomical characters that can be compared with their homologues in jawed vertebrates.\textsuperscript{57,88,97} However, the relationships of other ‘ostrocarid’ groups is poorly supported because they are devoid of a calcified endoskeleton, and their exoskeleton, which is sometimes entirely micromeric, provides indirect information about their internal anatomy in the form of faint impressions of, for example, gill pouches, brain, olfactory organs or labymorphin.\textsuperscript{97} As is the case for heterostracans, but there are no data for anaspids, and only a few tholodonts provide some information.\textsuperscript{57,90,94} Heterostracans are characterized by a single pair of common branchial openings, and are garned with aspistras and arandaspids in the pteraspidomorphs (Fig. 4)\textsuperscript{57,72}. However, apart from the presence of large median dorsal and ventral head plates made of accular bone, and a similar honeycomb-like layer in the exoskeleton of heterostracans and arandaspids, shared derived characters that are unique to these three groups are scarce.

For almost a century, most debates about the relationships of the various ‘ostrocarid’ groups have been centred on the structure of the rostral part of the head: the olfactory organs, their relation to the hypophysis (pituitary) and the oral region. Classically, the dorsal position of the common nasal and hypophyseal duct of osteostracans and anaspids was compared with the condition in lampreys\textsuperscript{92,93}. However, the recent description of the same region of the head in galeaspid has provided new insights.\textsuperscript{94} The still elusive heterostracan and tholodont internal anatomy could possibly be reconstructed on the basis of that of galeaspid, with paired nasal sacs and an anteriorly directed hypophyseal duct. This would mean that a galeaspid-like anatomy might have been widespread among stem gnathostomes, and that the alleged lamprey-like nasohypophyseal complex of osteostracans is independently derived from such a condition.

Fossils, phylogeny and technologies

It is sometimes said that fossils never, or rarely, overturn patterns of relationships based on extant organisms. Patterson\textsuperscript{57} mentioned a few possible exceptions, notably the ‘calcichordate theory’\textsuperscript{99}, which assumed that an ensemble of Palaeozoic echinoderm-like groups classically referred to as stylophorans are a paraphyletic array of stem chordates, stem cephalochordates, stem tunicates and stem vertebrates, the calcitic skeleton of which has been lost several times. It also suggested that tunicates, and not cephalochordates, were the closest extant relatives of vertebrates (contra to the then accepted relationships). This theory has raised heated controversies\textsuperscript{92}, but all stylophorans are now regarded as stem echinoderms. However, recent molecular phylogenies strongly support this tunicate–vertebrate relationship\textsuperscript{100}. Tunicates and vertebrates are therefore gathered in a group called Olfactores, a name that, paradoxically, was erected in the framework of the calcichordate theory\textsuperscript{92}, because some stylophorans that were thought to be stem tunicates display internal structures that resemble vertebrate ophistosomal organs. Patterson\textsuperscript{57} predicted that molecular sequence data would be the best test of the ‘calcichordate theory’, and, coincidently, the test seems to have been positive regarding tunicate relationships.

Regarding vertebrates, the hypothesis of living cyclostome paraphyly (that lampreys are more closely related to gnathostomes than to hagfishes) was only based on phenotypic data derived from extant species\textsuperscript{57}. Palaeontological data have been merely adapted to this pattern of relationships, because of the long-lasting conviction that certain ‘ostrocarids’ (osteostracans and anaspids) were most closely related to lampreys\textsuperscript{92,93}. More accurate character analyses later showed that ‘ostrocarids’ were exclusively stem gnathostomes, and the recent revival of cyclostome monophyly had no major bearing on their interrelationships\textsuperscript{92,93}. None of the fossils discussed earlier, be they soft-body imprints, bone fragments, scales or articulated skeletons, seems currently liable to overturn the interrelationships of the major extant vertebrate groups. However, they provide a minimal age for certain characters (thus the groups they define), and may reveal unsuspected character combinations that allow the reconstruction of the stepwise assembly of novel body plans that foreshadow major evolutionary transitions. This is, for example, what ‘ostrocarids’ document with the succession of characters that make up the jawed vertebrate body: the rise of the exoskeleton, cellular bone, endoskeletal bone, enlarged cerebellum or pectoral fins, but they are still rather powerless in providing a scenario for the rise of jaws, which is largely left in the hands of evolutionary developmental biologists. Nevertheless, the recent consideration of braincase anatomy in the basal-most ‘placoderms’ suggests that the anatomical gap between such ‘ostrocarids’ as galeaspid and osteostracans, and the earliest jawed vertebrates, may not have been that large, and that the prerequisites to the rise of jaws were already there\textsuperscript{210}.

The future of early vertebrate palaeontology rests on the quality of the data it can provide, especially on fossils derived from crucial periods, such as the Late Cambrian, Early Ordovician and early Silurian. Early vertebrates are generally difficult material, compressed or crushed in hard rocks. Throughout the twentieth century, some early vertebrate palaeontologists gave much weight to the then new preparation techniques and, coincidently, data would be the best test of the ‘calcichordate theory’, and, coincidently, soft tissues preserved as mere stains can be studied by element mapping that provides information on the fossilization process and sometimes the nature of the preserved tissues themselves. Armed with these non-destructive techniques, early vertebrate palaeontologists can considerably refine their observations and must not be afraid of proposing audacious interpretations of these miserable remains, even though ‘squashed slugs’ may be slippery!}

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This is an updated review of exoskeletal hard tissue in early vertebrates.


Patrick, R. S. The Ancestry of the Vertebrates (British Museum (Natural History), 1996).

This is an extensive account of the calcichordate theory.


This text is a review of fossil-based theories about the origin of vertebrates.


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The origin and early phylogenetic history of jawed vertebrates

Martin D. Brazeau1 & Matt Friedman2

Fossils of early gnathostomes (or jawed vertebrates) have been the focus of study for nearly two centuries. They yield key clues about the evolutionary assembly of the group’s common body plan, as well the divergence of the two living gnathostome lineages: the cartilaginous and bony vertebrates. A series of remarkable new palaeontological discoveries, analytical advances and innovative reinterpretations of existing fossil archives have fundamentally altered a decades-old consensus on the relationships of extinct gnathostomes, delivering a new evolutionary framework for exploring major questions that remain unanswered, including the origin of jaws.

Jawed vertebrates (gnathostomes) comprise more than 99% of living vertebrate species, including humans. This diversity is built on features including jaws, teeth, paired appendages, and specialized embryonic and skeletal tissues (Box 1); centuries of research have attempted to explain their origins1-3. In particular, jaws and paired appendages have become flagship systems in the study of evolutionary novelty4 — a key research programme in evolutionary biology5.

The deepest split in the modern gnathostome tree is that between the chondrichthyans (sharks, rays and chimaeras) and the osteichthyans (bony fishes and tetrapods). This divergence occurred in the Palaeozoic era, at least 423 million years ago (Ma)26, leaving a vast temporal and evolutionary gulf between modern lineages, with ample time for new innovations to overwrite primitive conditions. These complexities compel researchers to turn to the Palaeozoic fossil record to elucidate the origin of jawed vertebrates. A few well-preserved fossil taxa from a handful of Silurian–Permian sites in Europe and North America27 shaped late nineteenth- and early twentieth-century hypotheses of gnathostome evolution1,11,12 (Fig. 1). Many of these narratives persist to this day, either implicitly or explicitly. However, fossils once hailed as avatars for scenarios of jaw2,23 or fin origins turn out to be specialized rather than primitive after phylogenetic investigation15-16. Until they are placed in a evolutionary tree, Palaeozoic fossils are mute on the question of gnathostome origins.

In this Review, we examine the progress made in the past two decades on the study of early gnathostome interrelationships, focusing on key fossil discoveries that have prompted a renewed intensity of phylogenetic investigation. Although tremendous advances have been made, much work remains before this research can deliver finely atomized transformations of living species and gnathostomes to the exclusion of hagfishes and gnathostomes to the exclusion of hagfishes and gnathostomes. A series of remarkable new palaeontological discoveries, analytical advances and innovative reinterpretations of existing fossil archives have fundamentally altered a decades-old consensus on the relationships of extinct gnathostomes, delivering a new evolutionary framework for exploring major questions that remain unanswered, including the origin of jaws.

Diversity of Palaeozoic jawed vertebrates

Putative examples of jawed vertebrates date to the Ordovician period27-29, but the first definitive remains are of early Silurian age30. Early Devonian (419 Ma) mandibulate gnathostomes were already ecologically diverse31 and, by the close of the Devonian (360 Ma), the first tetrapods and many of their adaptations for terrestrialization had emerged32.

Early jawed fishes are divided into four broad categories: ancient representatives of chondrichthyans and osteichthyans, along with two exclusively extinct assemblages: acanthodians and placoderms. The early chondrichthyan record is dominated by isolated denticles (scales), teeth and spines. The oldest records of scales attributed to chondrichthyans are from the earliest Silurian (around 443 Ma)33, such as mongolepids34. Sina-canthids, represented by isolated spines that share histological similarities with chondrichthyans35, are also known from the early Silurian (about 438 Ma)36. The oldest universally accepted chondrichthyans are substantially younger, represented by Early Devonian body fossils (around 400 Ma; Fig. 2e). Some of these specimens derive from the Malvinokaffric Realm, a cold-water Southern Hemisphere palaeobiogeographic province that yields distinctive jawed vertebrate faunas almost exclusively composed of acanthodians and chondrichthyans37. Articulated chondrichthyan fossils are rare throughout the Devonian, with most specimens known from the exceptional Late Devonian Cleveland Shale Lagerstätte (Fig. 1).

The late Silurian–Devonian osteichthyan record is considerably better than that of chondrichthyans owing to the armour of dermal plates and...
Crown-, total- and stem-group concepts provide a useful framework for navigating evolutionary trees that include fossils. The tree shown in the figure reflects the most basic splits among living vertebrates. Crown groups comprise the last common ancestor of a group of living species plus all of its descendants, both fossil and modern. The gnathostome crown group includes the last common ancestor of osteichthians (represented by a salmon) and chondrichthians (represented by a shark) plus all of its descendants, and comprises all the green and orange parts of the tree. Total groups include the crown group of interest plus all extinct forms more closely related to that lineage than to any other living species. Here, the gnathostome total group is represented by all coloured parts of the tree. Stem groups are equal to a clad’s total group minus its crown group, shown here by the pink lineage connecting the vertebrate and gnathostome crown nodes. Jawed vertebrates include all of the gnathostome crown, and the upper reaches of the gnathostome stem. The lower part of the gnathostome stem is populated by jawless osteostracans, which are more closely related to jawed vertebrates than they are to modern jawless fishes. The principal task faced by palaeontologists is to fit fossil groups (such as acanthodians and placoderms; the dagger symbol indicates that they are extinct) within the genealogical framework for modern species. Monophyly of jawed vertebrates is evidenced by a series of shared morphological specializations including, but not limited to, jaws. Key gnathostome features are illustrated here for Eusthenopteron (Cleveland Museum of Natural History CMNH 8158, image courtesy of D. Chapman), an osteichthian and relative of land vertebrates. These traits must have evolved along the gnathostome stem lineage, but without fossils it is impossible to determine the order in which — or when — they arose.

The evolution of gnathostome phylogeny

The current picture of Palaeozoic gnathostome relationships is the product of three phases of study. Throughout, researchers have benefitted from high-quality data, thanks to the early application of physical tomography by Stensiö and the ‘Stockholm school’ and the maturation of acid-preparation techniques in the middle of the twentieth century and the non-destructive computed tomography of the past 15 years. The modern phase of research into gnathostome relationships began with the introduction of phylogenetic systematics to vertebrate palaeontology, which had previously focused on linking species from successive geological strata as an approximate ancestor–descendant chain. Monophyly of the major taxonomic divisions of early gnathostomes was assumed, and their relative relationships were largely inferred using evidence from European and North American fossils. Within a decade of the initial application of cladistics to early vertebrates, an imperfect consensus emerged that acanthodians were a clade of stem osteichthians and that placoderms were the immediate sister group of crown gnathostomes. This framework would persist for more than 30 years, despite the intervening discovery and detailed description of fossils from Australia, China and northern Canada that provided fresh morphological information beyond the stagnating stable of classic Euramerican taxa.
The second phase began in the 1980s with a cladistic reinterpretation of the ostracoderms. Detailed anatomical reinvestigations of ostracoderm sublineages and numerical phylogenetic analysis resulted in the recognition of this assemblage as a paraphyletic gnathostome stem group.\(^{35-49}\) Reconfiguration of the agnathan menagerie permitted reconstructions of evolutionary patterns in fin morphology and skeletal hard tissues, and identified the extinct jawless sister group of jawed vertebrates. Although many ostracoderm lineages have been considered contenders for this position, anatomical evidence overwhelmingly supports osteostracans. Like jawed vertebrates, but unlike other agnathans, osteostracans bear well-developed pectoral fins with associated girdles, a epicercal tail, and perichondral and cellular bone (Box 1).

The third and ongoing phase is the detailed scrutiny of the pioneering cladistic framework relating acanthodians and placoderms to modern jawed vertebrate lineages. Traction on this problem arose indirectly, beginning around the turn of the century with the development of expanded numerical phylogenetic analyses targeting relationships within osteichthyans\(^ {70-73}\) and chondrichthyans\(^ {74-76}\), but employing acanthodian and placoderm outgroups. These studies introduced the use of increasingly larger data sets, and provided the character information that would seed analyses targeting not individual lineages, but early jawed vertebrates as a whole. At the same time, a series of new fossil discoveries (outlined later) revealed unexpected anatomical combinations that raised serious questions about the coherence of acanthodians and placoderms. This set in motion a series of refined analyses of early jawed vertebrates bent on testing the supposed monophyly of these groups.\(^ {42,49,56,77,78}\) This final phase is a current debate and the setting for the following discussion.

**New fossil discoveries and their importance**

In this section, we highlight key finds since the 1980s that have challenged embedded perceptions and explain their importance in light of what is or was known about early jawed vertebrate evolution. Presented in approximate phylogenetic order, ascending from jawless members of the stem lineage, to placoderms, to members of the gnathostome crown, these discoveries provide a broad summary of the emerging picture of major evolutionary patterns in early gnathostomes. Detailed accounts of character transformation are provided elsewhere.\(^ {20}\)

**Shuyu and Romundina and their noses for success**

The neurocranium, or braincase, is a primitively cartilaginous structure that houses the brain and paired sensory organs in vertebrates. When coated with a mineralized rind, structurally complex braincases can be

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**Figure 1 | Fossils relevant to early jawed-vertebrate evolution derive from major fossil sites in North America and Europe, and increasingly China and Australia.** Palaeogeographic positions of localities bearing early jawed vertebrates and characterized by abundant fossils, high-fidelity preservation or both. Taxonomic breakdown of gnathostome diversity within sites is indicated by the associated pie charts and size-scaled to reported species richness.

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**MOTH, Man on the Hill.** The vignettes depict scenes based on key fossil sites: Gogo, Australia (left) and Cleveland Shale, USA (right) in the late Middle–Late Devonian; the Xitun Formation, China (left) and Orcadian Basin, UK (right) in the Early–early Middle Devonian; and the late Silurian Kuanti Formation, China (left and right). Illustrations by B. Choo, Flinders University. Palaeogeographic reconstructions by R. Blakey, Colorado Plateau Geosystems.
A tantalizing mosaic of cyclostome-like features generally associated with placoderms and chondrichthians. e, Braincase of Psarolepis, an Early Devonian lobe-finned osteichthyan from China represented by isolated bones, including spines of the kind associated with chondrichthians, placoderms and acenthians. f, The surprising reconstruction of Psarolepis was corroborated by the discovery of the more complete and even more ancient Guiyu, from the late Silurian of China. Images courtesy of a, Z. Gai; b, g, J. Long; c, i, M. Zhu; e, R. Miller; f, S. Cumbaa.

Claspers and their evolutionary implications

The Ptyctodontid placoderms have long been known to possess claspers8, intromittent organs associated with the pelvic fins and evidence of internal fertilization. This trait factored in early cladistic investigations of placoderm intra- and interrelationships, tying placoderms to chondrichthians52 and fuelling arguments that ptyctodonts are the sister group of all other placoderms58. The discovery of arthrodire embryos within adult specimens prompted renewed investigation of this group in which long-overlooked evidence of claspers was finally discovered82–84, followed by the realization that antiarchs also possessed these structures85. The palaeobiological and reproductive importance of claspers has been well considered82, but their full phylogenetic importance is unresolved. Current phylogenetic consensus does not regard placoderm and chondrichthyan claspers to be homologous85, but the homology of claspers within placoderms seems likely. Placoderm paratype demands the loss of internal fertilization before the origin of crown gnathostomes, signalling an unprecedented shift in reproductive biology within vertebrates85. Thus, we face two problematic alternatives: either internal fertilization was lost in a crownward segment of the gnathostome stem, defying observational data on the reproductive biology of living vertebrates85, or placoderms with claspers form a clade, contradicting the apparent signal of other traits85.
Entelognathus reframes ancestral conditions

The perceived ‘primitiveness’ of chondrichthyan anatomy entrenched in many general introductions to vertebrate biology has deep pre-Darwinian roots. Faced only with living species, this view seems reasonable enough: with their shagreen of tiny scales and cartilaginous internal skeletons, chondrichthyans seem to be tailor-made morphological intermediates between the naked hagfishes and lampreys on the one hand and the internally and externally bony osteichthyans on the other. The fossil record subverts this tidy picture by showing that both large dermal plates and a bony internal skeleton are innovations that arose long before the divergence of osteichthyans and chondrichthyans. However, the condition of the skeleton in the last common ancestor of jawed vertebrates has remained controversial thanks to two mutually reinforcing phenomena: a reluctance to make explicit comparisons between the bony plates of osteichthyans and placoderms, and repeated interpretations of at least some acanthodians as early osteichthyan relatives. Together these factors paint a picture of an ancestral crown gnathostome covered in a ‘micromeric’ outer skeleton of tiny scales, with a ‘macromeric’ skeleton composed of large plates reappearing in the osteichthyan lineage. This view was turned on its head by the discovery of the late Silurian Entelognathus in China (about 423 Ma; Fig. 2c). Although Entelognathus broadly resembles a standard-issue placoderm, its cheek and upper and lower jaws are covered with bones that match the pattern seen in osteichthyans, rather than other placoderms. This remarkable correspondence suggests that there is evolutionary continuity between the large dermal plates of placoderms and those of bony fishes.

Man on the Hill brings acanthodians into the light

The Man on the Hill (MOTH) locality in the Northwest Territories of Canada is an Early Devonian (about 419 Ma) Konrastov Lagerstätte yielding articulated early vertebrates. Originally discovered in the 1970s, new collections and advances in chemical preparation have since revealed exquisitely preserved fossils (Fig. 2d). Jawed vertebrates from MOTH are mostly acanthodians (Fig. 1), providing important anatomical detail on this enigmatic assemblage. Previously, the record of complete acanthodian fossils was mostly restricted to crudely prepared specimens from low-diversity, fluvial–lacustrine Early Devonian deposits of the United Kingdom. By contrast, acid-prepared acanthodians from the species-rich marine MOTH locality reveal crisp anatomical details. In particular, a host of these species have umbellate and denticle-like scales such as those found in chondrichthyans. Perhaps more importantly, the MOTH fauna includes examples of acanthodian-like fishes covered in scales with growth patterns and structure previously known only from isolated fragments, but conventionally assigned to chondrichthyans. This simultaneously suggests a position for acanthodians in the jawed vertebrate tree, while undermining confidence that they comprise a natural group.

The inside story on acanthodian morphology

Several early placoderms, osteichthyans and chondrichthyans yield detailed braincases, but acanthodian examples are rare. Subject to many re-interpretations over the past 100 years, the neurocranium of the Permian Acanthodes is central to debates on the evolutionary affinities of acanthodians. Various authors have been impressed by what they perceived as either particularly osteichthyan-like or chondrichthyan-like features of Acanthodes, triggering contrasting views on the placement of acanthodians as a whole. The Early Devonian (around 419 Ma) Ptomacanthus also preserves a braincase, although detail is obscure to the degree that this structure was initially ignored. Re-examination of Ptomacanthus revealed a neurocranium with a gross architecture that is more similar to that of placoderms or chondrichthyans than that of Acanthodes and osteichthyans, providing evidence in the first explicit argument for acanthodian paraphyly.

A sneak peek at early shark anatomy

With a sparse early record, interpretation of primitive chondrichthyan conditions drew heavily on body fossils from the latest Devonian, all of which are probably highly specialized. This changed with two stunning finds in the early 2000s. First was the discovery of more complete neurocranial of Pucapampella from the Early Devonian of Bolivia and a similar South African form. Previously named on the basis of an isolated neurocranial base, Pucapampella bears a chondrichthyan-specific hard tissue (prismatic calcified cartilage) in combination with a ventral fissure: a persistent division between two embryonic braincase components. Absent in ostracoderms, placoderms and other chondrichthyans, but present in Acanthodes and bony fishes, the ventral fissure was long considered key evidence for a close relationship between acanthodians and osteichthyans. Pucapampella suggests that this trait is a general feature of crown-group gnathostomes. Subsequent discoveries provided additional anatomical details for Pucapampella, revealing peculiar teeth and jaws to accompany its unanticipated neurocranial architecture. Hot on the heels of Pucapampella came the discovery of the oldest articulated chondrichthyan, Doliodus, from the Early Devonian of New Brunswick, was known for more than a century only by isolated teeth, and assigned to acanthodians. Recovery of an articulated head and forequarters revealed the signature chondrichthyan trait of prismatic calcified cartilage occurring in a fish with stubby spines along the leading edges of its pectoral fins (Fig. 2e), casting further doubt on acanthodian monophyly. Subsequent analysis of the braincase and dentition of Doliodus revealed primitive character states, such as fused tooth bases, not widely seen in crown chondrichthyans and certainly absent in modern sharks and rays, but common to acanthodians and early osteichthyans.

Rosetta stones for fragmentory bony fish remains

Fossil bony fishes have conventionally been deposited in one of the two living divisions: actinopterygians or sarcopterygians. This leaves the osteichthyan stem bref of fossils that document the origin of this enormously successful clade. A series of isolated scales of late Silurian–Early Devonian age were loosely tethered to actinopterygians as their representatives, but the discovery of more complete material attributed to Dialipina and Ligulalepis refames evidence of an eyestalk, a cartilaginous plinth that supports the eye in chondrichthyans and placoderms, but that is absent in modern osteichthyans. This might suggest Ligulalepis is a stem osteichthyan, but reports of eyestalks in early sarcopterygians argue for parallel loss in the two bony fish divisions. Complete specimens of Dialipina are even more puzzling, marrying a tail geometry found only in lobe-finned fishes with a cheek comprising tiny bones that bear no clear resemblance to the large plates of other osteichthyans or even Entelognathus. Ligulalepis and Dialipina vacliate between Actinopterygii and the osteichthyan stem in many analyses, and solid placements are likely to be elusive until these taxa are more completely documented.

Psarolepis and Guituy encapsulate the revolution

Perhaps more than any other discovery, Psarolepis represents the principal instigator of the current revolution in early jawed-vertebrate systematics. Recovered from late Silurian and earliest Devonian rocks of Canada, it is one of the earliest bony fishes (Fig. 1). First identified as a stem lungfish on the basis of jaw and braincase material, subsequent investigation of Psarolepis and the discovery of isolated cheek and shoulder bones highlighted more interesting affinities. Psarolepis exhibits two hallmarks of the lobe-finned fishes: a braincase divided into front and hind units by an articulating joint and a pore–canal complex in its dermal bones. However, the cleaver-shaped cheek and maxilla (upper external jaw bone) bear an uncanny resemblance to those of early ray-finned fishes, suggestive of a shared primitive condition for bony fishes. More surprisingly, Psarolepis bristled with spines: the shoulder girdle bears a pronounced spine over the fin articulation area, reminiscent of acanthodians and some placoderms, whereas the dorsal fins were preceded by spines like those of chondrichthyans and acanthodians. Psarolepis is most reasonably interpreted as a
The re-shaping of early jawed vertebrate phylogeny

This panoply of new taxa and unexpected character distributions fuelled doubts about the status of classic early jawed vertebrate categories9,38, but early studies did not match these queries with cladistic tests. In the past five years, the field has witnessed a spate of numerical analyses giving rise to rapidly shifting perspectives on phylogenetic relationships9,47-49,58, but were matched by dismissals citing ‘funny data’. However, the disarticulated nature of these fossils raised the troubling possibility that the combination of characters in Psarolepis was chimaeric: parts of different species misattributed to a single one. This concern was rejected, albeit indirectly, by the discovery of Guiyu9 (Fig. 2j). Broadly similar to Psarolepis, but from even older Silurian rocks in China (about 423 Ma), Guiyu provides exceptional corroboration that traits such as a jointed external pelvic girdles and a skull combed of mostly undifferentiated plates, with spines preceding the fins. Interpreted as an early sarcopterygian, Guiyu also shows that the last common ancestor of all modern osteichthyan arose no later than the Silurian, before the Devonian ‘Age of Fishes’.

The unfortunate complication of this hypothesis was that it implied non-orthogonality of osteichthyan and placoderm armoured exoskeletons. Similarities between osteichthyan and placoderm skulls and shoulder girdles had not gone unnoticed36,37, but were matched by dismissals citing ‘fundamental differences’ in construction28. The discovery of Entelognathus (already discussed) deals a blow to the latter perspective. Phylogenetic analysis accompanying the discovery36 unsurprisingly led to a wholesale debate about the status of classic early jawed vertebrate categories9,38, but early studies did not match these queries with cladistic tests. In the past five years, the field has witnessed a spate of numerical analyses giving rise to rapidly shifting perspectives on phylogenetic relationships9,47-49,58. However, some stable patterns are apparent and key areas of ongoing research are now coming into focus.

The monophyly of fossil osteichthyans and chondrichthyans is universally supported. Placoderms are repeatedly recovered as stem-group gnathostomes and acanthodians are generally agreed to be members of the gnathostome crown, with some noteworthy exceptions9,38. Major differences with previous hypotheses stem from important shifts in approach, such as abandoning earlier assumptions of placoderm and acanthodian monophyly. In all cases so far, the monophyly of placoderms has been rejected and, in all but one38, acanthodian monophyly has also been rejected.

In the earliest iterations, acanthodians were inferred to be massively paraphyletic, with some members associated with chondrichthyan, osteichthyan and gnathostome stem branches9,38. This configuration helped to explain the odd conjunction of osteichthyan, chondrichthyan and more primitive characters found in acanthodians. Furthermore, it implied an acanthodian-like appearance of the ancestral crown gnathostome: a small fusiform fish, covered in a denticle shagreen, a skull composed of mostly undifferentiated plates, with spines preceding the fins. The unfortunate complication of this hypothesis was that it implied non-orthogonality of osteichthyan and placoderm armoured exoskeletons. Similarities between osteichthyan and placoderm skulls and shoulder girdles had not gone unnoticed36,37, but were matched by dismissals citing ‘fundamental differences’ in construction28. The discovery of Entelognathus (already discussed) deals a blow to the latter perspective. Phylogenetic analysis accompanying the discovery36 unsurprisingly led to a wholesale
shift of acanthodian-type taxa to the chondrichthyan total group. Every subsequent analysis has corroborated this outcome. This key rearrangement eliminates the need to invoke convergence between placoderm and osteichthyan exoskeletons. By viewing the fragmented dermal skeletons of chondrichthyan and acanthodians as a derived condition, no special sister group relationship between osteichthyan and placoderms is implied, as had been assumed in the past.

Current analyses universally reject placoderm monophyly, with arthropods (and similar forms such as *Entelognathus*) resolved closest to the gnathostome crown (Fig. 3). This arrangement suggests that resemblances between arthropods and modern gnathostomes are homologous — a point reinforced by the arthropod gestalt of *Entelognathus*. Likewise, it suggests that the similarities between the more flat-headed and presumably benthic placoderms, such as antiarchs and peltichthyids, and jawless outgroups reflect a shared primitive condition. This has the convenient effect of stretching the placoderms into an array of jaw-bearing stem gnathostomes, although mandibles remain unknown in forms such as *Brindabellaspis* and peltichthyids.

The consistency of placoderm paraphyly across recent analyses suggests that this is well supported. However, available solutions are not wholly independent, with each data set incrementally updated from a core original study. Perhaps notably, the addition of taxa and characters has not increased support for the paraphyletic placoderm backbone. Instead, successive analyses have seen a winnowing of branch support for the deepest divergences among jaw-bearing stem gnathostomes, coupled with inconsistent arrangements of major placoderm lineages crownward of antiarchs and *Brindabellaspis*. This instability, combined with potential placoderm synapomorphies such as pelvic claspers and a persistent fissure between the nasal capsules and the remainder of the braincase, indicate that the 'placoderm problem' is far from resolved. A satisfactory resolution of the relationships of placoderms will have profound consequences for our understanding of the origin of modern jawed vertebrates.

**Future directions**

Early jawed vertebrate phylogenetics is in a state of infancy, but rapid progress is being made. Present discourse on early jawed vertebrate phylogenetics is marked by a growth of healthy debate and a relative lack of the kind of dogmatism that held back the field for nearly half a century. The question of the origin of the jaws themselves remains open. So far, the problem has been debated in terms of highly idealized archetypal scenarios, such as the transformation of gill arches into jaws. From both palaeontological and neontological perspectives, this scenario has proved deficient. Little direct evidence of the visceral skeleton of fossil jawless fishes is known; even the proximate outgroups of the jawed vertebrates — osteostracans and galeaspid — are presumed to have been jawless, but remains of the oral skeleton remain absent. What is known of the oral regions of osteostracans and galeaspid suggests that they possessed mouths that were specialized relative to the branchial arches, a condition consistent with modern jawless fishes. Placoderm paraphyly raises some hope that relevant data could be sourced from this assemblage (for example, *Brindabellaspis* or peltichthyids). The discovery of additional fossils will hopefully help to fill these gaps, but they will not be sufficient by themselves. Rigorous phylogenetic analysis must accompany these new finds to avoid simply shoehorning fossils into appealing narratives.
This first-generation application of cladistic methodology to early jawed vertebrates placed the ‘spiny sharks’ as early relatives of bony fishes, a perspective that profoundly influenced perceptions of the ancestral crown gnathostome family tree. 

Few have shifted the evolutionary paradigm as much as this study was the first to rigorously test—and, in doing so, to reject—placoderm-like creature with jaw bones resembling those of bony fishes. Of the many remarkable early gnathostome fossils to emerge from China, few have shifted the evolutionary paradigm as much as Entelognathus, a placoderm-like creature with jaw bones resembling those of bony fishes.

This article provided an explicit argument for the status of placoderms as stem gnathostomes that has not been seriously challenged in the following three decades. This article highlighted weak points in existing phylogenies of early jawed vertebrates, and triggered a resurgence in systematic studies.