Chordates

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Biologists, in a taxonomic sense, are human and, more broadly, they are members of the phylum Chordata. As humans have a strong tendency to worry about the place they occupy in Nature, biologists have devoted considerable effort toward understanding the evolution of the chordates (Figure 1). To put chordates into context, it is convenient to begin by focusing on the branch of the animal kingdom to which they belong — namely the deuterostomes.

The shrinking of the deuterostomes

According to the current majority view, the deuterostomes comprise three phyla — the echinoderms, the hemichordates and the chordates—with the last being subdivided into the tunicates (urochordates), amphioxus (cephalochordates), and vertebrates (Figure 2). The defining morphological character of deuterostomes is that their embryos develop the anus first and the mouth later (in practice, this sequence is often undetectable). In spite of this unsatisfying morphological definition, analysis of large and small subunit ribosomal DNA (rDNA) strongly supports the deuterostomes as a monophyletic clade, i.e., a group of all the descendants that have arisen from a single ancestor. Also, on the basis of rDNA data, as well as posterior *Hox* gene divergence, there is general agreement that the lophophorate phyla (phoronids, bryozoans and brachiopods), which have frequently been included in the deuterostomes — and still reside there in the pages of some current textbooks — should be removed and instead allied with the likes of annelid worms and mollusks.

This opens a gulf between the deuterostomes and the rest of the animal kingdom that has been made even wider by molecular data relocating the pterobranchs from the base of the deuterostome tree to a derived position within the hemichordates. Thus, the tentacles of pterobranchs (Figure 1C) and lophophorates (Figure 1H–J) are evidently instances of convergent evolution.

Sequence-based phylogenetic rearrangements and deuterostome evolution

Although sequence-based phylogenetic analyses have disrupted the lophophorate linkage between deuterostomes and other animals, some ideas about deuterostome evolution have not been negatively affected. Examples are scenarios proposing that the ancestor was a sea anemone-like animal (solitary or colonial) or a larva-like creature swimming with cilia. Garstang proposed such a larva-like ancestor in his early scenario for deuterostome evolution. He gave most attention to a hypothetical derivation of the chordate central nervous system from nerve-rich ciliated bands on either side of the larva (Figure 1L) by proposing that these bands migrated dorsally and rolled inward (Figure 1M) to form a dorsal nerve cord. On the other hand, the distancing of the lophophorates from the deuterostomes has seriously compromised theories proposing that an adult lophophorate was the ancestor of the deuterostomes.

In one instance, recent phylogenetic rearrangements have actually provided a more plausible explanation for the origin of the deuterostomes. Sequence-based phylogenetic trees strengthen the idea that the basal deuterostome was an acorn worm-like creature that, in turn, originated from an acoelomorph flatworm ancestor. Whereas the older, morphology-based trees usually had echinoderms branching off separately before the hemichordates, newer sequence-based analyses place the Ambulacraria — a clade of hemichordates plus echinoderms — at the base of the deuterostomes (Figure 2).

Moreover, acorn worms and not pterobranchs are now considered to be the basal hemichordates.

Finally, the sequence-based phylogenetic analyses have impacted the calcichordate theory of Jefferies. In its original version, the most basal deuterostome was pterobranch-like. Now, however, with the pterobranchs repositioned further up in the branches of the Ambulacraria, it has been proposed that the most basal deuterostomes were solutan carpoids — globular fossils with a heavily calcified body wall bearing a stalk at one end and a probable feeding arm at the other. This revision, however, lacks a good candidate ancestor for the solutan carpoids themselves.

Palaeontological attempts to reconnect the deuterostomes with other animals

Soon after molecular biology shrank the deuterostomes and disrupted their traditional linkage to the lophophorates, some palaeontologists responded by adding a new phylum at the base of the deuterostomes and proposing a new scenario for the origin of the group. The new discoveries are soft body fossils from the Early Cambrian, known as vetulicolians, which have been interpreted by some palaeontologists as having an inflated anterior half bearing large gill slits and a segmented posterior half (Figure 1P). It has been proposed that vetulicolians are the earliest deuterostomes, and that they arose from segmented, annelid-like creatures by evolving gill slits. If the vetulicolians are really basal deuterostomes they not only share many of the attributes of chordates, but also could have given rise to echinoderms and hemichordates by suppressing their segmentation. At present, however, the taxonomic affinities of vetulicolians are very controversial. In addition to being vigorously promoted as basal deuterostomes, vetulicolians have also been variously interpreted as fossils of arthropods, chordates, or more specifically pelagic tunicates.
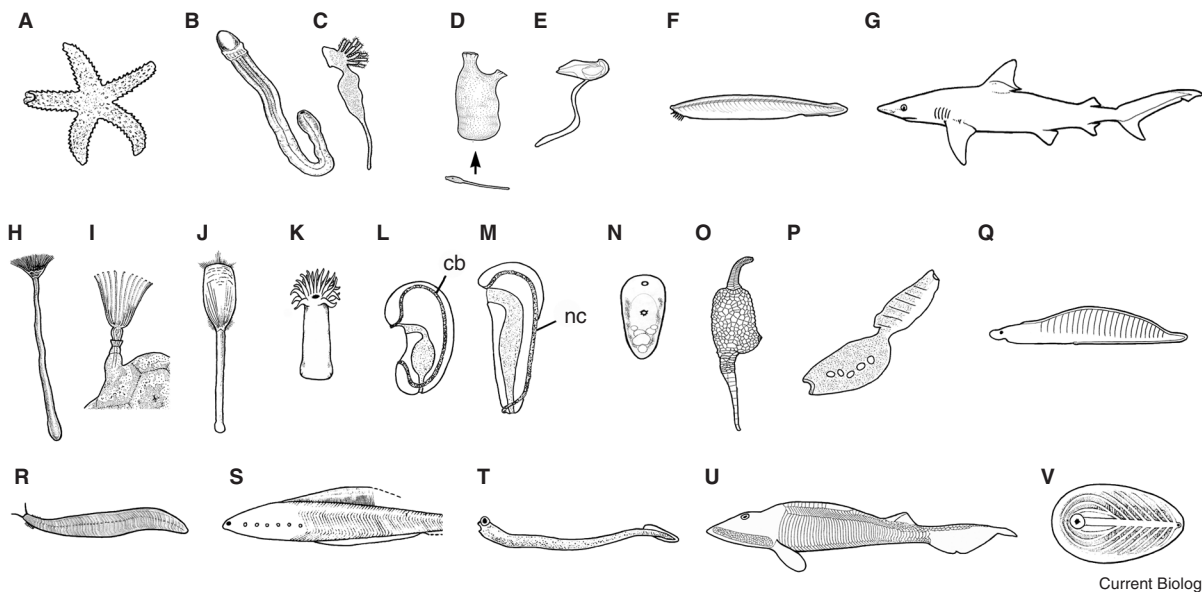
Figure 1. Representatives of the chordates and other relevant animal groups.

(A) Echinoderm (starfish); (B) Hemichordate (acorn worm); (C) Hemichordate (pterobranch); (D) Tunicate (ascidian: tadpole larva below, adult above); (E) Tunicate (appendicularian); (F) Amphioxus; (G) Vertebrate (shark); (H) Lophophorate (phoronid); (I) Lophophorate (bryozoan); (J) Lophophorate (brachiopod); (K) Sea anemone; (L-M) Hypothetical conversion of ciliated bands (cb) of a larva-like ancestor into the nerve cord (nc) of vertebrates; (N) Acoelomorph flatworm; (O) Solutan carpoid; (P) Vetulicolian; (Q) Yunannozoan (*Haikouella*); (R) *Pikaia*; (S) *Haikouichthys*; (T) Conodont animal; (U) Ostracoderm; (V) Dipleurozoan.

The chordates become unstable

Chordates are characterized by the presence of a notochord — at least transiently during embryogenesis — and a dorsal hollow nerve cord. The long accepted branching order within the chordates positioned the tunicates basal to a clade comprising amphioxus and vertebrates (Figure 2). More recently, however, molecular sequence analyses have had trouble resolving the relative positions of the tunicates and amphioxus. This uncertainty stems from the rapid evolution of tunicates and the consequent introduction of long-branch attraction artefacts into molecular phylogenetic analyses. In addition, genetic comparisons between tunicates and other animals can be complicated because tunicates have lost numerous gene groups, including some *Hox* genes and *Gbx*, an important rostrocaudal patterning gene in other chordates. At the same time, other gene groups have increased in number. It thus remains possible that the majority view may eventually reverse the positions of tunicates and amphioxus within the chordates (Figure 2).

Switching the positions of tunicates and amphioxus in the phylogenetic tree would have some interesting consequences: it would indicate that two features of tunicates — the striated muscles of the heart and a recently discovered neural crest-like tissue — are innovations of the common ancestor of tunicates and vertebrates rather than instances of convergent evolution between tunicates and vertebrates. Moreover, substituting amphioxus for tunicates at the base of the chordates would somewhat strengthen the idea that the chordates arose from segmented ancestors (perhaps annelid-like) and that this segmentation has been secondarily reduced in the head region of vertebrates. By the same token, removal of tunicates from the base of the chordates would correspondingly weaken the notion of Romer that the ancestors of chordates had muscular somites only posteriorly and that any muscular segmentation in the vertebrate head region must have evolved secondarily.

Credible and incredible chordate fossils

Some recently discovered fossils strongly indicate that chordates — at least tunicates and vertebrates — were present by the Early Cambrian. Many of these are soft body fossils from the Chengjiang formation in Southern China. Fossils are important for the big picture of evolution, because they can permit the recognition of ancestral character states and extinct character combinations. The Early Cambrian vetulicolian fossils have already been mentioned as putative basal deuterostomes, as well as basal chordates or even pelagic tunicates. In addition, Early Cambrian deposits have yielded two fossil species of more conventional tunicates, with bodies resembling the adults of modern solitary ascidians. Although one of these appears to be a fragment of a larger animal, possibly a lophophorate, the second species is based on complete specimens and provides plausible evidence that ascidians resembling modern ones were present at least in the Early Cambrian. Unfortunately,

these fossils represent the adult stage in the life history and, as such, lack most chordate features, except a perforate pharynx, and thus give no clear insights into the possible anatomy of early chordates.

In contrast to tunicates, no unequivocal amphioxus-like fossils have yet been found from the Early Cambrian. There was initially some suggestion that fossils of the yunnanozoan/*Haikouella* type resembled amphioxus, but now they are variously regarded as vetulicolians, hemichordates, or, as seems most likely, basal vertebrates. Another soft body fossil, the much discussed *Pikaia* from the Middle Cambrian, has been described sometimes as a close relative of amphioxus, although more often as an early chordate. In recent years, however, enthusiasm for viewing *Pikaia* as a chordate has been declining, and some palaeontologists have even suggested that the fossil may be an annelid or alternatively an arthropod. The lack of any plausible fossils of amphioxus-like animals prior to the late Paleozoic is a serious obstacle to reconstructing the anatomy of the ancestor of the chordates. Amphioxus and the vertebrates have been evolving independently for half a billion years, and, without the relevant fossil evidence, one cannot always be sure which amphioxus characters are primitive and which are derived.

The Early Cambrian deposits of South China have also yielded *Haikouichthys*, which almost (not quite) all palaeontologists consider to be a vertebrate. This creature, now known from numerous specimens, resembles a jawless fish without external skeletal armour. One conspicuous feature they do not share with jawless fish is that the putative gonads are serially repeated along the rostrocaudal axis of the trunk, as they are in amphioxus. Interestingly, *Haikouichthys* has now taken the title of world's oldest known vertebrate from the conodonts (jawless eels with pharyngeal teeth), which held that

Figure 2. The living groups of deuterostomes.

The tree is based on phylogenies constructed from molecular sequence data. It has recently been found that the hemichordates and echinoderms form a single clade, the Ambulacraria (a). Moreover, it is currently uncertain whether the tunicates and amphioxus should be positioned as shown here or change positions, as indicated by the arrow (b).
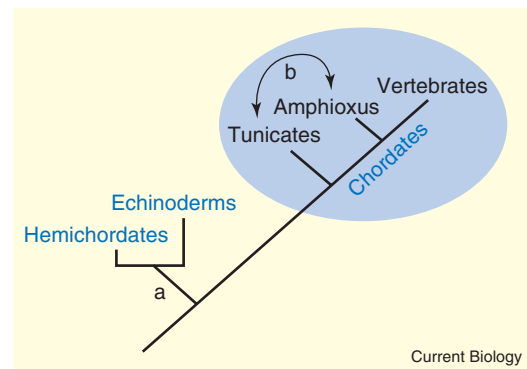
distinction only for a few years after gaining it from the ostracoderms.

Scenarios for chordate origin and evolution

Some scenarios for the origin of the chordates are based primarily on fossil evidence. The best developed of these is the 'calcichordate theory', already mentioned, according to which amphioxus, tunicates, and vertebrates separated individually from bottom-dwelling creatures looking like armor-plated tadpoles — dissenting palaeontologists claim that they are echinoderms. A less comprehensive fossil-based scenario is Dzik's proposal that the earliest vertebrates —in his view the conodonts — are derived from enigmatic metameric animals (dipleurozoans) that lived about 30 million years before the Early Cambrian.

In contrast to the above, most evolutionary explanations of the origin of the chordates are based on little or no fossil evidence. In addition, they may or may not consider the relationship of the three extant groups of chordates — tunicates, amphioxus, and vertebrates. There are numerous ideas about the origin of the chordates that start with the adult body plan of some non-deuterostome — say an arthropod or an annelid — and morph it directly or indirectly into a vertebrate. Frequently, such hypotheses create vertebrates and pursue the matter no further, although Dohrn proposed that, once evolved, some vertebrates degenerated to invertebrates

(amphioxus). In the light of what is now known about the *Hox* genes, such invertebrate-to-vertebrate conversions are virtually impossible, because there is no known genetic mechanism that could neatly remove all but one *Hox* cluster (the invertebrate condition) from the multiple *Hox* clusters characteristic of all vertebrates.

Another class of scenarios proposes a swimming tunicate as the basal chordate, which subsequently gives rise to amphioxus and vertebrates. Yet another type of scenario proposes that the tunicate ancestor of the chordates was an appendicularian tunicate (Figure 1E), which had a tadpole-like body throughout its life, perhaps derived from a vetulicolian precursor. The idea that appendicularians were the original chordates would be strengthened if molecular phylogenetic analyses could robustly show that appendicularians occupy a basal position within the tunicates. Unfortunately, however, the rapid evolution of tunicates has resulted in much disagreement about the branching order within the group. Other scenarios claim that the basal chordate was not an appendicularian, but an ascidian tunicate, more exactly, an ascidian larva that swam by undulating its tail. There are two explanations for the origin of such a larva. According to Garstang's later theory, an echinoderm-like larva added gill slits and sprouted a muscular tail to evolve into an ascidian larva. By contrast, Berrill and Romer assumed that the ascidian larva was suddenly

integrated into the life cycle of a benthic adult tunicate that had previously developed directly. All these ascidian tadpole scenarios are referred to as neoteny theories because they invoke precocious sexual maturity of the tunicate tadpole on its way toward becoming an amphioxus or a vertebrate.

Evolutionary developmental biology and chordate evolution

About twenty years ago, it was unexpectedly found that the genes directing development of distantly related animals tended to be conserved structurally and functionally. Beyond facilitating sequence-based phylogenetic analyses, this discovery allowed for a comparison of the genetic basis of developmental processes in different organisms. Eventually, this should help explain how developmental mechanisms themselves evolved. This new discipline, known as evo-devo, is especially concerned with detailing how morphological novelties arise in evolutionary lineages, such as, for instance, the innovation of neurogenic placodes in the early vertebrates. To date, most evo-devo studies of chordates have fallen into two categories: the first is the use of developmental gene expression to determine body part homologies among the major chordate groups, and the second is structural comparison of gene families to study gene duplication as an evolutionary mechanism.

Many developmental genes are highly conserved and it is not controversial to use molecular sequences to identify homologous genes from divergent organisms. However, developmental genes often function pleiotropically and can be co-opted for new functions during evolution. It is, therefore, a contentious issue whether conserved expression domains of such genes are useful characters for suggesting body part homologies between different animals. Certainly, basing homologies on such data is questionable when the overall body plans of the animals being compared are highly divergent. At

the other extreme, when overall body plans are almost identical, body part homologies are usually already obvious on morphological grounds and not in much need of support from molecular genetic data. Between these extremes, the major chordate groups have fairly similar overall body plans, so comparing developmental gene expression has worked quite well to bolster previously suggested homologies and suggest new ones. For example, comparison of developmental gene expression patterns has greatly strengthened the homology between the endostyles of tunicates and amphioxus and the thyroid gland of vertebrates. Similarly, the correspondence between the major brain regions of tunicates, amphioxus, and vertebrates has been well established. In addition, less extensive gene expression studies have given insights into several other homologies between the major chordate groups: namely, the heart, pronephric kidney, photoreceptors, gill slits, notochord, tail bud, and adenohypophysis. Strengthening these body part homologies is important, because tunicates and amphioxus are the best available proxies for the invertebrate ancestor of the vertebrates and provide insights into the genetic and morphological starting points for vertebrate evolution.

Even before the advent of evo-devo, Ohno pointed out that the vertebrate line of descent, unlike any other in the animal kingdom, has undergone extensive polyploidization and that this greatly increased genetic content could well have facilitated the spectacular radiation of the vertebrates. Subsequently, even before completely sequenced genomes became available, comparative molecular genetics largely confirmed Ohno's ideas. Most vertebrates appear to have undergone two rounds of genome duplication, although it is still controversial whether genes were actually duplicated on a large (but not genome-wide) scale or whether whole genomes were duplicated at once and then underwent some gene loss. The

accumulation of complete chordate genome sequences should help settle this controversy by revealing the chronology of duplication events and the amount of congruence in gene order. Such data should give insights into the amount of synteny in pre-duplicated genomes and the extent of post-duplication gene rearrangements. By now, the genomes of one tunicate and several vertebrate species have been completely sequenced and annotated, and similar projects are well advanced for two additional tunicates and amphioxus. The comparative study of chordate genomes should resolve current controversies about gene duplication and, more consequentially, raise new questions.

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