

# The Evolutionary Origin of the Vertebrate Body Plan: The Problem of Head Segmentation

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## Keywords

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## Abstract

The basic body plan of vertebrates, as typified by the complex head structure, evolved from the last common ancestor approximately 530 Mya. In this review, we present a brief overview of historical discussions to disentangle the various concepts and arguments regarding the evolutionary development of the vertebrate body plan. We then explain the historical transition of the arguments about the vertebrate body plan from merely epistemological comparative morphology to comparative embryology as a scientific treatment on this topic. Finally, we review the current progress of molecular evidence regarding the basic vertebrate body plan, focusing on the link between the basic vertebrate body plan and the evolutionarily conserved developmental stages (phylotypic stages).

Der ganze Mensch ist nur ein Wirbelbein (The entire human body is nothing but a backbone).

—Lorenz Oken (56, p. 371), 1807

If . . . we now compare the stages of development of the frog's skull with the persistent conditions of the skull in the *Amphioxus*, the Lamprey, and the Shark, we shall discover the model and type of the latter in the former. The skull of the *Amphioxus* presents a modification of that plan which is exhibited by the frog's skull, when its walls are still membranous and the notochord is not as yet imbedded in cartilage. The skull of the lamprey is readily reducible to the same plan of structure as that which is exhibited by the tadpole, while its gills are still external and its blood colorless. And finally, the skull of the shark is at once intelligible when we have studied the cranium in further advanced larvae, or its cartilaginous basis in the adult frog.

—Thomas H. Huxley (29, p. 424), 1858

## INTRODUCTION

### The Head as an Archetype

The question of how we can formulate and explain the vertebrate body has been a central topic of comparative zoology since the late eighteenth century, when the Romantic movement and German idealism were influential (**Table 1**). Immanuel Kant, a philosopher in German idealism, acknowledged in his *Kritik der Urteilskraft* that one animal species can be transformed into another. In agreement with Kant's aesthetics and teleology, Goethe proposed a model he called *Urtypus*, an idealized archetype, from which any type of living animal could be derived by simple modifications. In the pre-Darwinian background, recognition of the vertebrate head was based on this ideal morphology of Goethe. Goethe (20) and Oken (56) first introduced metameric views of the animal body (the animal body consists of segments equivalent to vertebrae) and considered the vertebrate skull to be a modified part of the vertebral column (vertebral theory) (**Figure 1**).

This vertebral theory was once mathematized by Carus, an anatomist who strongly appreciated Goethe's theory (61). This was how the vertebral theory was imported to Britain. Carus was a minimalist and constructed a highly simplified, invented vertebra as an archetype. Owen, a British zoologist, was strongly influenced by this idealistic archetype theory and subsequently constructed an extreme archetype for vertebrates in which the cranium was composed of several modified vertebrae, such that the scheme could derive all vertebrate skulls. However, this scheme resulted in the construction of a monster-like figure not found in nature, because it was an assemblage of characteristics that represented both derived and ancestral traits. Thus, the arguments on the head segmentation theory were a battle of epistemology in terms of how to perceive and define the archetype through the comparison of various animal forms.

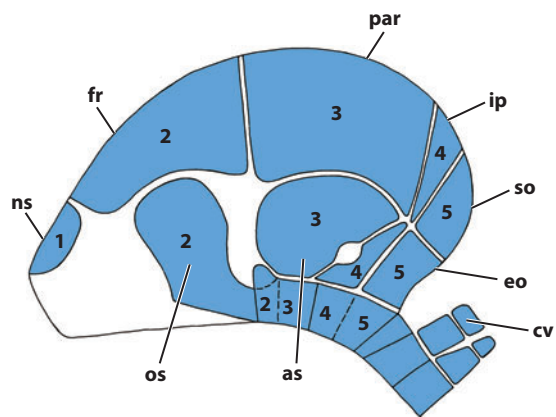
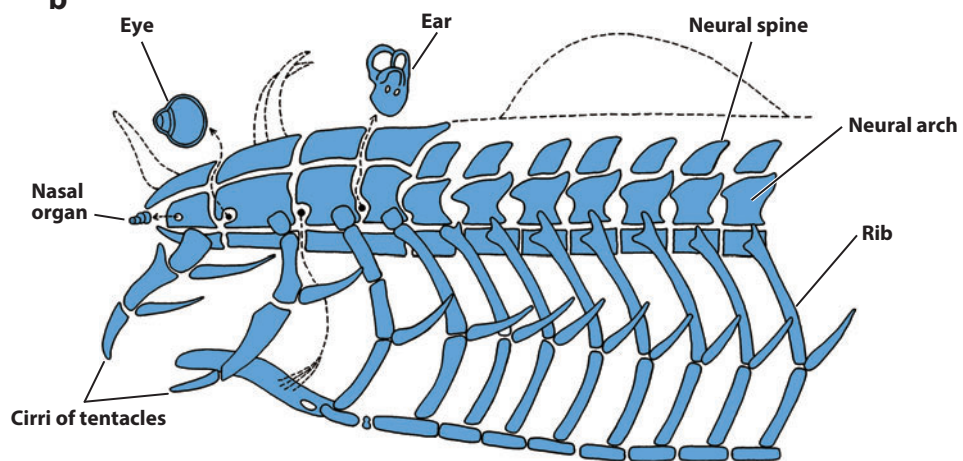
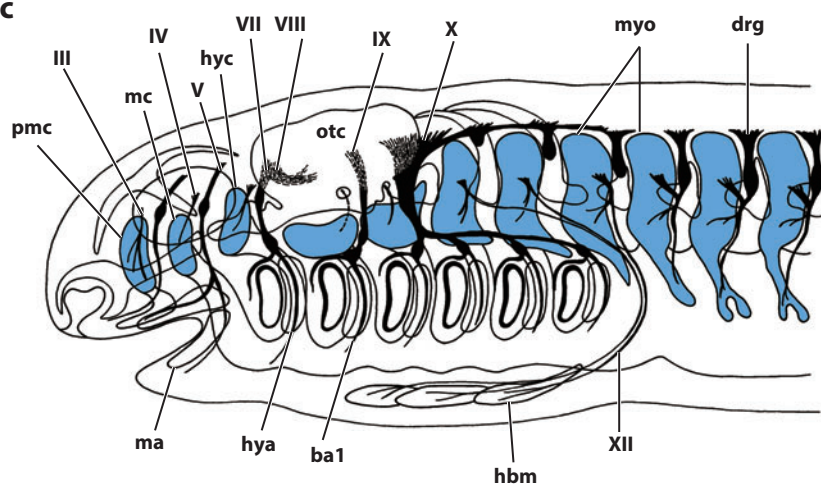
It is generally thought that the above argument of head segmentation as comparative osteology was finally refuted by Huxley's Croonian Lecture in 1858 (29), the same year that Darwin and Wallace put forth the theory of natural selection (see below). This, however, did not mean the end of the head segmentation theory, because the debate found another arena in comparative embryology.

### The Second Movement of the Head Segmentation Theory in Comparative Embryology

After the establishment of the archetypal theory of vertebrate head, scientists discussed this topic from the view of comparative embryology. The members of one major school believed that the

**Table 1 Theories on the origin of the vertebrates**

Theory	Clade	Author	Year	Reference	Primary claim
Annelid (ringed worm) theory	Lophotrochozoa	Semper	1874	69	Annelids have an organ organization similar to that of vertebrates (e.g., shark embryos). Specifically, the segmental organs in the trunk portions of annelids and vertebrates are similar.
Annelid (ringed worm) theory	Lophotrochozoa	Dohrn	1875	12	The annelid gills in each segment turn into the paired fins of fish.
Arthropod theory	Ecdysozoa	Leydig	1864	48	The ventral parts of insects are homologous to the dorsal parts of vertebrates (i.e., the upside-down theory). The vertebrate brain is homologous to insect supra- and subesophageal ganglia.
Arthropod theory	Ecdysozoa	Gaskell	1889	17	Vertebrates originated with a right-side-up arthropod, turned the gut into the neural tube, and then formed a new gut by fusing the appendages with each other laterally and at their tips.
Amphioxus theory	Chordata	Goodsir	1844	23	The anterior part of the amphioxus nerve cord is homologous to the vertebrate brain. The amphioxus connects vertebrates to annulose animals (earthworms and crustaceans) and symmetric ascidians.
Amphioxus theory	Chordata	Huxley	1874	30	The nerve cord in front of the seventh myotome is homologous to the preotic part of the vertebrate brain. The amphioxus is considered a protovertebrate.
Tunicate theory	Chordata	Kowalevsky	1871	42	Ascidians are the closest relatives of vertebrates because they possess a neural tube and a notochord.
Tunicate theory	Chordata	Morse	1872	53	The trunk portion of tunicate tadpoles is segmented. However, this theory does not clearly mention the evolutionary relationship between tunicate segments and vertebrate trunk somites.
Nemertean (ribbon worm) theory	Lophotrochozoa	Hubrecht	1883	28	The nemertean proboscis is homologous to the hypophysis cerebri, and the nemertean proboscidean sheath is homologous to the vertebrate notochord.
Anthozoan theory	Cnidaria	Sedgwick	1884	68	The mesoblastic somites of segmented animals (chordates) are derived from a diploblastic coelenterate-like ancestor, and the mouth of actinozoans differentiated into the mouth and the bilaterian anus.

**a****b****c**

vertebrate head is entirely segmented, and they were therefore called segmentalists; the members of the other major school believed that the vertebrate head is never segmented, and they were therefore called anti-segmentalists.

**The views of the segmentalists.** There are many comparative embryological studies on head segmentation theory. Gegenbaur (18, 19), for example, confirmed segmental correlations among some anatomical units in the heads of sharks, such as cranial nerves and pharyngeal arches. Gegenbaur believed that these units represented the fundamental body plan of the vertebrate head (i.e., a primitive feature of all vertebrate heads) (**Figure 2**). From this period forward, the major focus of the debate regarding the vertebrate head shifted from the adult skeleton to soft tissues or embryonic primordia, and from “higher” animals (such as amniotes) to “lower” animals (including amphioxus).

In 1874, Balfour (2) examined shark embryos and observed that the head mesoderm developed into three pairs of segments (the three preotic cavities referred to as the premandibular, mandibular, and hyoid cavities), which he believed to be comparable to somitic coeloms in the trunk. Along with the mesodermal segments, Balfour identified cranial nerves and pharyngeal clefts, which he believed were also segmented. In 1881, Marshall (49) confirmed Balfour’s results and agreed with the above segmental scheme. Marshall also later suggested that the head cavities could be divided into two populations, one for the eye muscles and one for the branchial muscles. Van Wijhe (73) further divided the head segments into dorsal and ventral moieties and established segmental assignments among the cranial nerve innervations, the extrinsic eye muscles, and the branchial muscles.

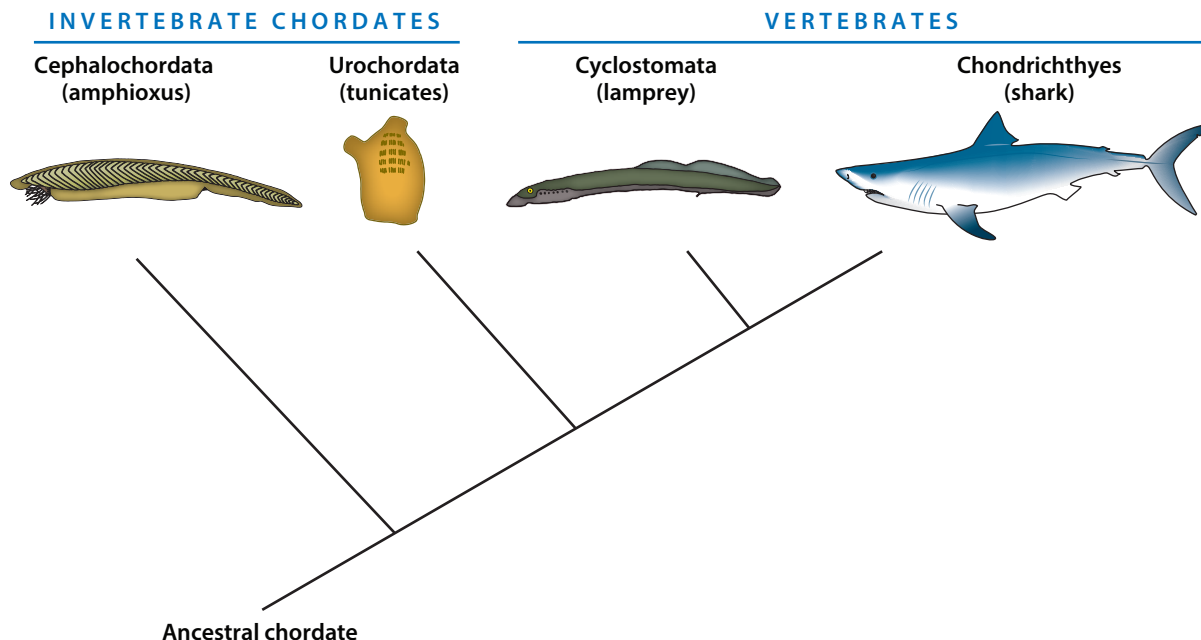
In the early twentieth century, the vertebrate head was viewed as a segmented pattern, as was observed for the trunk. The strongest proposal was put forth by Goodrich (21, 22), who revised the segmental plan of the vertebrate head that had been established by van Wijhe (73) by emphasizing the presence of primarily mesodermal segments in the head that were comparable to the somitic segments in the more posterior portions of the vertebrate body, namely, the trunk. The conceptual bases employed by Goodrich were similar to those recognized by Goethe and Oken (idealism): The head consisted of modified segments that were serially homologous with segments in the trunk.

Under the concept of head segmentation, Goodrich attempted to integrate various segmental embryonic elements, including somites, cranial nerves, and branchial arches, into one unit, and

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### Figure 1

Early hypotheses of head segmentation. (a) The vertebral theory of Goethe, in which the skeletal element of the mammalian skull is segmented, similarly to the vertebrae. Thus, different bones in the head represent five head segments, as numbered. Abbreviations: fr, frontal; ns, nasal; os, orbitosphenoid; par, parietal; as, alisphenoid; ip, interval parietal; so, supraoccipital; eo, exoccipital; cv, cervical. Adapted from Jollie (33) with permission from Oxford University Press. (b) The vertebrate archetype of Owen, who proposed that the anterior portion of the vertebrate body is a serial homolog of the trunk structure. The key difference from Goethe’s model is that Owen believed that all vertebrates arose from the archetypal animal, whereas Goethe considered the mammal head to be segmented, similarly to the vertebrae. Adapted from Owen (57). (c) The head metamerism theory of Goodrich. In contrast to Goethe’s and Owen’s ideas, Goodrich’s model was influenced by evolutionary and embryological perspectives. Moreover, Goodrich integrated nerve, muscle, and pharyngeal elements into one segmental unit, whereas Goethe and Owen considered only skeletal elements when claiming that the head is segmented. In the head region, each compartment contains a nerve, a head somite, and a branchial arch. Abbreviations: pmc, premandibular cavity; mc, mandibular cavity; hyc, hyoid cavity; otc, otic vesicle; myo, myotome; drg, dorsal root ganglia; ma, mandibular arch; hya, hyoid arch; ba1, branchial arch 1; hbm, hypobranchial muscle. Adapted from Goodrich (21) with permission from The Company of Biologists.



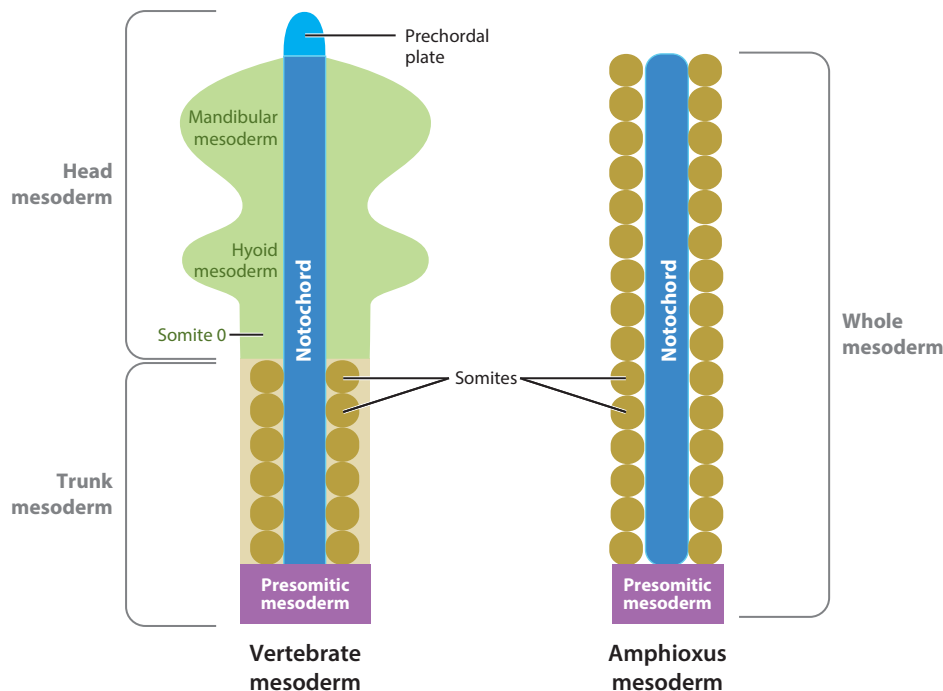
**Figure 2**

Chordate phylogenetic tree. The phylum Chordata consists of invertebrate chordates (Cephalochordata and Urochordata), jawless vertebrates (Cyclostomata), and jawed vertebrates (Chondrichthyes). Urochordata is the sister group to vertebrates, and Cephalochordata comprises the most basal living chordates. Recent genome studies of amphioxus suggest that it retains high synteny to the human genome (orthologous genes from some scaffolds are concentrated in specific areas of the chromosome) and is thus a good model animal with which to compare the vertebrate body plan. The amphioxus image is adapted from a drawing by Dr. N. Adachi (University of Chicago) and used with permission.

claimed that a head that was segmented into eight units represented a primitive condition of jawed vertebrates and that an amphioxus-like creature was the ancestor of the vertebrates (**Figures 2 and 3**). Goodrich assumed an evolutionary trend of “cephalization” that involved certain rostral segments. In this evolutionary context, the vertebrate head was defined as an anterior, highly specialized part of the trunk that contained the chief organs of sense—the brain, mouth, and gill slits. Goodrich also thought that higher vertebrates underwent a more pronounced cephalization process than did lower vertebrates. Goodrich’s ideas on head segmentation originated in Goethe’s idealist morphology and were based on the archetypal way of thinking, as represented in *Urtypus*.

**The views of the anti-segmentalists.** Generally, segmentalists regarded the vertebrate head as simply modified vertebrae. Concomitant with the rise of segmental views, anti-segmentalism arose. The anti-segmentalists held that the vertebrate head is never segmented like the trunk of the body.

For example, Fries (15, 16) doubted the serial homologies of preotic, postotic, and more posterior trunk somites, referring to von Kupffer’s (75, 76) study of lamprey embryos, in which the head segments arose from the endoderm rather than from the mesoderm. Another view was raised by Kingsbury and Adelman (37–39), who claimed that the metamerism of the vertebrate head is not anteroposteriorly continuous, because the vertebrate head components, such as the neuromeres (segments of the central nervous system), somitomeres (segments of trunk somites), and branchiomeres (segments of branchial arches), could not be integrated into a single series of units.



**Figure 3**

Mesoderm formation in vertebrates and amphioxus. In vertebrates, the mesoderm is divided into the head and the trunk mesoderm. At the axial level, the prechordal plate is located at the anterior tip, and the notochord is located immediately posterior to the prechordal plate. In amphioxus, the mesoderm does not have head and trunk identity, and the notochord runs from the rostral to the caudal end.

Another theory was introduced in 1972 by Romer (64), who claimed that the vertebrate head consists of somatic and visceral units and that these segmental units evolved independently at different times. One of the difficulties in accepting the idea of the eumetameric head (i.e., that branchiomeres and somitomeres, and possibly neuromeres as well, represent manifestations of a single metameric architecture) is that the head segmentation is organized, at least in part, around the existence of neural crest cells and placodes during embryogenesis. The neural crest and placodes are distributed in a U-shaped manner around the anterior neural plate, and the majority of the ventral portion of the skull in chickens and mice is derived from neural crest cells, in contrast to the dorsal part of the skull, which, except for its rostral end, is derived from the paraxial mesoderm. Therefore, it is unlikely that skull formation in vertebrates follows a simple metameric plan along the anterior/posterior axis that is comparable to trunk metamerism (43, 55).

In the 1970s, a groundbreaking technical advance in microscopy, the scanning electron microscope, was applied to understand head mesoderm morphology. Meier (52) and others found segmental patterns in the head mesoderm of chick embryos. Furthermore, a recent study of lampreys using scanning electron microscopy demonstrated that (a) there are no overt head cavities in the premandibular, mandibular, or hyoid mesoderms and (b) the developmental sequence of the head mesoderm is completely different from that of trunk somites (46). These results are in contrast to the findings of Koltzoff (41) and Damas (9), who identified head somites in developing lamprey embryos. Although lamprey head mesoderm appears to arise by enterocoely, the head cavities in



sharks often develop through secondary epithelialization of the mesenchymal head mesoderm (1). However, introducing new technology did not solve the head segmentation problem.

In sum, to discuss this problem in the context of science, we must present real evidence of developmental programs or ancestral forms.

### **Scientific Treatment of the Head Segmentation Theory**

Contrary to the original metaphysical concept of the vertebrate head, Huxley first attempted to refute the vertebral theory by a scientific procedure. Huxley considered embryos to develop through the common forms that are shared with both higher and lower animals. For Huxley, the archetype was not merely a concept; it should represent a hypothetical ancestral form with no derived traits specific to certain lineages. He viewed it as, in modern terms, an assemblage of sympleisiomorphies (ancestral traits) (29). Additionally, he used a graded series of chordate species for comparisons with the developing embryos of a frog, laying the groundwork for the ancestral traits to be found in earlier stages of development. Thus, he resorted to logic similar to recapitulation theory (later developed by Haeckel) and looked for vestigial segments in the embryonic skull, which he failed to discover. In this manner, Huxley refuted the vertebral theory of Goethe.

Although Huxley is often regarded as the scholar who terminated the vertebral theory, this was not actually the end. Huxley himself went back to the study of head segmentation, creating in essence the second act of the head segmentation theory. Thus, Huxley was not very successful in his Croonian Lecture *per se*, but it should be remembered that comparative embryology has been recognized ever since as a tool to look for solid evidence of segments, and the use of comparative osteological methods thus ended.

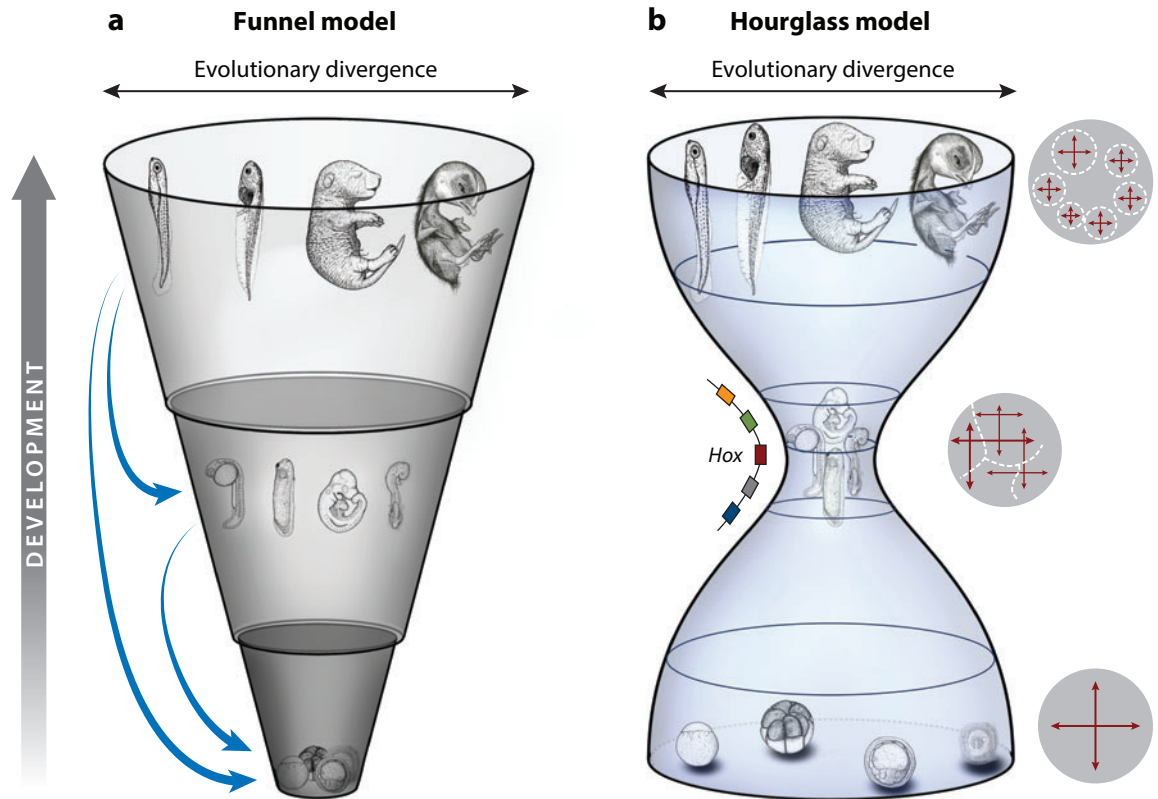
It should also be noted that, even before Darwin and Haeckel, there was the generally accepted concept of recapitulation, i.e., the idea that each embryonic stage of a certain animal should reflect the anatomical traits of an array of ancestors, which Huxley employed in his observation of frog embryos. Goethe's and Owen's tradition of archetypes was more closely affiliated with the "type of animal group" that used to be thought to be embodied at the intermediate stage of embryogenesis, as advocated by von Baer, a nonevolutionary embryologist. Huxley's method is more like Haeckel's, or, more appropriately, like the method of the first generation of recapitulationists, such as Meckel (51) and Tiedemann (72), who saw parallel relationships between the adult forms of lower to higher animals in the developing stages of embryos. It was Haeckel (25) who first introduced modern phylogenetic relationships and compared embryos to embryos (rather than to adults) belonging to different lineages, producing the final version of recapitulation theory in an evolutionary context.

In the context of this review, Huxley's 1858 paper (29) stands out because he used a recapitulation-like method (rather than relying on the natural philosophical archetype) to put the argument about segmentation on a semiscientific (rather than epistemological) background. Thus, the theory of head segmentation has seen the transition from archetype to embryo as well as the death and rebirth of recapitulation theories as their own contexts of debates from the late eighteenth to early twentieth centuries. This is where the history of head segmentation theories has relevance to modern concepts of evolutionary developmental biology, especially in our recognition and scientific definition of phylotypes (if there are any), as well as the relationships between phylogenetic trees that are now molecularly based and developmental stages that are now accessible in the form of gene regulation and expression profiles (discussed below).

### **Scientific Revision of the Recapitulation Theory**

The archetype as an epistemological concept implies an idealized pattern of animals, and head segments are also regarded as inherent and latent. Thus, it is not logically refutable: Even if the





**Figure 4**

The (a) funnel and (b) hourglass models of vertebrate embryogenesis, which describe how different embryonic stages diverged among different vertebrate species. In both models, embryogenesis proceeds from the bottom to the top, and the width represents the phylogenetic diversity of the developmental processes. (a) The funnel model predicts that diversity increases additively and progressively during embryogenesis, and thus that the most conserved stage is the earliest stage of embryogenesis. The hypothetical mechanisms that cause this relationship are based on the extreme case of developmental burden (63) and generative entrenchment (78), in which the viability of any developmental feature depends on an earlier feature (arrows). (b) The hourglass model, in contrast, predicts diverging early stages and the highest level of conservation in the organogenesis stage (14, 60). A highly intricate signaling network (circles to the right of the model), including the *Hox* cluster genes, leads to the conservation of this mid-embryonic or phylotypic period. Adapted from Irie & Kuratani (31) with permission.

head segments are invisible in adults or embryos, there may be an inherent pattern of segments that simply failed to be embodied as actual entities. A biological archetype, in contrast, can be seen in von Baer's concepts of *Urtyp* or *Haupttyp*, actually embodied in the form of developing embryos that we recognize today as phylotypes. As shown below, internal selection tends to stabilize such a conserved embryonic shape specific to each animal phylum (60, 66), defining the basic body plan of all the forms belonging to that particular taxon. The evolutionarily conserved stage of development, which appears at the organogenetic period of development, builds up an hourglass-shaped pattern of morphological diversity over developmental time, with the most constrained part at the phylotypic period (Figure 4).

Importantly, in the phylotype-based comparison, the phylotypic-stage embryo per se defines the global anatomical configuration of the taxon; therefore, if the phylotypic embryo does not possess the head mesodermal segments, then that taxon does not possess a segmented head either.

(Here, the vertebrates are defined as animals that do not have mesodermal segments in the preotic domain, similar to the definition of insects as arthropods with six legs. Ancestral possession of more appendages is irrelevant to this taxonomic definition based on the synapomorphy.) Thus, the presence or absence of head segments is now evaluated by the new patterns (synapomorphies) of developmental constraint that govern embryogenesis after the phylotypic period. (For a discussion of head segmentation from the perspective of developmental constraints, see 44, 45.)

Examinations of the segmented head as an ancestral embryonic type are also treated scientifically in a modern context. Here, the argument is more plesiomorphy oriented, and, as Huxley once did, the ancestral pattern is sought in the earlier stages of embryogenesis, in the hopes that it will reflect a more ancestral developmental program. As can be easily seen, this conjecture rests on the assumption that the morphological diversity of development will manifest in a Haeckel-like funnel pattern, with the most conserved embryonic patterns materializing first and then becoming gradually more specified, leading to the diversity of adult forms (**Figure 4**). This argument will never answer the question of the nature of the vertebrate head, but it will ultimately end up at the very beginning of the segmentation of some animal forms that evolved long before the rise of vertebrates. Thus, the evaluation of vertebrate head segmentation will depend on which of the above models fits actual embryogenesis and which of these models our current analyses and data are potentially able to support.

### The Hourglass Model in Relation to the Basic Body Plan of Vertebrates

Despite the apparent consistency between the funnel model and cascade-like molecular developmental processes (e.g., embryogenesis starts with a single-celled egg and then develops it into a complex body by putting genetic programs into motion one after another), certain researchers have noted that the earliest stages of development are more divergent among different species. In the 1990s, based on the conserved, anteroposterior pattern of gene expression, or *Hox* cluster expression, it was proposed that the common ancestor of the animal kingdom may have possessed a similar anteroposterior pattern of gene expression in mid-embryogenesis [i.e., the zootype hypothesis (70)]. Following these findings, an alternative model, the hourglass model (**Figure 4**), was proposed to explain the relationship between the basic body plan of vertebrates and their evolutionary history (14, 60).

Unlike the funnel model, the hourglass model posits that early embryogenesis is divergent among different species and that progressively increasing divergence occurs only after the most conserved mid-embryonic period (**Figure 4**). The essence of this model resides at this bottleneck stage, which is referred to as the vertebrate phylotypic period. The current understanding of the basic body plan of vertebrates (4) is based on a shared anatomy of adult morphology, whereas the concept of the phylotypic stage states that this conserved mid-embryonic stage is the source of the basic vertebrate body plan (14, 60). Nevertheless, even among biologists who support this model, no consensus stage or period has been defined for the phylotypic stage. Owing to the difficulty of quantitatively evaluating the conserved nature of embryonic stages, Ballard (3) proposed the pharyngula stage as the phylotypic stage, whereas Wolpert (79, pp. 183–87) proposed the early somite segmentation stage, and Slack et al. (70) proposed the tail-bud stage.

Another important implication of the hourglass model is that the number of shared features (or evolutionarily conserved features) among different species increases when looking back into their embryogenesis. However, in contrast to the funnel model, this tendency applies only until the phylotypic period, beyond which earlier stages reveal fewer shared features and decreased information regarding the species' evolutionary trajectory.

Along with studies of other proposed models (5, 58), studies that tested the funnel and hourglass models were based on qualitative comparisons of morphological elements (62) or on a select number of genes (26, 32). These models therefore remained to be tested until the recent advances in both comprehensive and quantitative molecular approaches.

## Recent Molecular Studies That Support the Hourglass Model

Testing models is important to understanding how embryogenesis reflects evolutionary history; we still do not understand why embryogenesis appears to reflect broader phylogenetic relationships than adult shapes do or which developmental stage is the best to study in order to understand the body plan and its evolution. Empirically, however, we already realize that embryonic similarity reflects phylogenetic affinity—for example, the embryogenesis of mice is more similar to that of rats than to that of chickens.

The primary obstacle that prevented biologists from testing models (14, 24, 60, 63, 74, 78; reviewed in 34) was the difficulty of quantitatively evaluating the evolutionary divergence or evolutionary distances between different embryonic stages. Despite substantial advancements and findings in the field of genomics [for example, the finding that whole-genome duplications took place in the vertebrate lineage (36)] and widely conserved developmental tool-kit genes (7), comparisons of genomic DNA sequences have provided little information about which embryonic stage retains the most conserved (or ancestral) state. After realizing that many animals, including humans and *Drosophila* species, have similar numbers of coding genes, researchers in the field of comparative genomics began focusing on noncoding regions of DNA. One remarkable fact is that the proportion of noncoding DNA in a genome seems to positively correlate with the apparent complexity of organisms (50), implying that the acquisition of new gene regulatory networks plays a more fundamental role than the acquisition of new coding genes. In accordance with this, some studies have attempted to test the funnel and hourglass models by using comprehensive gene expression profiling, paving the way for quantitative testing of the proposed models (8, 13, 26, 31, 32, 35, 47, 65). Although the majority of these expression-based studies have supported the hourglass model (13, 26, 31, 32, 35, 47, 77), some studies from the same group (8, 65) have supported the funnel model, demonstrating the conservation of the earliest stage (zygote–neurula) in zebrafish.

Some of the studies that supported the hourglass model provided evidence for a highly sequence-conserved status of genes expressed in mid-embryonic stages (13, 26, 32). However, perhaps more convincing evidence was provided by studies that demonstrated similar or conserved expression profiles of mid-embryonic stages between different species (31, 35, 47, 77). Meanwhile, investigators performing studies that supported the funnel model (8, 65) insisted that their results revealed the constrained status of early embryos from features of genes expressed in early stages—for example, a high ratio of essential genes expressed in the earlier stages, a sequence-conserved tendency of genes expressed in earlier stages, and a predicted complexity of protein–protein interaction network in the earliest stages. However, these studies did not demonstrate that genes expressed in earlier stages are actually conserved or coexpressed in the embryos of different species. Below, we discuss further how cross-species comparisons are important in evaluating evolutionary conservation.

Broadly speaking, the molecular, comparative approaches fall into two groups. One method is to evaluate the age, or “ancestralness,” of the sequences of genes expressed during development in each species (13, 26, 32, 65), and the other is to evaluate the conserved expression of orthologous genes in the embryos of different species (31, 35, 47, 77). The first method is based on the

perspective of molecular evolution, evaluating the sequence conservation of expressed genes or how widely conserved gene sets are used for each stage (for example, taking the ratio of vertebrate-conserved expressed genes to all the genes expressed at a certain developmental stage); however, it does not evaluate whether the “conserved genes” are expressed similarly in the embryos of different species. Given that animal development can be interpreted as a process of producing various cell types from a single fertilized egg, comparing the cell-type composition in vertebrate embryos with various marker genes could aid in identifying the most conserved stages. Analogous to this, analysis of the conserved gene expression profiles of the whole embryo between different species is expected to reflect the conservation of the composition of various cell types (albeit indirectly, and lacking in topological information) in the species being compared. Moreover, because cross-species comparison is essential to estimating the conserved nature of common ancestors when using extant animals, it is fair to say that cross-species expression analysis is a reasonable approach to test these models. After all, most molecular studies that are based on cross-species gene expression comparisons have shown highly similar expression in mid-embryonic stages, with earlier and later stages showing less similarity. For example, our previous results have indicated that pharyngular embryos are the period of highest conservation among four vertebrate species (*Mus musculus*, *Gallus gallus*, *Xenopus laevis*, and *Danio rerio*).

The cross-species molecular approach succeeded not only in verifying the hourglass model at the level of conserved gene regulation (namely, evaluating the evolutionary distance of developmental stages by conserved expression profiles) but also in narrowing the possible vertebrate phylotypic period in mice (at approximately embryonic day 9.5), chickens (at approximately stage HH16), *Xenopus laevis* (at approximately stages 28–31), and zebrafish (at approximately 24 hours after fertilization) (31). The shared features of the identified stages (31) correspond well with the basic vertebrate body plan (4) in that they can be divided into the tail, trunk, and head regions and have certain organ primordia that lead to the following structures: the pharyngeal structures, the eyes, the ears, the derivatives of the otic placode, an axially segmented body, the notochord, the kidneys, the heart, the digestive tube, and the epidermis, with the clear exception of teeth. Overall, provided that the hourglass model explains the divergence among vertebrate embryos, the model has a single important implication regarding the study of vertebrate head evolution: Important evolutionary information can be lost by searching evolutionary traces further back or by examining stages earlier than the phylotypic period.

With respect to the evolution of the basic vertebrate body plan from the possible metameric ancestor, it is noteworthy that the conserved stage of other animal phyla, typically that of arthropods, has also been depicted as embryonic patterns with rather clear segmental compartments that initiate at the germ-band stages (35). This similarity suggests a bilaterian ancestor with a metameric pattern (10, 11) and conserved molecular and genomic features that are found in embryos of various bilaterian species, especially those with a segmentation pattern (10, 11).

## Remaining Questions Regarding Vertebrate Evolution and Development

A consensus has not been reached on why the embryonic processes of different vertebrates exhibit hourglass-like conservation. Certain studies have proposed a mechanism that may lead to hourglass-like conservation (14, 60, 70), such as *Hox* colinearity or a complex interdependent signaling network among primordial organs in the phylotypic embryos that makes the embryos difficult to change, leading to the evolutionary conservation of these stages. Assuming that *Hox* cluster genes are the crucial factors that make embryos conserved or constrain the divergence of embryos, would whole-genome-duplicated species such as vertebrates show more strictly conserved mid-embryonic stages? Through comparative epigenomics, Tena et al. (71) uncovered a

pool of conserved regulatory regions that are active during the vertebrate phylotypic period in zebrafish and medaka species, suggesting that the gene regulatory network would be an attractive candidate that makes the mid-embryonic period conserved; however, this needs further investigation. In particular, no empirical evidence has been provided for any of these ideas, and thus the exact mechanisms that explain hourglass-like divergence remain to be elucidated.

Another question concerns the number of species or animal groups included in the hourglass model. Originally, the model was proposed for vertebrates, and consistent with this, recent studies based on vertebrate species demonstrated the highest expression similarity in mid-embryonic stages, especially in pharyngular embryos (31, 77). Meanwhile, a cross-species comparative gene expression study of six *Drosophila* species (35) suggested that the highest expression similarities at mid-embryonic stages can also be found in these species, and another study even implied hourglass-like conservation in *Caenorhabditis elegans* by showing the conserved nature of genes expressed in mid-embryonic stages (47). These studies have raised the question of how distantly related species can be included in the hourglass. Specifically, does the hourglass include not only vertebrates but also bilaterians? One study suggested that the segmentation period of the mosquito *Anopheles gambiae* exhibits the highest expression similarity for orthologous genes at previously defined mid-embryonic vertebrate stages, namely, the pharyngula stage (31). However, the exact answer to the question of whether all bilaterian species are included in the hourglass requires further investigation. Answering this question would shed light on how the vertebrate body plan evolved from invertebrates, particularly with respect to the link between vertebrates and the common ancestor of bilaterians, or the urbilaterians (11, 27, 54), and on the origin of the head structure.

Finally, one group recently reported that the plant *Arabidopsis thaliana* also exhibits hourglass-like gene expression divergence during development (59). Because plants and animals established multicellularity independently (40), this report may imply that hourglass-like divergence during embryogenesis is an inevitable feature of multicellular development; however, this remains to be confirmed.

## THE CURRENT SCARCITY OF MOLECULAR DATA

Despite recent advancements in the comprehensive acquisition of gene expression profiles, such as microarrays and massively parallel sequencing technologies, only a few studies have tested the proposed models of embryonic evolution. In particular, there is still a need for comprehensive expression data sets based on similar platforms (e.g., data sets based on RNA sequencing from the Illumina HiSeq platform) for embryos at different developmental stages from many more species. In chordates, the expression profiles of only six species (around 15 early-to-late stages of *Mus musculus*, *Gallus gallus*, *Pelodiscus sinensis*, *Xenopus laevis*, *Xenopus tropicalis*, and *Danio rerio*, essentially covering all the coding genes) have been studied so far, a group that does not include any cyclostomes or nonvertebrate chordates such as cephalochordates and urochordates. Moreover, in light of the focus on noncoding genes and gene regulatory networks when studying the evolution of complex body plans (50), studies that cover noncoding regions will be necessary.

Overall, more studies of both coding and noncoding regions of genetic elements of nonmodel (non-genome-sequenced) species are needed, and new genomic technologies that enable investigators to easily obtain high-quality genome sequences will substantially boost this field. Currently, even with the use of massively parallel sequencers, one of the limiting steps for obtaining a well-assembled animal genome sequence is the production of large numbers of long-insert libraries; this challenge could perhaps be overcome by new technologies such as genomic assembly using the HiC (chromatin interaction) approach (6).

## OPEN QUESTIONS ON THE ORIGIN OF THE VERTEBRATE HEAD

The following are three open questions on the origin of the vertebrate head along with possible ways that transcriptome age indices could help to answer them:

- *Question 1:* Is there homology between the head mesoderm (premandibular, mandibular, and hyoid mesoderm) of vertebrates and the rostral part of the somites in amphioxus?
- *Possible resolution:* If there is homology between the vertebrate head mesoderm and amphioxus rostral somites, studies should detect vertebrate head mesoderm components but not trunk somite components in the amphioxus rostral somites.
- *Question 2:* Is there homology between vertebrate, amphioxus, and tunicate pharyngeal regions?
- *Possible resolution:* Because the vertebrate pharyngeal region consists of an endodermal pouch, head mesodermal core, neural crest, and ectoderm, whereas the amphioxus lacks a neural crest and tunicates seem to not have a neural crest contribution to the pharyngeal region, studies should find genes expressed in neural crest and genetic networks that are essential for the interactions between neural crest cells and the other tissues. Thus, these studies should reveal a new genetic system introduced in the vertebrate pharyngeal region.
- *Question 3:* Is there homology between vertebrate, amphioxus, and tunicate neural tubes?
- *Possible resolution:* The vertebrate central nervous system is subdivided into the fore/mid/hindbrain and spinal cord. In addition, three major signaling centers (anterior neural ridge, zona limitans intrathalamica, and midbrain–hindbrain boundary) are essential to the formation of the vertebrate central nervous system. Thus, compartments in the central nervous system and the three major signaling centers in amphioxus and tunicates should not be exactly the same as those in vertebrates.

## CONCLUSION AND FUTURE PROSPECTS

This review has described how recent molecular-based comparative embryology studies have elucidated the origin of the vertebrate body plan and head structure. By reflecting on the historical arguments, we attempted to highlight the major advance of recent studies: the discovery that the general formulation of how vertebrate embryogenesis changes during evolution can be explained by the hourglass model. Meanwhile, precisely how the vertebrate body plan evolved is unclear and awaits further investigation, including (a) detecting the phylotype precisely; isolating several tissues that are chordate ancestral traits, such as the notochord and neural tube from different developmental stages from amphioxus, tunicates, and vertebrates; and examining the transcriptomes of these tissues (see sidebar, Open Questions on the Origin of the Vertebrate Head) as well as (b) determining the relationship between the evolution of the body plan and whole-genome duplications. If we consider that the concept of the body plan arose from structuralism—originally proposed by the linguist Saussure (67), who developed his theory by critically reconsidering the concept of evolution as scientific empiricism in the late nineteenth century—then evolutionary changes are transformations of an archetype. If so, then when each gene in gene regulatory networks (one of the factors of the transformation) duplicates and acquires new functions or obtains new *cis*-regulatory domains, how do these phenomena affect the body plan? To answer this question, is the current transcriptomic strategy useful enough? These questions should be addressed in the future.



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