



Asembling the Tree of Life

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Chordate Phylogeny and Development

Chordata, our own lineage (fig. 23.1), belongs to the successively more inclusive clades Deuterostomata, Bilateria, Metazoa, and so forth. The organization of chordates is distinctively different from that of its metazoan relatives, and much of this distinction is conferred by unique mechanisms of development (Slack 1983, Schaeffer 1987). Throughout chordate history, modulation and elaboration of developmental systems are persistent themes underlying diversification. Only by understanding how ontogeny itself evolved can we fully apprehend chordate history, diversity, and our own unique place in the Tree of Life. My goal here is to present a contemporary overview of chordate history by summarizing current views on relationships among the major chordate clades in light of a blossoming understanding of molecular, genetic, and developmental evolution, and a wave of exciting new discoveries from deep in the fossil record.

Chordates comprise a clade of approximately 56,000 named living species that includes humans and other animals with a notochord—the embryological precursor of the vertebral column. Chordate history can now be traced across at least a half billion years of geological time, and twice that by some estimates (Wray et al. 1996, Ayala et al. 1998, Bromham et al. 1998, Kumar and Hedges 1998, Hedges 2001). Chordates are exceptional among multicellular animals in diversifying across eight orders of size magnitudes and inhabiting virtually every terrestrial and aquatic environment (McMahon and Bonner 1985). New living chordate

species are still being discovered both through traditional explorations and as molecular analyses discover cryptic taxa in lineages whose diversities were thought to be thoroughly mapped. But it is unknown whether the pace of discovery is now keeping up with the pace of extinction, which is accelerating across most major chordate clades in the wake of human population growth (Dingus and Rowe 1998).

Many chordate clades have long been recognized by characteristic adult features, for instance, birds by their feathers, mammals by their hair, or turtles by their shells. But owing in large part to such distinctiveness, few adult morphological features have been discovered that decisively resolve the relationships among the chordate clades, and even after 300 years of study broad segments of chordate phylogeny remain *terra incognita*.

Much of the hypothesized hierarchy of higher level chordate relationships has been deduced from paleontology and developmental biology (Russell 1916). Thanks to the advent of phylogenetic systematics, both fields are expressing resurgent interest and progress on the question of chordate phylogeny. And, as they are becoming integrated with molecular systematic analyses, a fundamental new understanding of chordate evolution and development is emerging.

In most other metazoans, the adult fate of embryonic cells is determined very early in ontogeny. However as chordate ontogeny unfolds, the fates of embryonic cells are plastic for a longer duration. Chordate cells differentiate as signals pass

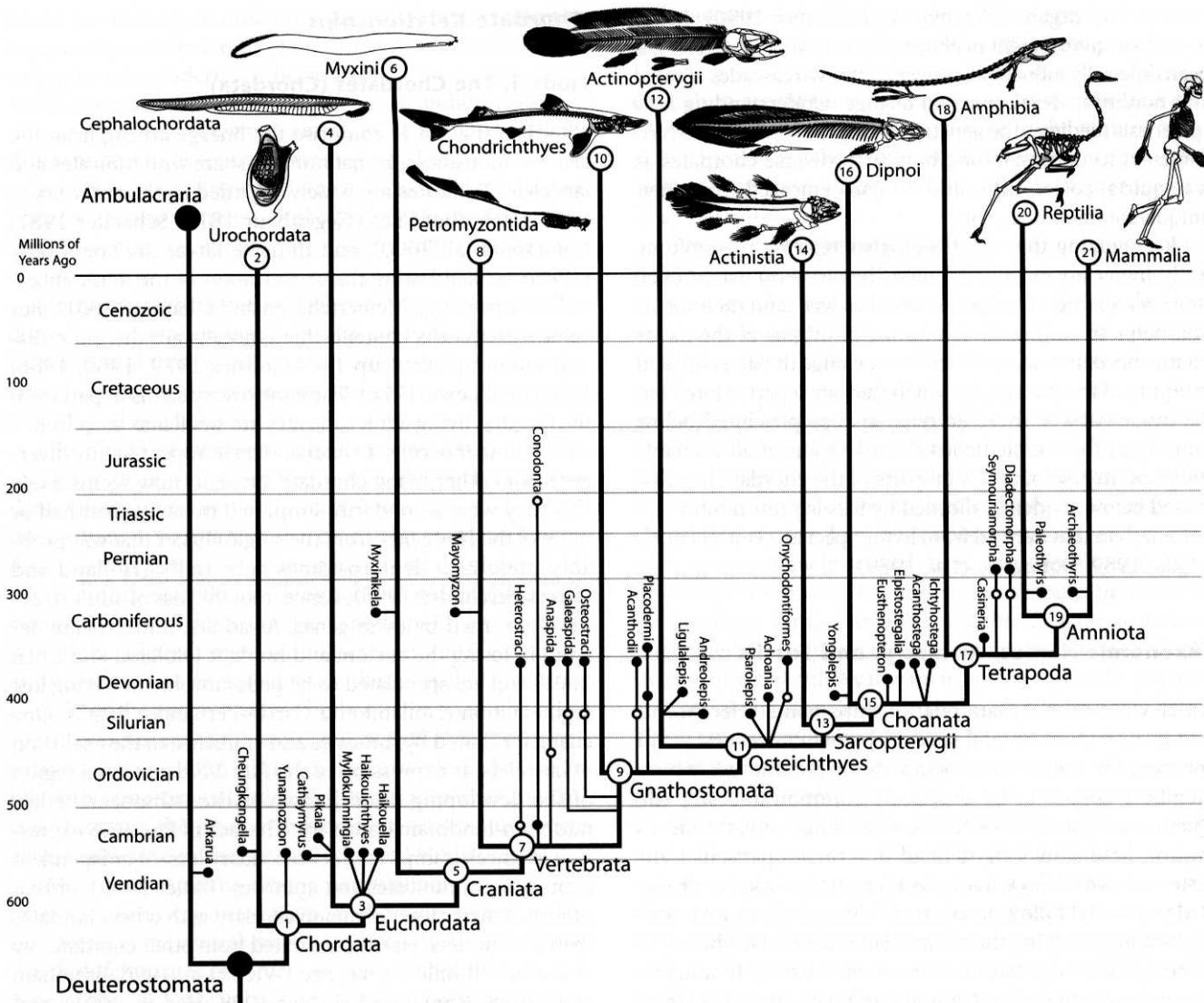


Figure 23.1. Chordate phylogeny, showing the relationships of extant lineages and the oldest fossils, superimposed on a geological time column. Nodal numbers are keyed to text headings.

between adjacent cells and tissues during the integration of developing cell lineages into functioning tissues, organs, and organ systems. Seemingly subtle modulations in early ontogeny by this information exchange system have occurred many times over chordate history to yield cascades of subsequent developmental effects that underlie chordate diversity (Hall 1992). Molecular and developmental genetic studies are now revealing the intricate details of this unique, hierarchical system of information transfer as genes are expressed in cells and tissues in early ontogeny. These analyses, moreover, generate data that possess a recoverable phylogenetic signal and are yielding fundamental insights into the evolution of development.

An important conclusion already evident is that major innovations in chordate design were generally derived from

preexisting genetic and developmental pathways, whose alteration transformed ancestral structures into distinctive new features with entirely different adult functions (Shubin et al. 1997). Increase in numbers of genes was a primary mediator of this change, and the inductive nature of chordate development amplified that change via epigenesis, which occurs as familiar physical forces and dynamic processes interact with the cells and tissues of a developing organism. These include gravity, adhesion, diffusion, mechanical loading, electrical potentials, phase separations, differential growth among tissues and organs, and many others (Rowe 1996a, 1996b). Morphogenic and patterning effects are the developmental outcomes of these recognized physical phenomena, because they affect interactions among virtually all developing cells,

tissues, and organs (Newman and Comper 1990). In the inductive environment of chordate ontogeny, epigenesis has been especially influential, triggering its own cascades of rapid and nonlinear developmental change. Understanding how epigenesis mediates the genetic blueprint of ontogeny is fundamental to understanding how such diverse chordates as sea squirts, coelacanths, and humans emerged from their unique common ancestor.

Recognizing that most biologists reading this volume study living organisms, the focus below is on extant taxa. However, extinct taxa are discussed as well, and their inclusion helps to emphasize the timing of origins of the major extant chordate clades and to acknowledge the diversity and antiquity of the lineages of which they are a part. Moreover, the framework of chordate relationships presented below came from the simultaneous consideration of all available evidence. In resolving several parts of the chordate tree discussed below, evidence afforded by fossils proved more important than that derived from living species (Gauthier et al. 1988a, 1989, Donoghue et al. 1989).

Taxonomic Names, Ancestry, and Fossils

Older views of chordate relationships make reference to groups united on general similarity or common gestalt. In contrast, the names used below designate lineages whose members appear to be united by common ancestry (de Queiroz and Gauthier 1992). To avoid ambiguity, the meanings of these names are defined in terms of particular ancestors of two or more living taxa (i.e., node-based or crown clade names). I follow an arbitrary but useful narrative convention in specifying the crown clade names used below in terms of their most recent common ancestry with humans. For example, the name Chordata refers to the clade stemming from the last common ancestor that humans share with living tunicates and lancelets; the name Vertebrata designates the clade stemming from the last common ancestor that humans share with lampreys; and so on (fig. 23.1). This is arbitrary in the sense that many other possible living specifiers among amniotes (viz., birds, turtles, crocodilians, lizards) in place of humans would designate the same clades.

Stem-based names are used in reference to a node or terminal taxon, plus all extinct taxa that are more closely related to it than to some other node or terminal taxon. In the interests of simplifying the complex taxonomy that evolved under the Linnaean system, I follow a convention now gaining popularity that employs the prefix "Pan-" to designate stem + crown lineages (Gauthier and de Queiroz 2001). For example, Pan-Mammalia refers to the clade Mammalia, plus all extinct species closer to Mammalia than to its extant sister taxon Reptilia. The clade Pan-Vertebrata includes Vertebrata plus all extinct taxa closer to Vertebrata than to hagfishes, and so forth.

Chordate Relationships

Node 1. The Chordates (Chordata)

Chordata (fig. 23.1) comprise the lineage arising from the last common ancestor that humans share with tunicates and lancelets. Tunicates are widely regarded as the sister taxon to all other chordates (Gegenbaur 1878, Schaeffer 1987, Cameron et al. 2000), and tunicate larvae are commonly viewed as manifesting the organization of the adult ancestral chordate (e.g., Meinertzhagen and Okamura 2001). But some systematists contend that lancelets are the more distant outgroup (Løvtrup 1977, Jeffries 1979, 1980, 1986, Jeffries and Lewis 1978). The controversy stems in part from the fact that living adult tunicates are small and built from a small number of cells. Even their larvae appear highly divergent from other living chordate larvae. It now seems likely that they were secondarily simplified in having lost half or more of the *Hox* genes from the single cluster that was probably present in deuterostomes ancestrally (Holland and Garcia-Fernández 1996), hence, too, the loss of adult structures governed by these genes. As adults, tunicates are derived in losing the coelom and hindgut (Holland and Chen 2001) and are speculated to be pedomorphic in having lost segmentation (Holland and Garcia-Fernández 1996). One character shared by tunicates and craniates, to the exclusion of lancelets, is expression of the *Pax 2/5/8* gene in a region of the developing brain known as the isthmocerebellar-midbrain-hindbrain boundary. The lack of *Pax 2/5/8* expression in lancelets implies either secondary loss, or independent expression in tunicates and craniates (Butler 2000), or that tunicates share closer common ancestry with other chordates than do lancelets. Having separated from other chordates by at least a half-billion years ago (Wray et al. 1996, Bromham et al. 1998, Kumar and Hedges 1998, Hedges 2001), and without a useful fossil record (below), relationships among these chordates must be viewed as tenuous (Gauthier et al. 1988a, Donoghue et al. 1989). More for narrative convenience than conviction, I follow current convention in treating tunicates as sister lineage to all other chordates.

Chordate Characters

The notochord. The namesake feature of chordates is a premiere example of embryonic induction and patterning, in which differentiation of the embryo along a dorsoventral axis launches a cascade of subsequent developmental events (Slack 1983, Schaeffer 1987). "Dorsalization" is controlled by the *Hedgehog* gene and signaling by bone morphogenesis protein, or BMP (Shimeld and Holland 2000). As in other bilaterians, chordates develop from three primary embryonic layers. These are the outer ectoderm, the inner endoderm, and the mesoderm, which arises from cells that migrate between the inner and outer layers. Chordate mesoderm develops in the upper hemisphere of the embryonic gastrula, its identity being induced partly as its cells stream across the

dorsal lip of the primordial opening (blastopore) into the inner cavity (archenteron) of the embryo, and partly by signaling from endoderm at the equator of the embryo (Hall 1992). Mesoderm cells reaching the dorsal midline condense into a strip of cells known as chordamesoderm, which later differentiates to become the notochord. The notochord in turn induces overlying ectoderm to form the dorsal neural plate, triggering another morphogenic chain of events as the chordate central nervous system (CNS) differentiates and begins to grow. In most chordates, the mesoderm immediately adjacent to the notochord takes on special properties, as does the ectoderm immediately adjacent to the neural plate. Elaboration of these dorsal structures is tied closely to evolution of the organs of information acquisition and integration, as well as to locomotion.

The chordate central nervous system. Induction of a dorsal neural plate is directed by the underlying chordamesoderm (above). This is the first step of neurulation, in which the nervous system arises, becomes organized, and helps direct the integration of other parts of the developing embryo. During neurulation, longitudinal neural folds arise along the edges of the neural plate, perhaps under the direction of the adjacent mesoderm (Jacobson 2001), and meet on the midline to enclose a space that initially lay entirely outside of the embryo. This “hollow” comprises the adult ventricular system of the brain and central canal of the spinal cord. It is lined with ciliated ependymal cells and its lumen fills with cerebrospinal fluid. This original “periventricular” layer becomes the primary region from which subsequent neural cells arise in the brain (Butler and Hodos 1996).

Molecular signaling during neurulation also produces anteroposterior regionalization in chordate embryos. The rostral end of the central nerve cord swells to form the brain, which differentiates into three regions that express distinct gene families and which have distinct adult fates. The rostralmost (diencephalic) domain of the neural tube expresses the *Otx* gene family and is connected to specialized light-sensitive cells. Behind this is a caudal (hindbrain–spinal cord) division, in which *Hox* genes are active and which receives nonvisual sensory inputs. Between the two lies an intermediate region marked by expression of the *Pax 2/5/8* patterning gene that is more problematically compared with a region known as the isthmocerebellar-midbrain-hindbrain boundary and involves the ear (Meinertzhagen and Okamura 2001, Butler 2000, Shimeld and Holland 2000). *Pax 2/5/8* is expressed in tunicates and craniates, but not lancelets (below).

Other bilaterians have a longitudinal nerve cord and brain but it is ventrally positioned; hence, biologists long maintained that the chordate dorsal nerve cord arose independently. However, both brains express orthologous homeobox genes in similar spatial patterns. For instance, the fruit fly has a regionalized neural tube with similarities in rostrocaudal and mediolateral specification to chordates (Arendt and Nübler-Jung 1999, Nielsen 1999, Butler 2000; for alternative view, see Gerhart 2000). Its rostral brain is specified by

the regulatory gene *Orthodenticle*, a homologue to the chordate *Otx* family genes, and it receives input from paired eyes. This suggests a common blueprint. Biologists long found it difficult to accept the two nerve cords as homologous owing to their different positions relative to the mouth, but it now appears that the deuterostome mouth is a new structure and not homologous to the mouth in protostomes (Nielsen 1999).

Special sensory organs of the head. An eye and ear of unique design were probably present in chordates ancestrally. The master control gene *Pax6* is expressed during early development in paired neural photoreceptors—eyes—in chordates and many other bilaterians. Paired eyes and ears, however rudimentary, were almost certainly present in chordates ancestrally (Gehring 1998). However, *Pax6* expression in chordates is manifested in eye morphogenesis that follows a unique hierarchy of pathways and inductive signals, and in which considerable diversity evolved among the different chordates lineages. Living tunicates, lancelets, and hagfish each appear uniquely derived, leaving equivocal exactly what type of eye was present in chordates ancestrally. In tunicates, the larval eye forms a small vesicle that contains a sunken, pigmented mass. Internal to the pigment lies a layer of cells that are directed radially toward it, and overlying the pigment are two hemispherical refractive layers (Gegenbaur 1878). These same relationships occur in all other chordates. However, in tunicates an optic vesicle is present only in larvae and is generally unpaired. Nevertheless, it is an outgrowth of the *Otx*-expressing region of the forebrain and it expresses *Pax6*, as do the paired eyes of vertebrates and unlike the median pineal eye (Meinertzhagen and Okamura 2001). In lancelets there is a single, median frontal eye, which also expresses *Pax6*, and like the bilateral eyes of vertebrates it is linked with cells in the primary motor center (Lacalli 1996a, 1996b, Butler 2000). In the case of lancelets, the forward extension of the notochord may be implicated in secondary fusion of the single eye. Hagfish have paired eyes, but they are poorly developed compared with most vertebrates.

The chordate ear or otic system eventually differentiated into the organs of both balance and hearing in vertebrates. Adult tunicates have sensory hair cells that support a pigmented otolith and are grouped into gelatinous copular organs located in the atrium of the adult. These cells express members of the *Pax 2/5/8* gene family, as do the otic placodes in craniates (but not lancelets), and in early development they are topographically similar to craniote otic placodes. However, placodes themselves are not yet present. Similar gene expression, cellular organization, and topography point to the probable homology of the otic organ in all chordates (Shimeld and Holland 2000, Jeffries 2001, Meinertzhagen and Okamura 2001).

Hormonal glands. Two hormonal glands arose in chordates ancestrally to exert novel control over growth and metabolism. The pituitary is a compound structure that forms via the interaction between neurectoderm, which descends from

the developing brain toward the roof of the pharynx, and oral ectoderm that folds inward to line the inside of the mouth. Ectoderm forms Rathke's pouch and becomes the glandular part of the pituitary, whereas neural tissue from the floor of the diencephalon becomes its infundibular portion. The infundibulum is present in lancelets and craniates, but its homologue in tunicates is unclear. However, in tunicates the homologue of the glandular portion, known as the neural gland, lies in the same position with respect to both brain and pharyngeal roof (Barrington 1963, 1968, Maisey 1986).

The second hormonal gland, the endostyle, develops in a groove in the floor of the larval pharynx in tunicates, lancelets, and in larval lampreys. Its cells form thyroid follicles that secrete iodine-binding hormones. Its homologue in gnathostomes is probably the thyroid gland, which also develops in a median out-pocketing in the floor of the pharynx, and also forms thyroid follicles that secrete iodine-binding hormones (Schaeffer 1987). Thyroid hormone production is controlled in large measure by the pituitary gland and affects growth, maintenance of general tissue metabolism, reproductive phenomena, and in some taxa metamorphosis.

Tadpole-shaped larva. Unlike the ciliated egg-shaped larvae of hemichordates and echinoderms, the chordate larva is tadpole shaped, with a swollen rostral end and a muscular tail. The rostral end houses the brain, beneath which lie the rostral end of the notochord, and the pharynx and gut tube. Behind the pharynx is a tail equipped with muscle deriving from caudal mesoderm (Maisey 1986, Schaeffer 1987). Although lacking tails as adults, the larvae of many species have tails of comparatively simple construction with muscle that form bilateral bands, in contrast to the segmental muscle blocks found in euchordates (below). A recent study of tailed and tailless tunicate larvae (Swalla and Jeffery 1996) found that the *Manx* gene is expressed in the cells of the tailed form but it is down-regulated in the tail-less species, and that complete loss of the tail can be attributed to disrupted expression of the single gene. Whether *Manx* was central to the origin of the tail in chordates is unknown, but this study highlights the potential genetic simplicity underlying complex adult structures.

Pan-Chordata

Although an extensive fossil record is known for many clades lying within Chordata, no fossils are known at present that lie with any certainty on its stem.

Node 2. The Tunicates or Sea Squirts (Urochordata)

Chordate species all can be distributed between the tunicates and euchordates, its two principal sister clades (fig. 23.1). The tunicates comprise a diverse marine clade that includes roughly 1300 extant species distributed among the sessile ascidians, and the pelagic salps and larvaceans (Jamieson 1991). Tunicate monophyly is well supported (Gegenbaur

1878, Maisey 1986). As adults, the tunicate body is enclosed within the tunic, an acellular membrane made of cellulose-like tunicin. It is derived from ectoderm, and in tunicates it may contain both amorphous and crystalline calcium carbonate spicules (Aizenberg et al. 2002). Echinoderms possess crystalline calcium in ectodermal structures, raising the question of whether biomineralization was present in deuterostomes ancestrally (see below). The tunic presents an outwardly simple body, but it cloaks a much more complex and derived organism. The pharynx is perforated by two pairs of slits and is enormously enlarged for suspension feeding. The pharynx size obliterates the coelom, a cavity inside the body walls that surrounds the gut in tunicate larvae and most adult chordates. Unique incurrent and excurrent pores supply a stream of water through the huge pharynx, which in some species serves in locomotion. All tunicates are mobile as larvae, but not all species have larval tails. The pelagic salps and larvaceans are thought to be more basal and to reflect the primitive adult lifestyle.

Pan-Urochordata

The fossil record of tunicates is sparse and tentative, but potentially long. The oldest putative tunicate, *Cheungkongella ancestralis*, from the Early Cambrian of China (Shu et al. 2001a) is known from a single specimen. It evidently preserves a two-fold division of the body into an enlarged pharyngeal region with pharyngeal openings, a large oral siphon surrounded by short tentacles, and a smaller excurrent siphon. The body appears wholly enclosed in a tuniclike outer covering. It has short tail-like attachment structure, a derived feature placing *Cheungkongella* among crown tunicates. This fossil, if properly interpreted, marks the Early Cambrian as the minimum age of divergence of tunicates from other chordates and implies a Precambrian origin for Chordata.

A possible stem tunicate fossil was brought to light through a reinterpretation of *Jaekelocarpus oklahomensis*, a Carboniferous "mitrate" (Dominguez et al. 2002). High-resolution X-ray computed tomography (e.g., Rowe et al. 1995, 1997, 1999, Digital Morphology 2003) provided new details of internal anatomy and revealed the presence of paired tunicate-like gill skeletons. *Jaekelocarpus* and a number of similar, tiny Paleozoic fossils have a calcite exoskeleton over their head and pharynx and are generally thought to lie as stem members of echinoderms or various basal chordate clades (Jeffries 1986, Dominguez et al. 2002). The mitrates may prove to be paraphyletic, and its members assignable to different deuterostome clades. The eventual placement of all of these fossils will have bearing on our interpretation of basal chordate relationships, and on the structure and history of mineralized tissues.

Node 3. Chordates with a Brain (Euchordata)

Euchordata comprise the last common ancestor that humans share with lancelets (but see caveats above), and all of its

descendants (fig. 23.1). Apart from the tunicates and a single ancient fossil of uncertain affinities (below), all other chordates are members of Euchordata. Expanding on the innovations that arose in chordates ancestrally, euchordates manifest more complex genetic control over development. This was accompanied by further elaboration of the CNS and special sense organs, and a fundamental reorganization of the trunk musculature and locomotor system.

Euchordate Characters

Increased genetic complexity I. Euchordates express *Msx*, *HNF-3*, and *Netrin* genes, whereas only *Hedgehog* is expressed in tunicates. This evident increase in homeobox expression corresponds to elaborated dorsoventral patterning in the CNS. Additional genes are also expressed in more elaborate anteroposterior regionalization, including *BFI* and *Islet* genes (Holland and Chen 2001). Tunicates express only one to five *Hox* genes, whereas lancelets express 10 *Hox* genes in one cluster, affecting broader regions of the brain and nerve cord. Although poorly sampled, at least one hemichordate (*Saccoglossus*) expresses nine *Hox* genes in its single cluster. Tunicates therefore may have lost genes that were present in deuterostomes ancestrally (Holland and Garcia-Fernández 1996).

Elaboration of the brain I. Lancelets were long thought to have virtually no brain at all, but recent structural studies reveal an elaborate brain and several unique resemblances to the brain in craniates (Lacalli 1996a, 1996b, Butler 2000). Reticulospinal neurons differentiate in the hindbrain, where they are involved in undulatory swimming and movements associated with the startle reflex. Also present in lancelets are homologues of trigeminal motor neurons, which are involved in pharyngeal movement, and possibly other cranial nerves (Fritzsche 1996, Butler 2000). Additionally, the neural tube is differentiated into an inner ependymal cell layer (gray matter) and synaptic outer fibrous layer (white matter; Maisey 1986) and is innervated by intermyotomal dorsal nerve roots that carry sensory and motor fibers (Schaeffer 1987). Several of these features lie partly or wholly within the expression domain of *Hox* genes.

Elaboration of the special senses I. An olfactory organ occurs in lancelets, in the form of the corpuscles of de Quatrefages. These are a specialized group of anterior ectodermal cells that send axonal projections to the CNS via the rostral nerves. They are marked by expression of the homeobox gene *AmphiMsx*, which is also expressed in craniate ectodermal thickenings known as placodes (below), but no true placodes have been observed in lancelets or tunicates (Shimeld and Holland 2000). The olfactory organ is highly developed in nearly all other euchordates.

Segmentation. Segmentation arises when mesoderm along either side of the notochord subdivides to form somites. These are hollow spheres of mesoderm that mature into muscle blocks known as myomeres, which are separated by sheets of connective tissue (myocomata). Only the mesoderm lying

close to the notochord becomes segmented, whereas more laterally the mesoderm produces a sheet of muscle that surrounds the coelomic cavity. The segmented muscles enable powerful locomotion, producing waves of contraction that pass backward and propel the body ahead. Segmentation is accompanied by *Fringe* (or its homologue) expression and signaling by the Notch protein, features shared with other segmented bilaterians. These regulate the timing and synchronization of cell-to-cell communication required of segmental patterning and the formation of tissue boundaries (Evrard et al. 1998, Jiang et al. 2000).

Other features. Also arising from mesoderm is a blood circulatory system of stereotyped arterial design, with a dorsal and ventral aorta linked by branchial vessels, and a complementary venous system (Maisey 1986). Other transformations traceable to the ancestral euchordate yielded a larva that is essentially a miniature, bilateral adult. As adults, a median fin ridge increases thrust area while helping to stabilize movement through the water (Schaeffer 1987).

Pan-Euchordata

The oldest stem euchordate fossil may be the Early Cambrian *Yunannozoon* from the Chengjiang lagerstätte of southern China (Chen et al. 1995, Shu et al. 2001b, Holland and Chen 2001). It is known from a single specimen that shows evidence of segmental muscle blocks, an endostyle, a notochord, and a nonmineralized pharyngeal skeleton. Little more than a flattened smear, the chordate affinities of this problematic fossil are debatable.

Node 4. The Lancelets (Cephalochordata)

The lancelets, sometimes known as amphioxus, form an ancient lineage that today consists of only 30 species (Gans and Bell 2001). *Branchiostoma* consists of 23 species and *Epigonichthys* includes seven (Poss and Boschung 1996, Gans et al. 1996). Lancelets are suspension feeders distributed widely in tropical and warm-temperate seas. The larvae are pelagic, and one possibly pedomorphic species remains pelagic as an adult. Adults of the other species burrow into sandy substrate, protruding their heads into the water column to feed.

Adult lancelets lack an enlarged head. They are unique in the extent of both the notochord and cranial somites, which extend to the very front of the body. A single median eye also distinguishes them, which, based on *AmphiOtx* expression, may be homologous to the paired eyes of other chordates and bilaterians (Lacalli 1996a, Butler 2000). Their feeding apparatus involves a unique ciliated wheel organ surrounding the mouth, and a membranous antrum that surrounds the pharynx (Maisey 1986, Holland and Chen 2001).

Pan-Cephalochordata

A single fossil from the Early Cambrian of China, known as *Cathaymyrus* (Shu et al. 1996), may be a stem cephalochordate and the oldest representative of the clade. *Pikaia gracilens*

from the Middle Cambrian Burgess Shale is known from numerous specimens and is popularly embraced as a cephalochordate (Gould 1989), but this is now questionable (Holland and Chen 2001). A mitrate known as *Lagynocystis pyramidalis*, from the lower Ordovician of Bohemia, may also be a stem cephalochordate (Jeffries 1986). In all cases, more specimens and more detailed anatomical preservation are needed to have any confidence in these assignments.

Node 5. Chordates with a Head (Craniata)

Craniata contain the last common ancestor that humans share with hagfish, and all its descendants (fig. 23.1). Even contemporary literature often confuses this clade name with the designation Vertebrata. However, Vertebrata are properly regarded as a clade lying within Craniata (Janvier 1996). Compared with their euchordate ancestors, craniates have increased genetic complexity, a larger brain, and more elaborate paired sense organs. Larvae probably persisted as suspension feeders (Mallatt 1985), but adults shifted to active predation with higher metabolic levels, more powerful locomotion, and a sensory system perceptive to multiple modes of environmental signal (Jollie 1982, Northcutt and Gans 1983; but see Mallatt 1984, 1985). A rigid skull protects and supports the brain, special sense organs, and feeding apparatus. Most important, the neural crest blooms in early development as a unique population of motile cells that induce new structures and assist the many parts of the increasingly complex head and pharynx to integrate as a functional whole.

Craniate Characters

Increased genetic complexity II. Craniates have at least two *Hox* gene clusters, and perhaps three or four clusters were present ancestrally (Holland and Garcia-Fernández 1996). This increase in number is correlated with further elaboration of the neurosensory system over that of lancelets and tunicates. Several additional gene families increased in number, including those encoding transcription factors (*ParaHox*, *En*, *Otx*, *Msx*, *Pax*, *Dlx*, *HNF3*, *bHLH*), signaling molecules (hh, IGF, BMP), and others (Shimeld and Holland 2000). The mechanism of duplication is uncertain.

Elaborated brain and sensory organs II. The craniate brain includes new cell types and neuronal groups. It now integrates input from elaborated special sensory organs that develop from paired ectodermal thickenings known as placodes, with the assistance of cells of the neural crest (Northcutt and Gans 1983, Webb and Noden 1993, Butler 2000, Shimeld and Holland 2000). Placodes are typically induced by the underlying mesoderm, and they develop into organs and structures that contribute sensory input to the brain. Although there is evidence for olfactory, optic, and otic organs earlier in chordate history, the integration of placodes with neural crest cells marks a first blossoming of acute, highly complex special sense organs. At least two placode types can now be distinguished. Sensory placodes are involved in the olfactory sacs,

lens, ear vesicles, and lateral line system, whereas neurogenic placodes contribute sensory neurons to cranial ganglia. Both categories include some rather different structures, and the different placodes probably had separate histories (Northcutt 1992, Webb and Noden 1993).

The craniate brain is also fully segmented in early ontogeny and differentiates into discrete adult regions associated with special cranial nerves that have specific sensory functions, motor components, or both. Up to 22 cranial nerves are known in some craniates (Butler 2000). The fore- and midbrain regions are expanded and compartmentalized to degrees not seen in other chordates. The forebrain differentiates from segmented prosomeres into an anterior telencephalon that receives input from highly developed olfactory nerves, and the diencephalon to which project the paired eyes (Butler and Hodos 1996). The pineal eye was probably also a part of this system ancestrally. Adult hagfish lack a pineal eye, evidently an ontogenetic loss as the entire visual system degenerates (Hardisty 1979, Forey 1984b). The midbrain arises from segmental mesomeres (Butler and Hodos 1996). The hindbrain develops from segmental rhombomeres controlled by *Hox* genes via *Krox-20* and *Kreisler* expression (Shimeld and Holland 2000). Also elaborated is the otic system, which functions in both vestibular and acoustic reception. Two semicircular canals were present ancestrally (Maisey 2001, Mazan et al. 2000). A lateral line system also arises from head and body placodes (Northcutt 1992). Its functions in electroreception (Bodsnick and Northcutt 1981), and also in mechanoreception by sensing water currents and turbulence, aiding locomotion and hunting (Pohlmann et al. 2001). Also, an autonomic nervous system helps control the endocrine system and other internal functions, and the spinal cord is equipped with dorsal root ganglia.

The internal skeleton. The cartilaginous precursor of an internal skeleton was present in the head, and along the notochord as paired neural and hemal arches. These elements develop via induction between the mesodermal sclerotome and the adjacent notochord and/or spinal chord (Maisey 1986, 1988), but only later in chordate history do they become mineralized or ossified (below). Although lacking jaws and teeth, the ancestral craniate probably had specialized hard mouthparts built from noncollagenous enamel proteins that formed mineralized denticles along the pharyngeal arches at the borders of the gill clefts. These are sites where endoderm and ectoderm interact, and neural crest may also contribute to their mineralization (Smith and Hall 1990). Even in hagfish, high molecular weight amelogens are associated with pharyngeal tissues (Slavkin et al. 1983, Delgado et al. 2001) and the calcium regulatory hormone calcitonin is present (Schaeffer 1987, Maisey 1988).

The neural crest. Origin of the neural crest was perhaps the most remarkable morphogenic event in deuterostome history, owing to the diverse structures that these cells induce or contribute to directly, and help to integrate (Northcutt and Gans 1983, Schaeffer 1987). Neural crest cells are

themselves induced by mesoderm along the edges of the overlying neural plate. They migrate to new locations throughout the head, where they produce the cartilaginous neurocranium, a unique structure housing the expanded brain and providing a rigid armature that suspends the special sense organs. Neural crest cells also form a cartilaginous branchial arch system. Neural crest cells also arise from the developing spinal cord to form spinal ganglia, the sympathetic nervous system, pigment cells, and adrenalin glands.

Neural crest cells do not differentiate nor are the structures that they build present in tunicates or lancelets. However, several neural crest cell-inducing genes occur in lancelets. These include the *Msx*, *Slug/Snail*, and *Distalless* gene families, which are expressed in lateral neural plate, and *Pax-3/7*, which is expressed in immediately adjacent ectoderm (Butler 2000, Shimeld and Holland 2000). *Hox* regulatory elements have also been identified in lancelets that in craniates drive spatially localized expression of neural crest cells in the derivatives of placodes and the branchial arches (Manzanares et al. 2000). Thus, well before the emergence of the ancestral craniate, the relative spatial expression patterns of several genes involved in neural crest induction were present.

Pharyngeal arch elaboration. In lancelets, there is a more or less stiff framework of several pairs of collagenous arches. Between adjacent arches are branchial clefts that function primarily in suspension feeding (Mallatt 1984, 1985). In contrast, craniate pharyngeal arches are major structural elements, composed of segmented cartilage or bone that suspend heavily vascularized gills within the clefts. The arches are muscular, and under CNS control they power a pump involved in both respiration and feeding. In craniates, for the first time, the pharyngeal clefts may properly be called gill slits (Schaeffer 1987, Maisey 1988). Each arch develops from an outer covering of ectoderm, an inner covering of endoderm, and a mesenchymal core derived from neural crest and mesoderm (Graham and Smith 2001). The majority of the neural crest cells forming the arches arise adjacent to the hindbrain rhombomeres, each arch with a neural crest population tied to a specific group of rhombomeres. This ensures the faithful transfer of segmental patterning information from the CNS to the arches, establishing a correspondence between innervations and effector muscles. The neural crest segregates into discrete arch populations partly through apoptosis, or preprogrammed cell death, in a process similar to that which sculpts the discrete digits in the tetrapod hands and feet (below). In both instances, key components in the cell death program are the genes encoding *Msx2* and *BMP4* (Graham and Smith 2001, Zhou and Niswander 1996).

Elaborated muscular system. Muscle ontogeny follows a unique pathway in craniates. First, mesodermal somitomeres appear in strict rostral to caudal order during gastrulation, as segmental arrays of paraxial mesenchymal cells condense along the length of the embryo (Jacobson 1988, 2001). Cranial somitomeres then disperse to form the striated muscles

of the head, including extrinsic muscles of the eye (except in hagfish, which may have lost them secondarily), and branchial musculature. In the trunk, the somitomeres gradually condense to form somites. Lateral to the developing somites the mesoderm differentiates into three separate populations of cells. These are the sclerotome, which later forms part of the cranium and much of the vertebral column, the dermatome, which forms the connective tissues of the dorsal trunk, and the myotome, which forms the striated muscles of the trunk. The adult trunk musculature consists of sequential chevron-shaped myomeres. Finally, the unsegmented lateral plate splits and the coelomic cavity forms between its two layers. The gut, which is no longer ciliated internally, becomes invested by a layer of smooth muscle that provides peristaltic contractions for the movement of ingested food (Schaeffer 1987, Maisey 1986).

Powerful heart and circulatory system. A powerful two-chambered heart is present in craniates along with red blood cells, hemoglobin, and vasoreceptors that monitor pressure and gas levels of the blood passing through the heart. Associated with the elaborated circulatory system is a highly innervated kidney (Schaeffer 1987, Maisey 1988).

Additional endodermal derivatives. The liver and pancreas arise from endoderm through new inductive signals from mesoderm. Also deriving from this source are elaborate endocrine glands including the parathyroids, which control calcium and phosphate metabolism with the plasma calcium-regulatory hormone (calcitonin), and the adrenal glands, all of which are controlled to varying degrees by the autonomic nervous system. The larval endostyle metamorphoses into the adult thyroid gland, becoming a true endocrine gland, directing its secretions into the circulatory rather than digestive system (Schaeffer 1987).

Paired and median fin folds. Primordia of the paired lateral and median appendages arise in craniates via mesodermal-epithelial induction, whereas the dorsal fin arises via interaction between the epidermis and trunk neural crest. A median fin fold is present in lancelets, but it develops without the neural crest interaction.

High metabolic capacity. Craniates possess a well-developed capacity for anaerobic metabolism, resulting in the formation of lactic acid. This probably evolved in association with burst activity that is unobtainable by relying solely on aerobic metabolism (Ruben and Bennett 1980).

Pan-Craniata

The oldest putative pancraniate is *Haikouella lanceolata*, known by more than 300 specimens from the Chengjiang lagerstätte of southern China (Chen et al. 1995, 1999, Shu et al. 2001a, 2001b, Holland and Chen 2001). It has a three-part brain and paired eyes. Its mouth has 12 oral tentacles, and the pharynx has six nonmineralized pharyngeal arches bearing gill filaments that lie in separate visceral clefts. A pair of grooves in its floor suggests an endostyle. There may be several mineralized denticles on the third arch, but preser-

vation leaves this uncertain. About two dozen paired straight myomeres are separated by myosepta behind the 5th visceral arch. Stains are preserved that may represent a heart with ventral and dorsal aorta, and anterior branchial artery. The notochord extends about 85% the length of the body, stopping short of the rostrum, and slight banding can be seen resembling the immature vertebral elements of lampreys (Holland and Chen 2001). It also has dorsal, caudal, and ventral midline fins. *Haikouella* has also been hypothesized to lie on the lamprey stem (Chen et al. 1999), but support is weak (Janvier 1999). From the same deposits, possibly lying on the craniate stem, are *Haikouichthyes* and *Myllokunmingia*, each known from a single fusiform fossil (Shu et al. 1999a, 1999b). The rostral two-thirds of their bodies comprises the pharyngeal region, with Z-shaped myomeres making up the rest. A median dorsal fin shows faint striations that may be fin rays. There are also paired lateral structures, but it is doubtful whether they are homologous with the fins of gnathostomes (below). In *Haikouichthyes* are nine pharyngeal arches and a complex skull, probably built of cartilage, suggesting the presence of neural crest cells. Neither specimen shows evidence of mineralization (Shimeld and Holland 2000, Holland and Chen 2001).

Node 6. The Hagfish (Myxini)

Hagfish comprise a poorly known chordate lineage that includes 58 living species (Froese and Pauly 2001). Throughout their life cycles, hagfish generally occupy deep marine habitats in temperate seas, ranging from 25 to 5000 m in depth (Moyle and Cech 2000). They scavenge large carcasses, burrow into soft substrate for invertebrates, and pursue small prey through the water column. But they are difficult to observe and little is known of their development.

The monophyly of Myxini is well supported. They have three pairs of unique tactile barbels around the nostril and mouth, and a single median nostril of distinct structure. Many other features distinguish them from other craniates, but some may reflect secondary loss, including absence of the epiphysis and pineal organ, reduction of the eyes, presence of only a single adult semicircular canal, and a vestigial lateral line system confined to the head (Hardisty 1979, Maisey 1986, 2001).

Pan-Myxini

Only three fossil species have been allied to the hagfish. The least equivocal is *Myxinikela siroka*, from the Carboniferous Mazon Creek deposits of Illinois (Bardack 1991). A second specimen from these same beds, *Pipiscus zangerli* (Bardack and Richardson 1977), is more problematically a hagfish and has also been allied to lampreys (below). *Xidazoon stephanus*, known by three specimens from the Lower Cambrian of China, has been compared with *Pipiscius* (Shu et al. 1999a, 1999b). Its mouth is defined by a circle of about 25 plates, and it may have a dilated pharynx and segmented tail. But

other assignments are equally warranted by the vague anatomy it preserves, and whether it is even a chordate remains questionable.

Node 7. Chordates with a Backbone (Vertebrata)

Vertebrata comprise the last common ancestor that humans share with lampreys, and all its descendants. The relationship of hagfish and lampreys to other craniates is long debated. Hagfish and lampreys were once united either as Cyclostomata or Agnatha, jawless fishes grouped by what its members lacked instead of by shared unique similarities, and they were considered ancestral to gnathostomes (e.g., Romer 1966, Carroll 1988). This grouping was largely abandoned as diverse anatomical data showed lampreys to share more unique resemblances with gnathostomes than with hagfish (Stensiö 1968, Løvtrup 1977, Hardisty 1979, 1982, Forey 1984b, Janvier 1996). But controversy persists, and recent studies of the feeding apparatus have resurrected a monophyletic Cyclostomata (Yalden 1985, Mallatt 1997a, 1997b). Cyclostome monophyly is also supported by ribosomal DNA (rDNA; Turbeville et al. 1994, Lipscomb et al. 1998, Mallatt and Sullivan 1998, Mallatt et al. 2001), vasotocin complementary DNA (cDNA; Suzuki et al. 1995), and globin cDNA (Lanfranchi et al. 1994). However, the results from small subunits of rDNA were overturned when larger ribosomal sequences were used, and morphological analyses that sample many different systems also refute cyclostome monophyly (Philippe et al. 1994, Donoghue et al. 2000). The question may not be settled, but I follow current convention and treat lampreys and hagfish as successive sister taxa to gnathostomes.

Vertebrate Characters

Increased genetic complexity III. A tandem duplication of *Hox*-linked *Dlx* genes occurred in vertebrates ancestrally, encoding transcription factors expressed in several developing tissues and structures. They are expressed in an expanded forebrain, cranial neural crest cells, placodes, pharyngeal arches, and the dorsal fin fold. An additional duplication evidently occurred independently in lampreys and gnathostomes (Amores et al. 1998, Niedert et al. 2001, Holland and Garcia-Fernández 1996).

Elaboration of the brain and special senses III. In vertebrates, exchange of products between blood and cerebrospinal fluid occurs via the choroid plexus, a highly vascularized tissue developing in the two thinnest parts of the ventricular roof of the brain. Vertebrate eyes are also enhanced by a retinal macula, a small spot of most acute vision at the center of the optic axis of the eye, and by synaptic ribbons that improve retinal signal processing. Extrinsic musculature originating from the rigid orbital wall provides mobility to the bilateral eyeballs. The pineal body is also photosensory, and in some vertebrates differentiates into a well-developed pineal eye with retina and lens. In addition, the lateral line system extends along the sides of the trunk (Maisey 1986).

Correspondingly, an extensive cartilaginous braincase that includes embryonic trabecular cartilages arises beneath the forebrain, and an elaborate semirigid armature supports the brain and its special sensory organs.

Locomotor and circulatory systems. Vertebrates have dorsal, anal, and caudal fins that are stiffened by fin rays, increasing thrust and steering ability. The circulatory and muscular systems were also bolstered. The heart comes under nervous regulation and a stereotyped vascular architecture carries blood to and from the gills. Myoglobin stores oxygen in the muscles, augmenting scope and magnitude in bursts of activity. The kidney is also elaborated for more sensitive osmoregulation and more rapid and thorough filtration of the blood (Maisey 1986).

Pan-Vertebrata

The oldest putative stem vertebrates are the heterostracans, an extinct lineage extending from Late Cambrian (*Anatolepis*) to the Late Devonian (Maisey 1986, 1988, Gagnier 1989, Janvier 1996). Their skeleton consists of plates of acellular membranous bone. Precise relationships of this clade are controversial, but if correct the position of heterostracans as the sister taxon to Vertebrata may suggest that lampreys may have secondarily lost a bony external skeleton. However, in the absence of direct evidence that lampreys ever possessed bone, heterostracan fossils and the characteristics of bone are treated below (see Pan-Gnathostomata, below).

Node 8. The Lampreys (Petromyzontida)

There are approximately 35 living lamprey species, all but three of which inhabit the northern hemisphere (Froese and Pauly 2001). In most, larvae hatch and live as suspension feeders in freshwaters for several years, then migrate to the oceans as metamorphosed adults, where they become predatory and parasitic. Nonparasitic freshwater species are known (Beamish 1985) and in some cases the metamorphosed adults are nonpredatory and do not feed during their short adult lives (Moyle and Cech 2000).

Lamprey monophyly is diagnosed by a unique feeding apparatus. It consists of an annular cartilage that supports a circular, suction-cup mouth lined with toothlike keratinized denticles. A mobile, rasping tongue is supported by a unique piston cartilage and covered by denticles whose precise pattern diagnoses many of the different species. Lampreys attach to a host, rasp a hole in its skin, and feed on its body fluids. Lampreys also eat small invertebrates. The structure of the branchial skeleton (Mallatt 1984, Maisey 1986) and the single median nasohypophysial opening (Janvier 1997) are unique. Lampreys have a distinctive suite of olfactory receptor genes that serves in the detection of odorants such as bile acids (Dryer 2000). There is also evidence that lampreys are apomorphic in having undergone duplication of a tandem pair of *Dlx* genes, followed by loss of several genes, independent of a comparable du-

plication and subsequent loss that occurred in gnathostomes (Niedert et al. 2001)

Pan-Petromyzontida

Haikouichthyes ercaicunensis (Shu et al. 1999b) from the Early Cambrian of China is the oldest fossil lamprey reported, but the data for its placement are tenuous (Janvier 1999). *Mayomyzon pieckoensis*, known by several specimens from the Late Carboniferous Mazon Creek beds of Illinois (Bardack and Zangerl 1968), is the oldest unequivocal lamprey, preserving unique lamprey feeding structures, including the annular and piston cartilages. *Hardistiella montanensis* (Janvier and Lund 1983) from the Lower Carboniferous of Montana preserves less detail, and it is not clear whether either lies within or outside of (crown) Petromyzontida. *Pipiscus zangerli* (Bardack and Richardson 1977) from the same Mazon Creek beds as *Mayomyzon* is sometimes also tied to lampreys, as well as hagfish, but it preserves little relevant evidence.

Node 9. Chordates with Jaws (Gnathostomata)

Gnathostomata comprise the last common ancestor that humans share with Chondrichthyes, and all of its descendants (fig. 23.1). Its origin was marked by additional increases in complexity of the genome, which mediated several landmark innovations, including jaws, paired appendages, several types of bone, and the adaptive immune system. Although the positions of certain basal fossils are debated, there is little doubt regarding gnathostome monophyly.

Gnathostome Characters

Increased genetic complexity IV. Gnathostomes have at least four *Hox* gene clusters, and some have as many as seven. In addition to specifying the fate of cell lineages along the anteroposterior axis, these gene clusters mediate limb development and other outgrowths from the body wall. It is questionable whether as many as four *Hox* clusters arose earlier, either in vertebrates or craniates ancestrally (Holland and Garcia-Fernández 1996), but in gnathostomes their expression nevertheless manifests more complex morphology. There was also duplication of *Hox*-linked *Dlx* genes and several enhancer elements, leading to elaboration of cranial neural crest in the pharyngeal arches, placodes, and the dorsal fin fold (Niedert et al. 2001). Immunoglobulin and recombinase activating genes also arose in gnathostomes, marking the origin of the adaptive immune system.

Brain and sensory receptor enhancement IV. The gnathostome forebrain is enlarged, primarily reflecting enhancement of the olfactory and optic systems. The extrinsic muscles of the eyeball are rearranged and an additional muscle (the obliquus inferior) is added to the suite present in vertebrates ancestrally (Edgeworth 1935). In the ear, a third (horizontal) semicircular canal arises, lying in nearly the same plane as the synaptic ribbons of the eye, and correlates with *Otx1* expression (Maisey 2001, Mazan et al. 2000). In addition,

the lateral line system is elaborated over much of the head and trunk. On the trunk, it is developmentally linked to the horizontal septum and becomes enclosed by mineralized tissues that insulate and tune directional electroreception by the lateral line system (Northcutt and Gans 1983). The gnathostome lateral line system derives from neural crest and lateral plate mesoderm induction, heralding a new stage in developmental complexity. Myelination of many nerve fibers improves impulse transmission through much of the body (Maisey 1986, 1988).

Mineralized, bony skeleton. Many bilaterians produce mineralized tissues, and both echinoderms and tunicates generate amorphous and crystalline calcium carbonate spicules (Aizenberg et al. 2002). Biomineralization is thus an ancient property, although its erratic expression outside of Craniata affords only equivocal interpretations of its history in this part of the tree. Certain other components required for bone mineralization, such as calcitonin, were already present but did not lead to bone production. However, in gnathostomes, different types of bone form in the head and body (Maisey 1988). Bone development requires the differentiation of specialized cell types, including fibroblasts, ameloblasts, odontoblasts, and osteoblasts, which are derived from the ectoderm and cephalic neural crest. In the formation of membranous bone, fibroblasts first lay down a fibrous collagen framework around which the other cells deposit calcium phosphate as crystalline hydroxyapatite. Another type of bone development typically involves preformation by cartilage, followed by deposition of hydroxyapatite crystals around the cartilage (perichondral ossification), or within and completely replacing it (endochondral ossification). Chondral ossification occurred first in the head in the oldest extinct gnathostomes (see Pan-Gnathostomata, below), and it later spread to the axial skeleton and shoulder girdle. Ossification in the shoulder girdle is of interest because it is the first such transformation of the embryonic lateral plate mesoderm and because it signals the initiation of neural crest activity in the trunk (Maisey 1988). In the shark lineage, the internal skeleton consists of cartilage that is sheathed in a layer of crystalline apatite, but fossil evidence suggests that this is a derived condition (below).

Elaborated skull. Cartilage and/or chondral bone surround the brain and cranial nerves, providing a semirigid armature for the special sensory organs. At the back of the head, the cephalicmost vertebral segment is “captured” during ontogeny by the skull to form a back wall of the braincase. Thereby, it confines several cranial nerves and vessels to a new passage through the base of the skull, known in embryos as the metotic fissure. Cellular membranous bone was also present, covering the top and contributing to other parts of the skull (Maisey 1986, 1988).

Jaws. The namesake characteristic of gnathostomes arises in ontogeny from the first pharyngeal arch, known now as the mandibular arch. Its upper half is the palatoquadrate cartilage, which is attached to the braincase primitively by

ligaments, whereas the lower half of the arch, Meckel’s cartilage, forms the lower jaw and hinges to the palatoquadrate at the back of the head. Teeth and denticles develop on inner surfaces of these cartilages through an induction of ectoderm and endoderm. Neural crest cells populating the mandibular arch derive from the mesomeres and from hind-brain rhombomeres 1 and 2, whereas the second pharyngeal arch, the hyoid arch, derives its neural crest from rhombomere 4 (Graham and Smith 2001).

Paired appendages. Other bilaterians have multiple sets of paired appendages that serve a broad spectrum of functions. It was long believed that their evolution was entirely independent of the paired appendages in gnathostomes, but this appears only partly true today. Common *Hox* patterning genes were likely present in the last common ancestor of chordates and arthropods, if not a more inclusive group. The *SonicHedgehog* gene specifies patterning along anteroposterior, dorsoventral, and proximodistal axes of the developing limb, via BMP2 signaling proteins (Shubin et al. 1997). In gnathostomes, independent expression of orthologous genes occurs in the elaboration of fins, feet, hands, and wings. As expressed in gnathostomes, the distal limb elements are the most variable elements. In basal gnathostomes they comprise different kinds of stiffening rays, whereas in tetrapods they are expressed as fingers and toes (Shubin et al. 1997). Moreover, somite development transformed to provide for muscularization of the limbs, as certain somite cells became motile and moved into the growing limb buds (Galis 2001). Thus, although the *Hox* genes have a more ancient history of expression, in gnathostomes they are expressed across a unique developmental cascade.

The adaptive immune system. One of the most remarkable gnathostome innovations is the adaptive immune system (Litman et al. 1999, Laird et al. 2000). It responds adaptively to foreign invaders or antigens such as microbes, parasites, and genetically altered cells. Other animals have immune mechanisms, but unique to gnathostomes is a system that is specific, selective, remembered, and regulated. Its fundamental mediators are immunoglobulin and recombinase activation genes, which are present throughout gnathostomes but absent in lampreys and hagfish. The immune system is expressed in a diverse assemblage of immunoreceptor-bearing lymphocytes that circulate throughout the body in search of antigens. Gnathostome lymphocytes present an estimated 10^{16} different antigen receptors, which arose seemingly instantaneously as an “immunological big bang” (Schluter et al. 1999) in gnathostomes ancestrally.

New endodermal derivatives. In gnathostomes, the endoderm elaborates to form the pancreas, spleen, stomach, and a spiral intestine (Maisey 1986).

Pan-Gnathostomata

Several extinct lineages lie along the gnathostome stem. Their relationships remain problematic, and most have been allied with virtually every living chordate branch (Forey 1984a,

Maisey 1986, 1988, Donoghue et al. 2000). All preserve mineralized and bony tissues of some kind, and the phylogenetic debate revolves in large degree around interpreting the history of tissue diversification. The most ancient, if problematic extinct pangnathostome lineage is Conodonta. Known to paleontologists for decades only from isolated, enigmatic mineralized structures, conodonts range in the fossil record from Late Cambrian to Late Triassic. The recent discovery of several complete body-fossils demonstrated that these objects are toothlike structures aligned along the pharyngeal arches and bordering the gill clefts. They are built of dentine, calcified cartilage, and possibly more than one form of hypermineralized enamel (Sansom et al. 1992). Microwear features indicate that they performed as teeth, occluding directly with no intervening soft tissues. They formed along the same zones of endoderm-ectoderm induction as the pharyngeal teeth in more derived vertebrates. The mineralized oropharyngeal skeleton and dentition arose at the base of the gnathostome stem, Cambrian conodont fossils providing its oldest known expression (Donoghue et al. 2000).

Branching from or possibly below the gnathostome stem are the heterostracans (see Pan-Vertebrata, above), whose skeleton consists of external plates of acellular membranous bone. In heterostracans, bones formed around the head, and the cranial elements seemingly grew continually throughout life. Their bone is formed of a basal lamina, a middle layer of spongy arrays of enameloid, and an outer covering of enameloid and dentine. Heterostracan fossils suggest that bone was acellular at first.

The next most problematic taxon is Anaspida, which range from Middle Silurian to Late Devonian (Forey 1984a, Maisey 1986, 1988, Donoghue et al. 2000). Anaspids are diagnosed by the presence of branchial and postbranchial scales, pectoral plates, and continuous bilateral fin folds. Perichondral ossification occurred in neural and hemal arches, and the appendicular skeleton, whereas endochondral ossification occurred in fin radials and dermal fin rays in the tail. The anaspid trunk squamation pattern suggests the presence of the horizontal septum, a critical feature in the trunk-powered locomotion that is also tied developmentally to the lateral line system. Anaspid lateral fin folds may prove to be precursors of the paired appendages of crown gnathostomes.

Lying closer to the gnathostome crown clade is Galeaspida, which range through the Silurian and Devonian. Its members are distinguished by a large median dorsal opening that communicates with the oral cavity and pharyngeal chamber. Galeaspids also have 15 or more pharyngeal pouches. Their chondral skeleton appears mineralized around the brain and cranial nerves, however the bone is primitive in being acellular (Maisey 1988). Lying closer to the gnathostome crown is Osteostraci, a lineage with a similar character and temporal range as galeaspids. Osteostracans have a dorsal head shield with large dorsal and lateral sensory fields. They share with crown gnathostomes cellular calcified tissues and perichondral ossification of the headshield, which encloses the

brain and cranial nerve roots. Ossification surrounds the orbital wall, otic capsules, and calcified parachordal cartilages, structures developing in extant gnathostomes via inductions between the CNS, notochord, and the ectomesenchyme. Perichondral mineralization of the otic capsule implies interaction between mesenchyme and the otic placode (Maisey 1988). Also present are lobed, paired pectoral fins that are widely viewed as homologous to the pectoral appendages in crown Gnathostomata (Forey 1984a, Maisey 1986, 1988, Shubin et al. 1997, Donoghue et al. 2000). Supportive of this view is the ontogenetic sequence in most extant gnathostomes, in which pectoral appendages arise before pelvic.

Node 10. Sharks and Rays (Chondrichthyes)

Chondrichthyes includes sharks, skates, rays, and chimaeras (fig. 23.1). The chimaeras (Holocephali) include roughly 30 living species, and there are about 820 living species of skates and rays (Batoidea) plus sharks (Moyle and Cech 2000). Morphology suggests that the species commonly known as sharks do not by themselves constitute a monophyletic lineage, and that some are more closely related to the batoids than to other “sharks” (Maisey 1986).

Earlier authors argued that these different groups evolved independently from more primitive chordates, and that Chondrichthyes was a grade that also included several cartilaginous actinopterygians (below). Cartilage is an embryonic tissue in all craniates, and it persists throughout life in sharks and rays (and a few other chordates), but the perception that “cartilaginous fishes” are primitive is mistaken. In its more restricted reference to sharks, rays, and chimaeras, the name Chondrichthyes designates a monophyletic lineage. Histological examination reveals bone at the bases of the teeth, dermal denticles, and some fin spines. This suggests that this restricted distribution of bone is a derived condition in chondrichthyans (Maisey 1984, 1986, 1988).

Other apomorphic characters include the presence of micromeric prismatically calcified tissue in dermal elements and surrounding the cartilaginous endoskeleton. Chondrichthyans also possess a specialized labial cartilage adjacent to the mandibles, the males possess pelvic claspers, and the gill structure is unique. The denticles (scales) possess distinctive neck canals (but these may not be unique to chondrichthyans), and the teeth have specialized nutrient foramina in their bases with a unique replacement pattern in which replacing teeth attach to the inner surface of the jaws as dental arcades (Maisey 1984, 1986). Fin structure also presents a number of unique modifications (Maisey 1986). Relationships among chondrichthyans have received a great deal of attention (Compagno 1977, Schaeffer and Williams 1977, Maisey 1984, 1986, Shirai 1996, de Carvalho 1996).

Pan-Chondrichthyes

The extinct relatives of chondrichthyans have a long, rich fossil record. The oldest putative fossils are scales with neck

canals from the Late Ordovician Harding Sandstone of Colorado (Sansom et al. 1996). Although present in extant sharks and chimeroids, most well-known Paleozoic sharks lack them. From the Silurian onward, chondrichthyan teeth are abundantly preserved, although in most cases their identification rests on solely phenetic grounds, and they provide little useful information on higher level phylogeny. The oldest anatomically complete fossils are the Late Devonian *Symmoriidae* and *Cladoselache*, which are known from numerous skeletons that in some cases preserve body outlines and other evidence of soft tissues. Both are stem chondrichthyans.

Node 11. Chordates with Lungs (Osteichthyes)

Osteichthyes (fig. 23.1) comprise the lineage stemming from the last common ancestor that humans share with actinopterygians. The name means “bony fishes” and was coined in pre-Darwinian times in exclusive reference to the fishlike members of this clade. In the phylogenetic system (de Queiroz and Gauthier 1992), the name now refers to all members of the clade, roughly half of which are the chordate species adapted to life on land.

Osteichthyan Characters

An extensive composite bony skeleton. All conclusions about skeletal evolution at this node are weak, because chondrichthyans lack an ossified internal bony skeleton that can be compared directly with that in osteichthyans. Nonetheless, the fossil record offers assistance and suggests that a bony skeleton likely arose in early panguathostomes, and that it was further elaborated in Osteichthyes. The membranous skeleton of the head forms laminae that descend from the braincase and offer attachment to muscles of the jaws and pharyngeal skeleton. The jaws themselves are invested in a layer of membranous bone, with teeth attached to their margins (Rosen et al. 1981, Maisey 1986). Around the pharyngeal chamber is an extensive series of dermal gular and opercular bones, which improve pharyngeal function as a suction chamber in both respiration and feeding. The pectoral girdle became ossified, primitively more through perichondral than endochondral processes. Lastly, in the fins are stiffening rays known as lepidotrichia, which represent rows of slender scales that replace the primitive covering of body scales (Maisey 1986, 1988).

Lungs. Lungs develop as ventral outgrowths from the rostral end of the gut tube and are often associated with skeletal structures of mesenchymal origin. Over the course of osteichthyan history, these diverticula become modified for radically different functions that range from respiration, to buoyancy regulation, to communication. In most terrestrial members of the clade, lungs completely replace gills. They are secondarily lost in some small living amphibian species, where cutaneous respiration takes over. Lungs develop as branching tubular networks constructed of sheetlike cellular epithelia. There can be hundreds to millions of branches

in the network, yet they must also have a regular patterning and structure to ensure proper function. A signaling pathway mediated by fibroblast growth factor (FGF) occurs in development of the branched lungs in the mouse, as well as in the branched respiratory tracheae in the fruit fly, raising the question of whether their common ancestor had a branched respiratory structure. But because the tracheal system lungs in insects are ectodermal and the osteichthyan lung is endodermal, this seems unlikely. Moreover, FGF is implicated in other branched structures and has probably been co-opted throughout metazoan history to produce different kinds of structures. The patterning mechanism is ancient, but its expression in the osteichthyan lung is unique (Metzger and Krasnow 1999).

Pan-Osteichthyes

Two problematic extinct lineages, Acanthodii and Placodermi, arguably lie along the osteichthyan stem, but the evidence is equivocal and a wide spectrum of other possibilities have been proposed. Although some gnathostomes went on to lose one or both sets of limbs, acanthodians are the only clade to exceed the primitive number of two pairs. An anterior spine stiffens each fin. Acanthodian fossils are known from the Late Silurian to the Late Devonian. Placoderms comprise a much more diverse clade whose fossil record extends from Early Devonian to Early Carboniferous. Placoderms are heavily armored, with a distinctive pattern of membranous bones forming a head shield that hinges to a membranous thoracic shield in a pair of ball-in-socket joints. Acanthodians and placoderms share with Osteichthyes the presence of the clavicle and interclavicles and other membranous elements in the pectoral girdle. Placoderms lie closer to Osteichthyes based on descending laminae of membranous bone in the neurocranium, lepidotrichia in the fins, and other features (Gardiner 1984). Difficulties in comparing skeletal features in these fossils with chondrichthyans, which largely lack a bony skeleton, complicate understanding the relationships of these extinct lineages (Maisey 1986).

Node 12. The Ray-Finned Fishes (Actinopterygii)

The ray-finned fishes (fig. 23.1) include nearly 23,000 living species and comprise nearly half of extant chordate diversity (Lauder and Liem 1983). The most basal divergence among extant actinopterygians is represented by the bichirs and reedfish (Polypteriformes), which commonly (but not unanimously) are regarded as sister taxon to all others. Next most basal was the divergence between the sturgeons and paddlefishes (Acipenseriformes), followed by gars (Ginglymodi) and bowfins (Halecomorpha). Among these basal clades alone are nearly 300 extinct genera named for fossils. However, this part of the actinopterygian tree remains a frontier, in large part because the fossil morphology is known only superficially (Grande and Bemis 1996). The rest of extant actinopterygian diversity resides among the teleosts (De Pinna

1996). Today actinopterygians occupy virtually every freshwater and marine environment. Their economic importance underlies the base of a huge global market, and actinopterygian conservation increasingly is involved in conflicts with development and use of the world's water resources. One member of this clade, the zebrafish, is growing in importance for biomedicine as an important model organism. Actinopterygian history and diversity are reviewed by Stiassny et al. (Stiassny et al., ch. 24 in this vol.).

Pan-Actinopterygii

The fossil record of stem actinopterygians extends tentatively into the Late Silurian (Long 1995, Arratia and Cloutier 1996). The Late Silurian *Andreolepis* and Early Devonian *Ligulalepis* are the oldest purported panactinopterygians fossils. They are known only from scales, which overlap in a seemingly distinctive tongue-in-groove arrangement often considered diagnostic of actinopterygians. However, an ossified Early Devonian braincase, possibly referable to *Ligulalepis* (Basden et al. 2000) closely resembles the braincase in the extinct Early Devonian shark *Pucapampella* (Maisey and Anderson 2001), although it is ossified. Hence, we may expect continued reassessment of character distributions and view as tentative the phylogenetic assignment of extinct taxa at this deep part of the tree. By the Early Devonian, actinopterygian fossils are found worldwide, but diversity is low. In the Middle and Late Devonian, only 12 species and seven genera are recognized. The best known is *Cheirolepis*, whose skeleton is known in detail (Arratia and Cloutier 1996). From Middle and Late Devonian rocks, abundant fossils of *Mimia* and *Moythomasia* have been recovered, representing the oldest members of crown clade Actinopterygii (Grande and Beamis 1996).

Node 13. Chordates with Lobe Fins (Sarcopterygii)

Sarcopterygians include the last common ancestor that humans share with coelacanth, and lungfishes and all its descendants (fig. 23.1). Just less than half of chordate diversity lies within this clade (Cloutier and Ahlberg 1996). Its early members were all aquatic, but from the Carboniferous onward most sarcopterygians have been terrestrial (Gauthier et al. 1989). Today only eight living species retain the ancestral life style. Two are coelacanth and the other six are lungfish, whereas the remainder of sarcopterygian diversity resides among the tetrapods.

Sarcopterygian monophyly is strongly supported, but relationships within are far from settled, especially when fossils are concerned. Leaving fossils aside for the moment, morphological, and molecular analyses continue to provide conflicting results (Marshall and Schultze 1992, Schultze 1994, Meyer 1995, Zhu and Schultze 1997). Older studies placed coelacanth outside of tetrapods + actinopterygians (von Wahlert 1968, Wiley 1979), and even with chondrichthans (Løvtrup 1977, Lagios 1979). Parvalbumin sequences

also support the placement of the *Latimeria* outside of Osteichthyes (Goodwin et al. 1987). Morphology consistently places Actinistia closer to tetrapods than to actinopterygians (Romer 1966, Rosen et al. 1981, Maisey 1986, Nelson 1989, Chang 1991), a position also supported by 28S rDNA (Hillis and Dixon 1989). But whether lungfish or coelacanth are closer to tetrapods, or whether lungfish and coelacanth together form a clade independent of tetrapods is still debated. A larger 28S sequence (Zardoya and Meyer 1996) found coelacanth and lungfishes to be the sister lineage to tetrapods. A genomic DNA analysis (Venkatesh et al. 1999, 2001) and morphology (Rosen et al. 1981, Maisey 1986, Cloutier and Ahlberg 1996) favor lungfishes and coelacanth as successive outgroups to tetrapods, the position that is followed here.

Sarcopterygian Characters

Lobe fins. The sarcopterygian pectoral and pelvic appendages form muscular lobes that protrude from the lateral body wall with a distinct skeletal architecture. In gnathostomes ancestrally there were multiple basal elements in each limb, but in sarcopterygians there is a single proximal element, followed distally by a pair of radial cartilages. This arrangement enables the insertion of muscles between the radials, giving the fin flexibility along its axis (Clack 2000). Fundamental similarities in branching occur within the embryonic digital arch in lungfishes and tetrapods, producing the familiar pattern of a single proximal element (humerus or femur), followed by a pair of elements (radius/ulna or tibia/fibula), followed by the more complex pattern of wrist and ankle bones. This branching sequence is known as the metapterygial axis, and it reflects further influence by *SonicHedgehog* (via BMP2 signaling proteins), which specifies patterning along anteroposterior, dorsoventral, and proximodistal axes of the developing limb. Expressed from the beginnings of gnathostome history in the development of fins, modified expression of orthologous genes lead to the elaboration of lobefins, feet, hands, and wings in sarcopterygians (Shubin and Alberch 1986, Shubin et al. 1997, Cloutier and Ahlberg 1996).

Enamel. A thin layer of enamel covers the teeth in sarcopterygians, and at their bases the enamel is intricately infolded into the dentine, in a pattern known as labyrinthodonty. Infolded enamel enhances tooth strength as well as the strength of attachment to the jaw (Long 1995).

Pan-Sarcopterygii

The acceptance by earlier researchers of paraphyletic groups such as the crossopterygians (e.g., Romer 1966) and the search for direct ancestors of tetrapods in these "amphibian-like fishes" left controversial the relationship among the extinct Paleozoic sarcopterygians (Rosen et al. 1981, Maisey 1986). However, most of these extinct taxa are now assignable as stem lungfish (Pan-Dipnoi) or stem tetrapods (Pan-Tetrapoda). However, two recent fossil discoveries lie on the sarcopterygian stem and provide the oldest evidence of the

clade. These are *Psarolepis romeri*, from the Late Silurian and Early Devonian of Asia (Ahlberg 1999, Zhu et al. 1999) and *Achoania jarvikii* (Zhu et al. 2001) from the Early Devonian of China. Lying at the base of either the sarcopterygian stem (Long 1995, Clack 2000) or the choanate stem is *Onychodontiformes*, a poorly known Devonian lineage whose members reached 2 m in length and are characterized by daggerlike tooth whorls. It is possible that *Psarolepis* lies within this clade.

Node 14. The Coelacanth (Actinistia)

Coelacanth history is at least 400 million years (Myr) long (Forey 1998), but only two species survive today. *Latimeria chalumnae* inhabits coastal waters along southeastern Africa, and a second population was recently discovered in the waters off Sulawesi (Erdmann et al. 1998). Divergent DNA sequences reportedly diagnose *Latimeria menadoensis* (Pouyaud et al. 1999), but it shows little morphological distinction. However, sequences from parts of two mitochondrial genes also diagnose the Sulawesi species, and molecular clock estimates suggest that it diverged from its common ancestor with the African species 5.5 Mya (Holder et al. 1999). Monophyly of the lineage has never been seriously questioned, and it is diagnosed by such features as the absence of the maxilla, absence of the surangular, absence of the branchiostegal rays, presence of a rostral electric organ, presence of numerous supraorbital bones, and a distinctive tassel on the tail.

Pan-Actinistia

The coelacanth fossil record ranges back to the Middle Devonian but it ends in the Late Cretaceous, or more tenuously the Paleocene (Cloutier and Ahlberg 1996). Approximately 125 extinct coelacanth species have been named (Cloutier 1991a, 1991b, Cloutier and Ahlberg 1996, Forey 1998). Although often described as a living fossil (Forey 1984b), a phylogenetic analysis of *Latimeria chalumnae* and its extinct relatives showed that the living species differ by many dozens of apomorphies from their Paleozoic relatives (Cloutier 1991a). Some of these characters represent losses of elements in the cheek and opercular region, leading to suggestions that coelacanth history was characterized by pedomorphosis (Lund and Lund 1985, Forey 1984b). However, there are also elaborations in complexity of skeletal elements, which indicate that the history of actinistians involved more than a single developmental trend and that living coelacanths are not "living fossils" (Cloutier 1991a).

Node 15. The Breathing Chordates (Choanata)

Choanata comprise the last common ancestor that humans share with lungfishes (fig. 23.1), and all its descendants (= Rhipidistia of Cloutier and Ahlberg 1996). Choanata monophyly is supported by genomic DNA (Venkatesh et al. 2001, Hyodo et al. 1997) and morphology (Rosen et al. 1981,

Maisey 1986, Cloutier and Ahlberg 1996), although it remains among the more controversial nodes within Chordata (above).

Choanata Characters

The choanate nose and respiratory system. Its namesake feature is a palatal opening called the choana that communicates externally via paired external nostrils to the lungs and pharynx. The interpretation of this region is controversial in both Paleozoic fossils and Recent taxa, and whether the choana was actually present ancestrally is in dispute (Rosen et al. 1981, Maisey 1986, Carroll 2001). Despite debate over this feature, other transformations of nasal architecture and function were underway. A nasolacrimal canal is present, connecting the orbit with the narial passageway (Maisey 1986). The snout in front of the orbits is elongated in association with these passageways. These facial changes appear related to modifications in the internal structure of the lung tied to increase in efficiency of air breathing with the addition of pulmonary circulation and augmentation of the heart with two auricles (Johansen 1970, Rosen et al. 1981).

Simplification of the pharyngeal skeleton. The opercular elements that enclosed the pharynx in osteichthyans ancestrally are reduced and the pharyngeal arches are simplified with the loss of their dorsal (pharyngobranchial) and ventral (interhyal) elements (Rosen et al. 1981). The upper division of the second arch, the hyomandibula, is reduced and freed from its primitive role as a support between the cranium and jaws. This may signal the beginning of its function in sound transduction.

Tetrapodous locomotion. Well-developed pectoral and pelvic skeletons with two primary joints are present, signaling the beginnings of stereotyped locomotor patterns (Rosen et al. 1981). In the forelimb, the humerus articulates with the shoulder girdle in a ball-in-socket joint. Distal to that is the radius and ulna, which articulate to the humerus in a synovial elbow joint. The presence of these elements represents the unfolding of fundamental patterning at a cellular level (Oster et al. 1988) that persists through most members of the clade. The pelvis is also strengthened by ventral fusion of its right and left halves to form a single girdle. In addition, the musculature that powers the limbs is segmented, paving the way for a blossoming of limb diversification.

Pan-Choanata

Lying along the stem of either Choanata or Sarcopterygii lies a poorly known lineage known as Onychodontida (Cloutier and Ahlberg 1996). If this placement proves correct, its Early Devonian fossils would be the oldest crown sarcopterygians yet discovered.

Node 16. The Lungfishes (Dipnoi)

The lungfishes (fig. 23.1) have a 400 Myr history but today include only six living species. Four live in freshwaters of tropi-

cal Africa (*Protopterus dolloi*, *P. annectens*, *P. aethiopicus*, and *P. amphibius*), one in South America (*Lepidosiren paradoxa*), and one in Australia (*Neoceratodus forsteri*). The monophyly of dipnoans has never been challenged. Their most distinctive features involve the feeding apparatus (Schultze 1987, 1992, Cloutier and Ahlberg 1996). Lungfish may have teeth along the margins of their jaws as juveniles, but they are lost in adults. The adult dentition consists of tooth plates that line the roof and floor of the mouth. The plates grow by the continual addition of new teeth and dentine, which consolidate into dental plates that are not shed (Reisz and Smith 2001).

Pan-Dipnoi (= Dipnomorpha)

Approximately 280 extinct species are known, their record extending back to the Early Devonian. The earliest dipnomorphs retain marginal teeth but also have palatal tooth plates. The earliest members of the lineage are from the Early Devonian and occupied marine waters, but by the mid-Devonian skeletal structures associated with air breathing had appeared and soon thereafter members of the lineage had moved to the freshwaters that all living species inhabit (Cloutier and Ahlberg 1996). *Yongolepis* and *Porolepiformes* are extinct lineages known from Devonian rocks that lie at the base of the stem of the lungfish lineage (Clack 2000).

Node 17. Chordates with Hands and Feet (Tetrapoda)

Tetrapoda (fig. 23.1) comprise the last common ancestor that humans share with amphibians, and all its descendants. The sister relationship between amphibians and amniotes (below) is supported by molecular (Hedges et al. 1993) and morphological data (Schultze 1970, 1987, Rosen et al. 1981, Cloutier and Ahlberg 1996). Historically, the name Tetrapoda designated all sarcopterygians possessing limbs with digits rather than fin rays, such as the Devonian *Ichthyostega* and *Acanthostega*. Although it is true that a wide morphological gap separates the fingers and toes of *Ichthyostega*, from more basal sarcopterygians that lack discrete digits such as *Eusthenopteron*, the limbs of *Ichthyostega* are quite different from those inferred to have been present in the last common ancestor of living tetrapod species. It was once believed that some of the extant tetrapod lineages arose independently from fishlike sarcopterygians, (Jarvik 1996), but recent phylogenetic analyses conclude that extant amphibians and amniotes share a more recent common ancestor that is not also shared with *Ichthyostega* or *Acanthostega*. The history of Tetrapoda was long considered to extend back to the Late Devonian, but under this more restrictive definition of the name, the oldest known tetrapods are Carboniferous fossils (Paton et al. 1999).

Tetrapod Characters

The tetrapod limb. In crown tetrapods, the shoulder girdle has a prominent scapular blade and a posterior coracoidal region, and the humerus has a discrete shaft. There are fully differentiated proximal and distal carpals in the wrist and

phalanges in the hand. The ankle also has separate proximal and distal tarsals and phalanges (Gauthier et al. 1988b). The evolution of fingers and toes is associated with changes in the timing and position of expression of the more ancient *Hox* genes that regulate development of the body axis and appendages (Shubin et al. 1997, Carroll 2001). In sampled actinopterygians, the *Hoxd-9* to *Hoxd-13* genes are expressed in an overlapping sequence from the proximal to distal ends of the posterior surface of the fin. In tetrapods the most distal gene, *Hoxd-13*, is expressed over a more anterior portion of the distal end of the limb, directing distal expansion of the limb and the formation of fingers and toes. Key components in the development of separate digits are cell death (apoptosis) programs directed by the genes encoding *Msx2* and *BMP4* (Graham and Smith 2001). These were first expressed in the development of separate pharyngeal arches. In tetrapods they are co-opted to direct apoptosis in the tissues that lie between the digits, to produce discrete fingers and toes (Zhou and Niswander 1996). Lost from the tetrapod limb are the ectodermal lepidotrichia, along with axial elements tied to axial locomotion through water, including the caudal fin rays.

Tetrapod skull. Reduction occurred in the dermal bones tied to aquatic feeding and respiration, including loss of the last opercular elements (subopercular, preopercular) and anterior tectal and internasal (Gauthier et al. 1988b). The braincase is further enclosed, as the metotic fissure becomes floored by the basioccipital and basisphenoid, and ossified lateral "wings" of the parasphenoid expand beneath the otic capsules. An elongated parasphenoidal cultriform process extends forward below much of the brain. Tetrapods also develop an ossified occiput and craniovertebral joint, heralding independence and mobility of the head on the neck. Also, the lateral line system of the skull lies almost entirely in open canals.

Vomeronasal organ. The vomeronasal organ is a paired structure located in the floor of the nasal chamber, on either side of the nasal septum. It is a chemoreceptor similar in general function to the olfactory epithelium and olfactory nerves and bulb. But unlike olfactory epithelium, its lining is nonciliated and it has separate innervation by the vomeronasal nerve, which projects to an accessory olfactory bulb, rather than to the main bulb as do the olfactory nerves. Its function is largely in reception of pheromones and other molecular mediators of social interaction. There is great elaboration of the vomeronasal organ in squamates, in which it takes on more general environmental functions. The vomeronasal organ was once thought to be absent in primates. But it is present in early development in nearly all mammals, and may be present in humans (Margolis and Getchell 1988, Butler and Hodos 1996, Keverne 1999).

Pan-Tetrapoda

The fossil record of stem tetrapods extends from the Middle Devonian through the Permian and is represented in many

parts of the world. However, the fossil record of its sister taxon (Pan-Dipnoi) suggests that the tetrapod stem extends to the Early Devonian or Late Silurian (Clack 2000). At the base of Pan-Tetrapoda lies Osteolepiformes, a diverse group that ranged from the Middle Devonian to Early Permian. One especially well-known member is *Eusthenopteron* (Jarvik 1996), long thought to be ancestral to tetrapods, now seen as a distant cousin. Monophyly of Osteolepiformes is not strongly defended, and some of its members may eventually find other positions near the base of this part of the chordate tree. Also near the base of Pan-Tetrapoda is Rhizontida, which ranged through much of the Devonian and Carboniferous. Its monophyly is well supported by pectoral fin morphology and scale composition (Cloutier and Ahlberg 1996). Some of its members were predators that grew to great size.

Still closer to the tetrapod crown is Elpistostegalia, which include only *Elpistostege* and *Panderichthyes*, from the Late Devonian of North America and Eastern Europe. These taxa are similar to Tetrapoda in having a cranial roofing pattern consisting of paired frontals that lie anterior to the parietals, and in the flattened shape of the head. They also have a straight tail lacking dorsal and ventral lobes, and the dorsal and anal fins are lost. All of these may indicate a shallow-water lifestyle (Clack 2000).

The Devonian taxa *Ichthyostega* (Jarvik 1996) and *Acanthostega* (Clack 1998) are still closer to the tetrapod crown and were long considered to be the basalmost tetrapods because they have hands and feet with discrete digits. However, their hands and feet were very different from those of extant tetrapods, as well as from the condition that was present in their last common ancestor (Gauthier et al. 1989). They have up to eight toes and retain primitive features such as a well-developed gill arch skeleton and lepidotrichia along the tail (lost in Tetrapoda), suggesting that they remained primarily aquatic (Coates and Clack 1990, Cloutier and Ahlberg 1996). One important feature *Ichthyostega* and *Acanthostega* share with crown tetrapods is the fenestra vestibuli, an opening through which the stapes communicates to the inner ear, signaling the beginnings of an airborne-impedance-matching ear.

Node 18. The Amphibians (Amphibia)

Extant amphibians (= Lissamphibia) comprise 4700 extant species that are all distributed among the distinctive frog, salamander (fig. 23.1), and limbless caecilian lineages. All are small and insectivorous and have wet skins that in many cases convey oxygen and other exogenous materials into the body. Hence, they are important as sensitive barometers of freshwater and riparian environments, and many species are facing decline. Their skeletons are pedomorphic in many respects, for example, in the maintenance of extensive cartilage in the adult skeleton, and in the absence of many membranous roofing bones (Djordović and Kalezić 2000). However, they are also highly derived in other respects, and none of the

extant species closely resembles its Paleozoic ancestors. Both molecular and morphological data suggest that frogs and salamanders are more closely related than either is to caecilians (Zardoya and Meyer 1996).

Pan-Amphibia

Relationships at the base of Pan-Amphibia are especially problematic, and more than 100 extinct species have been named for Permo-Carboniferous fossils alone. The problematic aistopods (Carroll 1998, Anderson et al. 2003), nectrideans, and microsaurids are often regarded as basal members of Pan-Amphibia. However, all are highly derived and their positions uncertain. The most basal divergence among pan-amphibians was that of the extinct Paleozoic loxammatids (Beaumont and Smithson 1998, Milner and Lindsay 1998). Temnospondyles are generally regarded to include all other panamphibians. Temnospondyles include large extinct *Edops*, *Eryops*, and mastodonsaurids (Damiani 2001) in addition to extant amphibians and a host of other fossils. These basal taxa include large and fully aquatic or amphibious carnivores, some exceeding 2 m in total length. They are distinguished by the opening of large fenestrae in the roof of the palate. However, the extinct lepospondyles have also been regarded as closer relatives of extant amphibians than temnospondyles (Laurin 1998a, 1998b), and the debate remains active. In either case, amphibians and amniotes had diverged from the ancestral tetrapod by the early Carboniferous.

By the Late Triassic, frogs, salamanders, and caecilians had diverged, and left a fairly detailed fossil record. One of the most exciting discoveries occurred in Late Jurassic sediments of northern China, where 500 exceptionally well-preserved salamander specimens were recently recovered. The new finds implicate Asia as the place of salamander diversification (Gao and Shubin 2001). Amphibian history is reviewed in detail by Cannatella and Hillis (ch. 25 in this vol.).

Node 19. Terrestrial Chordates (Amniota)

Amniota (fig. 23.1) comprise the last common ancestor that humans share with Reptilia, and all its descendants (Gauthier et al. 1988a). Although some members became secondarily aquatic, the origin of amniotes heralded the first fully terrestrial chordates. Its monophyly is strongly supported, and its membership is noncontroversial (with the exception of certain Paleozoic fossils). However, relationships among the major living amniote clades are debated. Of principle concern is whether mammals are closest to birds (Gardiner 1982, Løvtrup 1985) or are the sister taxon to other amniotes (Gauthier et al. 1988a, Laurin and Reisz 1995). Arguments linking birds and mammals are based on analyses confined to extant taxa alone, or they treat extant taxa primarily and then secondarily fit selected fossils to that tree. However, when all evidence is analyzed simultaneously, mammals are the sister taxon to other amniotes (Gauthier et al. 1988a, Laurin and Reisz 1995).

Amniote Characters

Amniote egg. The amniote egg and attendant equipment for internal fertilization present a complex of ontogenetic innovations affording reproductive independence from the water. Incubation of the amniote embryo is a more protracted process than before, because the larval stage and metamorphosis are lost, and instead a fully formed young emerges from the egg. Amniote eggs are larger than those of most nonamniotes, with larger volumes of yolk. As the embryo grows, its size produces special problems with respect to metabolic intensity, the exchange of respiratory gases, structural support, and the mobilization and transport of nutrients (Packard and Seymour 1997, Stewart 1997). The outer eggshell takes on an important role in mediating metabolism. It is made of semipermeable collagen fibers and varying proportions of crystalline calcite, which permits respiration while preventing desiccation. The eggshell also provides a calcium repository for the developing skeleton. The embryo is also equipped with several novel extra-embryonic membranes. The amnion encloses a fluid filled cavity in which the embryo develops. The allantois stores nitrogenous wastes, and the chorion is a respiratory membrane. A single penis with erectile tissue is also apomorphic of Amniota (Gauthier et al. 1988a).

The amniote skeleton and dentition. Amniotes have a ball-in-socket craniovertebral joint, which increases the mobility and stability of the head on the neck. They also have two coracoid ossifications in the shoulder girdle, an ossified astragalus in the ankle joint, and they lose fishlike bony scales from the dorsal surface of the body. Teeth are present on the pterygoid transverse process, but there is no infolding of enamel anywhere in the dentition. Also present is an enlarged caniniform maxillary tooth. These changes reflect fully terrestrial feeding and locomotor patterns (Gauthier et al. 1988b, Laurin and Reisz 1995, Sumida 1997).

Loss of lateral line system. The lateral line placodes fail to appear in amniotes (Northcutt 1992), and with their loss is the complete absence of a lateral line system. This is consistent with the view that amniote origins represent increasingly terrestrial habits.

Pan-Amniota

The amniote stem is represented by fossils that extend to the Early Carboniferous (Gauthier et al. 1988b), the oldest being *Casineria* (Paton et al. 1999). The best-known members of the amniote stem include the Carboniferous-Permian anthracosauroids, seymouriamorphs, and diadectomorphs, and a handful of other extinct taxa (Gauthier et al. 1988a, Sumida 1997). Many of the osteological transformations occurring among stem amniotes involved modifications of the dentition and palate, and specialization of the atlanto-axial joint between the head and neck. These modifications reflect an increased role of the mouth in capturing and manipulating terrestrial prey items. Also, there was increased

strengthening of the vertebral column via swelling of the neural arches, the girdles were expanded, the pelvis has an expanded attachment to the sacrum, and the limbs are elongated. Loss of the lateral line system was marked by the disappearance of the canals that it etches into the skull roofing bones. Collectively these features indicate that increasingly terrestrial patterns of locomotion, predation, and prey manipulation preceded the origin of Amniota.

Node 20. The Turtles, Lizards, Crocodylians, and Birds (Reptilia)

The Reptilia are the lineage stemming from the last common ancestor of birds and turtles (fig. 23.1). Reptilians comprise nearly 17,000 living species and enjoy a long and rich fossil record (Gauthier et al. 1988b, Laurin and Reisz 1995, Dingus and Rowe 1998). The name Reptilia was long used in reference to a paraphyletic assemblage of ectothermic amniotes, including turtles, lizards and snakes, crocodylians, and a host of extinct forms. Although long considered to have evolved from reptiles, mammals and birds were excluded from actual membership within it. More recently, the name Reptilia was brought into the phylogenetic system by defining its meaning in reference to the last common ancestor of turtles and birds, and by including birds within it. The name Reptilia has also been used to encompass the extinct relatives of mammals, once known as "mammal-like reptiles." But in the phylogenetic system, these taxa are now referred to under the term Pan-Mammalia (= Synapsida), and the name is rendered monophyletic by including mammals, plus all extinct taxa closer to mammals than to reptiles, within it. Reptile phylogeny is discussed elsewhere in this volume (Lee et al., ch. 26, and Cracraft et al., ch. 27).

Pan-Reptilia

The fossil record of Pan-Reptilia extends into the Late Carboniferous (Gauthier et al. 1988a). *Archaeothyris*, from the Joggins fauna of Nova Scotia, is the oldest panreptile that is known in some detail. In the Early Permian a diversity of poorly known forms are allied as Parareptilia (Gauthier et al. 1988a, Laurin and Reisz 1995, Berman et al. 2000), a tentatively monophyletic clade of extinct taxa that all differ considerably from one another. Their relationships to one another, and to extant turtles and diapsids remains unstable. Included among parareptiles are the Carboniferous-Permian mesosaurs, which seemed highly derived and adapted to a fully aquatic existence. Also often included are the small terrestrial bolosaurids, milleretids, and possibly also the procolophonids and pareiasaurs. The latter two are considered as possible extinct relatives of turtles, and the pareiasaurs are the only members of this basal part of the tree that grew to large adult weights (1000 kg). Pan-Mammalia (see below) dominates the early fossil record of amniotes, because many of its members expressed an early trend toward size increase. Panreptiles, with the exception of pareiasaurs, remained small. By the end of

the Triassic, however, these roles reversed, and from then on, the panreptiles dominate the fossil record and extant reptiles are far more numerous and diverse than mammals.

Node 21. Chordates with Hair (Mammalia)

Mammalia comprise the last common ancestor that humans (fig. 23.1) share with living monotremes, plus all its descendants (Rowe 1987, 1988, 1993, Gauthier et al. 1988a, Rowe and Gauthier 1992). It includes approximately 5000 living species and a long fossil record. The mammalian crown extends to the Middle or Early Jurassic, whereas the base of the mammalian stem (Pan-Mammalia or Synapsida) traces to the Late Carboniferous. Mesozoic mammals and their closest extinct relatives were tiny animals, and their fossils are notoriously difficult to collect. Most Mesozoic taxa are named from isolated dentitions or broken jaws, and the early history of mammals was long shrouded by incompleteness. But a host of exciting new discoveries from Asia and South America have yielded relatively complete ancient skeletons. Some were announced together with detailed phylogenetic analyses that are rapidly revising and detailing the early phylogeny of mammals (Hu et al. 1997, Luo et al. 2001a, 2001b, 2002, Rougier et al. 1998). Mammalia is apomorphic in the brain and special senses, body covering, musculature, skeleton, circulatory system, respiratory system, digestive system, reproductive system, metabolism, molecular structure, and behavior (see Rowe 1988, 1993, 1996a, 1996b, Gauthier et al. 1988a: appx. B). Only a few of these are discussed below.

Mammalian Characters

The neocortex. Compared with even their closest extinct relatives, mammals have large brains. The additional volume marks an episode of heterochrony (peramorphosis) in which the brain began to grow further into ontogeny and more rapidly than in their extinct relatives, marked by the origin of the mammalian neocortex. Its two hemispheres each have a columnar organization of six radial layers, generated in ontogeny by waves of migrating cells that originate from the ventricular zone and move radially outward to their adult positions. This inside-out pattern of neural growth produces a huge cortical volume in mammals. The developing mammalian forebrain hypertrophies into inflated lobes that swell backward over the midbrain and forward around the bases of the olfactory bulbs, which themselves are inflated. The cerebellum is also expanded and deeply folded. The neocortex supports heightened olfactory and auditory senses, and coincident, overlapping sensory and motor maps of the entire body surface. The enlarged cerebellum is related to acquisition and discrimination of sensory information, and the adaptive coordination of movement through a complex three-dimensional environment. These changes may reflect invasion of a nocturnal and/or arboreal niche and have been implicated in the evolution of endothermy (Rowe 1996a, 1996b).

The mammalian middle ear. In adults, the middle ear skeleton lies suspended beneath the cranium and behind the jaw. It is an impedance matching lever system that contains a chain of tiny ossicles connecting an outer tympanum to the fluid-filled neurosensory inner ear. Its parallel histories in ontogeny and phylogeny are among the most famous in comparative biology. The middle ear arose in premammalian history as an integral component of the mandible. Over a 100 Myr span of premammalian history, its bones were gradually reduced to tiny ossicles, reflecting specialization for increasingly high-frequency hearing, whereas the dentary undertook a greater role in the mandible. Hearing and feeding were structurally linked in premammalian history, but in mammals these functions became decoupled as the auditory chain was detached from the mandible and repositioned behind it, and a new craniomandibular joint arose between the dentary and squamosal bones. Separation of the ossicles from the mandible occurs in all adult mammals and was widely regarded as the definitive mammalian character under Linnaean taxonomy (Rowe 1987, 1988). In ontogeny the auditory chain differentiates and begins growth attached to the mandible. But the connective tissues joining them are torn as the brain grows, and the entire auditory chain (stapes, incus, malleus, ectotympanic) is carried backward during the next few weeks to its adult position behind the jaw. Transposition of the auditory chain is a consequence of its differential growth with respect to the brain. The tiny ear bones quickly reach adult size, whereas the brain continues to grow for many weeks thereafter. As the developing brain balloons, it loads and remodels the rear part of the skull, detaching the ear ossicles from the developing mandible. Many other features of the skull were altered by this dynamic epigenetic relationship between the rapidly growing brain and the tissues around it (Rowe 1996a, 1996b).

Enhanced olfactory system. The mammalian olfactory system is unique in the breadth of its discriminatory power. Approximately 1000 genes encode odorant receptors in the mammalian nose, making this the largest family in the entire genome (Ressler et al. 1994). Each gene encodes a different type of odorant receptor, and the individual receptor types are distributed in topographically distinct patterns in the olfactory epithelium of the nose. Their discriminatory power is multiplied by increased surface area provided by elaborate scrolling of the bony ethmoid turbinals. This rigid framework enhances olfactory discrimination by facilitating the detection of spatial and temporal information as odorant molecules disperse within the nasal cavity. Each odorant receptor transmits signals directly to a single glomerulus in the olfactory bulb without any intervening synapses; hence, the topographic distribution of odorant receptors over the ethmoid turbinals is mapped in the spatial organization of the olfactory bulb. Ossified turbinals occur only in mammals (and independently in a few birds), although there is ample evidence of unossified turbinals among their extinct relatives. Bone is fundamentally structural, and turbinal os-

sification may have arisen in response to tighter scrolling, increased surface area, and an increase in the number of olfactory odorant receptors in mammals compared with their closest extinct relatives. The ossified ethmoid turbinal complex may thus be viewed as the skeleton of the olfactory system, arising as an integral component of its distinctive forebrain.

Pan-Mammalia

The mammalian stem lineage, also known as Synapsida, contains mammals plus all extinct species closer to mammals than to Reptilia. Panmammalian fossils range back to the Late Carboniferous, and an exceptionally complete sequence of fossils links extant mammals to the base of their stem. Before phylogenetic systematics, the focus of study was to elucidate the reptile-to-mammal transition. The premammalian segment of this history was believed marked by rampant convergence in the evolution of mammal-like sensory, masticatory, and locomotor systems, and Mammalia itself was held to be a grade rather than a clade. The major debate involved rationalizing which character should mark the boundary between reptilian and mammalian grades. Few claims of homoplasy were substantiated when the characters were subjected to rigorous parsimony analyses, and as synapsids were placed in a taxonomy based on common ancestry (Rowe and Gauthier 1992).

Pan-Mammalia are diagnosed by the lower temporal fenestra and a forward-sloping occiput. Its early history saw enhancement of the locomotor system for fast, agile movement, and elaboration of the feeding system for macro predation. The primitive armature of a tympanic impedance matching ear also appeared early on (Kemp 1983).

A major node on the mammalian stem is Therapsida, whose fossils date back to the Late Permian. The temporal fenestra is larger than before, and there is a deeply incised reflected lamina of the angular (the homologue of the mammalian ectotympanic), and a deep external auditory meatus. These denote an ear more sensitive to a broader range of frequencies. Limb structure indicates a somewhat more erect posture and narrow-tracked gait, possibly facilitating breathing while running and a higher metabolic rate (Kemp 1983).

Cynodontia comprise a node within Therapsida whose monophyly is supported by numerous characters that were passed on to living mammals. The overriding feature of basal cynodonts is that their brain had expanded to completely fill the endocranial cavity, impressing its outer surficial features into the inner walls of the braincase (Rowe 1996a, 1996b, Rowe et al. 1995). Osteological synapomorphies include a broad alisphenoid (epipterygoid) forming the lateral wall to the braincase, and a double occipital condyle that permitted wide ranges of stable excursion of the head about the cranio-vertebral joint (Kemp 1983). The dentition is differentiated into simple incisiform teeth, a long canine, and postcanine teeth with multiple cusps aligned into a longitudinal row. The

dentary was elongated over the postdentary elements, which are reduced and more sensitive to higher frequencies.

Among nonmammalian cynodonts, those closest to crown Mammalia were tiny animals. Miniaturization involved elaborate repackaging of the brain and special sense organs, remodeling of the masticatory system, an accelerated rate of evolution in a complex occlusal dentition. The vertebral column became more strongly regionalized, and the limbs and girdles were modified for scansorial movement. Several episodes of inflation in the size of the brain occurred before the origin of mammals. The recent discovery of *Hadrocodon* (Luo et al. 2001b), from the Early Jurassic of China, may indicate that the neocortex and middle ear transformation originated just outside the mammalian crown, but it is questionable whether *Hadrocodon* lies outside or within the crown. In either event, inflation of the neocortex and detachment of the middle ear appear to coincide.

Discussion

Many of the innovations in chordates design described above arose as unique expressive pathways or as elaborations of preexisting genetic and developmental mechanisms. For example, in all chordates, molecular signaling during neurulation produces anteroposterior regionalization of the embryo, and a brain that divides into rostral, middle, and caudal divisions, each with its own region of unique genetic expression. The genes themselves are more ancient, being expressed in the same tripartite anteroposterior regionalization of the brain in arthropods and other bilaterians. But the inductive pathway of expression in chordates is unique, and it produces a nervous system radically different from that in arthropods, or in what was likely to have been present in bilaterians ancestrally.

Another pattern of morphogenesis and diversification corresponds to successive increases in the numbers of genes. The first episode occurred in either Chordata or Euchordata ancestrally, and in either case was associated with elaboration of brain and sensory organs, as well as with the appearance of mesodermal segmentation. The second occurred in craniates ancestrally and was accompanied by segmentation of the brain into prosomeres, mesomeres, and rhombomeres in early development, as well as enhancement of the adult brain and sensory organs. The third increase occurred in Vertebrata, and the fourth in Gnathostomata ancestrally, each in association with further elaboration of the brain and special senses. Mammalian origins also coincided with an unprecedented increase in the number of olfactory genes. Mammalian olfaction is the most sensitive of any chordate, and with up to 1000 genes coding for different odorant molecule receptors, olfactory genes comprise the largest single mammalian gene family. We can expect many similar examples of this pattern of gene increase and structural elaboration to be mapped in the near future.

The inductive nature of chordate ontogeny provided an especially rich substrate for evolutionary change. The most spectacular example is the neural crest, whose motile cells are induced by the underlying mesoderm and in turn induce many tissues and structures. The neural crest arose in craniates ancestrally, building the embryonic cartilaginous cranium, providing a rigid armature for the brain and special senses, and the skeleton of the pharynx, and providing a novel substrate for the tremendous range of evolutionary variation.

Epigenesis further multiplied these agents of morphogenesis. Origin of the mammalian middle ear may have been one such episode, in which early changes in the timing of development and rate of growth of the brain altered the adjacent connective tissues and the adult structures forming within them. In the wake of the ballooning brain, the rear of the developing mammalian skull is remodeled, and the middle ear ossicles and eardrum were detached and displaced backward from their embryonic attachment to the mandible. The differentiation of neurectoderm is one of the earliest events in ontogeny, and virtually anything that affects its pattern of development will set into motion a new dynamic in the surrounding connective tissues, potentially altering the adult structures that form within them. Just how much adult chordate morphology is epigenetically produced remains to be determined. These examples illustrate that mapping and understanding the relationship between molecules and morphology, as it unfolds in the course of ontogeny, is fundamental to chordate systematics and comparative biology, and understanding our place in the Tree of Life.

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