Early Development and Comparative Embryology

**Precis**

After a brief introduction to some concepts in developmental biology, we will examine the development of chordates from the structure of the gametes to the establishment of the embryonic axis and primordia of the organ systems. Our coverage emphasizes gastrulation; early development of the nervous system, neural crest, neurogenic placodes, and sense organs; mesoderm and coelom formation; and the basic structure and organization of the head and pharynx. These topics provide a foundation for understanding the subsequent development of the ten organ systems of vertebrates and introduce you to important developmental landmarks for the study of comparative vertebrate anatomy. We conclude with comments on the control of segmentation by Hox genes and how duplication of these regulatory genes early in vertebrate history may have profoundly influenced the course of vertebrate evolution.

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One of the greatest marvels of life is the transformation of one seemingly simple cell, a fertilized egg, into a structurally and functionally complex organism composed of multitudes of cells. Development is an exciting and dynamic process to observe, and its study has exploded in recent years as new techniques and approaches have been applied to classic questions. Development also offers many exquisite functional anatomical examples, from the match between the structure and function of the gametes to the patterns of interactions among cells and tissues during embryogenesis.

General Concepts in Developmental Biology

Beginning in the 19th century, comparative embryologists studied and named the succession of morphological changes during normal development (or ontogeny) of craniates. A summary of the succession of developmental stages during the life cycle of a typical vertebrate is shown in Figure 4-1. Embryogenesis is the series of stages during which a fertilized egg is converted into a self-sustaining individual organism. These stages are easily recognizable across the diversity of vertebrates and consist of fertilization, followed by cleavage, gastrulation, neurulation, organogenesis, and cytodifferentiation. Each of these six aspects of embryogenesis will be covered in this chapter, with specific examples from clasmobranchs, teleosts, amphibians, birds, and placental mammals. The end result of vertebrate embryogenesis is usually either a free-living larva, which will later metamorphose to achieve the general morphology of an adult (= indirect development), or a small "model" of an adult, which will change less dramatically during the course of its later development (= direct development). These aspects of later development (i.e., everything after embryogenesis) will not be treated here but will be covered in subsequent chapters.

The somatic cells of a vertebrate's body normally contain the same genes, but not all genes are active, or active to the same extent, during different periods of development or in the various cells and tissues of the adult. In contrast, a fertilized egg cell can be described as totipotent, in that it can develop the full variety of cell types present in an adult. As described in Chapter 1, the bodies of adult vertebrates are built from a great diversity of cell types. We are beginning to understand how gene activity is regulated during vertebrate development to produce cells with different fates. For example, differential distributions of certain key proteins and other materials in the cytoplasm of an egg cell can influence the way the genes contained in the nuclei of its daughter cells become expressed. Influencing the fate of a particular group of cells by means of such differential distributions of cytoplasmic materials is termed cytoplasmic specification. As cells with different properties begin to emerge during embryogenesis, their products, in turn, influence the way adjacent cells or tissues respond. This phenomenon is called induction, and it was discovered in 1901. In the 1920s, Hans Spemann, Hilde Mangold, and other experimental embryologists advanced our understanding of induction by observing the effects of transplanting bits of tissue from one amphibian embryo to another or from one part of an embryo to another. We now know that many of the substances responsible for inductions are proteins. Because these molecules can produce discernible morphological changes in the embryo, they often are known as morphogens (agents of morphogenesis). Cells that receive an inductive signal respond by regulating expression of their own genes. For example, a morphogen that stimulates cell division triggers a

![Figure 4-1](image-url)

**Figure 4-1**
Life cycle of a vertebrate showing the context for embryogenesis. Events of embryogenesis are the primary subject of this chapter; some comments on gametogenesis and gametes are included to set the stage for fertilization. Individual life marks the endpoint of embryogenesis; this point can be hard to specify. For present purposes, embryogenesis ends when the yolk or any other maternal nutrient is exhausted, and the young is capable of ingesting food.
neurons or muscles (see Chapters 10 and 13). The second method is by diffusion of small, typically soluble morphogens, in a fashion analogous to the way that circulating hormones can influence cells at a distance (see Chapter 15). When such signaling molecules diffuse directly through intervening tissues or extracellular matrix before acting on the target cells, they are said to be a paracrine secretion. In contrast, if signaling molecules are delivered to their target cells via the circulatory system, they are spoken of as endocrine secretions. Most morphogens important in early development act as paracrine secretions. The third method is by local changes in the composition of the extracellular matrix molecules around a cell, which then influence the differentiation of adjacent cells. This latter method may remain important in the self-organization and repair of connective tissues throughout life.

Much of embryogenesis appears to be regulated by series, or cascades, of inductions. Cells and tissues respond to inductive influences only during a limited period, when they are said to be competent to respond. As tissues differentiate, they express some of the genes associated with their final fate (Fig. 4-3). As differentiation proceeds, cells lose their competence to respond to some inductive influences but not to others. Their potential fates are said to have been restricted. Eventually, the fate of most cells is determined by subsequent inductive interactions. This final restriction in fate is followed by expression of the many genes associated with that particular cell type and eventual achievement of the morphology typical for that cell type. Developmental regulation may ultimately be viewed as a special case of the broader field of homeostatic regulation, by which all parts of an organism can respond to changes while continuing to function as an integrated whole. Studies of developmental regulation will surely prove to be among the most important aspects of comparative developmental biology because changes in the regulation of developmental events likely provided mechanisms for morphological diversification during vertebrate evolution. An example of this is the role of Hox genes in axial patterning of vertebrates, which is discussed at the end of this chapter.
To emphasize the importance of induction and developmental regulation, comparative embryologists often speak about epigenetics (Gr., epi = upon or on top of + genetics = in this case, the genome level). As a term, epigenetics refers to cell fates and the patterns of interactions and changes that occur at the cellular level during embryogenesis (Fig. 4-4). At the bottom of Figure 4-4 is the genetic level, which is a traditional focus of molecular and cellular biology. Genes code for proteins, such as structural proteins, enzymes, morphogens, or other regulatory molecules. Some of these proteins regulate the expression of other genes. Other proteins, chiefly structural proteins and enzymes, endow cells with their specific cell properties. The term cell properties covers an array of possible functions, from a cell’s ability to adhere to other cells; to its ability to synthesize and secrete morphogens that alter the function or state of differentiation of adjacent cells; to cell death, which is a normal developmental fate for a surprisingly large percentage of embryonic cells.

Exceedingly complex cell–cell interactions and morphogenetic movements can occur at the epigenetic

**FIGURE 4-4**
Examples of epigenetics. The shaded blue box represents epigenetic events, literally, on top of the genome. The small diagrams illustrate some of the many possible fates and interactions of cells and tissues during embryogenesis. These epigenetic phenomena act as a "filter" between the genes and the adult phenotype. (Based on a figure by Alberch.)
level. For example, if all of the cells in a flat sheet of epithelium change shape simultaneously, then the epithelium rolls up or folds into a tube or sphere that may serve as the precursor to an organ. The outcome of cell interactions at the epigenetic level also can regulate gene expression. In a mouse embryo, clusters of migrating neural crest cells (discussed later) come to lie beneath portions of the skin covering the jaw, where they participate in reciprocal inductive relationships with the overlying skin cells in the differentiation of a tooth. The skin cells determine what type of tooth will form (e.g., an incisor or a molar), and the neural crest cells then take over control of tooth morphogenesis (see Chapter 16). As another example, an alpha motor neuron that fails to establish contact with a muscle fiber will begin to express genes for cell death and die. As a concept, epigenetics is useful chiefly because it focuses our attention on the regulatory and cellular processes of development, which are a necessary filter between the genome and the completely constructed adult. In practice, however, we have much to learn about the specifics of most epigenetic interactions in most tissues of the vast majority of vertebrates, leaving plenty of room for basic research in this fascinating area.

Another important concept from research in cell and developmental biology concerns two basic ways in which cells can be organized into tissues in a vertebrate's body. We can contrast cells that are organized as an epithelium with those that are organized as a mesenchyme (Fig. 4-5). Cells in an epithelium form a closely packed array on top of a flexible foundation termed a basal lamina (Fig. 4-5A). A basal lamina is composed largely of extracellular materials secreted by the overlying epithelium, including proteins (particularly the mat-like form of collagen known as collagen type IV), glycoproteins (e.g., laminin and entactin), and proteoglycans rich in heparin sulfate. Together, these extracellular materials represent the extracellular matrix of the epithelium. Cells in an epithelium are usually polarized, which means that they exhibit basal specializations at the pole of the cell adjacent to the basal lamina and apical specializations at the free surface of the epithelium. As an example, the nucleus and mitochondria often are concentrated at the base of an epithelial cell, so that secretory organelles can be located closer to the free surface of the cell. Epithelial cells may bear cilia or microvilli on their apical surfaces that serve special functions of that cell type, such as mechanoreception or absorption of nutrients. Adjacent

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**FIGURE 4-5**

Models of epithelial and mesenchymal organization. Blue indicates extracellular matrices. A, Epithelial organization. In an epithelium, the cells are closely packed and arranged like bricks on a foundation. B, Mesenchymal organization. In a mesenchyme, the cells are loosely scattered in an extensive, gelatinous extracellular matrix. These two fundamental ways in which cells can be organized into tissues are important in embryogenesis because certain cell populations change from one type of organization to the other. Also, epithelial–mesenchymal interactions are important in the development of a great many organs.
cells in an epithelial sheet are linked by junctional complexes that can be thought of as fasteners that strengthen the continuity of the sheet and help limit passage of materials between them. In this way, epithelia often serve as barriers or filters. During embryogenesis, cells in an epithelium produce shape changes as a collective group. For example, if all of the cells in an epithelium are induced to narrow their apical surfaces, then the surface area of that epithelium will decrease, and folding may result (Fig. 4-4).

In contrast, a mesenchyme consists of a loosely packed array of stellate (= star-shaped) cells (Fig. 4-5B). The extracellular matrix of a mesenchyme has a very high water content, and contains fibrillar forms of collagen, such as collagen type I, and proteoglycans containing many chains of hyaluronic acid. These proteins give the extracellular matrix a gel-like consistency, so that the mesenchymal cells can be thought of as "raisins in Jell-O." Especially important is the protein fibronectin, which has attachment sites for cell surface proteins and other extracellular matrix molecules. By means of these attachment sites, fibronectin functions as a "glue" between cells and other molecules of the extracellular matrix. Mesenchymal cells generally lack cell surface specializations, such as cilia, and are not polarized. They have little or no physical contact with neighboring cells, so junctional complexes are absent. As a result of these characteristics, small molecules readily diffuse through the extracellular matrix of a mesenchyme. During morphogenesis, mesenchymal cells typically produce shape changes in an embryo by migrating as individual cells and then aggregating to form organ rudiments or primordia (or anlagen, from the German word for plan or design). Mesenchyme also is the source for various connective tissues and support structures of the body (see Chapter 5).

After drawing such a sharp distinction between epithelial and mesenchymal organization, it may come as a surprise to learn that many cell lines actually change from one mode of organization to the other and then back again during normal development. For example, as neural crest cells differentiate, they delaminate from the epithelium of the neural plate (discussed later) and then migrate as individual cells for some distance away from the parent epithelium before later reaggregating to form organ rudiments that may become reorganized as epithelia. The process of delamination involves breaking down the local epithelial organization, particularly the basal lamina and any cell junctions that held the cells in a sheet. Also, the organization of some tissues of adult vertebrates does not easily fit into either one of these two categories. Still, it is important to begin analysis of vertebrate embryogenesis with an understanding about these two extremes in tissue organization.

Having introduced some basic aspects of developmental biology, we now turn our attention to gametes and embryogenesis. Our coverage is designed to help you understand the later development, or organogenesis, of the organ systems. Knowledge of organogenesis helps us understand organs, their structural and functional complexity, and their homologies, which in turn positions us to interpret the evolution of organs and organ systems. Some of the variation in early development among different groups of vertebrates is considered in this chapter, but an extensive examination of this topic is beyond the scope of this book. Thus, we focus on a few taxa for understanding vertebrate embryogenesis, including elasmobranchs (Squalus and Rajah), frogs (Rana), salamanders (Ambystoma), and chickens (Gallus). (See Focus 4-1.)

Gametes and Fertilization

The reproductive cells, called gametes, develop in the gonads. More specifically, spermatozoa (or sperm) form in the seminiferous ampullae or seminiferous tubules of the testis, and ova (or eggs) form in the follicles of the ovary (for gonadal structure, see Chapter 21). The process of sperm formation is called spermatogenesis, the process of egg formation is called oogenesis, and together these events are termed gametogenesis (Fig. 4-6). Two major processes occur during gametogenesis: (1) reduction division, or meiosis, and (2) the acquisition of cellular specializations needed for fertilization and embryogenesis.

During gametogenesis, the gamete-producing cells undergo two meiotic divisions, so that mature gametes are haploid; that is, each has only a single set of chromosomes rather than the double set present in other body cells (Fig. 4-6). These two meiotic divisions are called meiosis I and meiosis II. Terms used to describe gametes during their differentiation are based on their meiotic state. Precursor cells of spermatozoa are spermatogonia; those for eggs are oogonia. When these cells enter into meiosis I, they are termed primary spermatocytes and primary oocytes, respectively. In males, completion of meiosis I yields two secondary spermatocytes; completion of meiosis II yields four spermatids. Spermatids are haploid (1N), which means that they have half the diploid complement of chromosomes. The spermatids continue to differentiate during the process known as spermiogenesis.

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1 Some key references, such as Nelsen (1953), are listed at the end of this chapter for those interested in this topic.
FOCUS 4-1  Model Species and Vertebrate Embryogenesis

What species should we study to understand the diversity of developmental mechanisms among vertebrates? As pointed out in Chapter 3, about 50,000 species of vertebrates are alive today. Yet very few of these species have been the subject of detailed embryological investigation. In fact, our knowledge of vertebrate embryogenesis is based on remarkably few "model species" that exhibit characteristics making them particularly suitable for the experimental techniques required for research in modern developmental biology. These few species are by far the overwhelming focus of current research on vertebrate development. Among the vertebrate species commonly studied by developmental biologists, six stand out: (1) a small freshwater teleost fish from southern Asia, the zebrafish (Danio rerio); (2) an aquatic salamander from Mexico, the axolotl (Ambystoma mexicanum); (3) a tadpole from the northern hemisphere (e.g., the grass frog, Rana pipiens); (4) various species of aquatic frogs, commonly called African clawed frogs (e.g., Xenopus laevis); (5) the domestic chicken (Gallus gallus); and (6) domesticated strains of the European house mouse (Mus domesticus). By almost any standard, these six species do not represent an ideal cross section of vertebrate taxa from the standpoint of phylogenetic breadth. Despite this, many people have generalized findings based on the embryology of these six model species to other taxa of vertebrates, usually by very informal phylogenetic analyses. This practice is now changing for two reasons. First, more researchers are studying and describing in detail the early development of species, such as various species of chondrichthyan fishes or metatherian mammals, that are not traditionally considered "model species" for developmental biology. Second, the types of comparisons being made have become more explicitly phylogenetic, in that researchers are treating embryological features as characters in phylogenetic data sets in exactly the same ways as any other characteristics of organisms. These two progressive changes seem certain to improve our insight into the evolution of development in vertebrates. Still, it is important to ask, why do we so restrict the taxa used for studies of vertebrate embryogenesis?

Primary considerations in selection of model species for embryological research have been convenience and practicality. For example, to simplify the husbandry of a captive breeding colony of vertebrates, researchers find it helpful to study small animals and especially helpful to study small, aquatic species that are broadly tolerant of varying environmental conditions. It is very useful to have a network of similarly minded colleagues and suppliers so that adults (or, even better, fertilized eggs) can be obtained throughout the year, a practice that tends to produce clusters of workers studying development of a particular model species. To help reduce the overall size of a laboratory colony, it is good to study species that produces large numbers of eggs or young and that can do this frequently (or, even better, constantly under laboratory conditions). Genetic studies needed for the development of mutations and molecular tools for research now are essential components for the most powerful technologies in experimental developmental biology. To apply these technologies, however, the animals must mature rapidly and be easily bred in the laboratory so that the maximum number of generations can be produced in a given time.

Each of the six species listed earlier—with the exception of tadpole frogs—meets most of these criteria for convenience and practicality. But can any other taxa be candidates for modern experimental research programs on the comparative embryogenesis of chordates?

The most important candidates from the perspective of improving our phylogenetic coverage of embryogenesis are non-tetrapods, and virtually all of the most interesting taxa fail the tests of convenience and practicality. For example, the recent development of methods by Linda Holland and Nick Holland for breeding amphioxus (Branchiostoma) in captivity has yielded many new insights into their early development, but the adults still must be collected in the wild, so that developmental genetic studies are limited, at least for now. As another example, directly developing amphibians (i.e., species that bypass the larval stage during development and hatch as miniature adults) have yielded many insights into head formation (see papers by Hanken et al. 1997 and Jennings and Hanken 1998). Nevertheless, most are not good candidates for developmental genetic studies. For the foreseeable future, then, we seem likely to study phylogenetic aspects of embryogenesis using descriptive, rather than experimental, analyses of species not traditionally used as models in developmental biology. This is not all bad, for careful description is fundamental to comparative biology. By means of such descriptions, we seem certain to discover new variations in vertebrate embryogenesis and perhaps to answer some of the most intriguing questions about the origin of these patterns.

into four mature spermatozoa. In females, completion of meiosis I yields one secondary oocyte and the tiny first polar body; completion of meiosis II yields a single haploid ovum and the second polar body (which is also haploid). The first polar body may also undergo meiosis II to yield two tertiary polar bodies that are genetically equivalent to the mature ovum, although they lack any reproductive future because their tiny size limits the amount of yolk and other materials needed for normal development.

Gametes are highly specialized cells, the structures of which closely match their functions (Fig. 4-7). Spermatozoa are specialized for mobility, whereas ova are essentially packages of materials and information that
FIGURE 4-6
Changes in chromosome number and DNA content during gametogenesis. A, Spermatogenesis and spermiogenesis. B, Oogenesis. The terminology for spermatogenesis and oogenesis is explained in the figure.

FIGURE 4-7
Human gametes. A, Spermatozoon. B, Oocyte. These are immature gametes. Before spermiogenesis is complete, spermatozoa lose most of the cytoplasmic droplet, visible as a bulge of cytoplasm in the middle piece. The oocyte shown is in an early stage of vitellogenesis, and it will accumulate additional materials before gametogenesis is complete. Major structures found in spermatozoa of other vertebrates generally resemble the condition in humans, but sperm shape varies greatly in different groups. Placental mammals have secondarily reduced the yolk content of oocytes, which thus are much smaller than eggs of other vertebrates. (Modified from Lentz.)
will be needed by the developing embryo. Thus, there is great disparity in the sizes of sperm and egg cells and a corresponding difference in the metabolic cost of producing them. Because of their smaller size, spermatozoa can be produced in extremely large numbers, and it is often the number of eggs that limits fecundity in species of vertebrates.

Spermatozoa

A mature mammalian sperm is a very small cell that has lost most of its cytoplasm during the process of spermiogenesis. It consists of a head, neck, and tail (Fig. 4-7A). The tail may be further divided into a principal piece, middle piece, and end piece. The head contains the greatly condensed nucleus and enzymes needed to penetrate the egg’s surrounding membranes and any adhering cells. These enzymes are located in a membrane-bounded vesicle, the acrosome, which covers the front of the nucleus. In some species, the acrosome also contains molecular components of species-specific recognition systems that help to ensure that spermatozoa of one species will not accidentally fertilize an ovum of another species. The middle piece contains coiled mitochondria that have the enzymes needed for the release of energy to the flagellum-like axial filament that powers locomotion. Because a spermatozoon is small, it lacks extensive stores of energy, and once it begins to move, it soon exhausts most of its potential for locomotion. Thus, spermatozoa are maintained inside a male’s reproductive tract in an immobile state and are activated to begin swimming only by changes encountered in a surrounding aqueous medium or by the fluids in the female’s reproductive tract. Often, the trigger that activates spermatozoa to swim is a change in pH.

Spermatozoa of different species of vertebrates vary greatly in the sizes and in the shapes of their heads, and sperm shape may be phylogenetically informative. For example, spermatozoa of rodents typically have sickle-shaped heads, whereas those of most other species of mammals are elliptical. Spermatozoa of some groups of bony fishes normally have two tails and are referred to as biflagellate spermatozoa.

If fertilization occurs in the environment, outside of the female’s reproductive tract, it is termed **external fertilization.** If fertilization occurs inside the reproductive tract of the female, it is termed **internal fertilization.** External fertilization is plesiomorphic for vertebrates and occurs in many extant species of aquatic vertebrates, such as lampreys, many species of bony fishes, and many species of frogs. In such species, a male releases spermatozoa directly from his reproductive tract near or onto eggs as they are extruded from the female’s reproductive tract into the environment. Most species of vertebrates that have internal fertilization have evolved specialized mechanisms that allow a male to introduce spermatozoa directly into a female’s reproductive tract by using an **intromittent organ** (see also Chapter 21). For example, many groups of fishes have specializations of the pelvic fins (e.g., claspers of chondrichthyans) or anal fins (e.g., gonopodia of many actinopterygians) that are used for intromission. In salamanders with internal fertilization, however, spermatozoa are packaged into **spermatophores** by the reproductive tract and cloacal glands of males. During courtship, a male salamander deposits spermatophores on the substrate, which may be either the bottom of a pond or leaf litter on land, depending on where that species breeds. The male then leads a female over the spermatophore, which she encloses with her cloacal lips to bring the packet into her reproductive tract. The package dissolves, liberating the spermatozoa to fertilize the eggs. Another interesting variant concerns **sperm storage,** which is remarkably common among vertebrates (the anatomy of reproductive tracts, including specializations for sperm storage, is described in Chapter 21). For example, in certain species of caecilians, the female reproductive tract has regions specialized for the storage and nourishment of sperm, so that sperm can be stored for extended periods before activation of their swimming mechanism—and fertilization—occur.

Egg Cells

Unlike spermatozoa, eggs are large, spherical, nonmotile cells. Within any given species, a mature egg, or ovum, is a larger cell than a spermatozoon chiefly because it contains the energy reserves and other materials in its cytoplasm to initiate embryonic development. Beyond that generalization, the sizes of vertebrate egg cells vary enormously: a human egg is about 0.15 mm in diameter, whereas the egg of a coelacanth (Latsimera) reaches 90 mm in diameter. This 600-fold difference in linear dimensions translates to a 200 million-fold increase in the total volume of materials inside the egg! Ova develop in **follicles** within an **ovary** (see Chapter 21). All egg-cells have, in addition to their plasma-cell membrane, a **primary egg cell membrane** immediately surrounding them. This extracellular, largely proteinaceous structure is secreted by the egg cell or by its surrounding follicle cells during oogenesis. The primary egg cell membrane has different names in different groups of vertebrates. One fre-
quently used term is **vitelline membrane**. In mammals, the primary egg cell membrane is known as the **zona pellucida**. In many actinopterygian fishes, it is toughened to withstand a harsh external environment and is known as a **chorion**. The thick chorion of actinopterygian fishes has special openings, termed **micropyles**, to admit spermatozoa. In some vertebrates, layers of follicle cells adhere to and surround the primary egg cell membrane. In mammals these adhering follicle cells are known as the **corona radiata**, and they pose an additional structural barrier that a spermatozoon must penetrate before fertilizing the egg.

The cellular organization of an ovum can be exceedingly complex (Fig. 4-7B). The cytoplasm of egg cells has a thick, gelatinous **cortex**, which is the zone of cytoplasm lying immediately adjacent to the egg’s plasma membrane. The cortex often contains pigment granules and **cortical granules**, which are important in fertilization, as described later. The interior cytoplasm of an egg cell may be more fluid than its cortex, but it is still highly structured and packed with materials needed for development. Ova contain variable amounts of yolk, which is composed primarily of protein, phospholipids, and neutral fats. The process of yolk deposition in the egg cell is termed **vitellogenesis**, and depending on the amount of yolk, vitellogenesis may require many months or even years. Yolk of vertebrate eggs is usually synthesized exogenously (i.e., not in the oocyte itself) in the mother’s liver and delivered to the ovary via the circulatory system. Once inside an egg cell, much of the yolk is organized into organelles known as **yolk platelets**.

The amount of yolk in an ovum determines how long the embryo will be nourished by food stored in the egg. There is little yolk in the **microlecithal eggs** (Gr., mikros = small + lekithos = yolk) of amphibians. These eggs hatch very soon in larvae that do not feed and that quickly metamorphose into feeding juveniles. Nonteleostean actinopterygian fishes, lungfishes, and amphibians have an intermediate amount of yolk in their **mesolecithal eggs**. Their eggs hatch into feeding larvae, which then metamorphose into juveniles. The eggs of most teleost fishes are physically small but have a very high proportion of yolk, which affects their cleavage (see Focus 4-2). Like other actinopterygians, teleosts follow the pattern of having feeding larvae. Much yolk is present in the large **macrolecithal eggs** of chondrichthyan fishes, coelacanths, reptiles, birds, and monotreme mammals, the embryos of which develop into miniature adults before hatching or birth. We also can describe the organization of the yolk itself. The term **plasmolecithal** (Gr., plasmo = fluid) describes an ovum in which the yolk is in suspension in the cytoplasm. In contrast is the descriptor **telolecithal** (Gr., telos = end), in which a great deal of yolk is packaged into yolk platelets.

Materials in an egg are not distributed randomly but establish and follow gradients. The small amount of yolk in the microlecithal egg of amphioxus is evenly distributed. In vertebrate eggs, yolk usually is most concentrated toward one end, the **vegetal pole**, and least concentrated at the other end, the **animal pole** (Fig. 4-8A). This gradient is particularly evident in macrolecithal eggs, where the egg nucleus and most of the cytoplasm are restricted to the animal pole. The future anteroposterior axis of the embryo is not the same as the egg axis but is related to it and is usually finally determined by the point of entry of sperm. Often the anterior end of the embryo is determined about midway between the animal pole and the equator of the egg (Fig. 4-8B).

In addition to yolk, vertebrate eggs are packaged with cytological necessities for early development of the zygote. Billions of ribosomes (related to protein synthesis) and thousands of mitochondria (related to ATP production) can be packed into a frog egg only a few millimeters in diameter. These organelles provide for future protein synthetic and energy needs of the embryo. Some **mRNA transcripts** of genes for later translation into proteins needed by the zygote are already prepositional in the egg cell’s cytoplasm. Still other information-containing molecules, such as the **germinal granules** of frogs, already are localized to parts of the oocyte, where they will help determine the differentiation of the germ cells in the adult.

At **ovulation**, an egg is discharged from its follicle and the ovary. A stimulus of some sort is needed to activate the egg and initiate further development. Normally, the stimulus is sperm penetration, but other chemical or physical stimuli are effective in some species, in which case the egg develops **parthenogenetically** (i.e., without the contribution of the DNA of the sperm). If an egg is not activated within a few hours of ovulation, its delicately balanced internal organization breaks down, and the egg degenerates.

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2The “chorion” of an actinopterygian egg (a primary egg cell membrane) is not homologous to the chorion of amniotes (which is a secondary egg cell membrane; see section, “Secondary Egg Cell Membranes and Extraembryonic Structures”).
As discussed in Chapter 3, Actinopterygii is an enormous group, including more than 25,000 living species. The species diversity of actinopterygians is reflected by many differences in their patterns of development. This focus compares aspects of the development of nonteleostean actinopterygians (the North American paddlefish, Polyodon spathula, and closely related sturgeons in the genus Acipenser), to derived teleosts (exemplified by zebrafish, Danio, and killifish, Fundulus). These taxa differ greatly in adult size, mode of life, spawning biology, and many developmental characteristics.

In the figure, A outlines the main periods of early development in the paddlefish; the terminology summarized in this figure can be applied to most actinopterygians. The embryonic period is defined as the period up to hatching. Much of early organogenesis is completed in this period, but the yolk has only been partially used. During the yolk sac larval period (B), the hatchling rapidly uses its yolk reserves to complete later organogenesis. The yolk sac larval period is usually a very dynamic time during embryogenesis of actinopterygians: the mouth opens, gill ventilatory and locomotory movements start, and the sensory systems finish their development, until, by the end of the period, the free-living larva is ready to start feeding (C). The free-living larva feeds on small plankton and grows very rapidly, and soon thereafter takes on the appearance of a miniature adult, at which time it may be called a juvenile (D; see Bernis and Grande, 1992).

The eggs of most actinopterygian fishes are small, usually no more than a few millimeters in diameter. Typically, they are surrounded by a tough outer chorion (ho-
mologous to the primary egg cell membrane), which has tiny openings, known as micropyles, to admit sperm. Teleostean eggs are usually yolk-containing and often have a large oil droplet floating in the cytoplasm. The oil serves as a nutrient for the embryo and as a buoyancy regulator in many taxa. In nonteleostean actinopterygians, such as paddlefishes or sturgeons, eggs are spawned directly onto the gravel bottom of a river. Eggs that develop in contact with the bottom are said to be demersal. The opposite condition is seen in many marine teleosts, in which the eggs are said to be pelagic because their enclosed oil droplet causes them to float at or near the water’s surface. The eggs of other species of marine teleosts and virtually all freshwater teleosts are denser than the water, and, if spawned in midwater, sink to the bottom. This is the mode of spawning seen in zebrafish. Still other teleosts, such as killifish, attach individual eggs to chosen spots in the environment, such as submerged leaves, relying on the sticky jelly coat surrounding the egg to hold it in place throughout development.

Cleavage in paddlefishes or sturgeons is unequal and holoblastic and generally resembles patterns seen in amphibia. Gastrulation involves movements of cells at both the animal and vegetal poles. The resulting embryo neurulates by rolling up the neural tube in a pattern very similar to that seen in amphibians. This type of cleavage and gastrulation is presumably the plesiomorphic pattern for osteichthyanas.

Teleosts exhibit many differences from these patterns. The egg cell nucleus in species such as the zebrafish (Danio rerio) undergoes repeated nuclear divisions, or karyokinetich divisions, yielding a yolky egg cell with many nuclei. Such yolks are termed syncytial yolk. In teleosts, cleavage is discolial and meroblastic, resulting in a blastoderm that rises as a cap of cells at the animal pole on top of an uncleaved mass of yolk (E).

During gastrulation in teleosts, the blastoderm expands greatly, a movement known as epiboly. Epiboly sweeps the margins of the blastoderm (known as the germ ring) around the egg (F–I). Epiboly simultaneously encloses the yolk and generates the three primary germ layers as well as the embryo’s axis. The rapid expansion of the blastoderm to cover the yolk is accomplished largely by the cells of the enveloping layer on the outer surface of the blastoderm. Note in Figure 1 that the embryo’s nervous system, brain, optic vesicles, and somites rapidly form before gastrulation is even completed (gastrulation is not complete until the yolk plug is no longer visible). Interestingly, neurulation in teleosts occurs by the formation of a solid rod of cells that subsequently develops its central canal via a process known as cavitation (Fig. 4-19B). This differs from the pattern of neural tube development seen in most other vertebrates. At first, the embryo’s head and tail are tightly bound to the underlying yolk. Soon, however, they will lift away during processes known as head formation and tail bud formation.
Fertilization

**Fertilization** involves sperm penetration, the combination of male and female nuclear material, and egg activation. Sperm penetration can be complicated because each egg cell is surrounded not only by its own plasma membrane but also by the primary egg cell membrane secreted during oogenesis. The sperm undergoes many changes as it approaches a **conspecific egg**. (The term conspecific refers to another individual of the same species.) Membranes around its acrosome break down, releasing enzymes that will break down the primary egg cell membrane, and a stiff acrosomal filament may form. A mammalian sperm is said to be **capacitated** when it is ready to penetrate the primary egg cell membrane and any other materials, such as adhering follicular cells of the corona radiata, that may surround the egg.

Contact of the sperm head with the plasma membrane of the egg initiates a complex **cortical reaction** in the egg. The cortical reaction draws the sperm head into the egg and concurrently releases materials from the cortical granules that raise the primary egg cell membrane from the egg surface, preventing other sperm from entering. The primary egg cell membrane is now called the **fertilization membrane** (Fig. 4-8B).

At the time of ovulation, the eggs of many vertebrates have not yet completed their meiotic divisions. For example, a human egg is fertilized while it is still a secondary oocyte because its nucleus is arrested midway through the second meiotic division. Sperm entry triggers the completion of this division, by which one set of chromosomes is discarded in the second polar body. This leaves the haploid egg nucleus ready to combine with the haploid sperm nucleus to form the diploid nucleus of the **zygote** (Gr., *zygon* = yoke or union).

Finally, fertilization triggers a redistribution of materials within the egg’s cytoplasm that activates the egg and establishes the plane of bilateral symmetry, if one was not already established in the unfertilized egg. Such redistribution is particularly evident in eggs of frogs in the genus *Rana*, in which the animal hemisphere is heavily pigmented. Cortical pigment granules shift after sperm entry and leave a **gray crescent** on one margin of the equator of the egg (Fig. 4-8B).
gray crescent marks the presumptive posterodorsal part
of the embryo. The first cell division, or cleavage,
of the zygote occurs soon after this redistribution
of materials.

Cleavage

Cleavage is a period of rapid cell division, during
which the unicellular zygote is converted into a multi-
cellular embryo known as the blastula (Gr., blastos =
germ or bud). Unlike typical mitotic divisions, no cy-
toplasmic growth occurs during cleavage, so the cells,
which are called blastomeres (Gr., meros = part), be-
come smaller and smaller. The rate of cell divisions
during cleavage is higher than that at any other stage
in development, and the number of cells in the zygote
increases rapidly (Fig. 4-9).

The pattern of cleavage is correlated with the amount
and distribution of yolk. In microlechtal and
mesolecithal eggs, the entire zygote divides, so cleav-
age is described as complete, or holoblastic. Cleavage
furrows mark the separation between daughter cells as
the egg divides. The first cleavage lies in the vertical
plane, extends from the animal to the vegetal pole, and
divides the embryo into prospective left and right sides
(Fig. 4-10 A and B). This cleavage bisects the gray cre-
cent in amphibian zygotes. The second cleavage, also
in the vertical plane (except in mammals3), is at right
angles to the first and results in the formation of four
cells (Fig. 4-10 A and B). The large amount of yolk in
mesolecithal eggs slows the separation of the blas-
томерез, so the first cleavage furrow does not reach the
vegetal pole before the second one begins at the ani-
mal pole (Fig. 4-10 B).

The third cleavage usually lies in the horizontal
plane, and it divides the embryo into eight cells. It lies
near the equator in embryos that developed from mi-
acrolechtal eggs (Fig. 4-10 A), but it is displaced to-
ward the animal pole in those that came from
mesolecithal eggs (Fig. 4-10 B). The resulting blasto-
 томерез in such embryos are unequal in size, with
those near the vegetal pole being much larger than
those near the animal pole. Cleavage continues in this
fashion, tending to alternate between the vertical and
horizontal planes. The cells that are formed remain
close to the periphery, where gas and other exchanges

3Most mammals exhibit a variant form termed rotational cleav-
age, in which the second cleavage passes through the horizontal
plane of one of the two blastomeres; see section, “Developmen-
tal Modifications of Eutherian Mammals” on page 144.

FIGURE 4-9
Rate of cell increase during cleavage. This diagram is based
on a frog embryo; similar patterns occur in other
vertebrates. (Based on a figure in Balinsky.)
FIGURE 4.10
Three patterns of cleavage from the two-cell stage to the early blastula of amphioxus, an amphibian, and a bird. A, Amphioxus (Branchiostoma). B, Frog (Rana). C, Chicken (Gallus). (Modified from figures in Balinsky.)
in the types of nucleic acid transcripts, organelles, enzymes, morphogens, and other substances that they contain. These differences influence the selection and sequence of expression of nuclear genes during subsequent stages of development.

**Fate Mapping**

The general term **fate mapping** is used to describe methods for tracing the fates of cells, tissues, or organ rudiments during development. These techniques provide some of our most powerful insight into the development of patterns in embryos. Vertebrate embryologists adopted standard colors for fate maps, using yellow for endoderm, red for mesoderm, green for chordamesoderm, and blue for ectoderm. Fate maps often are constructed by marking a population of cells and then following their subsequent development or by transplanting a portion of one embryo with different characteristics (i.e., pigmentation) into another embryo. A third general method is to remove, or extirpate, a portion of an embryo and observe what deficiencies exist in later stages.

In its simplest form, fate mapping can be used to study the fates of the surface cells of a blastula. To do this, parts of the surface of the blastula are stained with vital dyes, and the color spots are then traced as development proceeds. By this method, the general fates of different regions of the blastula stage were demonstrated long ago. Some regions will become gut, others, neural tube, epidermis, notochord, head mesoderm, and so on. More recently developed methods that offer precision tracing include injecting single cells with fluorescent carbocyanin dyes, such as DiI. In a few species, such as chickens, retroviruses also can be injected to infect a population of cells, which can be later identified as constituents within a complex organ, such as a muscle. Sometimes cell movements can be traced by noting the types of organelles that they contain. For example, it is possible to trace migrating neural crest cells in amphibian embryos because they have smaller yolk platelets than the stationary cells that they are passing.

One of the most powerful of the transplantation methods has been the **quail-chick chimera technique**, which allows for high-resolution study of the development of chick embryos. This has proved important for studies of organogenesis (especially of the nervous system and muscles) in amniotes. Another important transplantation method utilizes pigmented and albino axolots, *Ambystoma mexicanum*. Transplantation of cells or organ rudiments from pigmented embryos into albino embryos allows for easy visualization of their fate. This has been important in understanding the genesis of taste buds and lateral line sense organs (see Chapter 12).

It is useful to compare simplified fate maps of amphibian and avian blastulas because this will help you understand basic differences in their modes of gastrulation. The choice of amphibian species for this comparison is important, for not all species of amphibians have comparable fate maps. The generalized example shown in Figure 4-11.A is based on frogs of the genus *Rana*; it also approximates the condition in salamanders in the genus *Ambystoma*. Frogs of the genus *Xenopus*, however, are different from the species shown in that no cells fated to form mesoderm (red) are exposed on the surface of the blastula. The significance of this phylogenetic difference is unclear, but *Xenopus* is one of the most widely studied model species in developmental biology, which makes this difference of more than just passing interest (see Focus 4-1). The generalized example shown in Figure 4-11.B is based on the domestic chicken, *Gallus*. First, note that the blastula of the frog is spherical, whereas that of the chicken is flat. Next, notice that all three of the primary germ layers (i.e., ectoderm, mesoderm, and endoderm) are visible on the surface of both blastulas. In the frog, these primary germ layers form three horizontal bands around the egg. In the chick, the primary germ layers form three off-center targets, with cells fated to form endoderm in the middle, those fated to form mesoderm surrounding the endoderm, and those fated to form ectoderm forming the outermost circle.

**Gastrulation, Mesoderm Formation, and Early Neurulation**

Cleavage is followed by **gastrulation**, when cells with different fates move to appropriate parts of the embryo for further differentiation. Such **morphogenetic movements** result from active migration of cells, changes in the sizes and shapes of cells, and different rates of cell division in different parts of the embryo. The single-layered blastula is converted to a **gastrula** (L., *gastrula* = little stomach) with well-defined layers of tissue known as the **germ layers**. One germ layer, the endoderm, turns inward to form the gut, or **archenteron**, which at this stage opens to the surface

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4Recent research indicates that a few mesodermal cells may be exposed on the surface of the blastula of *Xenopus*.
only by a blastopore at the posterior end of the gastrula (Fig. 4-12D). The blastopore is at or near the prospective anus of the embryo, a condition that characterizes all deuterostomes (e.g., echinoderms, chordates, and relatives; see Chapter 2). The mouth will later break through at the opposite end of the embryo. The endoderm will form the lining of most of the digestive tract and the glandular cells that develop from it (Table 4-1). A second germ layer, the ectoderm, covers the surface of the embryo and will form the epidermis of the skin; the nervous system; and major sense organs, such as the nose, eye, taste buds, ear, lateral line, and electrosensory systems (Table 4-1). The neural crest is a special population of cells derived from the ectoderm that will form components of the nervous system as well as many other structures, including pigment cells, cartilages, and bones (see the section, “Neural Crest and Neurogenic Placodes”, page 149). Neurogenic placodes are another source of neurons for the developing nervous system (see later section, “Neural Crest and Neurogenic Placodes”). Cells that will form the third germ layer, the mesoderm, move into the roof of the archenteron (Table 4-1). The mesodermal cells that form the mid-dorsal part of the roof of the archenteron are referred to as the chordamesoderm (Fig. 4-12F), which will give rise to the notochord. Separation of the mesoderm from the other germ layers may occur during or after gastrulation. The mesoderm will form cartilages, bones, tendons, ligaments, muscles, the heart, blood vessels, the lining layers of the coelomic cavities, and many other tissues and organs (Table 4-1). As gastrulation ends, the ectoderm overlying the chordamesoderm is induced by the primordial notochord to thicken to become the neural plate, from which the brain, spinal cord, and neural crest will be derived (Fig. 4-12E). Gastrulation, neurulation, and mesoderm formation occur as three sequential and overlapping waves of development, generally in a cranial (anterior) to caudal (posterior) sequence.
The organization of the neurula stage of the embryo foreshadows the structure of the adult. Table 4-1 summarizes the developmental pathways leading to formation of the various organs. Each pathway leads from one of the three germ layers. Each organ is identified with a particular germ layer, although in nearly all cases more than a single germ layer contributes to the final adult form of the organ. Consider, for example, the pancreas: pancreatic secretory cells develop from embryonic endoderm cells lining the gut tube, but its blood vessels and connective tissue develop from mesoderm, and its nerve supply develops from neural crest.

Chordates differ in the mechanisms by which they gastrulate. Such differences are described in the following brief comparative overview. Bear in mind, however, that the end products of gastrulation are very comparable among all vertebrates. When such developmental stages are recognizably similar across broad phylogenetic gaps, they are known as phylotypic stages.

**Gastrulation by Invagination: Amphioxus**

The pattern of gastrulation is relatively simple in amphioxus, and its serves as a good model for the major events seen in other types of gastrulation. Its microlecithal egg develops into a blastula composed of small cells; those of the vegetal hemisphere are not much larger than others. The vegetal pole flattens and then folds inward by a process called invagination.
### TABLE 4-1  Derivatives of the Three Primary Germ Layers

| Somatic ectoderm | Epidermis  
|------------------|--------------------------  
|                  | Feathers, hair, skin glands, scales, horns in part  
|                  | Lens of eye  
|                  | Teeth, nails  
|                  | Notochord, dermophysial  
|                  | Anal canals  
|                  | Olfactory organ and cranial nerves 6 and 7  
|                  | Lateral line receptors and nerves (see text)  
|                  | Inner ear and cranial nerves will  
|                  | Ammonian, electroreceptors, and nerves (see text)  
|                  | Taste buds and nerves (see part)  
|                  | Core, somites  
|                  | Teeth, dentine  
|                  | Visceral skeleton  
|                  | Larynx  
|                  | Schwann cells  
|                  | Meninges of brain  
|                  | Central nervous system, cranial  
|                  | Nervous system, special sense organs, cranial  
|                  | Adrenal gland  
|                  | Brain and spinal cord  
|                  | Cortical and spinal nerves  
|                  | Retina and optic nerve  
|                  | Posterior auricular (neural crest)  
|                  | Lining of lungs  
|                  | Lining of urinary bladder  
|                  | Thyroid, parathyroid, thymus  
|                  | Liver, pancreas  
|                  | Lining of digestive tract  
|                  | Auditory tube  
| Neuronic placodes |  
|                  |  
|  
| Neural crest |  
|  
| Neural tube |  

| Endoderm | Archenteron (primitive gut)  
|----------|-----------------------------  
|  
| Chorda mesoderm |  
| Paraxial mesoderm (somites) |  
| Intermediate mesoderm (nephrotome) |  
| Mesoderm |  
| Lateral mesoderm (lateral plate) |  
|  
| Splanchnic layer |  
| Coelom |  
|  
|  

(Fig. 4-12A–D). The process of invagination in amphioxus resembles indenting the wall of a balloon or ball with your hand. The pocket that you create with your hand will be the embryo’s gut, and the two “layers” created by the indentation correspond to the ectoderm (on the outside of the balloon) and the endoderm plus mesoderm (the lining of the pocket). As invagination continues, the inward folding layer forms the archenteron, and the blastocoele becomes obliterated. The opening of the archenteron created by this process is the blastopore; it marks the future posterior end of the animal. Immediately following gastrulation,
Gastrulation by Involution: Amphibians

The large, yolk-filled cells that form in the vegetal hemisphere during the cleavage of a mesolecithal egg, as found in Rana or Ambystoma, cannot invaginate in the same way as in amphioxus. Instead, invagination of a few cells on the margin of the gray crescent of the embryo forms a cleft that is the beginning of the archenteron (Fig. 4-13A). The dorsal margin of the cleft is termed the dorsal lip of the blastopore. The cleft lengthens as time goes on, grows laterally and ventrally, and eventually forms a circular blastopore. As the blastopore develops, cells move from the dorsolateral surface of the embryo toward the blastopore, roll over its lip, and then continue to move forward beneath the ectoderm, thereby deepening and enlarging the archenteron (Fig. 4-13B). This process, in which a sheet of epithelium moves the reference point of the blastopore lips, is known as involution. Involution begins at the dorsal lip of the blastopore, but soon cells begin to involute at its lateral lips, and eventually a few cells involute at its ventral lip. The blastocoel becomes obliterated as the archenteron enlarges. Movement of surface cells toward the lips of the blastopore occurs faster than they can be involuted. As a result, the prospective ectodermal cells, which originally were limited to the animal hemisphere, overgrow the yolky cells of the vegetal hemisphere in a process called epiboly. Finally, only a small “plug” of yolky-filled cells can be seen through the blastopore (Fig. 4-13C). This stage is termed a yol plug gastrula.

Cells that involute form endoderm, chordamesoderm, and mesoderm proper (Fig. 4-13D). Prospective chordamesoderm cells in the mid-dorsal part of the archenteron roof will form the notochord (Fig. 4-13E, green). Prospective mesoderm cells lie more laterally (Fig. 4-13E, red). Mesodermal cells move forward as a sheet between the endoderm and the ectoderm (Fig. 4-13D). The notochord separates from the laterally placed mesoderm, and endodermal folds meet beneath it to complete the roof of the archenteron (Fig. 4-13D and E). At the boundary between the notochord and endoderm is a small patch of cells, termed the prechordal plate, that extends anterior to the notochord and will play a role in head formation (not shown in Fig. 4-13). The cells of the ectoderm overlying the notochord thicken to form the neural plate (Fig. 4-13D and E). Some other vertebrates with mesolecithal eggs, such as lungfishes, bichirs, sturgeons, and paddlefishes, exhibit comparable methods of gastrulation by involution.

Gastrulation by Ingression: Birds

The process of gastrulation is different in macrolecithal eggs, such as those of chondrichthyan fishes, reptiles, and birds. Because large, yolky eggs evolved independently in chondrichthyan fishes and amniotes, it should not be surprising that the methods of gastrulation differ. Gastrulation movements are best known in chickens, which have become a standard model for gastrulation in amniotes. The central part of the blastoderm in chick embryos becomes clear and forms the area pellucida (clear area); the peripheral part remains opaque and is termed the area opaca (opaque area; Fig. 4-14A). Cells separate as a sheet, or delaminate, from the deep surface of the area pellucida at its prospective caudal end and migrate forward, forming a layer known as the hypoblast (Fig. 4-14A). Between the hypoblast and the mass of uncleaved yolk is a small space known as the subgerminal cavity. The overlying cells of the chick blastoderm now constitute the epiblast, and the space between these two layers corresponds to the blastocoel. Cells then begin to migrate from the periphery of the epiblast toward its center, where they form a thickened, longitudinal ridge known as the primitive streak (Fig. 4-14B). As more cells move toward the primitive streak, cells that are already there turn inward ventrally and spread out laterally and anteriorly beneath the epiblast. Many of the cells that move through the primitive streak delaminate and migrate as individual mesenchymal cells. The first cells to move inward along the primitive streak displace hypoblast cells and form endoderm. Cells that move inward later form mesoderm. When the process is complete, epiblast cells that remain on the surface are the ectoderm. All three germ layers thus are derived from the epiblast. By comparing the fate map of a chicken blastula in Figure 4-11B with
Figure 4-13
Gastrulation and mesoderm formation in amphibians. A, Sagittal section and posterior view of an early stage of a frog embryo, just as invagination is beginning. B, Similar views of a later stage, when involution and epiboly are occurring. C, The blastopore now is complete, and the yolk plug protrudes through it. D, A sagittal section of a later gastrula of a salamander, showing the spread of the mesoderm between the ectoderm and endoderm. E, A transverse section of a later stage, when the notochord has formed, endodermal folds are completing the roof of the archenteron, and the neural plate has been induced. (A–C, Frog [Rana], from Balinsky and Fabian; D and E, salamander [Ambystoma], from Hamburger.)


Figure 4-14, you should be able to visualize how gastrulation via a primitive streak rearranges the cells of the blastula.

The inward movement of cells along the primitive streak has been called ingestion, but it is functionally comparable to the involution of cells in amphibian embryos. Indeed, the primitive streak has been considered the homologue of the blastopore since the 1870s. The process of ingestion of cells along the primitive streak is completed first near the anterior end of the embryo, and the wave of completion spreads caudally. As ingestion is completed, the primitive streak retreats caudally (from anterior to posterior), leaving the in-turned endoderm and mesoderm. Just as in amphibians, the notochord and mesoderm separate, and the ectoderm overlying the notochord thickens as the neural plate (Fig. 4-15A). Mesoderm immediately lateral to the notochord and developing neural tube, the paraxial mesoderm, differentiates into a series of somitomeres in the head (discussed in the section, “Basic Organization of the Vertebrate Head,” page 164) and somites in the trunk (Fig. 4-15B). These processes, too, progress from anterior to posterior. The coelom forms, as it does in amphibians, by cavitation in the mesoderm lying lateral to the somites.
**Mesoderm Differentiation**

As gastrulation ends, several other processes go on more or less concurrently. These are: (1) mesoderm differentiates into parts that will form primordia of many organ systems; (2) extraembryonic membranes develop in amniotes, surround the developing embryo, protect it, and serve other functions; and (3) neurulation occurs, and the nervous system and sense organs begin to differentiate. We consider these topics in this sequence, but keep in mind that these processes are going on at the same time.

Mesoderm, somites, and the coelom usually begin to form near the end of gastrulation, and mesodermal development continues through neurulation. Idealized cross sections through amphibian embryos at two different stages during neural tube formation are shown in Figure 4-16. By the time the neural tube is complete, three bands of mesoderm can be recognized on each side of the embryo (Figs. 4-15B and 4-16).

Thickened, block-like segmental somites, or paraxial mesoderm, lie lateral to the neural tube and notochord (Fig. 4-16). A broad, unsegmented lateral plate, or lateral plate mesoderm, extends laterally and ventrally between the archenteron and surface ectoderm. A nephric ridge, or intermediate mesoderm, which is segmented anteriorly, lies between the somites and lateral plate mesoderm. The definitive coelom develops by cavitation in the lateral plate. Portions of the coelom extend into the nephric ridge, and small, ephemeral spaces, known as somitocoelæs, may occur within the somites.

As development proceeds, each somite differentiates into three regions (Fig. 4-16B). Initially, somites are epithelially organized, but their cells will undergo epithelial–mesenchymal transitions and migrate to achieve their final locations in the body. The dorsolateral group of somite cells constitutes the dermatome. Cells in the dermatome differentiate into mesenchymal cells that migrate out beneath the surface ectoderm to form most of the dermis of the skin. Cells
FIGURE 4-16
Differentiation of trunk mesoderm in an idealized amphibian embryo. A, Early neurula. The inset shows a lateral view and the plane of section for the figure in A. It also diagrams the basic organization of the three divisions of trunk mesoderm. In amphibian embryos (and other vertebrates with mesolecithal eggs) the moderate amount of yolk is incorporated into yolk-laden cells in the floor of the archenteron. B, Later neurula, showing the structures that develop from each somite and each germ layer. Note that each of the three divisions of trunk mesoderm diagrammed in A in turn gives rise to subdivisions. See text for additional descriptions.
deep to the dermatome form the myotome, or embryonic muscle segment. The segmental myotomes extend ventrally between the ectoderm and lateral plate mesoderm and differentiate into muscle cells that form all (in amniotes) or most (in amniotes) of the somatic muscles of the body wall and appendages. The ventromedial part of the somite is the sclerotome. It differentiates into mesenchymal cells that migrate around the neural tube and notochord to form the vertebral column and occipital region of the skull. Other parts of the skull develop from mesenchymal cells derived from the cranial neural crest (ectomesenchyme, described later) and from the dermaterm. Other aspects of braincase and skull development will be treated in Chapter 7, and vertebral development will be described in Chapter 8.

The intermediate mesoderm develops two subdivisions, termed the nephric and gonadal ridges (Fig. 4-16B). The nephric ridge will differentiate into the kidney and its excretory ducts, as well as some portions of the reproductive ducts. The additional development of these organ systems will be treated in Chapters 20 and 21. The gonadal ridge forms medial to the nephric ridge. Gonadal development involves not only the mesoderm of the gonadal ridge but also migration of primary germ cells from endoderm, and is described in Chapter 21.

The lateral plate mesoderm is split into two layers, thereby creating the coelom (Fig. 4-16B). The medial splanchnic layer of the lateral plate mesoderm next to the endoderm will form the connective tissue and visceral muscles of the gut tube and heart walls. Splanchnic mesoderm forms the circulatory system, including the heart, arteries and veins, and circulating blood cells, with two important exceptions: (1) in the head region, neural crest cells contribute to blood vessel formation; and (2) the first generation of blood cells is made in the blood islands of the yolk sac. Part of the splanchnic mesoderm will form mesenteries and the coelomic epithelium (the visceral peritoneum) that covers the visceral organs. The lateral somatic layer of the lateral plate mesoderm forms the coelomic epithelium bounding the coelom laterally (the parietal peritoneum), and it also may contribute to the somatic musculature and other tissue of the lateroventral body wall. In contrast to the splanchnic layer, the somatic layer of lateral plate mesoderm does not form blood vessels or blood cells.

It is convenient to use the term splanchnopleure (Gr., splanchnomon = gut + pleure = wall) to refer to the mesodermal and endodermal components that form the wall of the gut (Fig. 4-16B). Similarly, the term somatopleure (Gr., somatikos = body + pleure = wall) refers to the mesodermal and ectodermal components that make up the body wall (Fig. 4-16B).

Secondary Egg Cell Membranes and Extraembryonic Structures

We described the primary egg cell membrane, or vitelline membrane, in the earlier section on gametes and fertilization. Secondary egg cell membranes may surround the primary egg cell membrane. All secondary egg cell membranes of vertebrates are produced by the female’s reproductive tract after an egg is ovulated from the ovary. Fertilization may occur after synthesis of the secondary egg cell membranes (e.g., frogs) or before they are added around the egg cell (e.g., chickens). Secondary egg cell membranes include such structures as adhesive jelly coats, egg capsules, egg cases, and egg shells. An example of the jelly coat of a frog’s egg is shown in Figure 4-8. Because they are produced outside of the ovary, secondary egg cell membranes may contain one egg cell (as in chickens) or many individual egg cells (as in the egg capsules of some elasmobranchs). As a general rule, secondary egg cell membranes provide a protective egg and embryo. In those species with secondary egg cell membranes, the individual “hatches” twice: first from its vitelline membrane and, usually much later, from its secondary egg cell membranes.

In the case of a chicken egg, fertilization occurs soon after ovulation, near the ostium or infundibulum of the oviduct (see Fig. 21-17). The fertilized egg travels down the oviduct to the shell gland. During this passage, the egg accumulates egg white, which consists of water (about 80% by weight) and proteins (mostly albumens). Initially, the egg is rotated, and at each end becomes coated with spirally wrapped layers of albumen, which form the chalaza. More layers of albumen adhere to the chalaza as the egg continues down the oviduct. Next, the egg and its adhering albumen are enclosed in a tough inner shell membrane composed of the protein keratin. The surrounding outer shell membrane entraps a small pocket of air known as the air space. Finally, the slightly porous calcareous shell is added in the shell gland. Secretion of all of these secondary egg cell membranes requires about 22 hours, after which the egg is laid, and its incubation can begin. The shell and shell membranes are protective layers, whereas the albumen (together with the yolk) provides raw materials, water, and energy needed by the embryo during its development.

With the evolution of the cleidoic egg (see Chapters 1 and 3), amniotes were able to bypass an aquatic larval stage and reproduce on land. This is possible because the embryos of amniotes possess four extraembryonic membranes that protect the embryo and
sustain its metabolism. Our model for the extraembryonic membranes of amniotes is based on chickens because, with only a few modifications, this model can be applied to amniotes in general.

As a chick embryo develops in the blastodisk on top of its large yolk mass, tissue layers extend from the embryonic body to form extraembryonic membranes (Fig. 4-17). First to develop is the yolk sac, which forms by the spreading of tissue layers over the yolk. Endoderm and splanchnic mesoderm eventually surround the yolk. Thus, the yolk sac of amniotes is referred to as a bilaminar (two-layer) yolk sac. This is in contrast to the yolk sac membrane of actinopterygian fishes, which contains all three germ layers and is termed a trilaminar yolk sac.

An extraembryonic coelom extends from the embryo between the somatic and splanchnic layers of the part of the lateral plate mesoderm that contribute to the yolk sac. As the yolk sac of a chick embryo develops, the ectoderm and somatic layer of the lateral plate mesoderm become elevated as amniotic folds that arch over the embryo and meet above it (Fig. 4-17B). As the head folds and tail folds deepen and undercut the embryo, and as lateral body folds form, the embryo is raised off the yolk mass, but its archenteron remains connected to the yolk sac by a narrow yolk stalk.

Blood vessels and blood cells begin their development in the splanchnic mesoderm of the yolk sac and spread into the embryo. Embryonic circulation is soon established, and a rich network of blood vessels conveys materials from the yolk to the embryo. These vessels also can bring in materials from the egg white because the periphery of the yolk sac that is spreading over the yolk is in contact with the egg white for a long time.

Two additional extraembryonic membranes form when the amniotic folds meet and fuse above the embryo’s body (Fig. 4-17C). The inner limbs of these folds form the amniotic membrane (or amnion), which surrounds the embryo’s body. The outer limbs of the fold form the chorionic membrane (or chorion), which surrounds the amnion and, eventually, the entire yolk sac. Amniotic fluid accumulates in the amniotic cavity between the amnion and the embryo, so that the embryo continues its development in a liquid environment. It consists chiefly of water with salts and a few proteins, as well as living cells sloughed off from the embryo’s ectoderm. Although a cleidoic egg must be laid on land, the embryo’s immediate environment remains aquatic, like that of fish and amphibian larvae. Amniotic fluid also provides an important protective liquid cushion around the embryo that buffers it against physical bumps. Additional protection is afforded by the chorion itself, as well as by the egg white and shell.

**Figure 4-17**

Schematic diagrams of the formation of extraembryonic membranes of a chick embryo. A. Early stage. B. Stage showing amniotic folds extending above the embryo’s body. C. Later stage, in which all four extraembryonic membranes (i.e., amnion, chorion, allantois, and bilaminar yolk sac) can be discerned. See text for additional description. *(Modified from Patten.)*
Amniotic fluid is increasingly important in the analysis of human genetics. It can be sampled after about 18 weeks of gestation using a method called amniocentesis. In this procedure, a long hypodermic needle is inserted through the mother's abdominal and uterine walls and into the amniotic cavity itself. The positions of the fetus and the tip of the needle are monitored continuously using ultrasound imaging. A small sample of amniotic fluid is withdrawn, from which it is possible to culture living cells that can be used to study the baby's chromosomes.

The third extraembryonic membrane of amniotes, the allantois, forms as an evagination of the posterior part of the archenteron, so its wall is composed of endoderm and vascularized splanchnic mesoderm (Fig. 4-17C). The allantois enlarges, nearly fills the extraembryonic coelom, and eventually contacts the chorion. Frequently, these two membranes fuse to form a chorioallantoic membrane. The chorion and amnion are avascular membranes because they develop before any blood vessels enter the somatic mesoderm. In contrast, the allantois is richly vascular, and it vascularizes the chorion, bringing embryonic vessels close to the inside of the porous inner and outer egg membranes and shell. Embryonic gas exchange occurs by this route. Nitrogenous excretory products, in the form of inert crystals of uric acid, accumulate in the lumen of the allantois. After hatching or birth, the base of the allantois remains in many amniotes as the urinary bladder (Chapters 20 and 21).

21-21). After a eutherian embryo's body form becomes apparent, it can be termed a fetus. Placentaion allows physiological exchange between the embryo and its mother. Because a eutherian mother will provide the embryo with nutrients and other materials via placental exchange, very little yolk is deposited in the egg cells of placental mammals during oogenesis. Eutherian eggs have become secondarily microlecithal, and this simplifies their cleavage. For development to proceed, it is essential that implantation occur very early, for without a placenta, the embryo's physiological needs cannot be met by the mother. Cleavage of a eutherian egg differs from that of other microlecithal eggs in that the second plane of cleavage is partially horizontal, a condition termed rotational cleavage. Cleavage continues, eventually yielding a ball of cells known as a blastocyst (Fig. 4-17A). The outermost cells of the blastocyst form a layer known as the trophoblast (Gr., trophe = nourishment + blastos = germ or bud), which is homologous to the chorionic ectoderm of the eggs of other amniotes. The trophoblast is the precursor of the fetal part of the placenta. The maternal part of the placenta is the vascularized and glandular uterine lining, or endometrium (Gr., metra = uterus). In the uterine environment, the trophoblast soon begins to grow and spread out on the endometrium. In human beings and other eutherians in which the fetal part of the placenta grows aggressively into the uterine lining, the trophoblast is said to be invasive. Contact or penetration of the endometrium by the trophoblast produces implantation of the embryo. In ways not fully understood, the trophoblast also provides an immunological barrier that prevents the mother from developing antibodies against the embryo, which otherwise would be rejected as foreign tissue because half of its genes are paternal. The trophoblast thus prevents immunological rejection of the embryo, a key innovation in the evolution of eutherian viviparity.

A small sphere of cells known as the inner cell mass lies within the trophoblast. All of the embryo's body will be derived from cells of the inner cell mass. The inner cell mass attaches to the trophoblast at the presumptive posterodorsal side of the embryo, but elsewhere it is separated from the trophoblast by a cavity, which is homologous to the blastocele of other vertebrates. Details of gastrulation and differentiation of the embryo, as well as its extraembryonic membranes, differ considerably among eutherian taxa; we will describe the human condition. Endodermal cells differentiate on the underside of the inner cell mass and spread laterally and ventrally within the cavity of the blastocyst to form the endodermal part of the yolk sac (Fig. 4-18B). There is no yolk in the yolk sac of a human embryo (or other eutherian mammals), but its
dorsal part eventually will become separated from the rest of the yolk sac to form the archenteron. A space that forms by cavitation among the cells in the dorsal part of the inner cell mass is the beginning of the amniotic cavity. Cells lining this cavity are regarded as ectoderm. The two-layered disk of cells (ectoderm and endoderm) between the cavities of the yolk sac and the amnion is the blastodisk, from which the embryo’s body will develop.

As in chick embryos (Fig. 4-14), gastrulation in a human embryo involves movements of prospective mesoderm cells toward a longitudinal primitive streak, through which they ingress and spread out between ectoderm and endoderm. Neurulation, mesoderm differentiation, and the separation of the embryo from the yolk sac by body folds resemble the corresponding processes in chick embryos. Mesoderm also spreads over the surfaces of the amnion and yolk sac, so that
these become typical two-layered extraembryonic membranes as in other amniotes (Fig. 4-18 C). A stalk of tissue persists near the posterior end of the embryo and connects it with the ectodermal trophoblast. Mesoderm spreads via this stalk over the underside of the trophoblast and converts it to a chorion that more closely resembles that of avian and reptilian embryos. An allantois grows from the posterior part of the archenteron into this stalk. The cavity inside the allantois never becomes large in human embryos, but its wall enables blood vessels from the embryo to reach the avascular chorion and to vascularize the fetal part of the placenta. This part of a eutherian placenta is homologous to the choioallantoic membrane of reptiles and birds. As the embryo enlarges, it rises above the yolk sac and allantois. Thus, the embryo is connected to its placenta by a long, cordlike stalk that also contains the allantois and yolk sac. This cordlike structure is the umbilical cord, which must be broken or cut at birth.

In summary, the development of a mammalian embryo resembles that of reptiles and birds except for (1) the reduction of yolk, (2) simplification and modification of early cleavage patterns, (3) production of a blastocyst, and (4) the précocious development of extraembryonic parts that contribute to the placenta and the umbilical cord.

### Organogenesis of the Nervous System and Sense Organs

Organ formation in the nervous and sensory systems provides several important examples of organogenesis and general concepts in embryology. The nervous system forms very early and is so pervasive in its structural and functional impact on other organ systems that an understanding of its early development will help you to understand many other aspects of the vertebrate body. Our first topic is neurulation, during which the neural tube forms and begins to differentiate into the brain and spinal cord. Next, we describe eye formation, which offers a particularly important demonstration of interactions of components of the central nervous system with other differentiating tissues to form a complex organ. In addition to the neural tube, vertebrates have two other neurogenic tissues: the neural crest and the neurogenic placodes. These embryonic precursor tissues are described, and some of their fates are discussed. Finally, we discuss the early development of other major cranial sense organs, including the nose, taste buds, ear, lateral line, and electroreceptive systems.

### Neurulation

As gastrulation nears completion and somites form, the chordamesoderm induces the overlying neural plate to differentiate into the neural tube characteristic of chordates (Fig. 4-19). Several stages in this process, termed neurulation, are defined based on pattern of development observed in sharks, frogs, and amniotes. First, the ectoderm overlying the notochord thickens and flattens to form a neural plate along the dorsal midline of the embryo. In this process, cells of the neural plate are said to palisade, which describes a change in their shape from a short, cuboidal form to a tall, columnar form. Second, the margins of the neural plate elevate as a pair of neural folds with a neural groove between them (Fig. 4-19 A). This is accomplished in part by a different type of shape change in the cells of the neural plate, in which the spines of the cells narrow or constrict to produce folding of the entire sheet. The neural folds are largest and most widely spaced at the anterior end of the embryo, in the region of the presumptive brain. Third, the neural folds meet dorsally, where the folds of the right and left sides fuse to form the neural tube. The neural groove becomes the central canal that characterizes the central nervous system of chordates. The epithelium immediately adjacent to the central canal is termed ependymal epithelium. The ependymal cells bear cilia that face the central canal. Initially, the walls of the neural tube may be one or two cells thick, but these cells rapidly divide to produce a wall several cell layers thick (see Fig. 13-9). Much of the central nervous system will differentiate from the walls of the neural tube, including portions of the eyes (see pages 146–149). Finally, the neural tube separates from overlying ectoderm, which is left to become the epidermis of the skin.

A variant form of neural tube formation occurs in teleost fishes (Fig. 4-19 B), and it has been regarded as a synapomorphy of teleosts. This is important because about half of all living vertebrates are teleosts, including a currently popular model for vertebrate development, the zebrafish (Danio rerio). In teleosts, a neural keel forms as a solid rod dorsal to the notochord, instead of the flat neural plate found in embryos of outgroup taxa. This neural keel subsequently cavitates to form the central canal of the central nervous system (Fig. 4-19 A). Soon after the left and right neural folds close in the head region, the three primary (or embryonic) brain regions can be distinguished: the prosencephalon, mesencephalon, and rhombencephalon (Fig. 4-20). In turn, the prosencephalon will form two of the five brain regions found in adults, which are the telencephalon and the diencephalon. The embryonic rhombencephalon will form the metencephalon and
myelencephalon of adults. The rhombencephalon shows evidence of segmental organization in its walls, in the form of a series of thickenings known as rhombomeres. The rhombomere segments are related to segments observed in the branchial region, and are discussed later in the section, “Organization of the Head in Amniote Embryos.”

As the neural tube forms and its anterior part differentiates into the brain, the embryo continues to lengthen along its anteroposterior axis. Head and tail folds form in the embryos of most vertebrates, and the embryo begins to separate from the large mass of yolk beneath it.

Eye Formation as an Example of Induction

Vertebrate eye formation offers excellent examples of epigenetic interactions among tissues to yield a complex organ. An understanding of some basic aspects of eye development will help you appreciate the ways in which we think cells and tissues interact throughout embryogenesis to produce a complex organism.

An idealized schematic diagram of eye development (based on the chick) is shown in Figure 4-20A–E. The first indication of eye development is the evagination of paired optic vesicles from the lateral walls of the diencephalon (Fig. 4-20A). As each vesicle grows toward the body surface, its proximal part narrows as an optic stalk, and its distal part invaginates to form a two-layered optic cup. The outer layer of the optic cup becomes the pigment layer of the retina, and the inner layer differentiates into the photoreceptive cells (rods and cones) and neuronal layers of the retina. Because of the way it develops, the retina can be considered to be a part of the brain. The outer, receptive segments of the rods and cones develop from cilia of the ependymal epithelium lining the optic cup. Because of the way the optic cup forms, these cilia are still directed toward its lumen, which was part of the body surface earlier in development. After the lumen has narrowed later in development, the receptive segments of the rods and cones lie next to the pigment layer of the retina, so that light must pass through the nervous layers of the retina to reach them (see Chapter 12).

As the optic cup approaches the body surface, it induces the surface ectoderm first to thicken as a lens placode and then to invaginate and form a lens vesicle that will differentiate into the lens. Adjacent mesenchyme encapsulates the lens and optic cup to form the strong, vascularized wall of the eyeball. Tissue from the ectodermal optic cup contributes to the iris, which regulates the amount of light entering the eye, and the ciliary body, which forms the muscles controlling the iris.
Figure 4-20 summarizes the epigenetic interactions that take place during eye formation, based largely on studies of amphibian embryos. Inductive interactions are shown by the orange arrows. In the early gastrula stage, the prospective ectoderm in the animal pole is induced by involuting chordamesoderm to form the neural plate. Chordamesoderm again induces a population of cells within the neural plate to differentiate into the prospective optic cup. This population of cells is induced again by chordamesoderm to bulge...
outward from the diencephalon to the optic vesicle, and eventually to form the optic cup. We know that chordamesoderm is necessary in all three of these inductions because, if it is extirpated prior to each of these stages, then the subsequent steps in differentiation will not occur.

Meanwhile, most of the surface ectoderm remaining after neural induction has been fated to form epidermis of the skin. By the time of early neurulation, a subpopulation of these cells has been designated as prospective lens. As the optic vesicle grows laterally, it induces these cells to form the lens placode, which soon invaginates to form the lens cup and eventually the lens vesicle. The lens vesicle receives an inductive signal from the neural retina that causes it to differentiate into the definitive lens.

As the lens placode invaginates, it leaves behind a population of cells in the prospective epidermis that now is fated to form the cornea. A final inductive signal from the lens vesicle is needed for corneal development to occur. Together, these cellular-level and tissue-level interactions constitute an inductive cascade (by analogy to a waterfall or rapids), in which a step in the differentiation of a tissue triggers a step in the differentiation of another tissue.

**Neural Crest and Neurogenic Placodes**

Neurogenic precursor tissues are those embryonic tissues that give rise to nerve cells or neurons during development. Amphioxus has only a single type of neurogenic precursor tissue, the neural tube itself (Fig. 4-12.F). Two general fates occur for neurons originating in the wall of the neural tube: (1) some will grow out from the neural tube to reach their target tissues, whereas (2) others will establish all of their connections to other neurons within the central nervous system. Unlike amphioxus, vertebrates have three types of neurogenic precursor tissues: (1) the neural tube, (2) the neural crest, and (3) neurogenic placodes (Fig. 4-21). Although the neural tube of vertebrates generates more neurons than the other two neurogenic precursor tissues, the other two sources of neurons are closely associated with many of the most remarkable structures—particularly the sense organs and cranial nerves—that are found in vertebrates.

The topography and developmental details of these three neurogenic precursor tissues have not been worked out for most species of vertebrates. During neurulation of an amphibian embryo, however, the neural folds of the head have distinct lateral and medial walls (Fig. 4-21A). Most of the medial wall will form the neural tube proper, which will develop into the brain and spinal cord. The crest of the fold will form the neural crest (Fig. 4-19), which will develop into many different structures (Table 4-2 and the section, “Migration and Fates of Neural Crest Cells,” page 151). Portions of the lateral wall of the neural fold will form neurogenic placodes (Fig. 4-21 and Table 4-3). The general definition of a placode is any ectodermal thickening caused by either increasing the basal-apical height of cells in that region or by increasing the number of cells. In addition to the lens of the eye (discussed earlier), many different tissues and organs of the integument originate at least partially as placodal thickenings, including scales, teeth, skin glands, and hair follicles (see Chapter 6). This is why the modifier “neurogenic” is used to distinguish those placodes that give rise to neurons.

The neural crest and the neurogenic placodes are among the most intriguing synapomorphies of craniates, for they form or participate in the formation of many structures not found in outgroups. In the 1980s, Glenn Northcutt and Carl Gans pointed out that neural
TABLE 4-2  Derivatives of Cranial and Trunk Neural Crest

<table>
<thead>
<tr>
<th>Cranial Neural Crest</th>
<th>Trunk Neural Crest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigment cells</td>
<td>Melanocytes</td>
</tr>
<tr>
<td></td>
<td>Xanthophores</td>
</tr>
<tr>
<td></td>
<td>Iridophores</td>
</tr>
<tr>
<td>Sensory systems</td>
<td>Spinal ganglia</td>
</tr>
<tr>
<td>Trigeminal nerve (V)</td>
<td>Vagal ganglia (X)</td>
</tr>
<tr>
<td>Facial nerve (VII)</td>
<td></td>
</tr>
<tr>
<td>Glossopharyngeal nerve (IX)</td>
<td></td>
</tr>
<tr>
<td>Vagal nerve (X)</td>
<td></td>
</tr>
<tr>
<td>Autonomic nervous system</td>
<td>Parasympathetic ganglia</td>
</tr>
<tr>
<td></td>
<td>Sympathetic ganglia</td>
</tr>
<tr>
<td></td>
<td>Adrenal medulla</td>
</tr>
<tr>
<td></td>
<td>Parasympathetic ganglia</td>
</tr>
<tr>
<td>Skeletal and connective tissues</td>
<td>Gill bars</td>
</tr>
<tr>
<td>Trabeculae</td>
<td>Walls of aortic arches</td>
</tr>
<tr>
<td>Parachordals</td>
<td></td>
</tr>
<tr>
<td>Odontoblasts</td>
<td></td>
</tr>
<tr>
<td>Membrane bones</td>
<td></td>
</tr>
<tr>
<td>Endocrine organs</td>
<td>Adrenal medulla</td>
</tr>
<tr>
<td></td>
<td>Calcitonin cells</td>
</tr>
<tr>
<td></td>
<td>Carotid body cells (Type I)</td>
</tr>
<tr>
<td></td>
<td>Parafollicular cells of thyroid</td>
</tr>
</tbody>
</table>

crest and neurogenic placodes are similar in that both (1) are derivatives of ectoderm, (2) migrate, and (3) form sensory neurons and special sense organs. Several key differences exist, however. Unlike neural crest, neurogenic placodes are initially restricted to the head, although some placodes may later migrate out onto the trunk (see section, “Sensory Systems and Nerves Derived from Neurogenic Placodes,” page 154). Neural crest can form motor neurons, but neurogenic placodes do not. Cells from neurogenic placodes can form sensory receptor cells, such as the olfactory epithelium or hair cells of the ear, but neural crest cells do not.

TABLE 4-3  Major Derivatives of Neurogenic Placodes

<table>
<thead>
<tr>
<th>Placode</th>
<th>Sensory Receptor Epithelium</th>
<th>Sensory Ganglion*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal placode</td>
<td>Olfactory epithelium</td>
<td>Profundal ganglion</td>
</tr>
<tr>
<td>Profundal placode</td>
<td></td>
<td>Geniculate ganglion</td>
</tr>
<tr>
<td>Geniculate placode</td>
<td></td>
<td>Anterodorsal lateral line ganglion</td>
</tr>
<tr>
<td>Anterodorsal lateral line placode</td>
<td>Induces taste buds</td>
<td>Anteroventral lateral line ganglion</td>
</tr>
<tr>
<td>Anteroventral lateral line placode</td>
<td>Supraorbital and infraorbital lateral lines and electroreceptor fields</td>
<td>Octaval ganglion</td>
</tr>
<tr>
<td>Otic lateral line placode</td>
<td>Mandibular lateral line and electroreceptors</td>
<td>Middle lateral line ganglion</td>
</tr>
<tr>
<td>Otic placode</td>
<td>Otic lateral line and electroreceptors</td>
<td>Membranous labyrinth</td>
</tr>
<tr>
<td>Middle lateral line placode</td>
<td>Middle lateral line</td>
<td>Posterior lateral line ganglion</td>
</tr>
<tr>
<td>Supratemporal lateral line placode</td>
<td>Supratemporal lateral line</td>
<td>Trunk ganglion</td>
</tr>
<tr>
<td>Trunk lateral line placode</td>
<td>Trunk line</td>
<td>Nodule ganglion</td>
</tr>
<tr>
<td>Petrosal placode</td>
<td>Induces taste buds</td>
<td></td>
</tr>
<tr>
<td>Nodose placodes(s)</td>
<td>Induces taste buds</td>
<td></td>
</tr>
</tbody>
</table>

*See Chapter 13 for additional details on these ganglia.

*This ganglion also known as the vestibulocochlear ganglion; see Chapter 13.
Fate maps of the neural crest and neurogenic placodes can be constructed using some of the methods described in the earlier section on fate mapping. For example, fate maps of neural crest cells in a chicken embryo are shown in Figure 4-22. Figure 4-22A shows the cranial neural crest that will form ectomesenchyme. In Figure 4-22B, the fate of the population of neural crest cells that will form sensory or autonomic ganglia is indicated. Pigment cells form from neural crest produced along the entire length of the neural tube; the fates of these pigment cells are not mapped in Figure 4-22.

Migration and Fates of Neural Crest Cells

Although they are derived from an epithelial layer, most neural crest cells transform into a loosely packed mesenchyme. Most mesenchymally organized cells in vertebrate embryos originate from mesoderm, but the neural crest is a primary source of mesenchymal cells in the head region. Mesenchyme of neural crest origin is called ectomesenchyme to reflect its derivation from the ectodermal neural crest. Ectomesenchyme participates in formation of many cranial structures (Table 4-2). Some neural crest cells remain close to their site.
of origin, but most migrate. Movements of neural crest cells can be influenced by local environmental conditions within the embryo. In the head, the streams of neural crest cells migrate ventrally around the developing eye and into the pharyngeal region (Figs. 4-23 and 4-24). These streams of neural crest cells contribute substantially to the branchiomeres, the serial structures in the pharyngeal wall that will give rise to branchial arches and many other cranial structures (see "Branchiomeres and Pharyngeal Organization," page 165).

Pathways of neural crest cell migration in the trunk of a vertebrate embryo are diagrammed in Figure 4-25. Many neural crest cells spread out between the surface ectoderm and the segmented somatic mesoderm to form dermal pigment cells. Other populations migrate a short distance and transform into spinal ganglion cells, which will send a short process into the wall of the neural tube and a longer process out into peripheral tissues (see Chapter 13 and Fig. 13-6). Some populations of neural crest cells migrate ventrally to form the sympathetic chain ganglia, a major portion of the autonomic nervous system of the trunk. Still others migrate between the neural tube and developing somites to reach the gut wall, where they form other components of the autonomic nervous system.

How do neural crest cells actually migrate through an embryo? First, the extracellular matrix of a vertebrate neurula-stage embryo consists chiefly of water and proteoglycans rich in hyaluronic acid, giving it a gelatinous consistency. Very little collagen is present at this stage. Thus, it is possible for an amoeboïd neural cell to migrate relatively easily through the extracellular spaces of the body. Second, it appears that at least some movements of neural crest cells can be guided by fibronectin "track ways" (Fig. 4-26). After neural crest cells adopt a mesenchymal organization, they decrease the number
**Figure 4.24**

Neural crest migration in the head of a generalized amniote (chick, Gallus) as seen in lateral view. A, An early stage of head development, in which the cranial neural crest (ectomesenchyme) still is located dorsally. B, A later stage of head development, when neural crest cells are migrating actively around the eye and down into the maxillary and mandibular branchiomes. The stream of hyoid crest migrates anterior to the ear; glossohypophyseal and vagal crest migrates posterior to the ear. The relationships between the streams of neural crest cells and the mesodermic somitomeres in the head region and the somites in the body also are indicated. (Based on Noden.)

**Figure 4.25**

Routes of migration of neural crest cells in the trunk of amniotes. This stereodiagram shows an idealized view of the routes of neural crest cells relative to the somites. In relation to a single somite, neural crest cells fated to form portions of the peripheral nervous system follow one of three paths. Path 1 carries the cells deep between adjacent somites or through the rostral half of each somite to form sympathetic chain ganglia or the adrenal medulla. Cells following Path 2 migrate between the sclerotome and overlying dermatome and will form portions of the sympathetic chain ganglia. Cells following Path 3 will form spinal (dorsal root) ganglia and sensory nerves for the skin and other somatic structures of the trunk. Pigment cells (short arrows) migrate out along the entire length of the neural tube. (Based on Le Douarin.)
FIGURE 4-26
Functional morphology of neural crest cell migration. A, Diagram showing delamination and migration of neural crest cells from the neural tube (left) to their reaggregation as organ rudiments (right). B, Corresponding levels of fibronectin and neural cell adhesion molecule (N-CAM) during the course of the migration. As the neural crest cells delaminate from the epithelium, they initially round up before assuming a stellate, migratory morphology. As they migrate through the body, they pass through regions rich in fibronectin. When fibronectin concentrations decrease, they stop migrating and reaggregate to form rudiments of ganglia and other structures. (Modified from Le Douarin.)

of neural cell-adhesion molecules (N-CAMs) on their cell membranes. N-CAMs provide a molecular way for cells to attach at their surfaces, so a decrease in the number of N-CAMs decreases their attachments to each other. In this phase of their migration, they are exposed to high levels of the extracellular matrix molecule, fibronectin. Other molecules on the surface of the neural crest cells recognize and bind to sites on fibronectin molecules. By binding to, and then releasing attachments to, fibronectin, the neural crest cells move through the extracellular matrix. A crude analogy might be hand-over-hand movement along a ladder or a series of bars. When the bars stop, that is, when the fibronectin concentration decreases, the neural crest cells stop moving. They then begin to express more NCAMs on their surfaces, adhere to each other, and form organ rudiments that have an epithelial organization.

Neural crest cells give rise to many tissues and structures (Table 4-2). These include the cartilaginous visceral arches in the pharyngeal wall, including the upper and lower jaws; much of the braincase; all pigment cells (except those of the retina of the eye); parts of the teeth and bony scales; portions of the heart; smooth muscle cells; the sensory neurons of all of the spinal nerves and many of the cranial nerves; large portions of the autonomic nervous system; endocrine organs, such as the adrenal medulla; and the myelinated cellular sheaths (Schwann cells) that surround most peripheral neurons. They also contribute to the connective tissue layers (meninges) that surround the brain and spinal cord. The diversity of neural crest derivatives also helps us make sense of several otherwise inexplicable medical syndromes, such as the frequent association of albinism with deafness or craniofacial malformations with heart defects. Neural crest cells not only contribute directly to many important structures but also appear to regulate many aspects of development. For example, as noted earlier in the discussion of epigenetics, neural crest cells participate in tooth formation by a reciprocal interaction with overlying ectoderm of the jaws. Neural crest is thus not only a key derived feature of vertebrates but also essential in organizing the vertebrate body plan.

Sensory Systems and Nerves Derived from Neurogenic Placodes
To frame this discussion, Figure 4-27 compares the developing nervous system of the spiny dogfish, Squalus, and the domestic chicken, Gallus. By this point in embryogenesis, the neural folds have completely closed along the entire length of the embryo, and the three primary brain regions have differentiated.

The position of neurogenic placodes on each side of the head also is indicated. Most of the neurogenic
placodes of special interest here give rise to specialized sensory receptor cells as well as to the nerves. For example, the nasal placode (Fig. 4-28) gives rise to the olfactory epithelium, and the nerves that grow out from this epithelium constitute the first cranial nerve (I). The fibers find their way to connections in the forebrain (Chapters 12 through 14). The terminal nerve also develops from the nasal placode. What the placodally derived sensory systems have in common is a network of sensory cells distributed in the skin or structures (e.g., the otic vesicle, which forms the inner ear) derived embryonically from the skin. General similarities also exist in the cell types associated with these sensory systems. For instance, in most cases, the sensory receptor cells are ciliated. According to this model, the inner ear is simply an in-folded piece of embryonic skin, an interpretation that is supported by its development.

Cells derived from dorsolateral neurogenic placodes form the otic vesicle and inner ear in all vertebrates and other important sensory systems in anamniotes, specifically, the lateral line mechanoreceptive and electroreceptive systems. Epibranchial neurogenic placodes (epibranchial placodes, for short) give rise to sensory nerves and ganglia for the chemoreceptive taste buds.

The details by which neurogenic placodes contribute cells to sensory systems are beginning to be un-
Figure 4-28
Ear formation and other aspects of head formation in a developmental series of skate embryos, *Raja erinacea*. A, Otic placode stage. B, Early otic pit stage. C, Otic pit stage. D, Late otic pit/early otic vesicle stage. E, Slightly later otic vesicle stage. F, Closing otic vesicle stage. Features of the branchiomeres and gill slits also are indicated.

...understood, particularly in axolotls (*Ambystoma mexicanum*). Essentially, subpopulations of placodal cells delaminate from the ectoderm and migrate as mesenchymal cells back toward the central nervous system. Sensory nerves and sensory ganglia associated with each of these sensory systems are formed from such placodally derived cells. Other subpopulations of placodal cells remain associated with the ectoderm, either staying where they formed or migrating before differentiation into sensory cells. Typically, sensory nerves...
derived from neurogenic placodes reach the brain stem in conjunction with cranial nerves derived from neural crest. For example, the **profundal placode** contributes sensory fibers to the profundal ramus of the trigeminal nerve (V₁), which enters the brain stem with the maxillary and mandibular rami of the trigeminal nerve (V₂, V₃; see Chapter 13).

By far the best studied of the dorsal series of neurogenic placodes is the **otic** (or octaval) placode, which gives rise to the otic vesicle and, eventually, the inner ear (Fig. 4-28). Like the other neurogenic placodes, the otic placode appears initially as a thickening of the ectoderm lateral to the rhombencephalon. The otic placode usually is apparent before the closure of the neural folds in the head region. The otic placode invaginates as an **otic pit** and eventually sinks beneath the overlying epithelium as the **otic vesicle**. Elasmo-branchs retain a duct, known as the **endolymphatic duct**, connecting the vesicle to the skin’s surface (see Chapter 12). Figure 4-29 shows the positions of the dorsolateral neurogenic placodes in a developmental series of a salamander (*Ambystoma*). Table 4-3 summarizes the different fates of the placodes diagrammed in Figure 4-29. In developing ambystomatid salamanders, it is easy to observe migration of the neurogenic placode that develops into the **main lateral line of the trunk**. This placode originates at the posterior end of the head and migrates as far back as the tail, “dropping off” small populations of cells en route. It is so large that this placode often can be seen even with a dissecting microscope as a small “bump” on the lateral surface of the trunk. Figure 4-30 shows the distribution of lateral line receptor organs, or **neuromasts**, in the skin of a juvenile axolotl (*Ambystoma mexicanum*). In addition to the array of neuromast organs that comprise the main lateral line, note the presence of lines of neuromasts on the head. Also note that fields of **ampullary organs**, specialized for electroreception, lie adjacent to the lines of neuromasts. Ampullary organs are restricted to the head in the axolotl and most other species of gnathostomes that have electroreception. On each side of the head, the cranial lines of neuromasts develop from five dorsolateral placodes. Three of these placodes originate anterior to the otic vesicle and elon-

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**FIGURE 4-29**
Simplified diagrams of neural crest and neurogenic placodes in lateral views of salamander embryos and larvae (*Ambystoma*) at different stages of development. The preotic lateral line placodes (indicated on the figure as a group to facilitate presentation at this scale) are anterodorsal lateral line placode, anterointernal lateral line placode, and otic lateral line placode (Table 4-3). The postotic lateral line placodes (indicated on the figure as a group to facilitate presentation at this scale) are the middle lateral line placode, supratemporal lateral line placode, and trunk lateral line placode (Table 4-3). The profoundal and epibranial placodes are not indicated. A, Stage 25. B, Stage 26-27. C, Stage 30. D, Stage 35. (Redrawn and modified from Stone; for more detailed descriptions of the neural crest and neurogenic placodes of Ambystoma, see Northcutt and Brändle, 1995.)
A. Skin of head

B. Skin of trunk

FIGURE 4-30
Distribution of neuromasts and electroreceptors in the skin of a juvenile axolotl. A, Skin of the head, cut in the dorsal and ventral midlines and flattened onto a glass slide. B, Skin of the trunk, prepared similarly. The lines of neuromast organs are shaded in blue. Note that the ampullary electroreceptors (orange) flank these lines. Pit organs shown in yellow are specialized mechanoreceptive components of the lateral line system. (Redrawn from Northcutt.)
gase (but do not migrate) to generate the supraorbital, infraorbital, and mandibular lines of neuromasts and their adjacent fields of electoreceptors. Behind the otic vesicle, two other dorsolateral placodes give rise to portions of the otic and supratemporal lines of neuromasts and adjacent fields of electoreceptors. Additional information concerning the structure, development, and function of these organs is included in Chapter 12.

**Development of the Coelomic Cavity and Mesenteries**

Early aspects of mesoderm differentiation were considered earlier (see page 140, "Mesoderm Differentiation"). This section traces the later development of the coelomic cavity that is defined by the wings of the lateral plate mesoderm. The definitive coelom is a space or network of spaces within the mesoderm that surrounds the visceral organs. It is lined by serosa, a thin membrane consisting of a simple squamous epithelium, which is called the coelomic epithelium, or mesothelium, and a thin layer of connective tissue beneath the mesothelium. The serosa secretes a small amount of watery, serous fluid into the cavity. Coelomic cavities allow room for the beating of the heart, changes in lung volume, the filling and emptying of the digestive tract, and other changes in the sizes and shapes of the organs. Thin membranes, the mesenteries, extend between the organs and from the organs to the body wall. Most mesenteries are identified by the prefix meso-, followed by the name of the organ to which they connect (e.g., the mesentery that extends between the dorsal body wall and the stomach is called the mesogaster; see later discussion for additional details). Some mesenteries are called ligaments (e.g., the falciform ligament extends between the ventral body wall and the liver). Although such ligaments are supporting structures, they differ both embryologically and structurally from the ligaments that link bones. Mesenteries consist of two layers of mesothelium with a thin layer of loose connective tissue between them. Mesenteries keep the organs in proper relationship to each other and provide passageways for ducts, blood vessels, and nerves going from one organ to another and between the organs and the body wall. Fat is stored in some mammalian mesenteries.

The coelom develops as a space within the part of the lateral plate mesoderm that extends from the level of the heart to the caudal end of the intestine (Fig. 4-31). As development continues, the splanchnic wall of the lateral plate mesoderm enfolds the gut and its derivatives (Fig. 4-31). These organs thus come to be surrounded by the coelom and are suspended by dorsal and ventral mesenteries formed by the coming together of lateral plate mesoderm dorsal and ventral to the gut tube. The liver diverticulum develops as a ventral outgrowth of the gut tube (Fig. 4-16). It grows into and expands within the ventral mesentery, dividing it into the lesser omentum that extends from the stomach and intestine to the liver, and the falciform ligament that extends from the liver to the ventral body wall (Fig. 4-31B). In a similar fashion, the dorsal pancreas grows into the dorsal mesentery. The heart develops by the fusion of a pair of blood vessels in the ventral mesentery just anterior to the liver (Fig. 4-31A; other aspects of heart development are traced in detail in Chapter 19). Continuous dorsal and ventral mesenteries are present early in development, but those supporting the heart, called mesocardia, as well as most of the ventral mesentery caudal to the liver are soon lost (Fig. 4-31C). Thus, the originally separate left and right halves of the coelom become continuous both anterior and posterior to the liver.

Most of the embryonic dorsal mesentery persists in adult vertebrates. The parts suspending different organs have different names. For example, the mesogaster supports the stomach. Parts of the dorsal mesentery may become complexly folded as the gut tube differentiates during later development. Additional mesenteries support the paired reproductive organs, which enlarge during development and "push" into the coelom from the dorsal body wall: a mesorchium goes to each testis; a mesovarium, to each ovary; and a mesotubarium, to each oviduct. The kidneys do not experience this same developmental "push" into the coelomic cavity but instead remain close to the body wall, plastered to it by the overlying peritoneal tissues. Thus, kidneys are described as retroperitoneal (= behind the peritoneal membrane).

In all vertebrates, a partition known as the transverse septum develops between the liver and the heart. It divides the coelom into an anterior pericardial cavity around the heart and a posterior pleuropertitoneal cavity around the abdominal viscera and lungs or swim bladder, if such organs are present (Fig. 4-32). Coelomic epithelium within the pericardial cavity is known as pericardium. The visceral pericardium covers the surface of the heart, whereas the parietal pericardium lies adjacent to the body wall (Fig. 4-32). The coelomic epithelium within the pleuropertitoneal cavity is called peritoneum, whereas that in the pleural cavities containing the lungs, when these cavities become distinct in amniotes, is known as the pleura.
Development of the Transverse Septum and Coelomic Divisions of Sharks

To visualize the development of the transverse septum, imagine the liver expanding laterally in the ventral mesentery (Fig. 4-33A) and carrying the coelomic epithelium that covers it laterally toward the body wall. When the liver touches the body wall (Fig. 4-33B), the visceral and parietal layers unite (Fig. 4-33C). Their unification forms a partition between the ventral parts of the pericardial and the pleuroperitoneal cavities. The liver grows caudally in the ventral mesentery during its subsequent enlargement, leaving the partition that it formed as the ventral part of the transverse septum (Fig. 4-33C). The ventral mesentery caudal to the liver disappears, but the liver remains connected to the transverse septum by a mesentery known as the coronary ligament, through which the large hepatic veins draining the liver enter the heart. Simultaneously, the paired common cardinal veins that carry blood from the dorsal part of the body wall to the heart push in medially from the dorsolateral parts of the body wall to the caudal end of the heart. They carry with them sheets of coelomic epithelium, which eventually form the dorsal part of the transverse septum. In sharks a small passage, the pericardioperitoneal canal, remains between the folds carrying the common cardinal veins. This passage interconnects the two parts of the coelom (Fig. 4-32A).
**FIGURE 4-32**
The coelom and its divisions in an idealized gnathostome, based on a shark (*Squalus*). *A*, Lateral view. *B*, Transverse section through pharynx and pericardial cavity.

**FIGURE 4-33**
The separation of the pericardial cavity from the pleuroperitoneal cavity is partly a by-product of the development of the transverse septum. However, the nearly complete separation of the two cavities also allows organs in one cavity to move independently of organs in the other cavity. For example, as we will discuss in Chapter 19, the presence of the pericardial cavity also allows the development of a reduced pressure around the fish heart, and this plays a role in the dynamics of blood circulation.

The heart and pericardial cavity of an adult shark are located far forward, ventral to the pharynx, and muscles of the body wall and the pharyngeal floor surround them (Fig. 4-32B). The heart thus is close to the gills, through which the heart must pump blood before it is distributed to the body. The transverse septum is located about the level of the pectoral girdle and lies in the transverse plane.

In some chondrichthians and bony fishes a pair of small abdominal pores lead from the caudal end of the pleuroperitoneal cavity to the cloaca. Their significance is uncertain, but they may represent an ancestral passage for sperm and eggs from the coelom to the outside. Gametes are discharged in this way in lampreys.

**Figures 4-34**
The coelom and its two divisions in a basal tetrapod, such as a salamander. A. Lateral view. B. Transverse section through pleural recesses and pericardial cavity.

**Modifications of the Coelom and Mesenteries of Tetrapods**
As in an adult shark, the heart and pericardial cavity of an embryonic tetrapod are located far forward in the body and ventral to the pharynx. During later development, however, the heart of tetrapods migrates caudally, closer to the lungs. The pericardial cavity thus comes to lie ventral to the anterior part of the pleuroperitoneal cavity, and the transverse septum assumes an oblique orientation, as seen in adult amphibians (Fig. 4-34A). The lungs are located ventrolateral to the digestive tract and its supporting mesenteries in the part of the pleuroperitoneal cavity overlying the pericardial cavity (Fig. 4-34B). The areas containing the lungs are called the pleural recesses. They frequently extend ventrally on each side of the pericardial cavity, thereby partly separating the pericardial cavity from the body wall (Fig. 4-34B). The membrane between pleuroperitoneal and pericardial cavities is homologous to the transverse septum of a shark, but the part of it separating the pleural recesses from the pericardial cavity often is called the pleuropericardial membrane.

In most living diapsids (some lizards, snakes, crocodiles, and birds) as well as all mammals, additional
folds of coelomic epithelium separate the paired pleural recesses from the rest of the pleuroperitoneal cavity. The coelom of these animals thus consists of four compartments: the pericardial cavity, two pleural cavities, and the peritoneal cavity (Fig. 4-35.A). The location of the lungs in distinct pleural cavities allows them to expand and contract independently of other organs.

In reptiles and birds, the folds separating the pleural cavities from the peritoneal cavity form the oblique septum. In mammals, the separation between the two pleural cavities and the peritoneal cavity develops by the pleuroperitoneal membranes, which push in from the dorsolateral body wall, and by other folds that extend laterally from the mesenteries and medially from the body wall to meet the pleuroperitoneal membranes (Fig. 4-35.A). Somatic muscles invade these membranes and part of the transverse septum separating the pericardial and peritoneal cavities to form the diaphragm (Fig. 4-35.A). This is the primary respiratory muscle of a mammal. Because these developmental processes occur early in organogenesis, when the pericardial cavity and heart are located far forward, the somatic muscles entering the diaphragm develop from somites in the cervical (neck) region. When these structures shift caudally later in development, branches of cervical spinal nerves that innervate the diaphragmatic musculature become greatly elongated and pass through the anterior part of the trunk as the phrenic nerves (Fig. 4-35.B). The enlarging lungs and pleural cavities of mammals grow laterally and ventrally to surround the pericardial cavity and heart, often meeting ventral to the pericardial cavity (Fig. 4-35.B). This effectively separates the pericardial cavity from contact with the body wall. In some mammalian species, the heart and pericardium also are separated from the diaphragm by a caudal and ventral growth of the lungs and pleural cavities. The
wall of the pericardial cavity thus consists of the pari-
etal pericardium and parietal pleura, with a thin layer
of conneting tissue sandwiched between them. This
combined wall often is called the **pericardium** or
**pericardial sac**.

Many organs lie between the pleural cavities of
mammals: the pericardial cavity and heart; the esoph-
agus; major arteries and veins; the phrenic and other
nerves; and, in embryonic and young mammals, the
thymus. The area between the two pleural cavities that
contains these structures is called the **mediastinum**.
The mesentery formed where the medial walls of the
two pleural cavities meet above and below these struc-
tures is the mediastinal septum. Other aspects of
mesentery formation and body cavities will be dis-
cussed in connection with the gut tube and its deriva-
tives (Chapter 17).

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**Basic Organization of the Vertebrate Head**

Embryogenesis of cranial structures is a major focus
in evolutionary morphology because the brain, major
sense organs, and many parts of the feeding and respira-
atory systems are located in the head. Also, the cran-
ial skeleton develops from several different sources,
which can be most easily understood from a develop-
mental perspective (see Chapter 7). Elasmobranchs
(sharks, skates, and rays) are convenient vertebrates in
which to study head development. In contrast to the
embryos of most bony fishes, which are typically small
and thus strongly curved around their yolk, elasmob-
branch embryos are large and have a “straight” pha-
ryngeal region that is easy to study (Fig. 4-36). In
embryos of amniotes (e.g., the chick) the develop-
ment of the pharyngeal region has been dramatically
altered from the basic plan seen in early vertebrates.
No amniotes retain gill slits as an adult, nor do they
develop the full complement of aortic arches, nor do
they have the lateral line mechanosensory and elec-
trosensory systems that are found in early vertebrates.
All three of these features are fundamental to under-
standing the plesiomorphic structure and organiza-
tion of the head, thus making elasmobranch embryos
the specimens of choice.

Figure 4-37 is a schematic lateral diagram of the
head of an idealized shark embryo, based on a fa-
mous illustration by an English comparative
anatomist and embryologist, Edwin S. Goodrich.
Goodrich was interested in detecting the role of seg-
mentation in patterning the structures of the verte-
brate head, and his interpretive drawing has influ-
enced generations of anatomists since its publication
in the 1930s. The basic arrangement of the cranial
nerves is particularly clear from his diagram, and it
will be a useful reference throughout your study of
vertebrate anatomy.

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**FIGURE 4-36**

Scanning electron micrograph
showing organization of the gill
arches in a skate embryo (**Raja
erinacea**). This specimen is at a
later stage of development than
the one shown in Figure 4-28F.
Note the well-developed eye;
the nearly complete series of
branchiomeratic and gill slits;
and the still small external gill
filaments forming on the hyoid,
glossopharyngeal, and vagal
branchiomeratic. The nasal
placode is beginning to
invaginate to form the nasal pit.
FIGURE 4-37
Semitransparent schematic view of vertebrate head development, based on a shark embryo. The brain is omitted. The diagram illustrates segmentation of the cranial somites and branchiomerces. Somites bear Arabic numerals and are innervated by cranial nerves III, IV, and VI. Branchiomerces located between the gill slits and including the jaws are innervated by cranial nerves V, VI, VII, IX, and X. The braincase and associated nasal and otic sensory capsules are indicated. The myotomes and somatic motor nerves of segments 4 and 5 degenerate and are indicated with dashed lines. (Based on a figure by Goodrich.)

Branchiomerces and Pharyngeal Organization

In many textbooks and in the primary literature, the terms “pharyngeal arch,” “visceral arch,” and “branchial arch” are used inconsistently. Noden (1991) considered the term “branchial arch” least prone to the misinterpretation that all of the cells forming the arches develop from the visceral tube. However, using the word “arch” leaves open the possibility of confusion with the aortic arches or the skeletal arch that forms within a “branchial arch.” Also, in adult fishes, “branchial arch” is used to refer to fully differentiated gill arches that carry gill filaments. In the absence of a single, wholly satisfactory term, we consider it best to refer to these embryonic structures collectively as the branchial segments, or branchiomerces (Bemis & Grande, 1992). This term branchiomerce reflects the obvious segmentation of the gill region while avoiding confusion about either the embryonic sources of their cells or the fully differentiated adult condition. By tradition and because they are good descriptors, we use
the terms visceral pouch or pharyngeal pouch to describe an outpocketing of the pharynx lying between two adjacent branchiomerces.

The basic pattern of the branchiomerces in a lateral view of an embryo of a skate (Raja) is apparent in Figure 4-36 (see also Fig. 4-28). Working from anterior to posterior, the mandibular, hyoid, glossopharyngeal, and vagal branchiomerces can be seen. Between each pair of branchiomerces are gill openings. These gill openings break through very early and never close in embryonic skates; this is different from the pattern in amniotes, in which gill openings are transitory structures if they develop at all.

An example of a transitory developmental feature in skates concerns the development and regression of external gills. Clear, functional reasons exist as to why external gills develop in many embryonic and larval fishes. In the case of the little skate, the embryonic period (from just after fertilization up to hatching from its vitelline membrane) and larval period are passed inside a proteinaceous capsule known as a mermaid's purse. The embryonic and larval periods can last more than 150 days, and at the end, a miniature adult breaks out of the mermaid's purse. How does efficient gas exchange occur within the confines of the mermaid's purse? In Figure 4-36, three small buds are visible on each of the postmandibular branchiomerces. Each of these buds will grow out to form a long, filamentous, capillary loop that serves to exchange respiratory gases. These external gills begin to develop as soon as the heart is sufficiently developed to circulate blood through the aortic arches. In order to have an efficient gas exchange surface, however, it is also necessary to move water over it. Larval skates generate a current by beating the tip of the tail to create a flow of aerated water through small openings in the egg case. This is functionally important because, in such a young larva, the development of the cranial muscles and skeleton is insufficient to allow for the generation of a respiratory current of water by the mechanism used in adults. About halfway through the developmental period, the mouth and relevant cranial skeletal muscular elements are fully functional, and it becomes possible to irrigate the gills by the normal buccal pumping mechanism used by adults (see Chapter 18). From this point on, the external gills regress until they are indistinguishable from the other gill filaments of the gill (i.e., the capillary loops that formed the external gills are retained as part of the regular series of gill filaments in an adult; see Pelster and Bemis, 1992).

A schematic diagram of a horizontal section through the pharynx of an elasmobranch embryo is shown in Figure 4-38. Note in Figure 4-38A that each branchiomere contains rami of the cranial nerve associated with that branchiomere, as well as branchiomatic muscle, a skeletal rod, and an aortic arch. In each branchiomere, the lateral surface is covered with ectoderm, and the medial surface is covered with endoderm.

The nomenclature of the branchiomerces in elasmobranchs is summarized in Figure 4-38B. The first two branchiomerces are so important in craniofacial development that they are given special names, the mandibular branchiomere and the hyoid branchiomere; these are also commonly referred to as the first arch and the second arch, respectively. Caudal to the hyoid branchiomere, each branchiomere may be best denoted by the name of its associated cranial nerve. Thus, the next in series is the glossopharyngeal branchiomere, followed by four or even five vagal branchiomeres (depending on the species of elasmobranch chosen for study). Branchiomeres also can be denoted by numbers, which correspond to the numbering system for their aortic arches (Fig. 4-38B). Note, however, that the numbering system for the cranial nerves does not match that of the aortic arches and branchiomerces. The trigeminal (Vth) nerve supplies the mandibular branchiomere. The facial (VIIth) nerve supplies the hyoid branchiomere. Branchiomere III is supplied by the glossopharyngeal (IXth) nerve, and branchiomeres IV to VII are supplied by rami of the vagal (Xth) nerve (Chapter 13).

In elasmobranchs, gill slits develop between each branchiomere and the one anterior to it (Figs. 4-36 and 4-38B). Each gill slit or pouch thus has the same number as the branchiomere immediately anterior to it, although these numbers are conventionally written as Arabic numerals instead of Roman numerals. The development of the gill slits is shown in the developmental series of skate embryos illustrated in Figure 4-28. Gill slit 2 is the first to open, followed by gill slit 3, and next by gill slit 1 (which receives the special name of spiracle). All but the last gill slit are open in the older skate embryo shown in Figure 4-36. The lack of strict anterior-to-posterior sequence in the opening of the gill slits is undoubtedly related to differences in size and function of the openings in adult elasmobranchs. Before each gill slit “breaks through,” we refer to the tissue that occupies the opening as the branchial membrane. The pocket on the medial side of each branchial membrane lined with endoderm is known as a pharyngeal pouch. Endodermal cells derived from these regions have many different fates, including formation of endocrine glands and organs of the immune system. These fates are traced in Chapter 15.

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5The post-trematic ramus of the appropriate nerve supplies the skeletal muscles of a branchiomere, so it is usually the largest nerve bundle in a branchiomere; see Figure 13-18C.
Organization of the Head in Amniote Embryos

Head development in amniote embryos differs in some important ways from what we have seen in elasmobranchs, and as already noted, elasmobranchs are far better for understanding plesiomorphic features of cranial organization. Much more research, however, has been conducted on amniote embryos, particularly chickens (Gallus) and mice (Mus). Figure 4-39 presents an idealized schematic diagram of the cranial organ systems in an amniote embryo, redrawn from...
A. Expression domains of Hox-b genes

B. Neural tube, notochord, optic vesicle and otic vesicle

C. Neural crest

D. Paraxial mesoderm

E. Heart and aortic arches

F. Veins

G. Pharyngeal pouches, thyroid gland and Rathke's pouch
the elegant synthetic work of Drew Noden (1991). Using gray tic lines to demarcate branchiormeric boundaries, the diagram presents major cranial tissues in registration with each other and with the expression domains of Hox genes known to play a role in cranial patterning (Fig. 4-39A).

Figure 4-39B shows dorsal axial structures, including the neural tube and notochord. The prosencephalon, mesencephalon, and rhombencephalon (the three primary brain regions) are labeled, and the seven rhombomeres in the wall of the hindbrain are numbered. The paired otic and optic vesicles also are shown; these are easily detected reference points when comparing embryonic vertebrates. The anterior tip of the notochord is another important reference point for comparing vertebrate embryos. It also marks the beginning of the “new head” postulated by Northcutt and Gans (1983; see Chapter 2). The hypophyseal lamina immediately anterior to the notochord; its location is demarcated in this diagram by the infundibulum, which contributes to formation of the hypophysis (see Fig. 15-4A).

Figure 4-39C diagrams the cranial neural crest migrating ventrally into the branchiormeres, in movements already introduced in Figure 4-24B. The many different fates of these cells were briefly discussed earlier and are summarized in Table 4-2.

Figure 4-39D shows the organization of paraxial mesoderm in the head. The anterior portion of the paraxial mesoderm is incompletely divided in amniote embryos. Instead, it consists of a series of seven somitomeres, which can be detected using scanning electron microscopy to study carefully dissected embryos. Somitomeres are transient, mesenchymally organized structures, which, unlike true somites, never become “epithelialized” and thus never contain somitocoele cavities. Note that the series of seven somitomeres in amniotes is numbered independently from the somites in the trunk. This convention differs from the standard nomenclature used for elasmobranch embryos, which is shown in Figure 4-37. This is because anterior portions of the paraxial mesoderm of elasmobranchs subdivide into well-developed, epithelially organized somites containing somitocoeles (somites 1–3 in Fig. 4-37). It has proved difficult for researchers to agree on a single alignment and consistent numbering system equating the anterior cranial somites of elasmobranchs with the somitomeres of amniotes. Despite this and many differences in their organization, cells derived from the paraxial mesoderm are thought to have similar fates in elasmobranchs and amniotes. Much of the paraxial mesoderm forms skeletal muscles of the head, including the extrinsic eye muscles and muscles of the branchiormeres. As determined by Noden (1991) and shown in Figure 4-39D, amniote somitomeres 4, 6, and 7, along with several anterior somites, contribute to branchiormeric muscles in branchiormeres I (mandibular), II (hyoid), and III (glossopharyngeal). Somitomeres 1 to 3 and 5 contribute to the extrinsic ocular muscles (see Fig. 13-20).

The major cranial blood vessels are diagrammed in Figure 4-39E and F. The numbering system for aortic arches used for elasmobranchs (Fig. 4-38B) also applies to amniotes, but because amniotes develop fewer vagal branchiormeres, they consequently have fewer aortic arches. Also, the fifth aortic arch never forms in amniotes, and so it is indicated with a dashed line (Fig. 4-39E). Major cranial veins, such as the anterior cardinal vein, develop by fusions of a plexus of smaller vessels. As in the trunk, cranial blood vessels derive from mesoderm. The precursor cells, known as angioblasts, migrate early from their sources and move invasively throughout the head mesenchyme. Angioblasts differentiate into the lining cellular layer of the blood vessels, which is known as endothelium. In contrast to veins, arteries tend to develop by branching of existing vessels, a process known as angiogenesis. This difference in their mode of formation helps to explain why veins are anatomically more variable than arteries.

The paired pharyngeal pouches are shown in Figure 4-39G. As in the scheme shown for elasmobranchs in Figure 4-38, the pharyngeal pouches are numbered with Arabic numerals. The rudiment of the thyroid

**Figure 4-39**
Anatomical relationships among the components of the head in an amniote embryo in lateral view. The diagram is comparable to a stage 14 chick (45 hours, 22 somites). Light gray lines demarcate the approximate boundaries of branchiormeres I, II, III, IV, and V. A, Expression domains of genes of the Hox-b cluster. B, Axial structures including the neural tube and notochord. C, Neural crest migrating ventrally into the branchiormeres. D, Paraxial mesoderm, incompletely divided into somitomeres anteriorly and completely divided into somites in the trunk; somitomeric contributions to branchiormeres I, II, and III also are indicated. E, Heart and aortic arches. F, Major veins. G, Pharyngeal pouches and Rathke’s pouch. Amniote embryos have four paired pharyngeal pouches lined with endoderm; in all gnathostomes, Rathke’s pouch forms as a single median diverticulum of ectoderm just anterior to the oral plate. (Modified from Noden.)
gland originates as a median ventral diverticulum from the floor of the pharynx between the mandibular and hyoid branchiomerres. It extends caudally during later development and ceases to be connected to the pharynx. The developing lung also originates as a median ventral diverticulum; its retained connection to the gut tube will become the trachea. Rathke's pouch (Fig. 4-39C) is a median dorsal diverticulum of ectoderm that forms just anterior to the oral plate, the region known as the stomodeum. As it extends dorsally, Rathke's pouch comes to lie adjacent to the infundibulum, which is a ventral outpocketing of the neural tube (Fig. 4-39B). Rathke's pouch and the infundibulum together form the master endocrine gland, the hypophysis. The dual embryonic origins of the hypophysis continue to be reflected in the types and mechanisms of hormone production and secretion used by its component parts (see Chapter 15, especially Fig. 15-4).

**Hox Genes, Segmentation, and the Evolution of the Vertebrate Body Plan**

The connection between development and evolution has drawn the attention of biologists and philosophers for more than a century. The actively growing field of evolutionary developmental biology examines connections between embryology and evolution using tools from molecular biology, phylogenetics, and comparative anatomy. It has long been thought that detailed knowledge of DNA regulatory genes, which are those that control expression of other genes, will prove essential to understanding major features of vertebrate evolution. Perhaps 10% of the genome of a vertebrate consists of DNA regulatory genes, and we still have much to learn about all aspects of the story. To illustrate the type of synthesis that may emerge, we discuss Hox genes and their role in the development of the vertebrate body plan.

A segment is a set of body parts that is present in a repeated series in an embryo or adult. Segmental organization is fundamental to the vertebrate body plan, and all vertebrates are more or less obviously segmented during their development. Segmentation is most obvious in embryonic stages but often becomes masked in adults, particularly in the cranial region. Nevertheless, we have seen that the head displays several types of interrelated segmentation. Branchiomerres provide clear evidence of segmental organization, as do the rhombomeres of the hindbrain and other components of the head (Fig. 4-39B). Cranial neural crest cells migrate ventrally to contribute to the branchiomerres, following the same segmental pattern (Fig. 4-39C). The paraxial mesoderm that lies to each side of the developing brain is also segmentally organized, whether as true somites found in elasmobranch embryos (Fig. 4-37) or as the transient somitomeres of amniotes (Fig. 4-39D).

The connection between development and evolution can be demonstrated by considering the role of Hox genes in regulating the development of such segmented structures. A series of these genes or their homologues is expressed differentially along the length of the body axis; this differential expression pattern helps establish the basic pattern of the segments and instruct the subsequent development of segment-specific features. In this way, Hox genes can be thought of as generating unique tags for specific locations in the body.

Hox genes code for helix-turn-helix transcription factors, which are DNA binding proteins (Fig. 4-40A and B). These proteins contain variable regions (green in Fig. 4-40) and highly conserved regions (red in Fig. 4-40). They fold in such a way that their tertiary structure consists of two helical regions separated by a short, variable “turn” region. The very highly conserved sequence of about 60 amino acids, known as the homeodomain, forms a recognition helix that can be presented to the major groove in the double-helical DNA molecule (Fig. 4-40B).

The portion of a Hox gene that codes for the homeodomain portion of the transcription factor is called a homeobox. Many families of genes contain homeoboxes. Thus, not all genes containing homeobox sequences are Hox genes, but these are the best known genes in this class. Wherever homeobox sequences occur in the genome, they code for remarkably similar amino acid sequences. These sequences also are preserved across remarkable phylogenetic gaps: 59 of the 60 amino acid residues in some homeodomains of vertebrates and fruit flies are identical. Such evolutionary conservation is an outstanding example of the tight link between structure and function at the molecular level because, if the homeodomain portion of the transcription factor does not fit precisely into the major groove of the DNA double helix, then it cannot function. The highly conserved homeodomain region, however, is just one part of the transcription factor. The remaining regions of the protein can be of variable length and differ from one Hox protein to the next. It is the regions outside the homeodomain that allow the transcription factor to bind with other specific factors in order to recognize specific genes along a DNA strand (Fig. 4-40C–E). By means

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6By convention, the word Hox is italicized to indicate a DNA sequence or gene; when not italicized, the word Hox indicates an amino acid sequence or protein.
of their variable regions, different Hox proteins can regulate expression of different genes.

Proteins made by a particular Hox gene are found inside cells in a restricted and specific set of segments of the body. The places where a particular gene is expressed are known as that gene’s expression domain. By regulating the expression of other genes, Hox genes determine the features that are characteristic of each body segment. This is the result of unique expression domains or unique combinations of overlapping expression domains. Perturbation experiments, known as “knockout” experiments, involve the deletion or inactivation of a particular gene, usually by mutation. A Hox gene that has been knocked out may result in a specific segmentation defect or defects; defects do not necessarily result because other genes can sometimes
take over the role of the missing gene. In contrast, the insertion of an extra Hox gene can result in the formation of additional segmental structures.

The Hox genes of gnathostomes are organized in four clusters, each of which is located on a separate chromosome (Fig. 4-40F). Each cluster consists of as many as 13 paralogous subgroups. The presence of these paralogous subgroups appears to be the result of tandem duplications of Hox genes that occurred prior to the common ancestry of arthropods and vertebrates. It is possible to align the Hox genes of one cluster with those of other clusters based on sequence similarity. We follow the nomenclature of Scott (1992), Krumlauf (1994), and others in naming the genes by their cluster and sequence order from the 3’ to 5’ end of the DNA.

A parologue is a copy of a gene within the same genome.

The opposite ends of a single strand of DNA are referred to as 3’ and 5’. At the 3’ end, a hydroxyl group is attached to the third carbon in the sugar (ribose) ring. At the opposite end of the DNA strand, the fifth carbon atom is joined to a phosphate group. This convention of naming genes in order from 3’ to 5’ ends is used because the normal direction of mRNA transcription proceeds from the 3’ end of a gene toward the 5’ end.

Some Hox genes have been lost in the evolutionary lines that lead to living amniotes, which is why genes such as Hox-a8 or Hox-d2 are missing from Figure 4-40F.

Within each cluster, the Hox genes occur in a highly conserved, linear order corresponding to their anterior-to-posterior expression domains along the body axis. This phenomenon is known as colinearity. Although each individual gene is transcribed from the 5’

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**Figure 4-41**
Phylogenetic tree of chordates showing position of Hox gene cluster duplications by Holland et al. (1994), and the subsequent losses of individual Hox genes proposed by Meyer (1998). Based on analyses of Hox genes in amphioxus, hagfish, lampreys, basal osteichthyans, and amniotes. Genes shown with dashed lines have been lost. (Modified from Holland et al. and Meyer.)
to 3' direction, those that lie toward the 3' end of each cluster are transcribed earlier in development than those nearer the 5' end. Because the *Hox* genes are expressed sequentially in this linear order, they can establish the pattern of segmentation along the anteroposterior axis of the embryo. Thus, for example, we can recognize a boundary between *Hox* genes expressed in tissues of the hindbrain and those expressed in the trunk.

Colinearity between the DNA transcription level and the appearance of segmentation ranks as one of the most intriguing aspects of the story of the *Hox* genes. For example, *Hox-b1*, *Hox-b2*, *Hox-b3*, *Hox-b4*, and *Hox-b5* are arranged along the DNA molecule in an order that closely corresponds to their pattern of expression in tissues of the head, with the boundaries of their expression domains corresponding to boundaries between rhombomeres (Fig. 4-39A). *Hox-b2* is expressed from rhombomere 3 caudally into the trunk, *Hox-b1* is expressed in alignment with rhombomere 4, *Hox-b3* is expressed from rhombomere 5 caudally into the trunk, *Hox-b4* is expressed from rhombomere 7 caudally into the trunk, and *Hox-b5* is expressed posterior to all of the rhombomeres (Fig. 4-39A). Within this series, note that only *Hox-b1* fails to follow strictly the colinear pattern between cluster order and expression domain.

The occurrence of homologues of *Hox* genes in taxa such as the fruit fly, *Drosophila*, supports the notion that these genes first evolved at least 600 million years ago in flatworms or other early bilateral metazoans. Their truly remarkable constancy suggests that once the molecular system for determining the anteroposterior axis of animals was established, it was retained throughout evolutionary history and has served as the basis for the establishment of diverse body plans in animal phyla.

Comparative studies on *Hox* gene clusters suggest that two rounds of gene duplication occurred in the line leading to craniates, with the first round of duplications between amphioxus and craniates, and the second between hagfishes and gnathostomes (Fig. 4-41). *Hox* genes also were lost in some lineages leading to extant vertebrates. It is tempting to attribute the great diversification of craniates to these duplications, for gene duplication makes it possible for copies of particular genes to evolve rapidly without impairing any existing functions. Then, at a later time, some altered duplicate sequences might be “recaptured” for use in generating new phenotypes. The comparative analysis of *Hox* genes has become an extremely exciting area for research because it combines genetics, development, and systematics in the search for explanations in vertebrate evolution.

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**Summary**

1. Gametes are haploid cells that contain only a single set of chromosomes. Sperm are small, motile gametes that are capable of reaching and penetrating an egg. Eggs are much larger, nonmotile gametes that contain the metabolic reserves and information molecules needed to initiate development. Materials in an egg are not distributed randomly. The amount of yolk in the egg correlates with the future pattern of nutrition of the embryo or larva.

2. Fertilization is the union of a haploid sperm with a haploid egg to form a diploid zygote. It involves several events: sperm penetration of the egg, combination of male and female nuclear material, and activation of the egg.

3. During cleavage, the single-celled zygote is converted to a multicellular embryo called a blastula, but no growth occurs. Because the materials in the egg were not distributed evenly, the cells (blastomeres) in different parts of the blastula contain different enzymes, mRNAs, and other components. As a result, the blastomeres differ in their presumptive fates.

4. During gastrulation, the single-layered blastula is converted into a two-layered gastrula. The gastrula is covered by ectoderm and has a simple gut cavity, the archenteron, which is lined by endoderm and opens to the surface posteriorly at the blastopore. Mesoderm spreads between the ectoderm and endoderm. Patterns of gastrulation and mesoderm formation are greatly influenced by the amount of yolk and vary considerably in different groups of craniates.

5. Formation of the neural tube is induced by the underlying chordamesoderm; the central nervous system develops from the neural tube.

6. In addition to the neural tube, vertebrates have two other neurogenic tissues: the neural crest and neurogenic placodes.

7. Ectodermal neural crest cells separate during the formation of the neural tube and spread throughout the embryo. They give rise to many structures (i.e., branchial skeleton, other cranial bones, pigment cells, many peripheral neurons, parts of teeth, and bony scales) and also help regulate development and the emergence of the
vertebrate body plan by patterning mesoderm and ectoderm.

8. Neurogenic placodes give rise to sensory epithelia, nerves innervating sensory epithelia, and other components of the cranial nerves. Major derivatives include the nose, ear, and lateral line system (including electroceptors in many groups of vertebrates).

9. Amniotes have lost the neurogenic placodes associated with the lateral line and electroreceptive systems and lack these sensory systems as adults.

10. The mesoderm differentiates into a series of segmental somites that lie lateral to the neural tube and notochord, a nephric ridge, and a lateral plate that spreads around the archenteron and yolk. The coelom develops by cavitation within the lateral plate, but it may enter the nephric ridge and somites.

11. Each somite breaks up into three regions. Laterally, the dermatome becomes reorganized as a mesenchyme, and its cells spread out beneath the ectoderm to form the dermis of the skin. Myotome gives rise to all or most of the somatic muscles of the body. Medially, the sclerotome reorganizes as a mesenchyme, and its cells migrate around the neural tube and notochord to form most of the axial skeleton.

12. The nephric ridge gives rise to the kidney tubules and the urinary and genital ducts.

13. The coelom divides the lateral plate mesoderm into an outer somatic and an inner splanchnic layer. The somatic layer forms the parietal peritoneum and contributes to the somatic muscles in many species. The splanchnic layer forms the connective tissue and visceral muscles of the gut and the heart walls and visceral peritoneum.

14. The yolk of macrolecithal eggs becomes suspended from the embryo in an extraembryonic membrane known as the yolk sac. The yolk sac is trilaminar in actinopterygian fishes, including all three germ layers, but is bilaminar in reptiles and birds, consisting only of the endoderm and splanchnic mesoderm.

15. The ectoderm and somatic mesoderm rise off the yolk in amniotes to form additional extraembryonic membranes. The amnion protects the embryo in a cushion of water, and a protective chorion surrounds the amnion, embryo, and yolk sac. Albumen, a shell membrane, and a shell, all of which are secreted by the oviduct, lie peripheral to the chorion.

16. The last extraembryonic membrane of amniotes, the allantois, extends into the extraembryonic coelom. It functions in gas exchange and serves as a site for the accumulation of excretory products. Its base may remain as the urinary bladder.

17. The development of eutherian mammals is similar to the pattern seen in birds except for the secondary reduction of yolk, the simplification of cleavage, and the precocious development of those extraembryonic membranes that contribute to the placenta. The placenta is composed of the embryonic chorioallantoic membrane and part of the maternal endometrium.

18. The definitive coelom is a space lined with epithelium in the lateral plate mesoderm. It surrounds the visceral organs and allows their functional movements.

19. Mesenteries consist of a double layer of coelomic epithelium. These membranes hold the visceral organs in place and provide routes for the passage of ducts, blood vessels, and nerves from the body wall to the organs and between the organs.

20. Early in development, a left and a right coelom are separated from each other by continuous dorsal and ventral mesenteries. Much of the ventral mesentry later disappears caudal and cranial to the liver.

21. The coelom of a fish becomes divided into an anterior pericardial cavity and a posterior pleuropitoneal cavity by a transverse septum, through which blood vessels enter the heart.

22. The heart and pericardial cavity of tetrapods such as amphibians shift caudally, from a position beneath the gills closer to the lungs. As a consequence, the pericardial cavity underlies the cranial part of the pleuropitoneal cavity, and the transverse septum assumes an oblique orientation.

23. In some reptiles and in birds and mammals, folds separate the portions of the pleuropitoneal cavity containing the lungs from the part containing the other visceral organs. The coelom of these tetrapods is thus divided into two pleural cavities—a peritoneal cavity and a pericardial cavity.

24. In mammals, the pleuropitoneal folds and the ventral part of the transverse septum are invaded by somatic musculature of cervical origin and form the diaphragm.

25. The pleural cavities of mammals extend ventrally during development, separating the pericardial cavity from the body wall. The area between the pleural cavities, which contains the pericardial cavity and other organs, is known as the mediastinum.

26. Many important landmarks in the head and pharynx can be recognized in embryonic vertebrates. Many of the patterns observed are conserved across vertebrate history, but some features, such as the patterning of cranial mesoderm or the occurrence of neurogenic placodes that contribute to the lateral line system, show marked differences between elasmobranchs and amniotes.
27. The expression of segmental structures in the hindbrain of vertebrates is regulated by clusters of Hox genes, which code for helix-turn-helix transcription factors. Homologous genes also occur in many groups of invertebrates. The homeobox region of a Hox gene codes for the large helix of the transcription factor, which fits into the major groove of a DNA double helix. Homeobox sequences are remarkably conserved across Metazoa. This transcription factor system must have evolved at least 600 million years ago, before arthropods and vertebrates diverged.

28. Hox genes of gnathostomes are basically organized as four clusters, a through d (variations exist that were not discussed in this chapter). The genes within a cluster follow a strictly conserved order along the DNA. The term colinearity describes the close association between a Hox gene's position within a cluster and its expression domain along the anterior-to-posterior axis of the embryo.

29. It has been proposed that two major rounds of gene duplication occurred early in vertebrate history. The duplications of regulatory genes, such as Hox genes, may have allowed rapid evolutionary change in vertebrates because the copies would be free to accumulate mutations silently before being "recaptured" for use by descendant taxa.

REFERENCES


