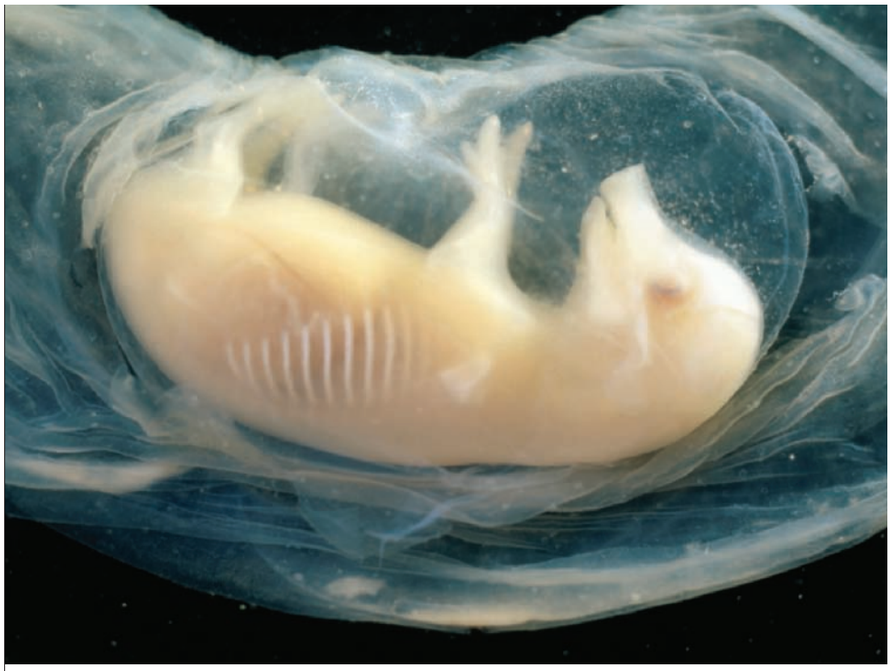


Daniel Sambraus/SPL/Photo Researchers, Inc.

Embryo pig *(Sus scrofa domestica)* after 33 days of development. The embryo is about 16 mm long and is surrounded by several membranous sacs, including the fluid-filled amnion (closest to the embryo), which cushions and protects it.

Study Plan

48 Animal Development

Why It Matters

The uterine contractions announcing birth are taking place at shorter intervals and with greater intensity. The mother-to-be endures the discomfort and apprehension with the knowledge that the child that has been growing in her body will soon come into the world. It began as a fertilized egg, about the size of a period on this page, and grew through a program of cell divisions, complex cell movements, and molecular interactions. She was unaware of these complexities except for movements of the fetus that became apparent about 14 weeks after she became pregnant.

Her baby's development required no conscious attention on her part: human development, like that of all animals, is programmed to proceed inexorably from fertilized egg to free-living offspring. Even childbirth is the result of programmed events that, once started, normally move to conclusion without requiring deliberate input from the mother.

Over the course of its development, the baby's body formed all the organ systems required for independent existence, and at its birth they are already working to sustain its life. Most astonishing, per-

haps, is the baby's brain. It began as a tube of nerve tissue that bulged outward and enlarged, continually adding nerve cells and connecting them into circuits until it attained what may well be some of the most complexly organized matter in the universe—all as part of the automated events of development. Still, the human brain is unique only in the degree of its complexity and integrative capacity; the brains of other mammals are basically similar and develop through the same embryonic pathways.

The baby enters the outside world passing head first through the cervix, and then the vagina. Soon the rest of the body slips through, aided and lubricated in its passage by release of the fluid that surrounded and cushioned it in the uterus. In the first indignity of life, the baby is briefly held upside down to drain fluid from its lungs. This action triggers its first breath, followed by a satisfyingly loud cry.

The baby is proudly displayed to the mother, who greets it with love, relief, joy, and realization of the responsibilities the baby will bring. It is a girl, who with further luck and good care will continue developing through childhood, puberty, adult life, and old age, all through programs built into her hereditary molecules. As part of these passages, she may bring her own child into the world.

People have tried since ancient times to understand how development and birth take place. The scientific quest began with Aristotle, who observed chick development and correctly interpreted the functions of the placenta and umbilical cord in humans. The investigators who followed Aristotle concentrated on describing developmental changes in **morphology**, which is the form or shape of an organism, or of a part of an organism. More recently, investigators began to trace the molecular underpinnings of the morphological events.

In this chapter we survey the results of these investigations. We take up the story of animal development where the previous chapter left off, with the fertilized egg. We continue with the early events leading from the fertilized egg to the primary tissues of the embryo, and then trace the development of organs from these tissues. Next, we describe human development as representative of the process in mammals. Then, we survey the cellular and molecular bases of these mechanisms. At the cellular level, the development of an adult animal from a fertilized egg involves cell division, in which more cells are produced by mitosis; **cell differentiation**, in which changes in gene expression establish cells with specialized structure and function; and **morphogenesis** ("form creation"), the generation of the body form of the animal as differentiated cells end up in their appropriate sites. Finally, we discuss the genetic and molecular mechanisms that are largely responsible for directing the course of development.

48.1 Mechanisms of Embryonic Development

Fertilization of an egg by a sperm cell produces a zygote. Embryonic development begins at this point and ultimately produces a free-living individual. All the instructions required for development are packed into the fertilized egg.

Developmental Information Is Located in both the Nucleus and Cytoplasm of the Fertilized Egg

Mitotic divisions of the zygote formed when egg and sperm nuclei fuse are the beginning of developmental activity (see Section 47.2).

Information Storage in the Egg. The information that directs the initiation of development is stored in two locations in the fertilized egg. Part of the information is stored in the zygote nucleus, in the DNA derived from the egg and sperm nuclei. This information directs development as individual genes are activated or turned off in a highly ordered manner. The rest of the information is stored in the egg cytoplasm, in the form of messenger RNA (mRNA) and protein molecules.

Because the fertilizing sperm contributes essentially no cytoplasm, nearly all the cytoplasmic information of the fertilized egg is maternal in origin. The mRNA and proteins stored in the egg cytoplasm are known as **cytoplasmic determinants**. They direct the first stages of animal development, in the period before genes of the zygote become active. Depending on the animal group, the control of early development by cytoplasmic determinants may be limited to the first few divisions of the zygote, as in mammals, or it may last until the actual tissues of the embryo are formed, as in most invertebrates.

Other Components of the Egg. In addition to cytoplasmic determinants, the oocyte cytoplasm also contains ribosomes and other cytoplasmic components required for protein synthesis and the early cell divisions of embryonic development. For example, the egg cytoplasm contains all the tubulin molecules required to form the spindles for early cell divisions. It also contains mitochondria, nutrients stored in granules in the yolk and in lipid droplets, and, in many animals, pigments that color the egg or regions of it.

The **yolk** contains nutrients. When the egg itself supplies all the nutrients for development of the embryo, as in the eggs laid by insects, reptiles, and birds, it contains large amounts of yolk. When the mother supplies most of the nutrients, as in the placental mammals, the egg has a small quantity of yolk that is used only for the earliest stages of development.

Depending on the species, the yolk may be concentrated at one end or in the center of the egg, or distributed evenly throughout the cytoplasm. Its distribution influences the rate and location of cell division during early embryonic development. Typically, cell division proceeds more slowly in the region of the egg containing the yolk. In the large, yolky eggs of birds and reptiles, cell division takes place only in a small, yolk-free patch at the surface of the egg.

Unequal distribution of yolk and other components in a mature egg is termed **polarity.** For example, in most species the egg nucleus is located toward one end of the egg. This end of the egg, called the **animal pole,** typically gives rise to surface structures and the anterior end of the embryo. The opposite end of the egg, the **vegetal pole,** typically gives rise to internal structures such as the gut and the posterior end of the embryo. Yolk, when unequally distributed in the egg cytoplasm, is most frequently concentrated in the vegetal half of the egg. The egg's polarity contributes to the generation of body axes. For example, egg polarity plays a role in setting the three body axes of bilaterally symmetrical animals (such as humans and dogs): the anterior–posterior axis, the dorsal–ventral (back–front) axis, and the left–right axis **(Figure 48.1).**

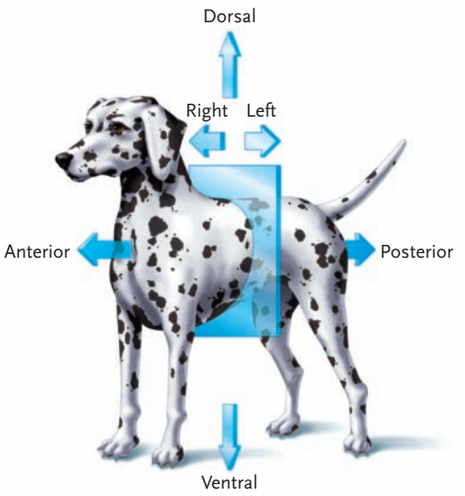


Figure 48.1
Body axes: anterior–posterior, dorsal–ventral, and left–right.

Cleavage, Gastrulation, and Organogenesis Are Early Events in Development

Soon after fertilization, the zygote begins a series of mitotic **cleavage** divisions, so called because cycles of DNA replication and division occur without the production of new cytoplasm. As a result, the cytoplasm of the egg is partitioned into successively smaller cells without increasing the overall size or mass of the embryo **(Figure 48.2).** These cells are called **blastomeres** (*blastos* = bud or offshoot; *meros* = part or division). In the frog *Xenopus laevis,* for example, a sequence of twelve cleavage divisions produces an embryo of about 4000 cells, which collectively occupy about the same volume and mass as did the original zygote.

Cleavage is the first of three major developmental processes that, with modifications, are common to the early development of most animals (described in detail for particular animals in the next section). Following cleavage, the second major process, **gastrulation,** produces an embryo with three distinct primary tissue layers. Following gastrulation, the development of the major organ systems, called **organogenesis,** gives rise to a free-living individual with the body organization characteristic of its species. Organogenesis involves the same mechanisms used in gastrulation—cell division, cell movements, and cell rearrangements. **Figure 48.3** outlines these stages in the life cycle of a frog.

The cleavage divisions lead to three successive developmental stages that are common to the early development of most animals. The first stage, called a **morula** (*morula* = mulberry), is a solid ball or layer of blastomeres. As cleavage divisions continue, the ball or layer hollows out to form the second stage, the **blastula** (*ula* = small), in which the blastomeres enclose a fluid-filled cavity, the **blastocoel** (*koilos* = hollow).

Once cleavage is complete, the cells of the blastula migrate and divide to produce the **gastrula** (*gaster* = gut or belly). This morphogenetic process, gastrulation, dramatically rearranges the cells of the blastula into the three primary cell layers of the embryo: the outer **ectoderm** (*ecto* = outside; *derma* = skin), the inner **endoderm** (*endo* = inside), and the **mesoderm** (*meso* = middle) between the ectoderm and the endoderm. Gastrulation establishes body pattern; that is,

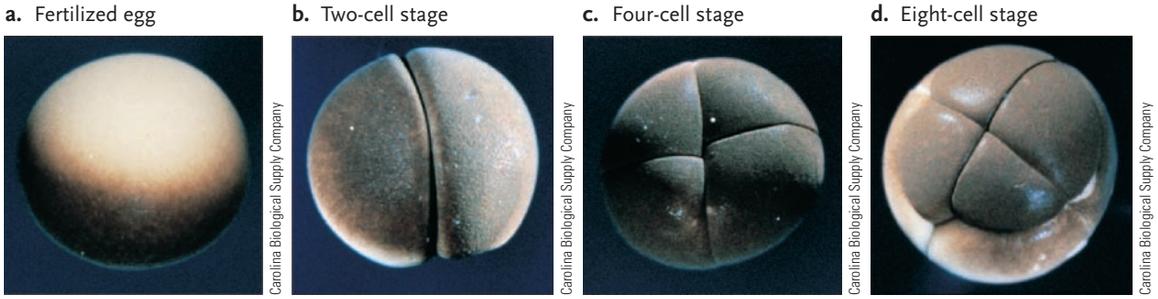


Figure 48.2
The first three cleavage divisions of a frog embryo, which convert the fertilized egg into the eight-cell stage. Note that the cleavage divisions cut the volume of the fertilized egg into successively smaller cells.

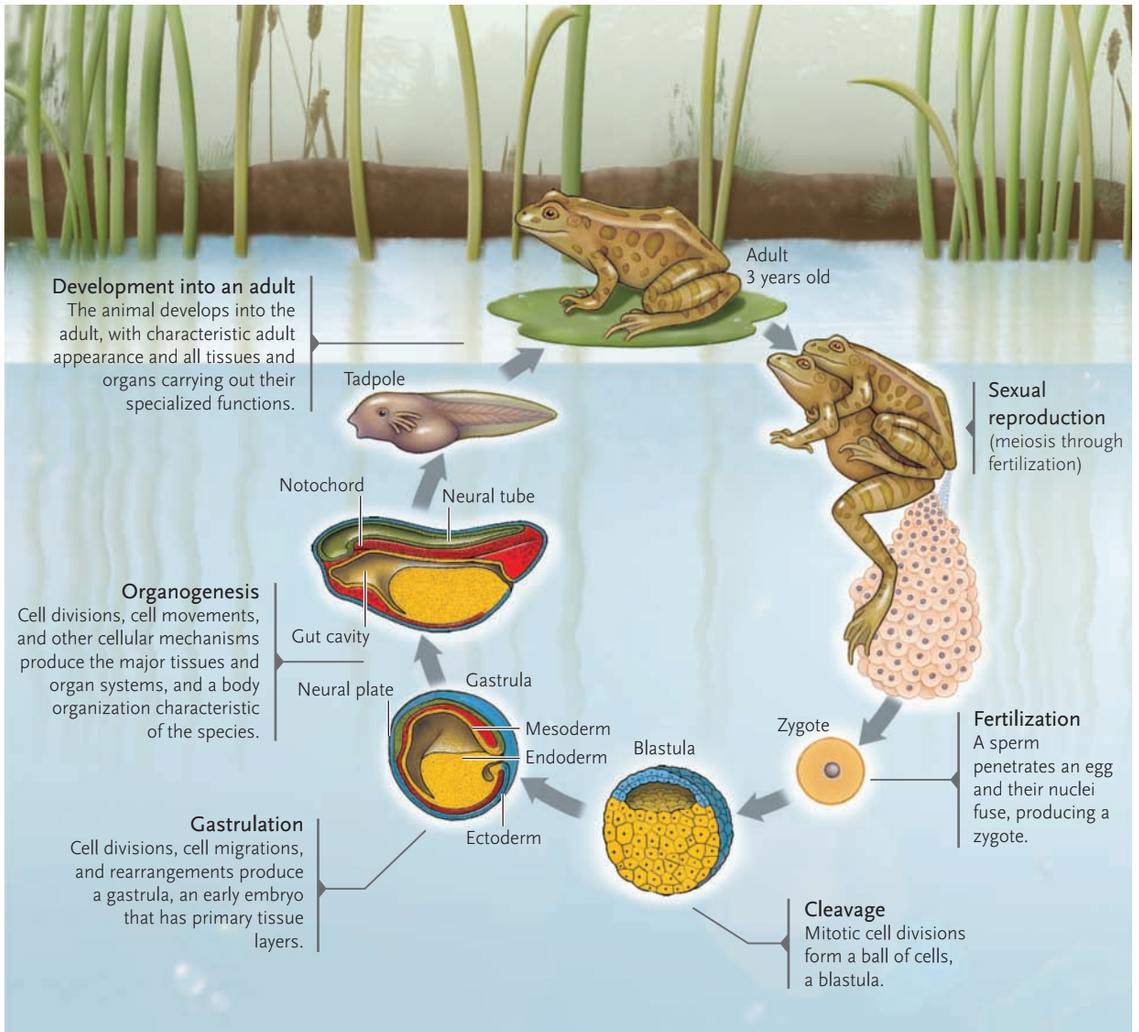


Figure 48.3
Stages of animal development shown in a frog.

each tissue and organ of the adult animal originates in one of the three primary cell layers of the gastrula **(Table 48.1).**

The cell movements also form a new cavity within the embryo, the **archenteron** (*arche* = beginning; *enteron* = intestine or gut), which is lined with endoderm. The archenteron forms the primitive gut of the embryo; an opening at one end, the **blastopore**, gives rise to the anus or mouth of the embryo, depending on the animal group (see Section 29.2). In the protostomes, which include annelids, arthropods, and mollusks, the blastopore develops into the mouth, and the anus forms at the opposite end of the embryonic gut. In the deuterostomes, which include echinoderms and chordates, the blastopore develops into the anus and the mouth forms at the opposite end of the embryonic gut. By the time gastrulation is complete, the embryo has clearly defined anterior, posterior, dorsal, and ventral regions.

As the blastula develops into the gastrula, embryonic cells begin to differentiate: they become recognizably different in biochemistry, structure, and function. The developmental potential of the cells also becomes more limited than that of the fertilized egg from which they originated. For example, although a fertilized egg is capable of developing into a complete embryo, a mesoderm cell may develop into muscle or bone but not normally into outside skin or brain. This restriction of developmental potential does not occur, as was once thought, because the cells have lost all their genes except those for the structure and function of the cell type they will become. Rather, the differentiating cells actually all contain complete genomes of the organism, but each type of cell has a different program of gene expression.

Development in all animals is accomplished by a number of mechanisms that are under genetic control but are influenced to some extent by the environment (for example, temperature affects the rate of cell division). The mechanisms are

1. Mitotic cell divisions.
2. Cell movements.

3. **Selective cell adhesions**, in which cells make and break specific connections to other cells or to the extracellular matrix.
4. **Induction**, in which one group of cells (the inducer cells) causes or influences another nearby group of cells (the responder cells) to follow a particular developmental pathway. The key to induction is that only certain cells can respond to the signal from the inducer cells. Induction typically involves signal transduction events (see Chapter 7). These events are triggered either by direct cell-to-cell contact involving interaction between a membrane-embedded protein on the inducer cell and a receptor protein on the surface of the responder cell, or by a signal molecule released by the inducer cell that interacts with a receptor on the responder cell. (The latter is an example of paracrine signaling; see Section 40.1.)
5. **Determination**, in which the developmental fate of a cell is set. Prior to determination, a cell has the potential to become any cell type of the adult but, after determination, that property is lost as the cell commits to becoming a particular cell type. Typically, determination is the result of induction, but in some cases it results from the asymmetric segregation of cellular determinants.
6. Differentiation, which follows determination, involves the establishment of a cell-specific developmental program in cells. Differentiation results in cell types with clearly defined structures and functions; those features derive from specific patterns of gene expression in cells.

You will see examples of these mechanisms in the examples of development discussed in the following three sections.

Study Break

1. How do cleavage divisions differ from cell division in an adult organism?
2. What are the primary cell layers of the embryo, and what process is responsible for producing them?

48.2 Major Patterns of Cleavage and Gastrulation

With the principles of early embryonic development established, we describe cleavage and gastrulation in three animal groups that have been models in *embryology* (the study of embryos and their development): sea urchins, amphibians, and birds. Later in the chapter, we describe cleavage and gastrulation in humans and other mammals, which resemble the pattern in birds.

Table 48.1 **Origins of Adult Tissues and Organs in the Three Primary Tissue Layers**

Primary Tissue Layer	Adult Tissues and Organs
Ectoderm	Skin and its elaborations, including hair, feathers, scales, and nails; nervous system, including brain, spinal cord, and peripheral nerves; lens, retina, and cornea of eye; lining of mouth and anus; sweat glands, mammary glands, adrenal medulla, and tooth enamel
Mesoderm	Muscles; most of skeletal system, including bones and cartilage; circulatory system, including heart, blood vessels, and blood cells; internal reproductive organs; kidneys and outer walls of digestive tract
Endoderm	Lining of digestive tract, liver, pancreas, lining of respiratory tract, thyroid gland, lining of urethra, and urinary bladder

Sea Urchin Gastrulation Follows a Symmetrical Pattern That Reflects an Even Distribution of Yolk

Cleavage divisions proceed at approximately the same rate in all regions of a sea urchin embryo **(Figure 48.4,** step 1), reflecting the uniform distribution of yolk in the sea urchin egg. These divisions continue until a blastula containing about a thousand cells is formed (step 2).

Gastrulation begins at the vegetal pole of the blastula. As a result of induction, some cells in the middle of that region become elongated and cylindrical, causing the region to flatten and thicken. Then, some cells break loose and migrate into the blastocoel (see Figure 48.4, step 3). These cells, called *primary mesenchyme cells* (mesenchyme means "middle juice"), move around inside the blastocoel, making and breaking adhesions, until eventually they attach along the ventral sides of the blastocoel. These cells will eventually become the mesoderm (see step 7), which will give rise to skeletal elements of the embryo. Next, the flattened vegetal pole of the blastula invaginates, pushing gradually into the interior (steps 4 and 5). The cells that invaginate are future endoderm cells. The inward movement, in effect much like pushing in the side of a hollow rubber ball, generates a new cavity, the archenteron. The opening of the archenteron is the blastopore.

As the archenteron is forming, the cells of the invaginated cell layer send out extensions that stretch across the blastocoel and contact the inside of the ectoderm (step 6). These extensions make tight adhesions and then contract, pulling the invaginated cell layer inward with them and thereby eliminating most of the blastocoel.

At this point the embryo has two complete cell layers. The outer layer remaining from the original blastula surface makes up the ectoderm of the embryo. The second, inner layer, derived from the cells forming the

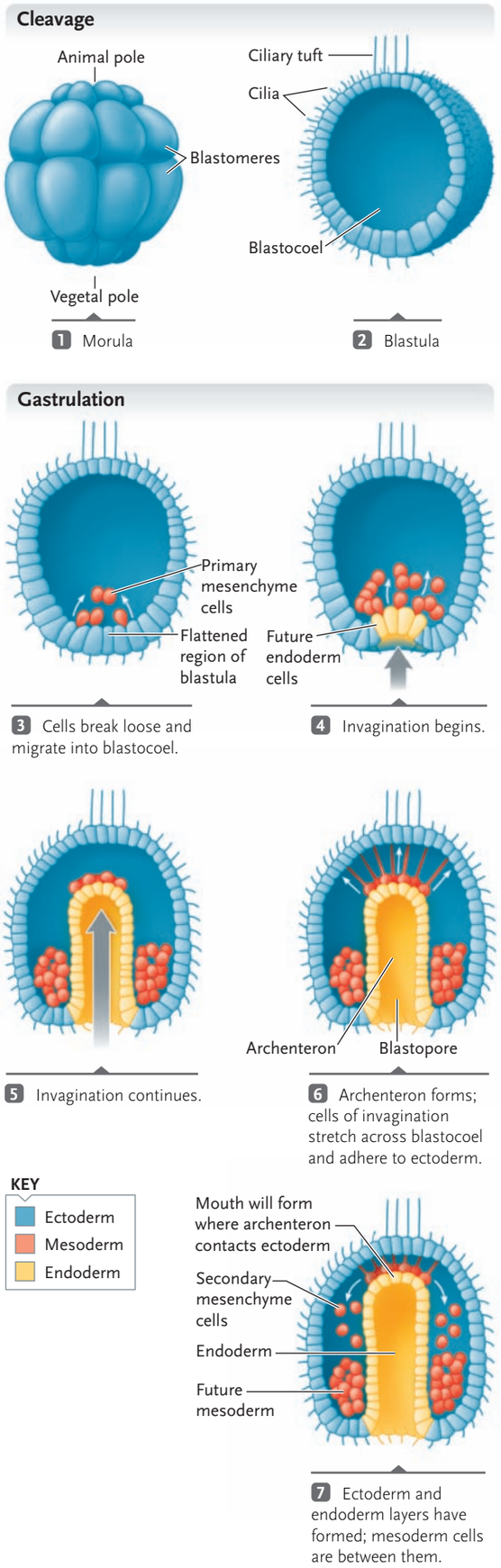


Figure 48.4
Cleavage and gastrulation in the sea urchin.

archenteron, makes up the endoderm. Mesodermal cells are also beginning to form a third layer, the mesoderm. Some are derived from the primary mesenchyme cells and others from *secondary mesenchyme cells*, cells that migrated into the space between ectoderm and endoderm (step 7). When the mesoderm layer is complete, the embryo has three complete layers: ectoderm, mesoderm, and endoderm. At this point, cells within each layer begin to differentiate, as evidenced by the synthesis of different proteins in each layer.

As the ectoderm, mesoderm, and endoderm develop, the embryo lengthens into an ellipsoidal shape with the blastopore marking the posterior end of the embryo. From this point on, organ systems differentiate through further cell division, cell movements, selective cell adhesions, induction, and differentiation. The blastopore forms the anus; a mouth will form at the opposite, anterior end of the gut.

Amphibian Cleavage and Gastrulation Are Influenced by an Unequal Distribution of Yolk

In amphibian eggs, such as those of frogs, yolk is concentrated in the vegetal half, which gives it a pale color. The animal half is darkly colored by a layer of pigment granules just below the surface. The sperm normally fertilizes the egg in the animal half (**Figure 48.5,** step 1). After fertilization, the pigmented layer of cytoplasm rotates toward the site of sperm entry, exposing a crescent-shaped region of the underlying cytoplasm at the side opposite the point of sperm entry (step 2). This region, called the **gray crescent**, establishes the dorsal–ventral axis of the embryo, with the gray crescent marking the future dorsal side.

Normally, the first cleavage division runs perpendicular to the long axis of the gray crescent and divides the crescent equally between the resulting cells (step 3). If one of the first two blastomeres does not receive gray crescent material, and the cells are separated experimentally, the cell without gray crescent divides to produce a disordered mass that stops developing. The cell receiving the gray crescent produces a normal embryo. Thus cytoplasmic material localized in the gray crescent is essential to normal development in frog embryos.

As cleavage of a frog embryo continues, cell divisions proceed more rapidly in the animal half, producing smaller and more numerous cells in this region than in the yolky vegetal half. By the time cleavage has produced an embryo with 15,000 cells, the animal half of the embryo has hollowed out, forming the blastula (**Figure 48.6,** step 1, and **Figure 48.7a**).

Gastrulation begins when cells from the animal pole move across the embryo surface and reach the region derived from the gray crescent. This site is marked by a crescent-shaped depression rotated clockwise 90°

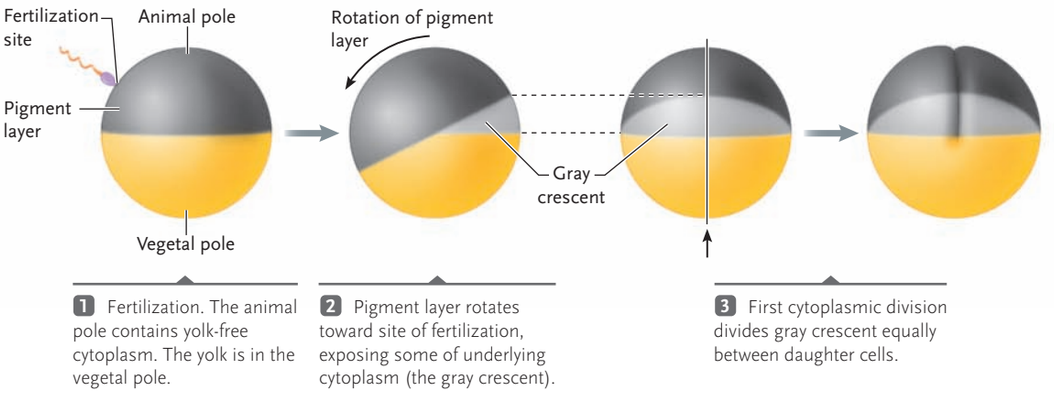


Figure 48.5
Rotation of the pigment layer and development of the gray crescent after fertilization in a frog egg. The gray crescent marks the site where gastrulation of the embryo will begin.

called the **dorsal lip of the blastopore.** Cells changing shape and pushing inward from the surface in a process called **invagination** produce the depression. With continued inward movement of additional cells, the depression eventually forms a complete circle (Figure 48.6, step 2, and **Figure 48.7b**), which is the blastopore.

As cells migrate into the blastopore by a process is called **involution**, the pigmented cell layer of the animal half expands to cover the entire surface of the embryo (Figure 48.6, step 3). The cells of the vegetal half are enclosed by the movement, and show on the outside as a yolk plug in the blastopore (Figure 48.6,

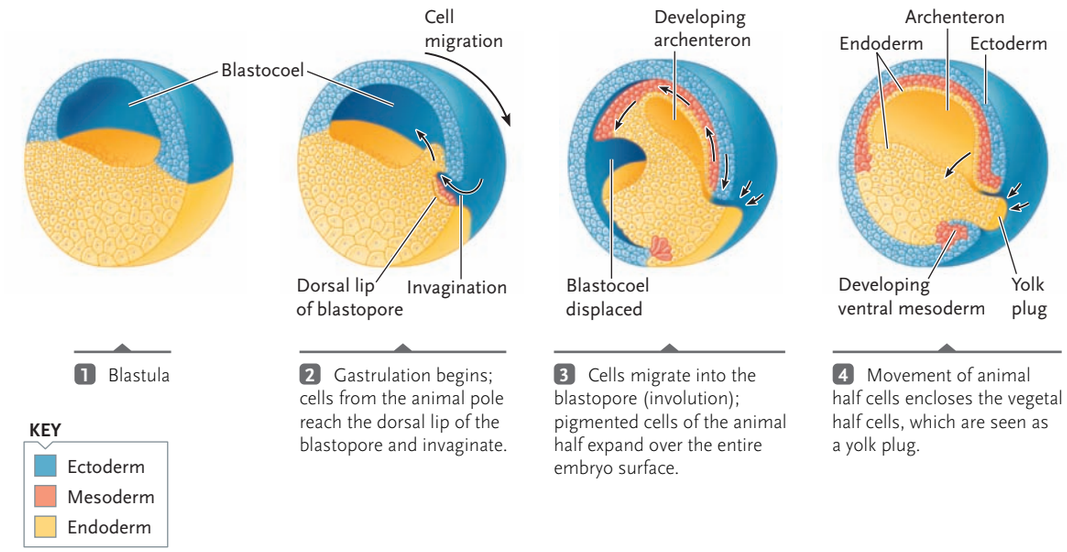


Figure 48.6
Gastrulation in a frog embryo. Yolk cells are shown in darker yellow.

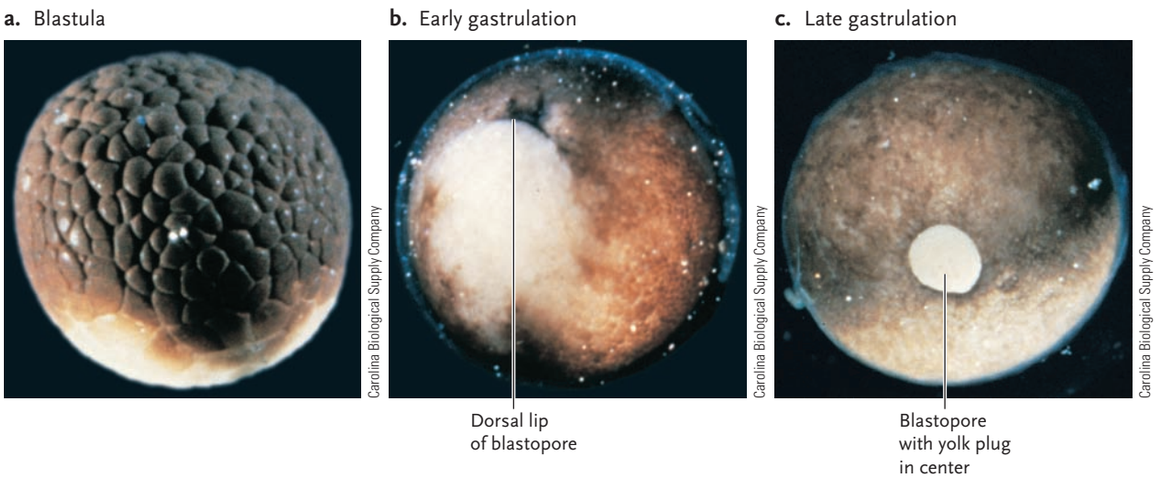


Figure 48.7
Formation of the dorsal lip of the blastopore and the completed blastopore, closed by a yolk plug, in photomicrographs of a frog embryo.

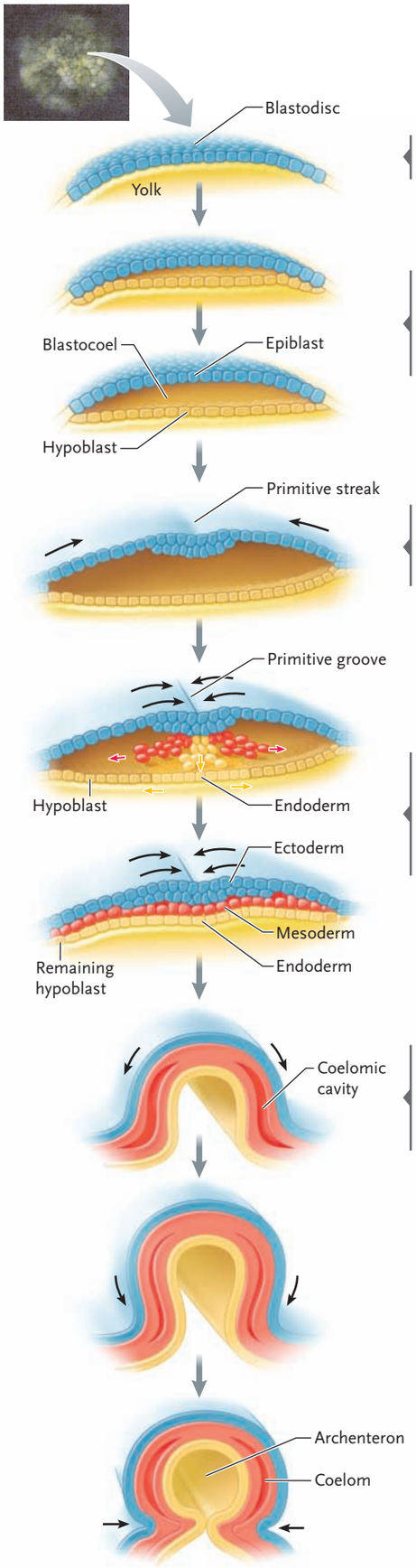


Figure 48.8
Gastrulation in a bird embryo.
(Photo: Peter B. Armstrong, University of California, Davis.)

step 4, and **Figure 48.7c**). As in other vertebrates, the blastopore gives rise to the anus.

Within the embryo, continuing involution moves cells into the interior and upward (see Figures 48.7b and c), forming two layers that line the inside top half of the embryo. The uppermost of these layers is induced to become the dorsal mesoderm (shown in red). The layer beneath it, which contains cells originating from both the outer surface of the embryo and the yolky interior, becomes the endoderm (shown in lighter yellow). The pigmented cells remaining at the surface of the embryo form the ectoderm (shown in blue). The ventral mesoderm begins to be induced near the vegetal pole.

As the mesoderm and endoderm form, the depression created by the inward cell movements gradually deepens and extends inward as the archenteron (see Figures 48.6c and d), which displaces the blastocoel. The cells of the three primary cell layers continue to increase in number by further movements and divisions as development proceeds.

During frog gastrulation, cells of the dorsal lip of the blastopore are inducer cells that control blastopore formation; if the cells in the dorsal lip are removed and transplanted elsewhere in the egg, they cause a second blastopore—and a second embryo—to form in this region (see Section 48.5).

The events of gastrulation in frogs thus include the same developmental mechanisms as in sea urchins—cell divisions, cell movements, selective adhesions, induction, and differentiation.

Gastrulation in Birds Proceeds at One Side of the Yolk

The pattern of gastrulation in birds and reptiles is modified by the distribution of yolk, which occupies almost the entire volume of the egg. (Birds and reptiles, as well as mammals, are all amniotes; see Section 30.7.) The portion of the cytoplasm that divides to give rise to the primary tissues of the embryo is confined to a thin layer at the egg surface. Although mammalian eggs have relatively little yolk, gastrulation follows a similar pattern in mammals, as discussed in Section 48.4.

Cleavage and Gastrulation in Birds. The early cleavage divisions in birds produce a disclike layer of cells at the surface of the yolk called the **blastodisc (Figure 48.8,** step 1). When blastodisc formation is complete, the layer contains about 20,000 cells. The cells of the blastodisc then separate into two layers, called the **epiblast** (top layer) and **hypoblast** (bottom layer). The flattened cavity between them is the blastocoel (step 2).

Gastrulation begins as cells in the epiblast stream toward the midline of the blastodisc, thickening the epiblast in this region. The thickened layer—the **primitive streak**—begins forming in the posterior end of the embryo and extends toward the anterior end as more cells of the epiblast move into it (step 3). The

thickening at the anterior end of the primitive streak, called the *primitive knot,* is the functional equivalent of the amphibian dorsal lip of the blastopore. The primitive streak initially defines the axes of the embryo: it extends from posterior to anterior, the region where the streak forms is the dorsal while beneath it is the ventral side, and it defines left and right sides of the embryo.

As the primitive streak forms, its midline sinks, forming the **primitive groove.** The primitive groove is a conduit for migrating cells to move into the blastocoel. The first cells to migrate through the primitive groove are epiblast cells (step 4), which will form the endoderm. Cells migrating laterally between the epiblast and the endoderm form the mesoderm. The epiblast cells left at the surface of the blastodisc form the ectoderm (see step 4). Thus all the primary tissue layers of the chick embryo arise from the epiblast.

Of the cells in the hypoblast, only a few, near the posterior end of the embryo, contribute directly to the embryo. These hypoblast cells form the *germ cells* that, later in development, migrate to the developing gonads and found the cell line leading to eggs and sperm (see Section 47.2).

Initially, the ectoderm, mesoderm, and endoderm are located in three more or less horizontal layers. During gastrulation, the endoderm pushes upward along its midline. At the same time, its left and right sides fold downward, forming a tube oriented parallel to the primitive streak (step 5). The central cavity of the tube is the archenteron, the primitive gut. The mesoderm separates into two layers, forming the coelom, a fluid-filled body cavity (see Section 29.2 and Figure 29.4c). These movements complete formation of the gastrula.

Formation of Extraembryonic Membranes. Each of the primary tissue layers of a bird embryo extends outside the embryo to form four **extraembryonic membranes (Figure 48.9),** which conduct nutrients from the yolk to the embryo, exchange gases with the environment outside the egg, and store metabolic wastes removed from the embryo. The **yolk sac** consists of extensions of mesoderm and endoderm that enclose the yolk. Although the yolk sac remains connected to the gut of the embryo by a stalk, yolk does not directly enter the embryo by this route. Instead, it is absorbed by blood vessels in the membrane, which transport the nutrients to the embryo. The **chorion,** produced from ectoderm and mesoderm, is the outermost membrane, which surrounds the embryo and yolk sac completely, and lines the inside of the shell. This membrane exchanges oxygen and carbon dioxide with the environment through the shell of the egg. The **amnion** is the innermost membrane, which closes over the embryo to form the *amniotic cavity.* The cells of the amnion secrete *amniotic fluid* into the cavity, which bathes the embryo and provides an aquatic environment in which it can develop. Reptilian and mammalian embryos are also surrounded by an amnion and amniotic fluid. By providing the embryo with an aquatic environment, this adaptation made possible the development of fully terrestrial vertebrates. The evolutionary importance of the amnion to the fully terrestrial vertebrates is recognized by classifying them together as **amniotes.** A membrane derived from mesoderm and endoderm that has bulged outward from the gut forms a sac called the **allantois.** This sac closely lines the chorion and fills much of the space between the chorion and the yolk sac. The allantois stores nitrogenous wastes (primarily uric acid) removed from the embryo. In addition, the part of the allantoic membrane that lines the chorion forms a rich bed of blood capillaries that is connected to the embryo by arteries and veins. This circulatory system delivers carbon dioxide to the chorion and picks up the oxygen that is absorbed through the shell and chorion.

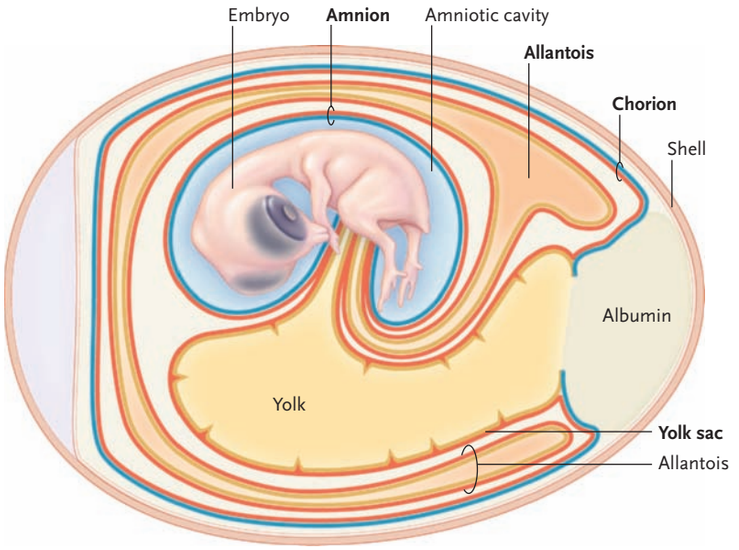


Figure 48.9
The four extraembryonic membranes in a bird embryo (in bold).

Study Break

1. What is the role of the gray crescent in amphibian development?
2. What evidence indicates that cells of the dorsal lip of the blastopore act as inducer cells?
3. What are the extraembryonic membranes in birds, and what are their functions?

48.3 From Gastrulation to Adult Body Structures: Organogenesis

Following gastrulation, organogenesis—the process by which the ectoderm, mesoderm, and endoderm develop into organs—gives rise to an individual with the body organization characteristic of its species. Organo-

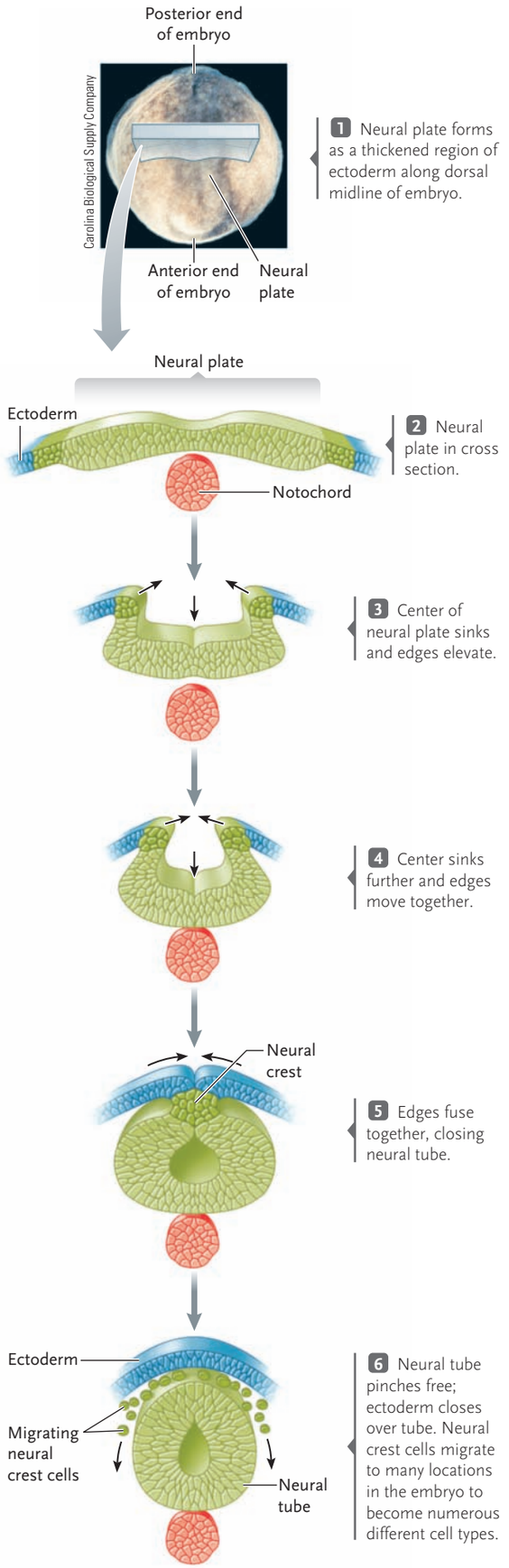


Figure 48.10 Development of the neural tube and neural crest cells in vertebrates. Photo is of an amphibian embryo; drawings show steps in a bird embryo.

genesis involves the same mechanisms used in gastrulation—cell division, cell movements, selective cell adhesion, induction, and differentiation—plus an additional mechanism, *apoptosis,* in which certain cells are programmed to die (apoptosis is also discussed in Section 43.2). To illustrate how the cellular mechanisms of development interact in organogenesis, we follow the formation of major organ systems in the frog embryo. Then we describe the generation of one organ, the eye, which follows a pathway typical of eye development in all vertebrates.

The Nervous System Develops from Ectoderm

In vertebrates, organogenesis begins with development of the nervous system from ectoderm, a process called **neurulation.** As a preliminary to neurulation, cells of the mesoderm form a solid rod of tissue, the **notochord**, which extends the length of the embryo under the dorsal ectoderm. Notochord cells carry out a major induction, in which they cause the overlying ectoderm to thicken and flatten into a longitudinal band called the **neural plate** (**Figure 48.10,** steps 1 and 2). Experiments have shown that if the notochord is removed, the neural plate does not form.

Once induced, the neural plate sinks downward along its midline (steps 2 and 3), creating a deep longitudinal groove. At the same time, ridges elevate along the sides of the neural plate. The ridges move together and close over the center of the groove (steps 4 and 5), converting the neural plate into a **neural tube** that runs the length of the embryo. The neural tube then pinches off from the overlying ectoderm, which closes over the tube (step 6). The central nervous system, including the brain and spinal cord, develops directly from the neural tube.

During formation of the neural tube, cells of the **neural crest**—the region where the neural tube pinches off from the ectoderm (shown in blue in Figure 48.10)—migrate to many locations in the developing embryo and become numerous different types of cells which contribute to a variety of organ systems. (The neural crest is one of the defining features of vertebrates.) Some cells develop into cranial nerves in the head; others contribute to the bones of the inner ear and skull, the cartilage of facial structures, and the teeth. Yet others form ganglia of the autonomic nervous system, peripheral nerves leading from the spinal cord to body structures, and nerves of the developing gut. Still others move to the skin, where they form pigment cells, or to the adrenal glands, where they form the medulla of these glands. The migration of neural crest cells occurs in the development of all vertebrates.

Other structures differentiate in the embryo while the neural tube is forming. On either side of the notochord, the mesoderm separates into blocks of cells called **somites**, spaced one after the other along both

sides of the notochord **(Figure 48.11).** The somites give rise to the vertebral column, the ribs, the repeating sets of muscles associated with the ribs and vertebral column, and muscles of the limbs. The mesoderm outside the somites, which extends around the primitive gut (lateral mesoderm in Figure 48.11), splits into two layers, one covering the surface of the gut, and the other lining the body wall. The space between the layers is the coelom of the adult (see Section 29.2).

Sequential Inductions and Differentiation Are Central to Eye Development

We now take up the development of the eye, to show how cellular mechanisms interact in organogenesis. Eyes develop by the same pathway in all vertebrates.

The brain forms at the anterior end of the neural tube from a cluster of hollow vesicles that swell outward from the neural tube (**Figure 48.12,** step 1). One paired set of vesicles, the *optic vesicles,* develop into the eyes. The figure depicts the optic vesicles in the brain of a frog embryo; note that the morphology of the forebrain, midbrain, and hindbrain in embryos differs among vertebrates.

The optic vesicles grow outward until they contact the overlying ectoderm, inducing a series of developmental responses in both tissues. The outer surface of the optic vesicle thickens and flattens at the region of contact and then pushes inward, transforming the optic vesicle into a double-walled *optic cup,* which ultimately becomes the retina. The optic cup induces the overlying ectoderm to thicken into a disclike swelling, the *lens placode* (step 2). The center of the lens placode sinks inward toward the optic cup, and its edges eventually fuse together, forming a ball of cells, the *lens vesicle* (step 3).

The developing lens cells begin to synthesize *crystallin,* a fibrous protein that collects into clear, glassy deposits. The lens cells finally lose their nuclei and form the elastic, crystal-clear lens.

As the lens develops, it contacts the overlying ectoderm, which has closed over it. In response, the ectoderm cells lose their pigment granules and become clear, developing into the cornea. Eventually, the developing cornea joins with the edges of the optic cup to complete the primary structures of the eye (step 4). Other cells contribute to accessory structures of the eye; for example, mesoderm and neural crest cells contribute to the reinforcing tissues in the wall of the eye and the muscles that move the eye. Figure 48.12, step 5, shows a fully developed vertebrate eye.

Many experiments have shown that the initial induction by the optic vesicle is necessary for develop-

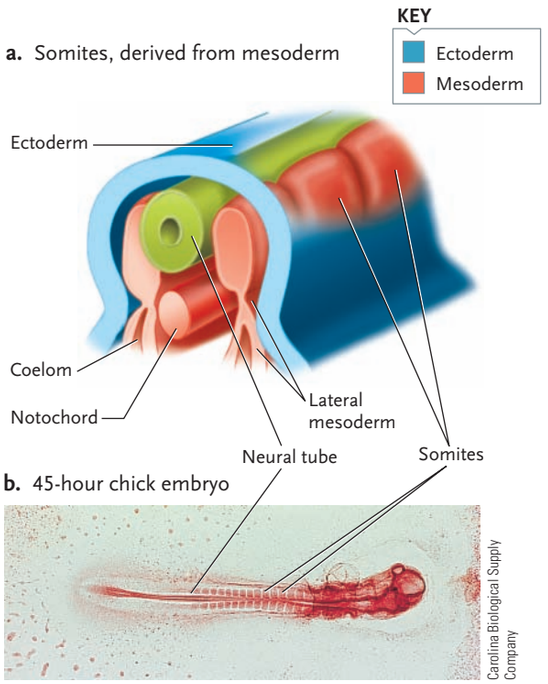


Figure 48.11
Later development of the mesoderm. **(a)** The somites develop into segmented structures such as the vertebrae, the ribs, and the musculature between the ribs. The lateral mesoderm gives rise to other structures, such as the heart and blood vessels and the linings of internal body cavities. **(b)** The somites in a 45-hour chick embryo.

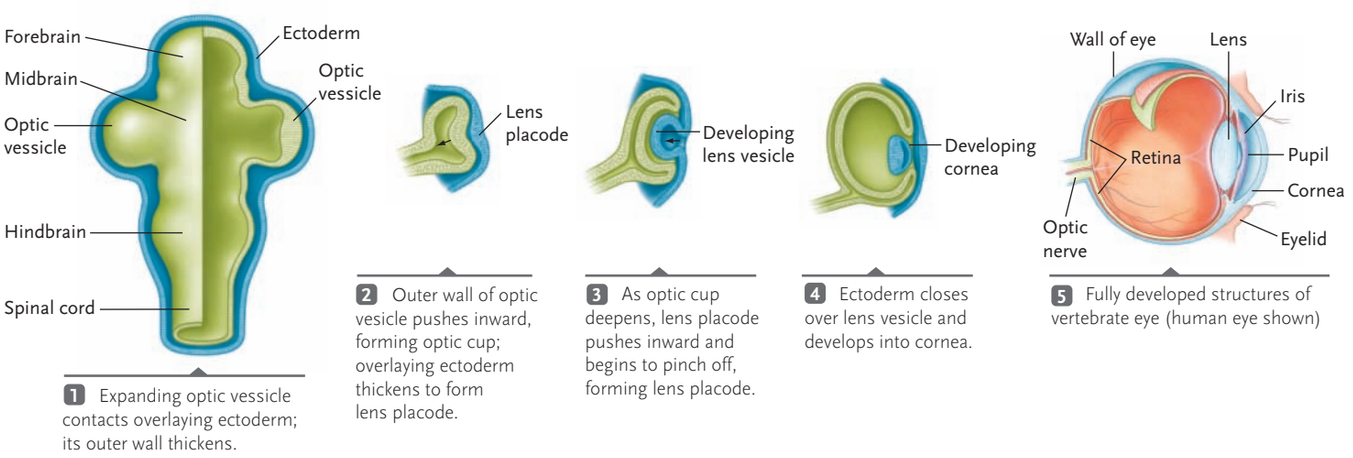


Figure 48.12
Stages in the formation of the vertebrate eye from the optic vesicle of the brain and the overlying ectoderm.

ment of the eye. For example, if an optic vesicle is removed before lens formation, the ectoderm fails to develop a lens placode and vesicle. Moreover, placing a removed optic vesicle under the ectoderm in other regions of the head causes a lens to form in the new location. Or, if the ectoderm over an optic vesicle is removed and ectoderm from elsewhere in the embryo is grafted in its place, a normal lens will develop in the grafted ectoderm, even though in its former location it would not differentiate into lens tissue.

Eye development also demonstrates differentiation. Ectoderm cells that are induced to form the lens of the eye synthesize crystallin; in other locations, ectoderm cells typically synthesize a different protein, *keratin,* as their predominant cell product. Keratin is a component of surface structures such as skin, hair, feathers, scales, and horns. In other words, as a response to induction by the optic vesicle, the genes of the ectoderm cells coding for crystallin are activated, while genes coding for keratin are not expressed.

Apoptosis Eliminates Tissues That Are No Longer Required

Induction and differentiation build complex, specialized organs from the three fundamental tissue types. Complementing these processes is *apoptosis,* programmed cell death, which in this case removes tissues present during development but not in the fully formed organ. Apoptosis plays an important role in the development of animals, both invertebrates and vertebrates. The best example of apoptosis in frog development occurs during metamorphosis, in which the tadpole changes into an adult frog. The tail of the tadpole becomes progressively smaller and finally disappears because its cells disintegrate and their components are absorbed and recycled by other cells. Cells that are eliminated by apoptosis, like those of a tadpole's tail, are typically parts of structures required at one stage of development but not for later stages.

In the next section, we describe cleavage, gastrulation, and organogenesis in human and other mammalian embryos.

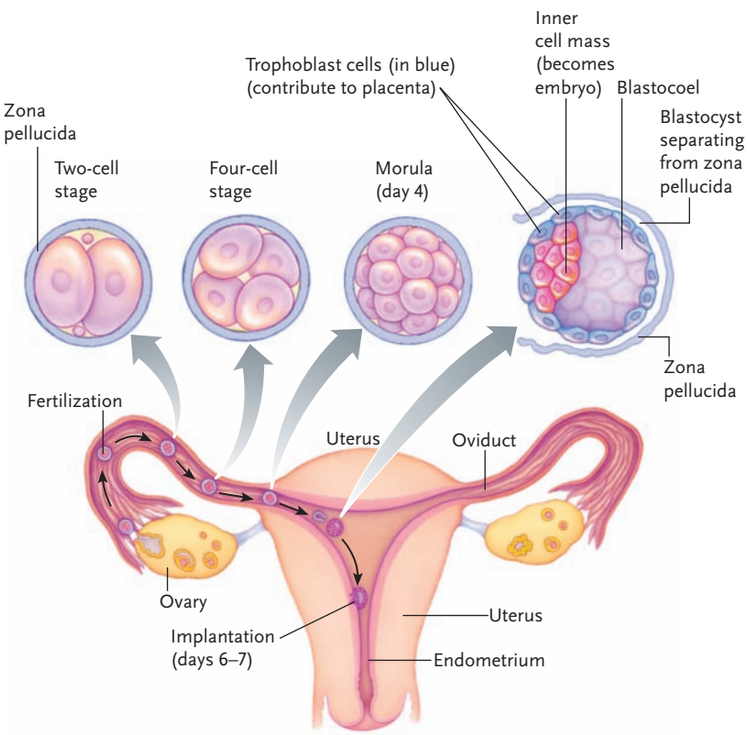


Figure 48.13 Early stages in the development of the human embryo.

Study Break

1. What is the outcome of organogenesis?
2. What tissues or organs develop from the neural tube and neural crest cells?

48.4 Embryonic Development of Humans and Other Mammals

Human embryonic development is representative of the placental mammals, in which the embryo develops in the uterus of the mother. In the uterus, the embryo is nourished by the placenta, which supplies oxygen and nutrients and carries away carbon dioxide and nitrogenous wastes.

The period of mammalian development that is called **pregnancy** or **gestation** varies in different species. Larger mammals generally have longer gestation periods; for example, gestation takes 600 days in elephants, about 1 year in blue whales, and a mere 21 days in hamsters.

In humans, gestation takes an average of 266 days from the time of fertilization, or about 38 weeks. Because the date of fertilization may be difficult to establish, human gestation is usually calculated from the beginning of the menstrual cycle in which fertilization takes place, giving a period of about 9 months. On this basis, human gestation is divided into three **trimesters,** each 3 months long.

The major developmental events in human gestation—cleavage, gastrulation, and organogenesis—take place during the first trimester. By the fourth week, the embryo's heart is beating, and by the end of the eighth week, the major organs and organ systems have formed. From this point until birth, the developing human is called a **fetus.** Only 5 cm long by the end of the first trimester, the fetus grows during the second and third trimesters to an average length of 50 cm and an average weight of 3.5 kg (or about 19.7 inches and 7.7 pounds). The period of gestation ends with birth.

Cleavage and Implantation Occupy the First 2 Weeks of Development

We noted in Section 47.3 that human fertilization occurs when the egg is in the first third of the oviduct leading from the ovary to the uterus. After fertilization, cleavage divisions take place during passage of the developing embryo down the fallopian tube and while it is still enclosed in the zona pellucida—the original coat of the egg **(Figure 48.13).**

By day 4, the morula, a 16- or 32-cell ball, has been produced. By the time the endometrium (uterine lining) is ready for implantation (about 7 days after ovulation; see Section 47.3), the morula has reached the uterus and has undergone further cell divisions and differentiation into a blastocyst. At this time, the **blastocyst** is a single-cell-layered hollow ball of about 120 cells with a fluid-filled cavity, the blastocoel, in which a dense mass of cells is localized to one side. This **inner cell mass** will become the embryo itself, while the rest of the blastocyst will become tissues that support the development of the embryo in the uterus. The outer single layer of cells of the blastocyst is the **trophoblast.**

When it is ready to implant, the blastocyst breaks out of the zona pellucida and sticks to the endometrium on its inner cell mass side **(Figure 48.14a).** Implantation begins when the trophoblast cells overlying the inner cell mass secrete proteases that digest pathways between endometrial cells. Dividing trophoblast cells fill in the digested spaces, appearing like fingerlike projections into the endometrium. These cells continue to digest the nutrient-rich endometrial cells, serving both to produce a hole in the endometrium for the blastocyst and to release nutrients that the developing embryo can use after the small amount of yolk contained in the egg cytoplasm is used up. While the blastocyst is burrowing into the endometrium, the inner cell mass separates into the *embryonic disc,* which consists of two distinct cell layers (see Figure 48.14a). The layer farther from the blastocoel is the epiblast, which gives rise to the embryo proper, and the layer nearer the blastocoel is the hypoblast, which gives rise to part of the extraembryonic membranes. When implantation is complete, the blastocyst has completely burrowed into the endometrium and is covered by a layer of endometrial cells **(Figure 48.14b).**

Mammalian Gastrulation and Neurulation Resemble the Reptilian–Bird Pattern

Gastrulation proceeds as in birds (see Figure 48.8), with the formation of a primitive streak in the epiblast. Some epiblast cells remain in place, becoming the ectoderm, while others enter the streak to form the endoderm and mesoderm. The ectoderm, mesoderm, and endoderm are located initially in three layers; from this initial arrangement, the endoderm folds to form the primitive gut, and becomes surrounded with ectoderm and mesoderm. Neurulation in human and other mammalian embryos takes place essentially as in birds (see Figure 48.10).

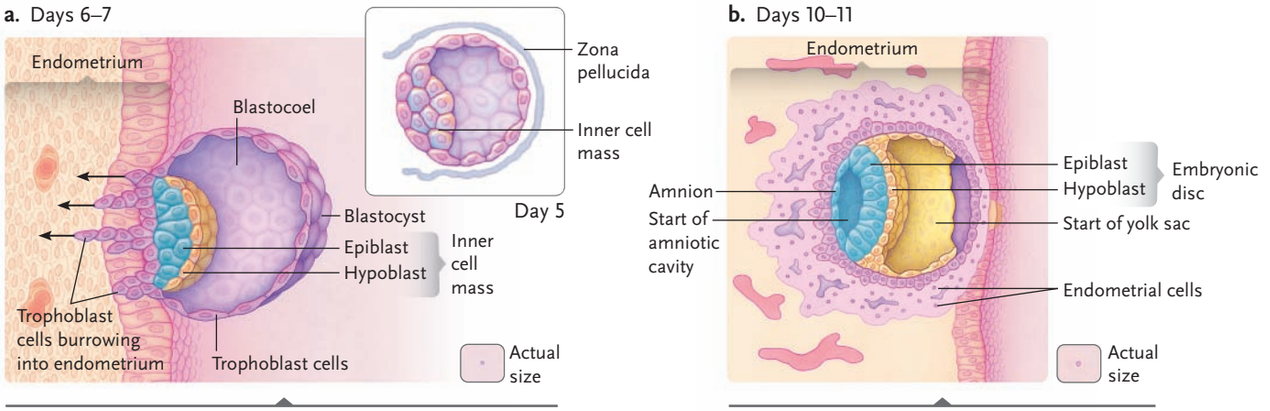
Extraembryonic Membranes Give Rise to the Amnion and Part of the Placenta

Soon after the inner cell mass separates into the epiblast and hypoblast, a layer of cells separates from the epiblast along its top margin (see Figure 48.14b). The fluid-filled space created by the separation becomes the amniotic cavity, and the layer of ectodermal cells forming its roof becomes the amnion, the extraembryonic membrane surrounding the cavity. The amnion expands until eventually it completely surrounds the embryo and suspends it in amniotic fluid. As in birds, the hypoblast develops into the yolk sac. However, in mammals, the mesoderm of the yolk sac gives rise to the blood vessels in the embryonic portion of the placenta.

While the amnion is expanding around the embryonic disc, blood-filled spaces form in maternal tissue, and trophoblast cells grow rapidly around both the embryo and amnion to form the chorion **(Figure 48.14c).** Next, a connecting stalk forms between the embryonic disc and the chorion, while the chorion begins to grow into the endometrium as fingerlike or treelike extensions called **chorionic villi (Figure 48.14d).** The chorionic villi greatly increase the surface area of the chorion. Where these villi grow into the endometrium is the area of the future placenta. As the chorion develops, mesodermal cells of the yolk sac grow into it and form a rich network of blood vessels, the embryonic circulation of the placenta. At the same time, the expanding chorion stimulates the blood vessels of the endometrium to grow into the maternal circulation of the placenta **(Figure 48.14e).**

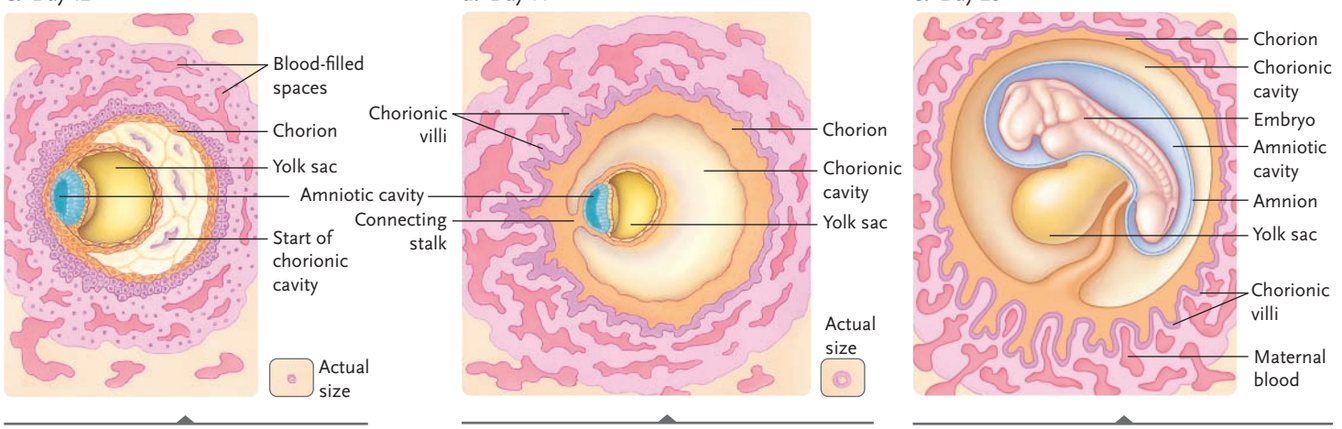
Within the placenta of humans, apes, monkeys, and rodents, the maternal circulation opens into spaces in which the maternal blood directly bathes the capillaries coming to the placenta from the embryo **(Figure 48.14f).** (Different types of placentas are found in other mammals.) The embryonic circulation remains closed, however, so that the embryonic and maternal blood do not mix directly. This prevents the mother from developing an immune reaction against cells of the embryo, which may be recognized as foreign by the mother's immune system. Eventually, the placenta and its blood circulation grow to cover about a quarter of the inner surface of the enlarged uterus and reach the size of a dinner plate.

As the embryonic blood circulation develops, this connecting stalk between the embryo and placenta develops into the **umbilical cord,** a long tissue with blood vessels linking the embryo and the placenta. The vessels in the umbilical cord are derived from the extraembryonic membrane, the allantois. They conduct



Surface cells of the blastocyst attach to the endometrium and start to burrow into it. Implantation is under way.

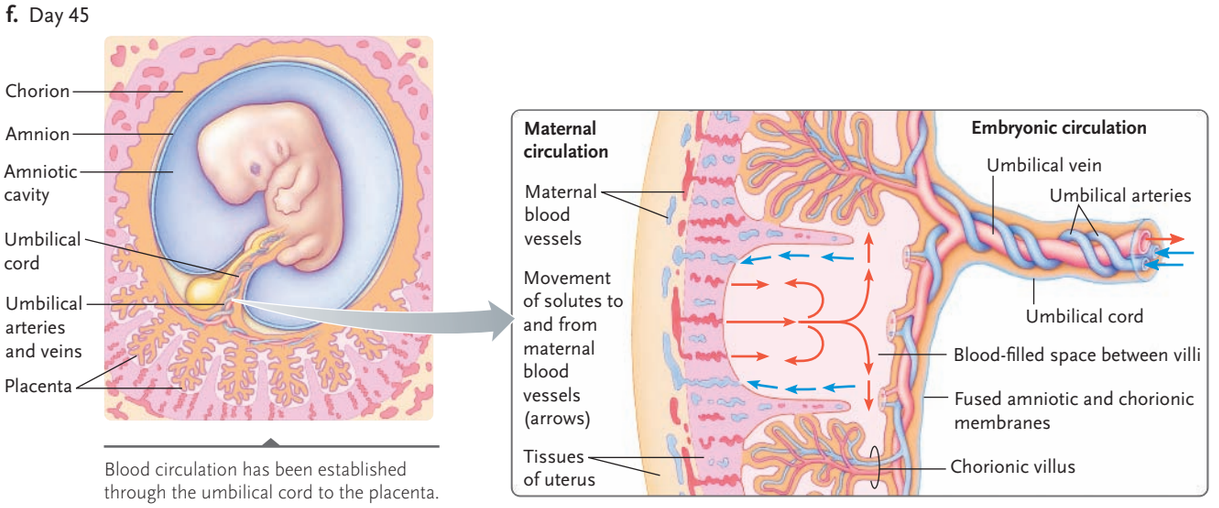
A layer of epiblast cells separates, producing the amniotic cavity. The cells above the cavity become the amnion, which eventually surrounds the embryo. The hypoblast begins to form around the yolk sac.



Blood-filled spaces form in maternal tissue. The chorion forms, derived from trophoblast cells, and encloses the chorionic cavity.

A connecting stalk has formed between the embryonic disk and chorion. Chorionic villi, which will be features of a placenta, start to form.

The chorion continues to grow into the endometrium, producing the chorionic villi. The chorion growth stimulates blood vessels of the endometrium to grow into the maternal circulation of the placenta.



Blood circulation has been established through the umbilical cord to the placenta.

Figure 48.14
Implantation of a human blastocyst in the endometrium of the uterus and the establishment of the placenta.

blood between the embryo and the placenta (shown in the inset for Figure 48.14f).

Within the placenta, nutrients and oxygen pass from the mother's circulation into the circulation of the embryo. Besides nutrients and oxygen, many other substances taken in by the mother—including alcohol, caffeine, drugs, and toxins in cigarette smoke—can pass from mother to embryo. Carbon dioxide and nitrogenous wastes pass from the embryo to the mother, and are disposed of by the mother's lungs and kidneys.

If the presence of a genetic disease such as cystic fibrosis or Down syndrome is suspected, tests can be carried out on cells removed from the embryonic portion of the placenta or from the amniotic fluid, which contains cells derived from the embryo. The test using cells of the placenta is called *chorionic villus sampling;* the test using cells derived from the amniotic fluid is called *amniocentesis* (*centesis* = puncture, referring to the use of a needle, which is pushed through the abdominal wall, to obtain fluid from the amniotic cavity). Chorionic villus sampling can be carried out as early as the eighth week, compared with 14 weeks for amniocentesis. Both tests carry some degree of risk to the embryo.

Further Growth of the Fetus Culminates in Birth

By the end of its fourth week, a human embryo is 3–5 mm long, 250–500 times the size of the zygote **(Figure 48.15a).** It has a tail and pharyngeal arches, which are embryonic features of all vertebrates (see Section 30.2). The pharyngeal arches contribute to the formation of the face, neck, mouth, nasal cavities, larynx, and pharynx. After 5 to 6 weeks, most of the tail has disappeared and the embryo is beginning to take on recognizable human form **(Figure 48.15b).** At 8 weeks, the embryo, now a fetus, is about 2.5 cm long **(Figure 48.15c).** Its organ systems have formed, and its limbs, with fingers or toes at their ends, have developed **(Figure 48.15d).**

Figure 48.16 shows the hormonal events and associated physical events of birth. As the period of fetal growth comes to a close, the fetus typically turns so that its head is downward, pressed against the cervix. A steep rise in the levels of estrogen secreted by the placenta at this time causes cells of the uterus to express the gene for the receptor of the hormone *oxytocin*. The receptors become inserted into the plasma membranes of those cells. Oxytocin—which is secreted by the pituitary gland—binds to its receptor, triggering the smooth muscle cells of the uterine wall to contract and begin the rhythmic contractions of labor. These contractions mark the beginning of **parturition** (*parturire* = to be in labor), the process of giving birth.

The contractions push the fetus further against the cervix and stretch its walls (see Figure 48.16, step 1). In response, stretch receptors in the walls send nerve signals to the hypothalamus, which responds by stimulating the pituitary to secrete more oxytocin. In turn, the oxytocin stimulates more forceful contractions of the uterus, pressing the fetus more strongly against the cervix, and further stretching its walls. The positive feedback cycle continues, steadily increasing the strength of the uterine contractions.

As the contractions force the head of the fetus through the cervix (step 2), the amniotic membrane bursts, releasing the amniotic fluid. Usually, within 12 to 15 hours after the onset of uterine contractions, the head passes entirely through the cervix. Once the head is through, the rest of the body follows quickly and the entire fetus is forced through the vagina to the exterior, still connected to the placenta by the umbilical cord (step 3).

After the baby takes its first breath the umbilical cord is cut and tied off by the birth attendant. Contractions of the uterus continue, expelling the placenta and any remnants of the umbilical cord and embryonic membranes as the afterbirth, usually within 15 minutes to an hour after the infant's birth. The short length of umbilical cord still attached to the infant dries and shrivels within a few days. Eventually, it separates entirely and leaves a scar, the **umbilicus** or navel, to mark its former site of attachment during embryonic development.

The Mother's Mammary Glands Become Active after Birth

Before birth of the fetus, estrogen and progesterone secreted by the placenta stimulate the growth of the mammary glands in the mother's breasts. However, the high levels of these hormones prevent the mammary glands from responding to *prolactin,* the hormone secreted by the pituitary that stimulates the glands to produce milk. After birth of the fetus and release of the placenta, the levels of estrogen and progesterone fall steeply in the mother's bloodstream, and the breasts begin to produce milk (stimulated by prolactin) and secrete it (stimulated by oxytocin).

Continued milk secretion depends on whether the infant is suckled by the mother. If the infant is suckled, stimulation of the nipples sends nerve impulses to the hypothalamus, which responds by signaling the pituitary to release a burst of prolactin and oxytocin. Hormonal stimulation of milk production and secretion continues as long as the infant is breastfed.

So far, we have followed the development of a generic human, but certain aspects of development differ depending on the offspring's sex. Next we look at the specifics of male and female development.

A Gene on the Y Chromosome Determines the Development of Male or Female Sex Organs

The gonads and their ducts begin to develop during the fourth week of gestation. Until the seventh week, male and female embryos have the same set of inter-

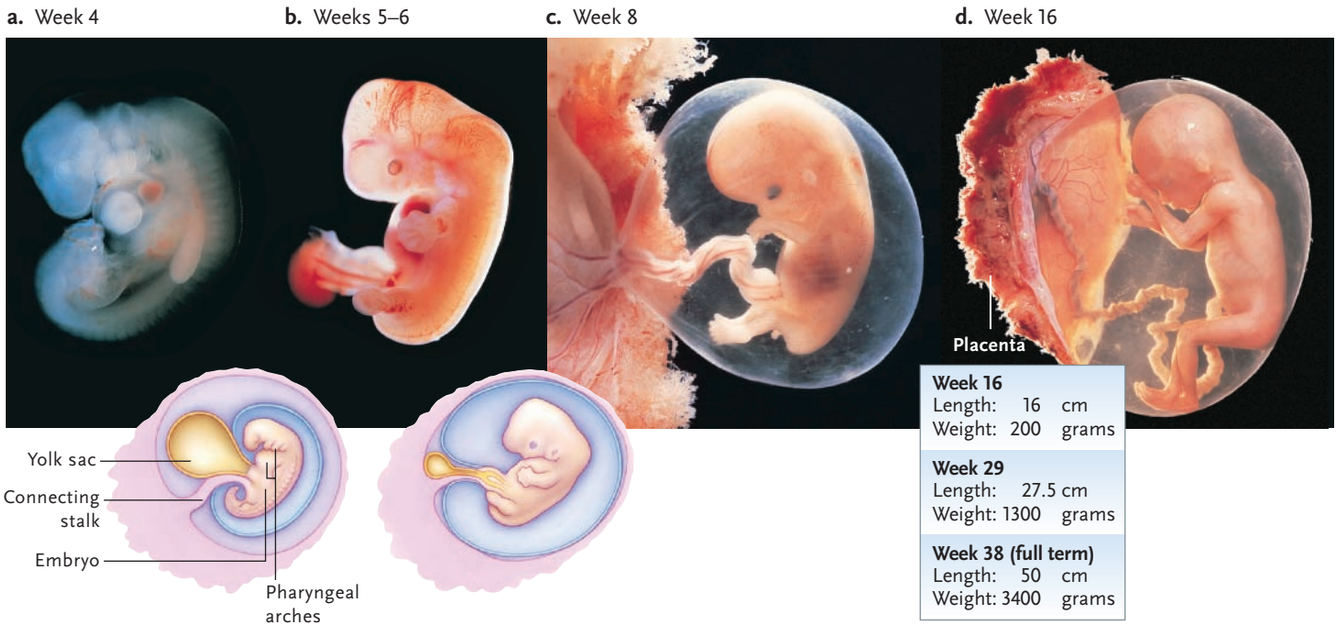
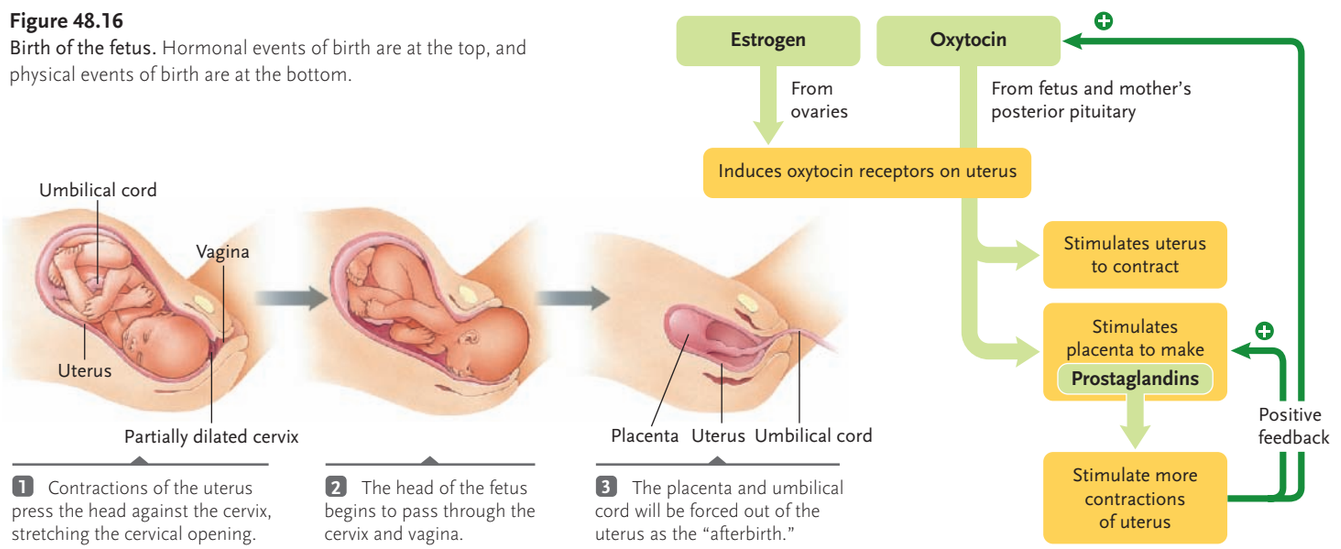


Figure 48.15
The human embryo at various stages of development, beginning at week 4. The chorion has been pulled aside to reveal the embryo in the amnion at week 8 and week 16. By week 16, movements begin as nerves make functional connections with the forming muscles.
(Photos: Lennart Nilsson, A Child Is Born, © 1966, 1977, Dell Publishing Company, Inc.)

nal structures derived from mesoderm, including a pair of gonads **(Figure 48.17a).** Each gonad is associated with two primitive ducts, the **Wolffian duct** and the **Müllerian duct**, which lead to a cloaca. These internal structures are *bipotential:* they can develop into either male or female sexual organs.

The presence or absence of a Y chromosome determines whether the internal structures develop into male or female sexual organs. If the fetus has the XY combination of sex chromosomes, a single gene on the Y chromosome, *SRY (Sex-determining Region of the Y)*, becomes active in the seventh week. The protein encoded by the gene sets a molecular switch that causes the primitive gonads to develop into testes. The fetal testes then secrete two hormones, testosterone and the *anti-Müllerian hormone (AMH)*. The testosterone stimulates development of the Wolffian ducts into the male reproductive tract, including the epididymis, vas deferens,

Figure 48.16
Birth of the fetus. Hormonal events of birth are at the top, and physical events of birth are at the bottom.



and seminal vesicles **(Figure 48.17b).** AMH causes the Müllerian ducts to degenerate and disappear. (*Insights from the Molecular Revolution* describes experiments that traced the activity of the *SRY* gene and its encoded protein in male development.) Testosterone additionally stimulates the development of the male genitalia.

If the fetus has the XX combination of chromosomes, no SRY protein is produced and the primitive gonads, under the influence of the estrogens and progesterone secreted by the placenta, develop into ovaries. The Müllerian ducts develop into the oviducts, uterus, and part of the vagina, and the Wolffian ducts degenerate and disappear **(Figure 48.17c).** The female sex hormones additionally stimulate the development of the female external genitalia.

Development Continues after Birth

Once fetal development is over, humans and other mammals, and indeed most other animals, follow a prescribed course of further growth and development that leads to the adult, the sexually mature form of the species. In humans, the internal and external sexual organs mature and secondary sexual characteristics appear at puberty. Similar changes occur in most mammals. There are, in fact, many examples among different animal groups of developmental changes that take place after hatching or birth. In some cases, offspring hatch that are distinctly different in structure from the adult. Examples among invertebrates include insects such as *Drosophila* and butterflies, in which eggs hatch to produce larva that undergo metamorphosis into the adult. Frogs similarly hatch as tadpoles, which undergo metamorphosis to produce the adult.

We have now described embryonic development in animals from a morphological perspective. In the rest of the chapter, we will focus on the cellular and molecular mechanisms that underlie development.

Study Break

1. Distinguish between the roles of the trophoblast and inner cell mass of the blastocyst in mammalian development.
2. What hormone would you use to induce labor in a pregnant woman?

48.5 The Cellular Basis of Development

In the preceding sections, you learned about the processes of development from a mainly structural point of view. Underlying those developmental processes are specific cellular and molecular events. In this section, you will learn about all the cellular events that underlie the stages of development.

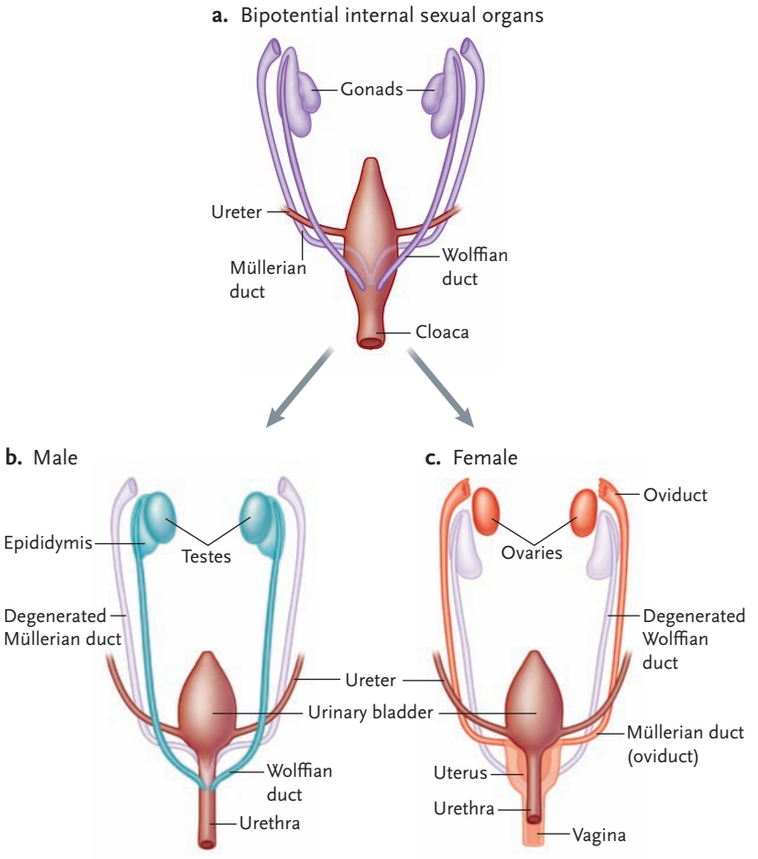


Figure 48.17 Development of the internal sexual organs of males and females from common bipotential origins.

Cell Division Varies in Orientation and Rate during Embryonic Development

The *orientation* and *rate* of mitotic cell division have special significance in the development of the shape, size, and location of the organ systems of the embryo. Regulation of these two features of mitotic cell division occurs at all stages of development.

The orientation of cell division refers to the angles at which daughter cells are added to older cells as development proceeds. It is determined by the location of a furrow that separates the cytoplasm after mitotic division of the nucleus (furrowing is discussed in Section 10.2). The furrow forms in alignment with the spindle midpoint. Therefore, when the spindle is centrally positioned in the cell, the furrow leads to symmetric division of the cell. However, when the spindle is displaced to one end of the cell, the furrow leads to asymmetric division of the cell into a smaller and a larger cell. Little is known about how spindle positioning is regulated.

The rate of cell division primarily reflects the time spent in the G_1 period of interphase (see Section 10.2); once DNA replication begins, the rest of the

cell cycle is usually of uniform length in all cells of the same species. As an embryo develops and cells differentiate, the time spent in interphase increases and varies in length in different cell types. As a result, different cell types proliferate at various rates, giving rise to tissues and organs with different cell numbers. Some cells, when fully differentiated, remain fixed in interphase and stop replicating their DNA or dividing. Nerve cells in the mammalian brain and spinal cord, for example, stop dividing once the nervous system is fully formed. Ultimately, the rate of cell division is under genetic control.

Frog egg cleavage provides examples of how both changes in orientation and rate of mitotic division affect development. The first two cleavages start at the animal pole and extend to the vegetal pole, producing four equal blastomeres (see Figure 48.2). The third cleavage occurs equatorially. However, because there is yolk in the vegetal region of the embryo, this cleavage furrow forms not at the equator but up higher toward the animal pole. The result is an eight-cell embryo with four small blastomeres in the animal region of the embryo, and four large blastomeres in the vegetal region. The blastomeres in the animal region of the embryo proceed to divide rapidly, while the blastomeres in the vegetal part of the embryo divide more slowly because division is inhibited by yolk. As a result, the morula produced consists of an animal region with many small cells, and a vegetal region with relatively few blastomeres.

Cell-Shape Changes and Cell Movements Depend on Microtubules and Microfilaments

We have seen that embryonic cells undergo changes in shape that generate movements, such as the infolding of surface layers to produce endoderm or mesoderm. Entire cells also move during the embryonic growth of animals, both singly and in groups. Both the shape changes and the whole-cell movements are produced by microtubules, powered by dyneins and kinesins, and microfilaments, powered by myosins (see Section 5.3). Movements are also produced by changes in the rate of growth or by the breakdown of microtubules and microfilaments. Generally speaking, changes in both cell shape and cell movement play important roles in cleavage, gastrulation, and organogenesis.

Changes in Cell Shape. Changes in cell shape typically result from reorganization of the cytoskeleton. For example, during the development of the neural plate in frogs, the ectoderm flattens and thickens; that is, microtubules within cells in the ectoderm layer lengthen and slide farther apart, causing the cells to change from a cubelike to a columnar shape **(Figure 48.18a).**

Once formed, the neural plate sinks downward along its midline. This change is produced by a change in cell shape from columnar to wedgelike **(Figure 48.18b).** As the tops of the cells narrow, the entire cell layer is forced inward—it invaginates. How does this occur? Each wedge-shaped cell contains a group of microfilaments arranged in a circle at its top. Research suggests that the microfilaments slide over each other, tightening the ring like a drawstring and narrowing the top of the cell. This mechanism is supported by experiments in which cytochalasin, a chemical that interferes with microfilament assembly, was added to the cells. As a result, the microfilament circle was dispersed, and no invagination of the ectoderm occurred.

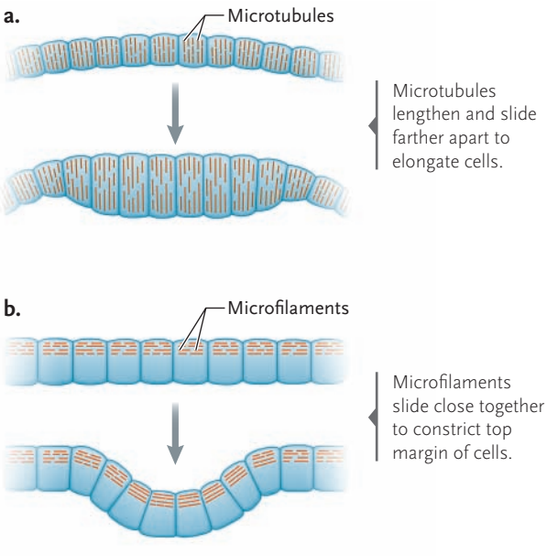


Figure 48.18
The roles of microtubules **(a)** and microfilaments **(b)** in the changes in cell shape that produce developmental movements.

Whole-Cell Movements. Among the most striking examples of whole-cell movements in embryonic development are the cell movements during gastrulation and the often long-distance migrations of neural crest cells. These whole-cell movements involve the coordinated activity of microtubules and microfilaments. The typical pattern of movement is a repeating cycle of steps that resemble how an amoeba moves. First, a cell attaches to the substrate (**Figure 48.19,** step 1) and moves forward by elongating from the point of attachment (step 2). The cell next makes a new attachment at the advancing tip (step 3), and then contracts until the rearmost attachment breaks (step 4). The front attachment now serves as the base for another movement.

How do the cells know where to go? Typically, cells migrate over the surface of stationary cells in one of the embryo's layers. In many developmental systems, migrating cells follow tracks formed by molecules of the extracellular matrix (ECM), secreted by the cells along the route over which they travel. An important

Insights from the Molecular Revolution

Turning On Male Development

The switch to male development in mammalian embryos is triggered by the protein encoded in the *SRY* gene, carried on the Y chromosome. Individuals with a mutation in which *SRY* encodes a faulty, inactive protein develop into females, even though they have the XY combination of sex chromosomes.

Molecular studies revealed that the mutant SRY proteins have changes in single amino acids or have a missing segment. All the single amino acid changes are concentrated in a region of the SRY protein known as the *HMG box,* which can bind to DNA. This discovery suggested that SRY is a regulatory protein that binds to the control regions of genes such as *AMH,* which encodes the anti-Müllerian hormone, and turns them on.

A group of investigators led by Michael Weiss at the Harvard Medical School and Massachusetts General Hospital in Boston carried out molecular studies testing whether SRY directly turns on the *AMH* gene. For their experiments, the researchers attached the control region of the *AMH* gene to the coding portion of a luciferase gene. Luciferase is the firefly enzyme that catalyzes a reaction with the substrate luciferin to produce light. In this experiment, luciferase was used as a reporter; measuring its activity indirectly informed the researchers about the molecular reactions occurring with the *AMH* gene. Luciferase activity is measured by breaking open cells to produce cell extracts, adding the substrate luciferin to samples of the extract, and quantifying the light emitted from the reaction using a special photodetector system. The composite *AMH*-luciferase gene was introduced into embryonic cells removed from the developing gonads of XY rat embryos, taken at the time when differentiation into a testis or ovary would normally begin.

A normal human *SRY* gene was then introduced into the gonad cells containing the artificial gene. High luciferase activity was seen, confirming that the normal SRY protein activates the *AMH* gene. The experiment was repeated with a mutant *SRY* gene isolated from a human patient who had developed into a female even though she had the XY combination of sex chromosomes. In her case, the mutation resulted from a change of a single amino acid in the HMG box of the SRY protein. When her *SRY* gene was added to the embryonic rat gonad cells, there was no luciferase activity, indicating that her altered SRY protein could not turn on the *AMH* gene.

Adding a normal SRY protein to the *AMH* gene in a test tube showed that the protein binds directly to the gene. Tests with DNA-digesting enzymes showed that combination with SRY protects a segment of the control region of the *AMH* gene from attack by the enzymes. This protection indicates that SRY binds in this region, as expected for a regulatory protein.

Current goals include finding the genes activated by SRY that direct development toward the male. The research promises to reveal the complete sequence of molecular events directing male development. It may also lead to treatments for developmental abnormalities produced when the sex-determining system goes awry.

track molecule is *fibronectin,* a fibrous, elongated protein of the ECM. Migrating cells recognize and adhere to the fibronectin; in response, internal changes in the cells trigger movement in a direction based on the alignment of the fibronectin molecules.

Some migrating cells follow concentration gradients instead of molecular tracks. The gradients are created by the diffusion of molecules (often proteins) released by cells in one part of an embryo. Cells with receptors for the diffusing molecule follow the gradient toward its source, or move away from the source.

Selective Cell Adhesions Underlie Cell Movements

Selective cell adhesion, the ability of an embryonic cell to make and break specific connections to other cells, is closely related to cell movement. As development proceeds, many cells break their initial adhesions and move, and then form new adhesions in different locations. Final cell adhesions hold the embryo in its correct shape and form. Junctions of various kinds, including tight, anchoring, and gap junctions, reinforce the final adhesions (see Section 5.5).

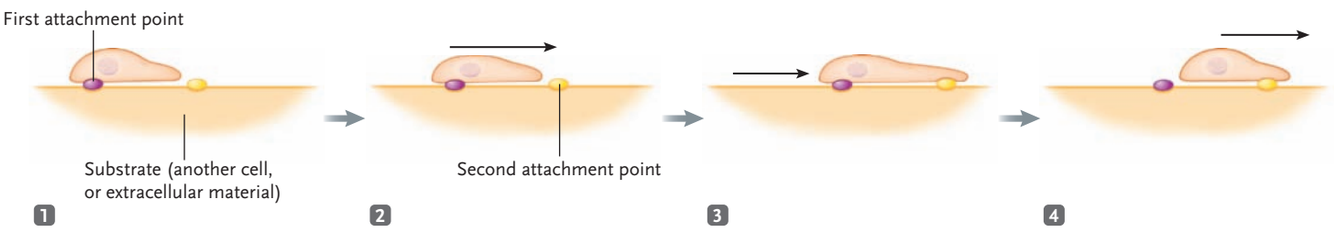


Figure 48.19 The cycle of attachments, stretching, and contraction by which cells move over other cells or extracellular materials in embryos.

The selective nature of cell adhesions was first demonstrated in a classic experiment by Johannes Holtfreter of the University of Rochester and his student P. L. Townes. In this experiment, the researchers removed pieces of ectoderm, mesoderm, and endoderm from living amphibian embryos in the neurulation stage, separated them into individual cells, and added the cells in various combinations to a culture medium. Initially the cells clumped together at random into a ball. After a few hours, they sorted themselves out and moved into arrangements resembling their normal locations in the gastrula **(Figure 48.20).**

Further research has identified many cell surface proteins responsible for selective cell adhesions, including **cell adhesion molecules** (CAMs; see Section 5.5) and **cadherins** *(calcium-dependent adhesion molecules)*. The cadherins are so named because they require calcium ions to set up adhesions. As cells develop, different types of CAMs or cadherins may appear or disappear from their surfaces as they make and break cell adhesions. The changes reflect alterations in gene activity, often in response to molecular signals arriving from other cells. For example, in the neural plate stage of neurulation in the frog, N-cadherin is on neural plate cells, keeping those cells together, while E-cadherin is on the adjacent ectodermal cells, keeping those cells together. The neural tube is produced when the neural plate cells separate from the ectodermal cells, while both cell types retain their respective cadherin type. The neural crest cells have neither cadherin bound to them, so they do not bind to each other and they disperse (as described earlier). However, if N-cadherin is expressed in the ectodermal cells through experimental manipulation, the forming neural tube does not separate from the flanking ectodermal cells because all of the cells are held together by N-cadherin.

Induction Depends on Molecular Signals Made by Inducing Cells

Recall from Section 48.1 that induction is the process in which a group of cells (the inducer cells) causes or influences a nearby group of cells (the responder cells) to follow a particular developmental pathway. Recall also that induction is the major process responsible for determination, in which the developmental fate of a cell is set. Many experiments have shown that induction occurs through the interaction of signal molecules with surface receptors on the responding cells. The signal molecules may be located on the surface of the inducing cells, or they may be released by the inducing cells. The surface receptors are activated by binding the signal molecules; in the activated form, they trigger internal response pathways that produce the developmental changes (surface receptors and their associated signal transduction pathways are discussed in Sections 7.3 and 7.4). Often, the responses include changes in gene activity.

A German scientist, Hans Spemann of the University of Freiburg, carried out the first experiments identifying induction in embryos in the 1920s. He and his doctoral student, Hilde Mangold, found that if the dorsal lip of a newt embryo was removed and grafted into a different position on another newt embryo, on the ventral side for instance, cells moving inward from the dorsal lip induced a neural plate, a neural tube, and eventually an entire embryo to form in the new location **(Figure 48.21).** On the basis of his pioneering research, Spemann proposed that the dorsal lip is an *organizer,* acting on other cells to alter the course of development. This action is now known as *induction,* and the cells responsible for induction are known generally as inducer cells. Spemann received the Nobel Prize in 1935 for his research. (In 1924, the year their research paper was published, Mangold died in an accident when her kitchen gasoline heater exploded. She would likely have also received the Nobel Prize, but they are never awarded posthumously.)

Spemann's findings touched off a search for the inducing molecules that must pass from the inducing cells to the responding cells. It took many years to achieve success. Finally, molecular techniques led to the identification of inducing molecules. For example, in 1992, researchers constructed a DNA library from *Xenopus* gastrulas by isolating and cloning the cellular DNA in gene-size pieces. They made mRNA transcripts of the cloned genes and injected them into early *Xenopus* embryos in which the inducing ability of the mesoderm had been destroyed by exposure to ultraviolet light. Some of the injected mRNAs, translated into proteins in the embryos, were able to induce formation of a neural plate and tube and lead to a normal embryo. More than 10 other proteins acting as inducing molecules have been identified in the *Xenopus* system.

Differentiation Produces Specialized Cells without Loss of Genes

Differentiation is the process by which cells that have committed to a particular developmental fate by the determination process (see Section 48.1) now develop into specialized cell types with distinct structures and functions. As part of differentiation, cells concentrate on the production of molecules characteristic of the specific types. For example, 80% to 90% of the total protein that lens cells synthesize is crystallin.

Research into differentiation confirmed that as cells specialize, they retain all the genes of the original egg cell; except in rare instances, differentiation does not occur through selective gene loss. Several definitive experiments supporting this conclusion were carried out several decades ago by Robert Briggs and Thomas King of Lankenau Hospital Research Institute in Philadelphia (now Fox Chase Cancer Center),

Figure 48.20 Experimental Research

Demonstrating the Selective Adhesion Properties of Cells

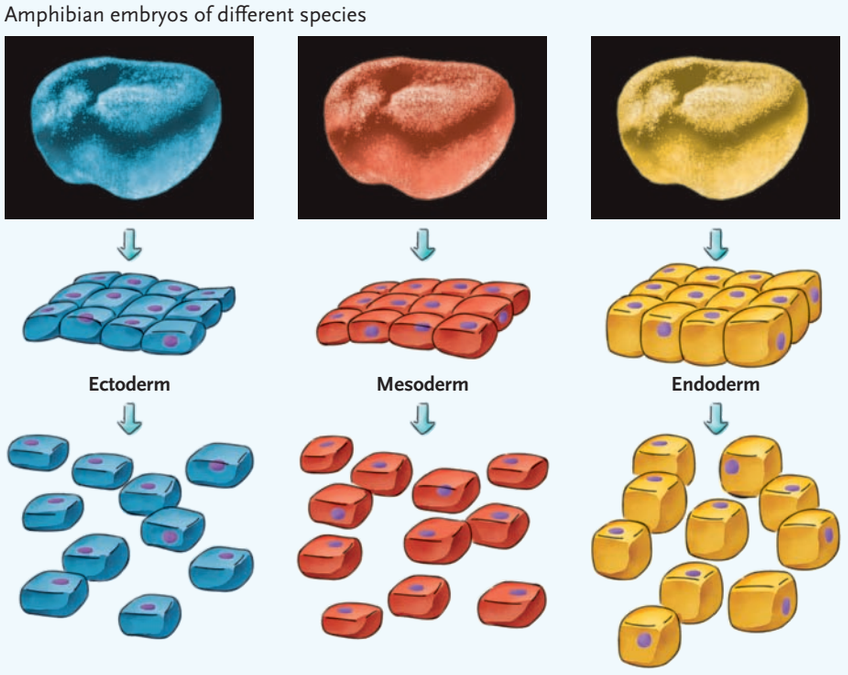
QUESTION: Do cells make specific connections to other cells?

EXPERIMENT: Johannes Holtfreter and P. L. Townes demonstrated that cells make specific connections to other cells, that is, that cells have selective adhesion properties.

1. Holtfreter and Townes separated ectoderm, mesoderm, and endoderm tissue from amphibian embryos soon after the neural tube had formed. They used embryos from amphibian species that had cells of different colors and sizes, so they could follow under the microscope where each cell type ended up. (The colors shown here are for illustrative purposes only.)

2. The researchers placed the tissues individually in alkaline solutions, which caused the tissues to break down into single cells.

3. Holtfreter and Townes then combined suspensions of single cells in various ways. Shown here are ectoderm + mesoderm, and ectoderm + mesoderm + endoderm. When the pH was returned to neutrality, the cells formed aggregates. Through a microscope, the researchers followed what happened to the aggregates on agar-filled petri dishes.



RESULTS: In time the reaggregated cells sorted themselves with respect to cell type; that is, instead of the cell types remaining mixed, each cell type became separated spatially. That is, in the ectoderm + mesoderm mixture, the ectoderm moved to the periphery of the aggregate, surrounding mesoderm cells in the center. In no case did the two cell types remain randomly mixed. The ectoderm + mesoderm + endoderm aggregate showed further that cell sorting in the aggregates generated cell positions reflecting the positions of the cell types in the embryo. That is, the endoderm cells separated from the ectoderm and mesoderm cells and became surrounded by them. In the end, the ectoderm cells were located on the periphery, the endoderm cells were internal, and the mesoderm cells were between the other two cell types.

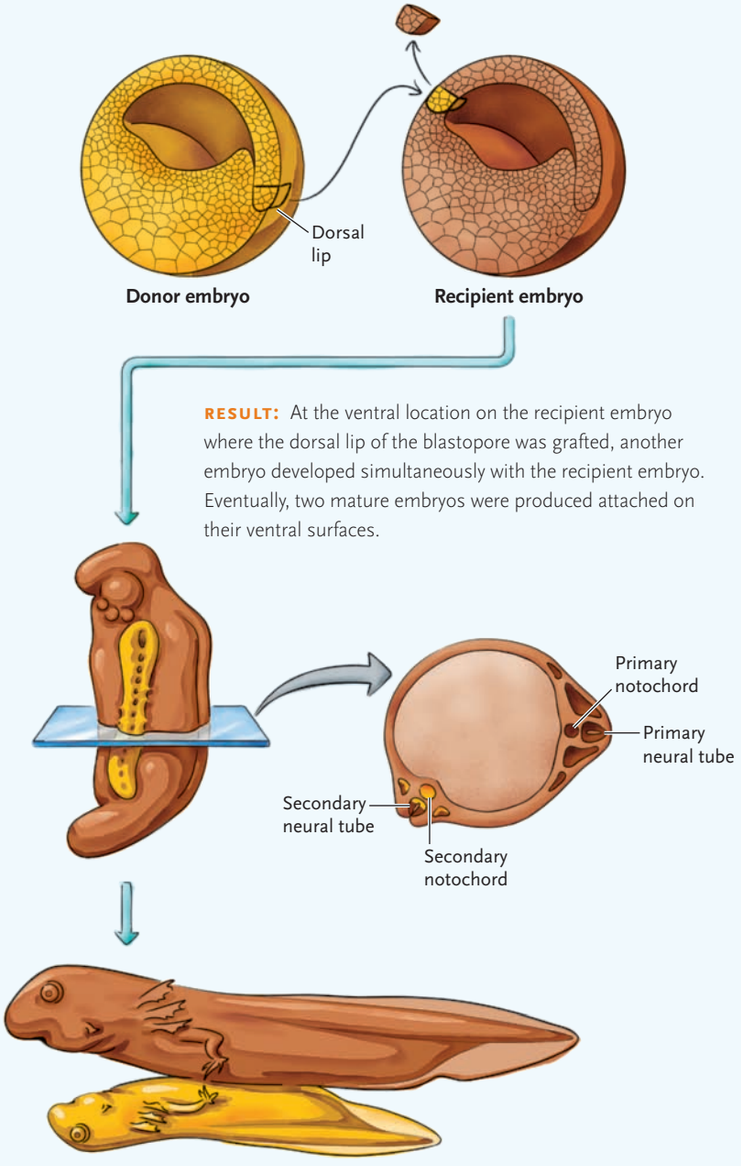
CONCLUSION: Holtfreter interpreted the results to mean that cells have selective affinity for each other; that is, cells have selective adhesion properties. Specifically, he proposed that ectoderm cells have positive affinity for mesoderm cells but negative affinity for endoderm cells, while mesoderm cells have positive affinity for both ectoderm cells and endoderm cells. In modern terms, these properties result from cell surface molecules that give cells specific adhesion properties.

Figure 48.21 Experimental Research

Spemann and Mangold's Experiment Demonstrating Induction in Embryos

QUESTION: Does induction occur in embryonic development?

EXPERIMENT: Hans Spemann and Hilde Mangold performed transplantation experiments with newt embryos, the results of which demonstrated that specific induction of development occurs in the embryos. The researchers removed the dorsal lip of the blastopore from one newt embryo and grafted it onto a different position—the ventral side—of another embryo. The two embryos were from different newt species that differed in pigmentation, allowing them to follow the fate of the tissue easily. The embryo with the transplant was allowed to develop.



RESULT: At the ventral location on the recipient embryo where the dorsal lip of the blastopore was grafted, another embryo developed simultaneously with the recipient embryo. Eventually, two mature embryos were produced attached on their ventral surfaces.

CONCLUSION: The grafted dorsal lip of the blastopore induced a second gastrulation and subsequent development in the ventral region of the recipient embryo. The result demonstrated the ability of particular cells to induce the development of other cells.

and extended by John B. Gurdon of the University of Cambridge, United Kingdom. In a typical experiment, the nucleus of a fertilized frog egg was destroyed by ultraviolet light. A micropipette was then used to transfer a nucleus from a fully differentiated tissue, intestinal epithelium, to the enucleated egg. Some of the eggs receiving the transplanted nuclei subsequently developed into normal tadpoles and adult frogs. This outcome was possible only if the differentiated intestinal cells still retained their full complement of genes. This conclusion was extended to mammals in 1997 when Ian Wilmut and his colleagues successfully cloned a sheep—Dolly—starting with an adult cell nucleus. (This experiment is described in Section 18.2.)

Fate Mapping Maps Adult Structures onto Regions of the Embryos from Which They Developed

From the early days of studying development, embryologists have focused on describing not only how embryos form and develop, but exactly how adult tissues and organs are produced from the cells of the embryo. Thus, an important goal in embryology was to trace cell lineages from embryo to adult. For most organisms it is not possible to trace lineages at the individual cell level, primarily because of the complexity of the developmental process and the typical opacity of embryos. However, it has been possible to map adult or larval structures onto the region of the embryo from which each structure developed. This type of study is called *fate mapping,* and the result is called a **fate map.** Experimentally, fate mapping is done by following development of living embryos under the microscope, either using species in which the embryo is transparent, or by marking cells so they can be followed. Cells may be marked with vital dyes (dyes that do not kill cells), fluorescent dyes, or radioactive labels. Fate maps have been produced for a number of organisms, including the chick, *Xenopus,* and *Drosophila*.

In most cases a fate map is not detailed enough to relate how particular cells in the embryo gave rise to cells of the adult. The exception is the fate map of the nematode *Caenorhabditis elegans,* an organism that has a fixed, reproducible developmental pattern. This animal has a transparent body, and scientists have been able to map the fate—trace the **cell lineage**—of every somatic and germ-line cell as the zygote divides and the resulting embryo differentiates into the 959-cell adult hermaphrodite or the 1031-cell adult male **(Figure 48.22).** They found that all somatic cells of the adult can be traced from five somatic *founder cells* produced during early development. Knowing the cell lineages of *C. elegans* has been a valuable tool for research into the genetic and molecular control of development in this organism, for mutants affecting development have an easily visualized effect.

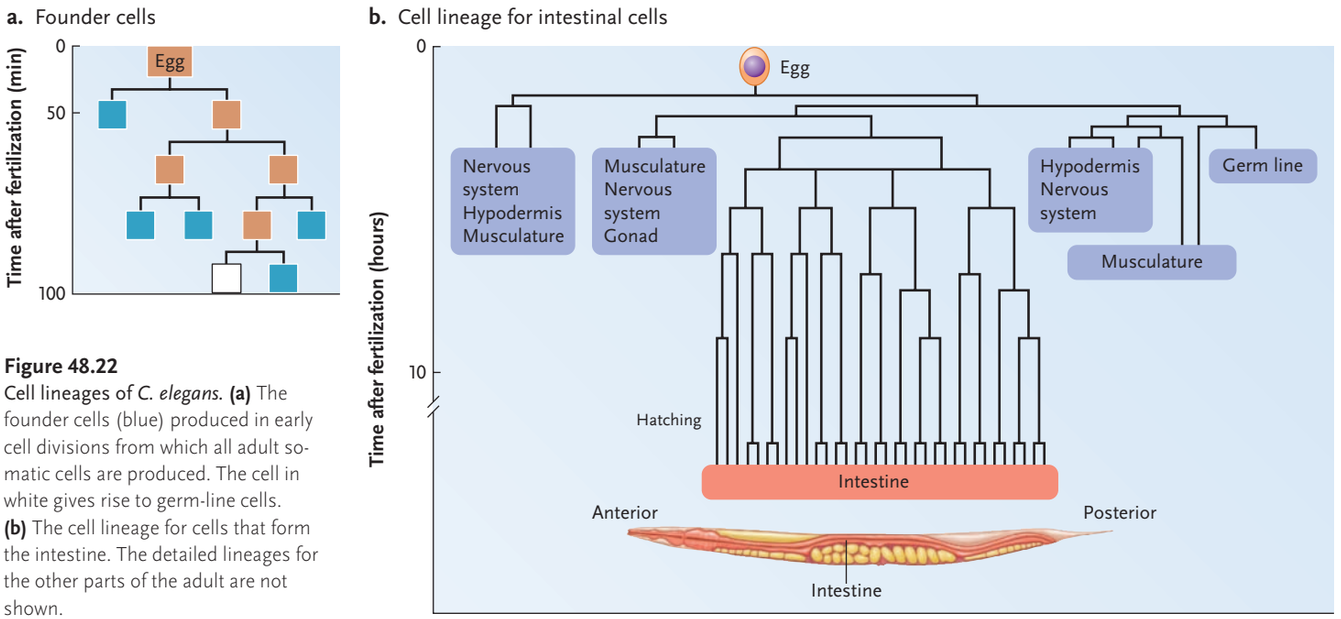


Figure 48.22
Cell lineages of *C. elegans*. **(a)** The founder cells (blue) produced in early cell divisions from which all adult somatic cells are produced. The cell in white gives rise to germ-line cells. **(b)** The cell lineage for cells that form the intestine. The detailed lineages for the other parts of the adult are not shown.

Study Break

1. What are the key cellular events that contribute to morphogenesis in animals?
2. What is induction? What molecules are involved?

48.6 The Genetic and Molecular Control of Development

We have now looked at development at the level of the whole organism, from the fertilized egg to the fully formed individual, and then at the cellular changes and movements that underlie this progression. We now turn to genetic and molecular mechanisms which, to a large extent, determine the course of development. In particular, these include the molecular mechanisms that control gene expression (see Chapter 16).

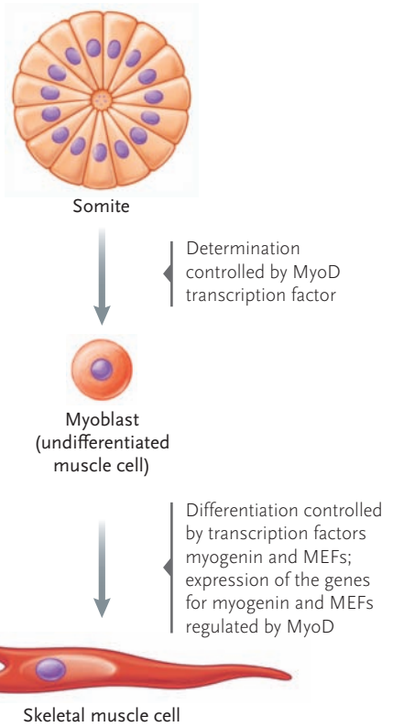
Developmental biologists are very interested in identifying and characterizing the genes involved in development, and defining how the products of the genes regulate and bring about the elaborate events we see. One productive research approach has been to isolate mutants that affect developmental processes. Researchers can then identify the genes involved, clone these genes, and analyze them in detail to build models for the molecular functions of the gene products in development. A number of model organisms are used for these studies because of the relative ease with which mutants can be made and studied and the ease of performing molecular analyses. These organisms include the fruit fly *(Drosophila melanogaster)* and *C. elegans* among invertebrates, and the zebrafish *(Danio rerio)* and the mouse *(Mus musculus)* among vertebrates. *Focus on Research* describes why the zebrafish is a valuable model organism for genetic and molecular studies of development.

Genes Control Cell Determination and Differentiation

As you have learned, determination, the setting of the developmental fate of a cell, in many cases is the result of induction. The end result of determination is differentiation, which produces cell types of particular kinds, such as skin cells or nerve cells. Both determination and differentiation involve specific, regulated changes in gene expression.

One well-studied example of the genetic control of determination and differentiation is the production of skeletal muscle cells from somites in mammals **(Figure 48.23).** Recall that somites are blocks of mesoderm cells that form along both sides of the notochord (see Figure 48.11). Under genetic control, particular cells of a somite differentiate into skeletal muscle cells. First, paracrine signaling from nearby cells induces those somite cells to express the master regulatory gene, *myoD*. The product of *myoD* is the transcription factor MyoD. By turning on specific muscle-determining genes, the action of MyoD brings about the determination of those cells, converting them to undifferentiated muscle cells known as **myoblasts.** Among the genes that MyoD regulates are the myogenin and MEF genes. These genes are also regulatory genes, expressing transcription factors in the myoblasts that turn on yet another set of genes. The products of those genes—which in-

Figure 48.23
The genetic control of determination and differentiation involved in mammalian skeletal muscle cell formation.



clude myosin, a major protein involved in muscle contraction—are needed for the differentiation of myoblasts into skeletal muscle cells.

Generally speaking, the molecular mechanisms involved in determination and differentiation depend on regulatory genes that encode regulatory proteins controlling the expression of other genes. In essence, the regulatory genes act as master regulators; expression of the regulatory genes is controlled by induction in most cases.

Genes Control Pattern Formation during Development

As a part of the signals guiding differentiation, cells receive positional information that tells them where they are in the embryo. The positional information is vital to **pattern formation:** the arrangement of organs and body structures in their proper three-dimensional relationships. Positional information is laid down primarily in the form of concentration gradients of regulatory molecules produced under genetic control. In most cases, gradients of several different regulatory molecules interact to tell a cell, or a cell nucleus, where it is in the embryo. Below, we describe in brief the results of studies of the genetic control of pattern formation during the development of the fruit fly, *Drosophila melanogaster*. The developmental principles discovered from these studies apply to many other animal species, including humans.

Embryogenesis in *Drosophila*. The production of an adult fruit fly from a fertilized egg occurs in a sequence of genetically controlled development events. Following fertilization, division of the nucleus begins by mitosis, but the cytoplasm does not divide in the early embryo (cytokinesis does not occur) **(Figure 48.24).** The result is a multinucleate blastoderm. At the tenth nuclear division, the nuclei migrate to the periphery of the embryo where, after three more divisions, the 6000 or so nuclei are organized into separate cells. At this stage, the embryo is a *cellular blastoderm,* corresponding to a late blastula stage in the animals we discussed earlier. The cellular blastoderm develops into a segmented embryo (an embryo with distinct segments); at that point, 10 hours have passed since the egg was fertilized. About 24 hours after fertilization, the egg hatches into a larva, which undergoes three molts, then becoming a pupa. The pupa undergoes metamorphosis to produce the adult fly, which emerges about 10–12 days after fertilization. As illustrated by the color usage in Figure 48.24, the segments of the embryo can be mapped to the segments of the adult fly.

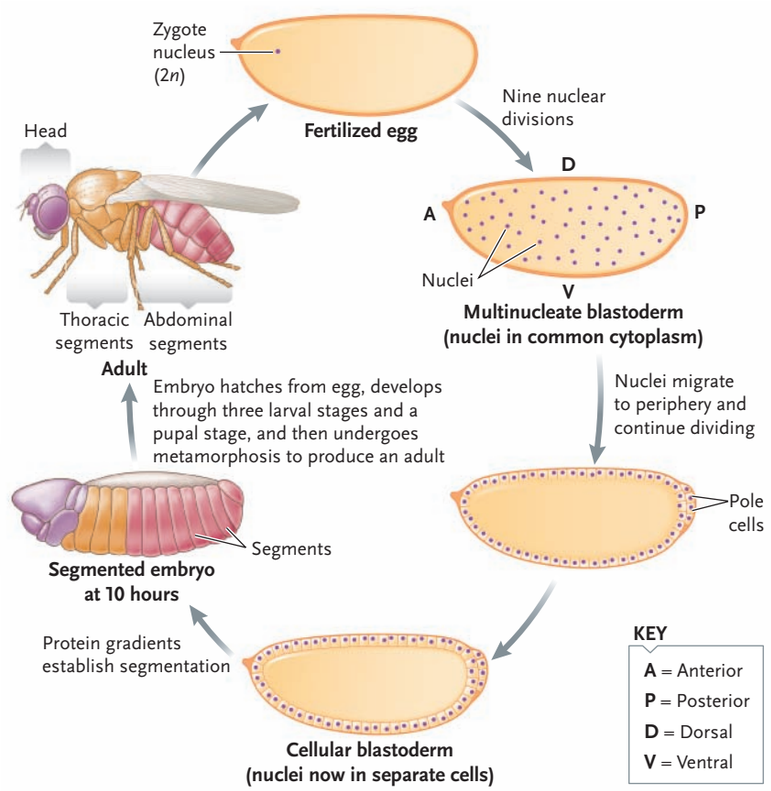


Figure 48.24
Embryogenesis in *Drosophila* and the relationship between segments of the embryo and segments of the adult.

Genetic Analysis of *Drosophila* Development. The study of developmental mutants by a large number of researchers has given us important information about

Focus on Research

Model Research Organisms: The Zebrafish Makes a Big Splash as the Vertebrate Fruit Fly


David M. Parichy

The zebrafish *(Danio rerio)* is a small (3 cm) freshwater fish that gets its name from the black and white stripes running along its body. Native to India, it has spread around the world as a favorite aquarium fish. Beginning about 30 years ago, it began also to be used in scientific laboratories as a model vertebrate organism for studying the roles of genes in development. Its use is now so widespread that it has been dubbed the "vertebrate fruit fly."

The zebrafish brings many advantages as a model research organism. It can be maintained easily in an ordinary aquarium on a simple diet. Although its generation time is relatively long (3 months for the zebrafish as compared with $1\frac{1}{2}$ months for the mouse), a female zebrafish produces about 200 offspring at a time, as compared with an average of 10 for the mouse.

Embryonic development of the zebrafish takes place in eggs released to the outside by the female. The embryos develop rapidly, taking only 3 days from egg laying to hatching. Best of all, the eggs and embryos are transparent, providing an open window that allows researchers to observe developmental stages directly, with little or no disturbance to the embryo. Observational conditions are so favorable that the origin and fate of each cell can be traced from the fertilized egg to the hatchling. Individual nerve cells can be traced, for example, as they grow and make connections in the brain, spinal cord, and peripheral body regions. Removing or transplanting cells and tissues is also relatively easy. Biochemical and molecular studies can be carried out by techniques ranging from the simple addition of reactants to the water surrounding the embryos to injection of chemicals into individual cells.

The zebrafish has some advantages for developmental studies compared with other vertebrate organisms used as developmental models, including the amphibian *Xenopus,* and the mouse. The early developmental stages of a zebrafish are remarkably like those of mammals, and adult structures such as the eye and skeletal system are typically vertebrate. *Xenopus* takes years to become developmentally mature and produce offspring, and it is not readily amenable to genetic analysis. Although the mouse is a mammal with development and anatomy closely related to those of humans, mouse embryos develop inside the mother and can be observed only by removing them from the mother's body; outside the body, they can be maintained only by demanding and elegant experimental techniques. Chemical studies while the embryo is inside the mother are difficult to perform. Additionally, maintaining colonies of mice is expensive.

The advantages of working with the zebrafish have spurred efforts to investigate its genetics, with particular interest in genes that regulate embryonic development. This work has already identified mutants of more than 2000 genes, including more than 400 genes that influence development. Most of the mechanisms controlled by the developmental genes resemble their counterparts in humans and other mammals.

For some of the zebrafish genes, developmental and physiological studies have revealed functions that were previously unknown for their mammalian equivalents. For example, Nancy Hopkins, a developmental geneticist at MIT, found a gene necessary for normal liver and gut development in the zebrafish. The gene is 80% identical in nucleotide sequence to a human gene; identification of the gene's role in zebrafish gave the first clues to its function in mammals. Other zebrafish mutants have been identified that affect development of the brain and spinal cord, the eyes and ears, the skeletal and digestive systems, and the circulatory system, including the heart, blood vessels, and blood cells. The mutants open these systems to biochemical and molecular study and experimentation.

Genetic studies with the zebrafish have been reinforced by a project to obtain the DNA sequence of its entire genome. The sequencing project, which was completed in 2005, will allow all the zebrafish genes to be located, identified, and correlated with their equivalents in humans and other model research organisms, including the mouse, *C. elegans,* and *Drosophila.* Undoubtedly, the zebrafish will continue to be a valuable model in developmental biology research.

Drosophila development. Three researchers performed key, pioneering research with developmental mutants: Edward B. Lewis of the California Institute of Technology, Christiane Nüsslein-Volhard of the Max Planck Institute for Developmental Biology in Tübingen, Germany, and Eric Wieschaus of Princeton University. The three shared a Nobel Prize in 1995 "for their discoveries concerning the genetic control of early embryonic development."

Nüsslein-Volhard and Wieschaus studied early embryogenesis. They searched for *every* gene required for early pattern formation in the embryo. They did this by looking for recessive *embryonic lethal* mutations. These mutations, when homozygous, result in the death of the embryo during development. By examining at what stage of development an embryo died, and how development was disrupted, they gained insights into the role of the particular genes in embryogenesis.

Lewis studied mutants that changed the fates of cells in particular regions in the embryo, producing structures in the adult that normally were produced by other regions. His work was the foundation of research identifying master regulatory genes that control the development of body regions in a wide range of organisms.

Maternal-Effect Genes and Segmentation Genes for Establishing the Body Plan in the Embryo. A number of genes control the establishment of the embryo's body plan. These genes regulate the expression of other genes. There are two classes: *maternal-effect genes,* and *segmentation genes* that work sequentially **(Figure 48.25).**

Many **maternal-effect genes** are expressed by the mother during oogenesis. These genes control the polarity of the egg and, therefore, of the embryo. Some control the formation of the anterior structures of the embryo, others control the formation of the posterior structures, and yet others control the formation of the terminal end.

The *bicoid* gene is the key maternal-effect gene responsible for head and thorax development. The *bicoid* gene is transcribed in the mother during oogenesis, and the resulting mRNAs are deposited in the egg, localizing near the anterior pole **(Figure 48.26).** After the egg is fertilized, translation of the mRNAs produces BICOID protein, which diffuses through the egg to form a gradient with its highest concentration at the anterior end of the egg, and fading to none at the posterior end of the egg. The BICOID protein is a transcription factor that activates some genes and represses others along the anterior–posterior axis of the embryo. Embryos with mutations in the *bicoid* gene have no thoracic structures, but have posterior structures at each end. Researchers concluded, therefore, that the *bicoid* gene in normal embryos is a master regulator gene controlling the expression of genes for the development of anterior structures (head and thorax).

A number of other maternal-effect genes, through the activities of their products in gradients in the embryo, are also involved in axis formation. The *nanos* gene, for instance, is the key maternal-effect gene for the posterior structures. When the *nanos* gene is mutated, embryos lack abdominal segments.

Once the axis of the embryo is set, the expression of at least 24 **segmentation genes** progressively subdivides the embryo into regions, determining the segments of the embryo and the adult (see Figure 48.25). Gradients of BICOID and other proteins encoded by maternal-effect genes regulate expression of the embryo's segmentation genes differentially. That is, each segmentation gene is expressed at a particular time and in a particular location during embryogenesis.

Three sets of segmentation genes are regulated in a cascade of gene activations. The first set to be expressed is the **gap genes,** for example, *hunchback* and *tailless*. These genes are activated based on their positions in the maternally directed anterior–posterior axis of the egg by reading the concentrations of BICOID and other proteins. Gap genes, through their activation of the next genes in the regulatory cascade, control the subdivision of the embryo along the anterior–posterior axis into several broad regions. Mutations in gap genes result in the loss of one or more body segments in the embryo **(Figure 48.27a).**

The products of gap genes are transcription factors that activate **pair-rule genes.** The actions of the prod-

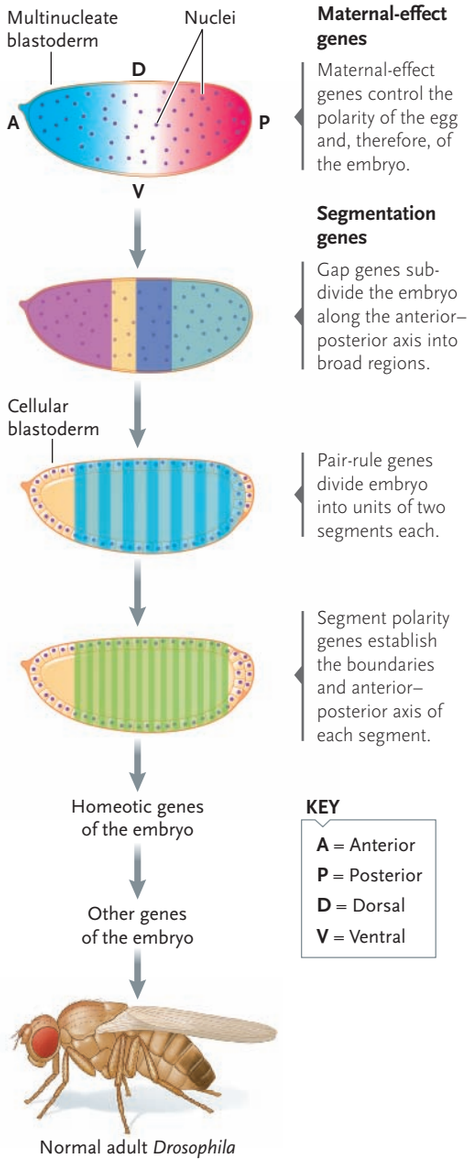


Figure 48.25
Maternal-effect genes and segmentation genes and their role in *Drosophila* embryogenesis.

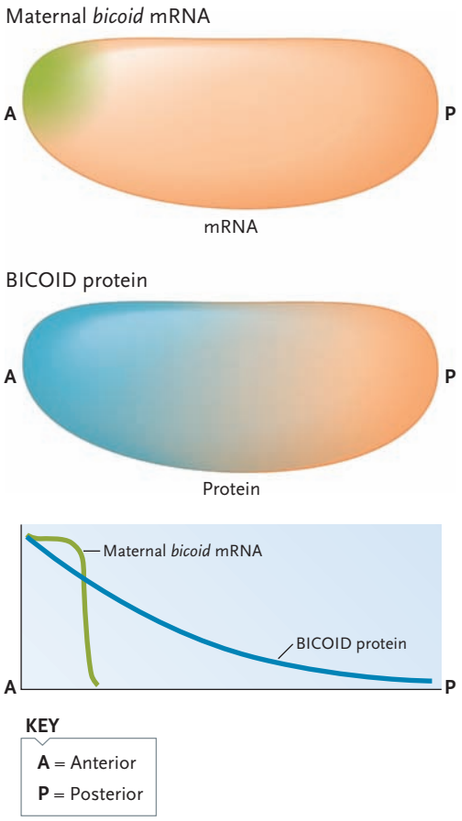


Figure 48.26
Gradients of *bicoid* mRNA and BICOID protein in the *Drosophila* egg.

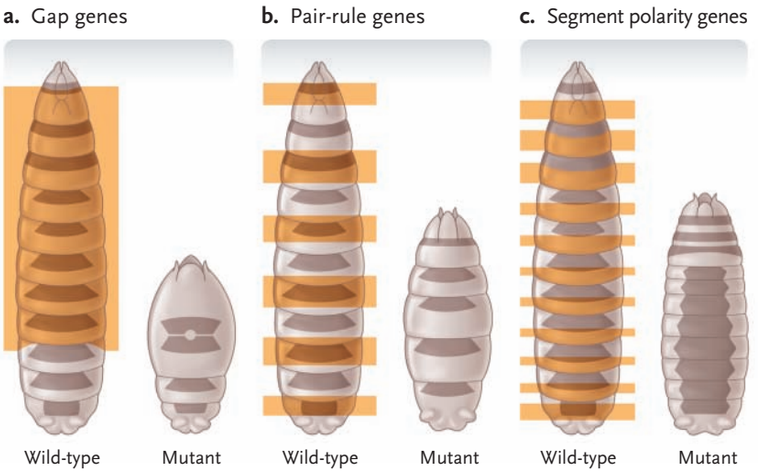


Figure 48.27
Examples of effects of mutations in the different types of segmentation genes of *Drosophila*. Blue highlights indicate wild-type segments that are mutated. **(a)** Gap gene mutants lack one or more segments. **(b)** Pair-rule gene mutants are missing every other segment. **(c)** Segment polarity gene mutants have segments with one part missing and the other part duplicated as a mirror image.

ucts of pair-rule genes divide the embryo into units of two segments each. Mutations in pair-rule genes delete every other segment of the embryo **(Figure 48.27b).**

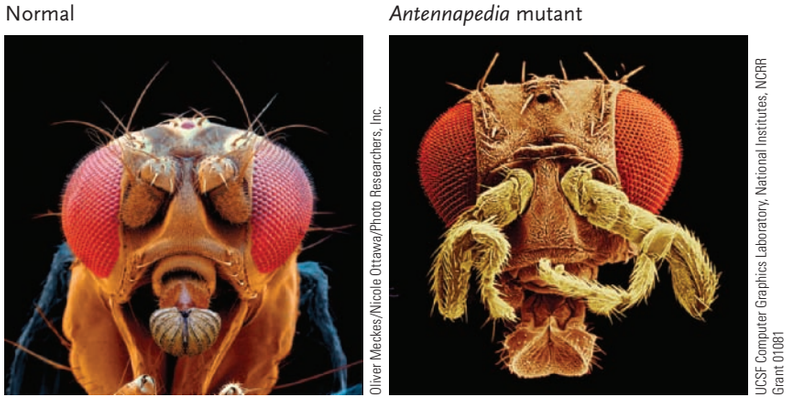
The products of pair-rule genes are transcription factors that regulate the expression of the last set of genes in the series, the **segment polarity genes.** The actions of the products of segment polarity genes set the boundaries and anterior–posterior axis of each segment in the embryo. Mutations in segment polarity genes produce segments in which one part is missing and the other part is duplicated as a mirror image **(Figure 48.27c).** The products of segment polarity genes are transcription factors and other molecules that regulate other genes involved in laying down the pattern of the embryo.

Homeotic Genes for Specifying the Developmental Fate of Each Segment. Once the segmentation pattern has been set, **homeotic** (structure-determining) **genes** of the embryo specify what that segment will become after metamorphosis. In normal flies, homeotic genes are master regulatory genes that control the development of structures such as eyes, antennae, legs, and wings on particular segments (see Figure 48.24). Researchers discovered the role of homeotic genes from the study of mutations in these genes; such mutations alter the developmental fate of a segment in the embryo in a major way. For example, in flies with a mutation in the *Antennapedia* gene, legs develop in place of antennae **(Figure 48.28).**

How do homeotic genes regulate development? Homeotic genes encode transcription factors that regulate expression of genes responsible for the development of adult structures. Each homeotic gene has a common region called the **homeobox** that is key to its function. A homeobox corresponds to an amino acid section of the encoded transcription factor called the **homeodomain.** The homeodomain of each protein binds to a region in the promoters of the genes whose transcription it regulates.

Homeobox-containing genes are called *Hox* genes. There are eight *Hox* genes in *Drosophila* and, interest-

Figure 48.28
Antennapedia, a homeotic mutant of *Drosophila*, in which legs develop in place of antennae.



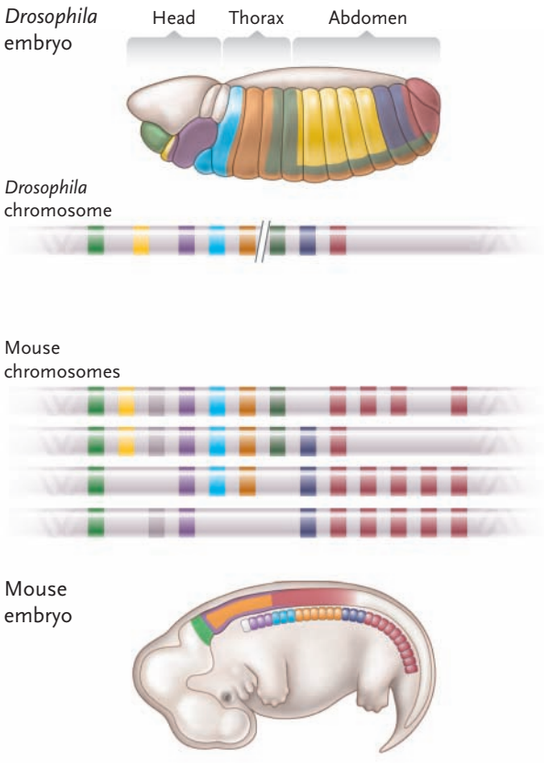


Figure 48.29
The *Hox* genes of the fruit fly and the corresponding regions of the embryo they affect. The mouse has four sets of *Hox* genes on four different chromosomes. Their relationship to the fruit fly genes is shown by the colors.

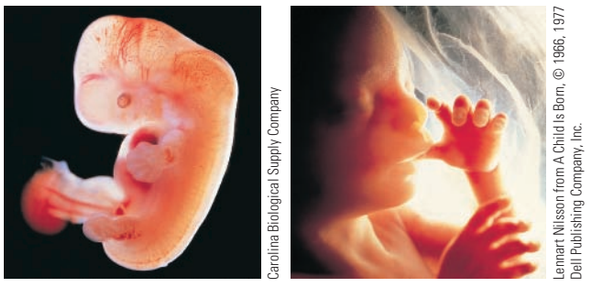
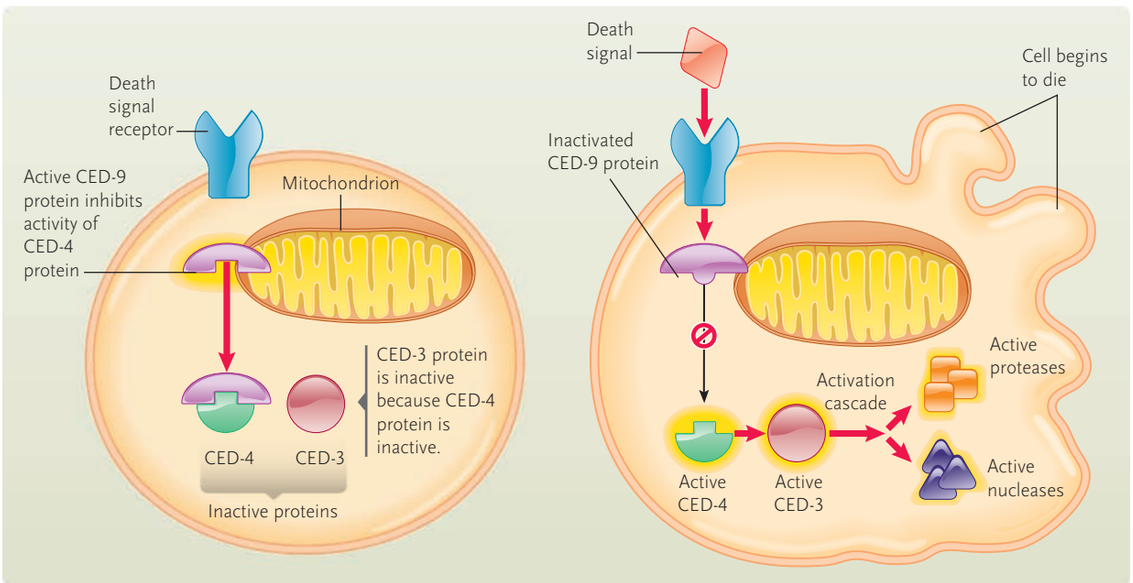


Figure 48.30
An illustration of apoptosis in humans: the removal of tissue between developing fingers and toes to produce the free fingers and toes later in development.

ingly, they are organized along a chromosome in the same order as they are expressed along the anterior–posterior body axis **(Figure 48.29).**

The discovery of *Hox* genes in *Drosophila* led to a search for equivalent genes in other organisms. The result of that search has shown that *Hox* genes are present in all major animal phyla. In each case, the genes control the development of the segments/regions of the body and are arranged in order in the genome. (This is a good example of how the results of studies in a model research organism are broadly applicable.) The homeobox sequences in the *Hox* genes are highly conserved,



Apoptosis is inhibited as long as CED-9 protein is active; cell remains alive.

When a death signal binds to the death signal receptor, it activates the receptor, which leads to inactivation of CED-9 protein. As a result, CED-4 protein is no longer inhibited and becomes active, activating CED-3 protein. Active CED-3 triggers a cascade of activations producing active proteases and nucleases, which cause the changes seen in apoptotic cells and eventually to cell death.

Figure 48.31
The molecular basis of apoptosis in *C. elegans.* **(a)** In the absence of a death signal, no apoptosis occurs. **(b)** In the presence of a death signal, activation of CED-4 and CED-3 proteins triggers a pathway that leads to the cell's death.

indicating common function in the wide range of animals in which they are found. For example, the homeobox sequences of mammals are the same or very similar to those of the fruit fly (see Figure 48.29).

Homeotic genes are also found in plants. For example, many homeotic mutations that affect flower development have been identified and analyzed in *Arabidopsis* (see Section 34.5).

Apoptosis Is Triggered by Cell-Death Genes

We have noted the role of apoptosis in the breakdown of a tadpole's tail; there are many other examples of apoptosis in both vertebrate and invertebrate development. In humans, for example, the developing fingers and toes are initially connected by tissue, forming a paddle-shaped structure; later in development, cells of the tissue die by apoptosis resulting in separated fingers and toes **(Figure 48.30).** Like many other mammals, kittens and puppies are born with their eyes sealed shut by an unbroken layer of skin. Just after birth, cells die in a thin line across the middle of each eyelid, freeing the eyelids to open. During the pupation stage that converts a caterpillar to a butterfly, many tissues of the larva break down by apoptosis and are replaced by newly formed adult tissues.

Apoptosis results from gene activation, in response to molecular signals received by receptors at the surfaces of the marked cells. In effect, the signals amount to a death notice, delivered at a specific time during embryonic development. For example, in the nematode *C. elegans,* division of the fertilized egg leads to a total of 1090 cells. Of these, exactly 131 die at prescribed times to produce a total of 959 cells in the adult hermaphrodite.

The molecular basis of apoptosis in *C. elegans* involves a molecule that can be considered a death signal binding to a receptor in the plasma membrane of a target cell for apoptosis. The receptor is activated and this leads to activation of proteins that kill the cell. In the absence of the death signal, the killing proteins remain inactive.

Let's walk through the two situations. In the absence of a death signal, the membrane receptor is inactive **(Figure 48.31a).** This allows a protein associated with the outer mitochondrial membrane, CED-9 (encoded by the *ced-9 ce*ll *d*eath gene) to inhibit CED-4 (encoded

Unanswered Questions

What gene or factor initiates sex determination in birds?

You learned in this chapter that the presence of the *SRY* gene on the Y chromosome determines that a mammal will develop as a male, but the molecular basis for sex determination in birds is unknown. It is known that avian sex determination is chromosomal and that birds have a ZW sex-determination system, which is reversed compared with the mammalian XY system. That is, female birds have two different sex chromosomes—they are ZW—and males have two identical chromosomes—they are ZZ. At least two candidate genes are being investigated for their possible role in avian sex determination: *DMRT1* (Z-linked *d*oublesex- and *m*ab-3-*r*elated *t*ranscription factor gene), which contains a highly conserved DM (cysteine-rich DNA binding) domain and encodes for a protein that is specifically enhanced more in male urogenital development than in female development, and *ASW* (*A*vian *S*pecific gene on the *W* chromosome, also known as *HINTW, PKC1W,* or *Wpkci*), an altered form of a protein kinase C inhibitor gene.

In mammals, one of the two X chromosomes in females is inactivated through the process of dosage compensation, a genetic regulatory mechanism that equalizes the phenotypic expression determined by X-linked genes so that they are equally expressed in XY males and XX females (as described in Section 13.2). But dosage compensation does not occur in birds; birds lack inactivation of one of the two male Z chromosomes and seem to compensate for a double dose of Z-linked genes by other mechanisms. How do these genes alone or together determine sex in birds? Recent work on tinamou (an ancient group of South American birds) and ratites (flightless birds) by Yayoi Tsuda and Yoichi Matsuda of Hokkaido University in Japan suggests that although all bird sex chromosomes evolved from the same pair of autosomes, the Z and W sex chromosomes have independently diverged from one another several times.

What initiates childbirth?

As discussed in this chapter, oxytocin receptors in the smooth muscle cells of the uterine wall stimulate the muscle to begin the rhythmic contractions of labor marking the beginning of birth or parturition. The initiation of labor is a complex process that has yet to be fully explained, however. What fetal-derived signals lead to the initiation of labor? How do these signals play a role in preterm labor? What roles do signals from the placenta, such as prostaglandins, play in regulating the timing of birth? What roles do the fetal hypothalamus and adrenal glands play in initiating childbirth? What hormones, such as estradiol from the mother or cortisol and corticotropin-releasing factor from the fetus, send the signals that begin the process of parturition? Dr. Louis J. Muglia at Washington University School of Medicine is studying the mechanisms that control normal-term labor and how these mechanisms malfunction to result in preterm labor. Dr. Muglia's research into the timing of birth and the challenges to prevent premature births has demonstrated that prostaglandins are essential for the initiation of parturition, and he has determined that regulation of the *COX-1* gene is important for the initiation of parturition in mice. The *COX-1* gene encodes cyclooxygenase-1, a protein that acts as an enzyme to speed up the production of prostaglandins in the stomach; expression of the *COX-1* gene increases in the uterus during pregnancy. Identifying other regulatory factors may provide critical information into the timing of the onset of parturition.

Laura Carruth, an assistant professor of biology at Georgia State University, studies the genetic and hormonal factors that lead to sex differences in brain development. Her work focuses on model systems in songbirds (the Australian zebra finch) and mice. To learn more about Dr. Carruth's research, go to http://biology.gsu.edu/people/faculty/person.cfm?person=2167.

by the *ced-4* gene) and CED-3 (encoded by the *ced-3* gene), the two proteins that are needed to turn on the cell death program. Cells in this situation, with the *ced-9* gene being expressed and its product CED-9 being active, are those that normally survive to form the adult nematode. If a death signal binds to the receptor, however, the receptor becomes activated and the events that follow are typical of signal transduction pathways **(Figure 48.31b)** (see Sections 7.1–7.4). In this case, the activated receptor leads to inactivation of CED-9. Because CED-9 no longer is inhibiting them, CED-4 is activated and, in turn, activates CED-3. Activated CED-3 triggers a cascade of reactions, including the activation of proteases and nucleases that degrade cell structures and chromosomes as part of the cell death program.

Studies of mutants helped understand the role of the cell death genes in *C. elegans*. In mutants lacking a normal *ced-3* or *ced-4* gene, the 131 marked cells fail to die, producing a highly disorganized embryo. In the nervous system, for example, 103 cells that die by apoptosis in normal embryos live to form neurons in the mutants. These extra neurons, which are inserted at random in the embryo, lead to a disorganized and nonfunctional nervous system.

Genes related to *ced-3* and *ced-4* have been found in all animals that have been tested for their presence. In humans and other mammals, the equivalent of *ced-3* is the *caspase-9* gene, which encodes a protease that degrades cell structures. The *caspase-9* gene becomes active, for example, in the cells that form the webbing between the fingers and toes, and causes the webbing to break down. The equivalent of *ced-4* is the *Apaf* gene (for *A*poptotic *p*rotease-*a*ctivating *f*actor). Mammalian cells are saved from death by the *Bcl* family of genes, which are the equivalent of *ced-9* in *C. elegans*. The genes are so closely related that they retain their effects if they are exchanged between *C. elegans* and human cells.

Study Break

1. In general, how are determination and differentiation controlled?
2. How do the segmentation genes and homeotic genes of *Drosophila* differ in function?

Review

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48.1 Mechanisms of Embryonic Development

- Developmental information is stored in both the nucleus and cytoplasm of the fertilized egg. The mRNA and protein molecules that direct the first stages of development are the cytoplasmic determinants.
- The unequal distribution of yolk and other components makes eggs polar. The animal pole typically gives rise to surface structures and the anterior end of the embryo, while the vegetal pole typically gives rise to internal structures of the embryo such as the gut (Figure 48.2).
- Following fertilization, cleavage divisions produce the morula. The morula hollows out to form the blastula, which develops into the gastrula, the stage in which rearrangements of cells produce the ectoderm, mesoderm, and endoderm. Gastrulation establishes the body pattern, in that the organs and other structures of embryo arise from these three tissue layers (Figure 48.3; Table 48.1).
- Development proceeds as a result of cell division, cell movements, selective adhesions, induction, determination, and differentiation.

Animation: Where embryos develop

Animation: Stages of development

Animation: Cytoplasmic localization

48.2 Major Patterns of Cleavage and Gastrulation

- In sea urchins eggs, yolk is distributed evenly. As a result, cleavage divisions take place at the same rate in all regions of the embryo, and gastrulation follows a symmetrical pattern (Figure 48.4).
- In amphibian eggs, yolk is distributed unequally, with most in the vegetal pole. As a result, the rate of cell division is more rapid in the animal pole, and gastrulation shows an asymmetric pattern (Figures 48.5 and 48.7).
- In bird and reptile embryos, the cleavage divisions give rise to a flat disc of cells at the top of the yolk, which divides into the epiblast and the hypoblast. In gastrulation, cells of the epiblast migrate to the interior to form the endoderm and the mesoderm. The epiblast cells left at the surface form the ectoderm (Figure 48.8).
- In birds and reptiles, the yolk sac, chorion, amnion, and allantoic membrane form from extensions of the primary tissue layers. These extraembryonic membranes conduct nutrients from the yolk to the embryo, exchange gases with the environment, and store metabolic wastes (Figure 48.9).

Animation: Process of gastrulation

48.3 From Gastrulation to Adult Body Structures: Organogenesis

- In organogenesis, the three primary tissues give rise to the tissues and organs of the embryo. Organogenesis begins with neurulation, the development of the nervous system from ectoderm (Figures 48.10 and 48.11).
- The mesoderm splits into somites, which give rise to the vertebral column and to the muscles of the ribs, vertebral column, and limbs (Figure 48.11).
- Development of the eye from optic vesicles is illustrative of the inductions and differentiations common to organogenesis in vertebrates (Figure 48.12).
- Apoptosis—programmed cell death—plays an important role in development by removing tissues present during development but not in the adult organ.

Animation: Neural tube formation

Animation: Embryonic induction

Animation: AER transplant

Animation: Formation of human fingers

48.4 Embryonic Development of Humans and Other Mammals

- In humans, as in other placental mammals, cleavage divisions produce a morula that differentiates into a blastocyst. The blastocyst implants into the endometrium of the uterus, and its inner cell mass separates into the epiblast and hypoblast. The epiblast produces the ectoderm, mesoderm, and endoderm of the embryo (Figures 48.13 and 48.14a, b).
- Gastrulation, neurulation, differentiation of cell layers, and formation of extraembryonic membranes occur by mechanisms similar to those of bird and reptile embryos. Differentiation of ectoderm, mesoderm, and endoderm into their final tissues and organs also occurs in a similar way to birds and reptiles.
- Extraembryonic membranes form in mammals by processes that are also similar to the reptilian–bird pattern. However, some of the membranes have altered functions, reflecting the minimal amount of yolk in mammalian embryos, and maintenance of the embryo by the placenta (Figure 48.14c–e).
- The placenta is connected to the embryo by the umbilical cord, which conducts blood between the embryo and the placenta (Figure 48.14f).
- Fetal growth proceeds until birth, when the fetus is forced from the uterine cavity and through the vagina by contractions of the uterus, stimulated by oxytocin (Figures 48.15 and 48.16).
- The mother's mammary glands secrete milk once the offspring is born. Suckling by the offspring stimulates prolactin and oxytocin release from the pituitary, which stimulates milk production and secretion from the glands, respectively.
- Embryos develop internal male or female sex organs from the same primitive structures. The presence or absence of a Y chromosome, which carries the key *SRY* gene, determines whether the internal structures develop into male or female sexual organs (Figure 48.17).
- Most animals continue development after hatching or birth, leading to the adult, the sexually mature form of the species.

Animation: Fertilization

Animation: Cleavage and implantation

Animation: First 2 weeks of development

Animation: Weeks 3 to 4 of development

Animation: Proportional changes during development

Animation: Structure of the placenta

Animation: Fetal development

Animation: Birth

Animation: Anatomy of the breast

48.5 The Cellular Basis of Development

- Development in animals involves the regulation of specific cellular events, including cell division, cell movement, and cell adhesion.
- Cell division in development varies in orientation and rate.
- Cell movements in development occur through changes in cell shape or the migrations of entire cells. Shape changes are produced by microtubules or microfilaments. In cell migrations, cells follow tracks in the embryo or move in response to gradients of signal molecules (Figures 48.18 and 48.19).
- Selective cell adhesions, which depend on surface glycoproteins including CAMs and cadherins, underlie many cell movements. The final cell adhesions hold the embryo in its correct shape and form (Figure 48.20).
- Induction results from the effects of signaling molecules of the inducing cells on the responding cells (Figure 48.21).
- In differentiation, cells change from embryonic form to specialized types with distinct structures and functions. Differentiation occurs by differential gene activation.
- For some organisms, the origins of adult or larval structures have been mapped to regions of the embryo from which each structure derived (Figure 48.22).

48.6 The Genetic and Molecular Control of Development

- Pattern formation derives from the positions of cells in the embryo. Typically, positional information is detected by the cells in the form of concentration gradients of regulatory molecules encoded by genes (Figures 48.24–48.27).
- *Hox* genes are evolutionarily conserved regulatory genes that control the development of the segments or regions of the body (Figures 48.28 and 48.29).
- Apoptosis, programmed cell death, typically eliminates structures required for earlier but not later stages of development (Figures 48.30 and 48.31).

Questions

Self-Test Questions

1. Major contributors to the axes of the animal body are the:
   a. sperm and egg cytoplasm.
   b. sperm and egg chromosomes.
   c. ribosomes and mitochondria
   d. egg nucleus and yolk.
   e. pigments.
2. The process by which cells undergo mitosis without a corresponding increase in cytoplasm is called:
   a. polarity.
   b. cleavage.
   c. gastrulation.
   d. organogenesis.
   e. induction.
3. Which of the following mechanisms does *not* contribute to zygote development?
   a. meiosis
   b. mitosis
   c. selective cell adhesions
   d. determination
   e. induction
4. A major event during gastrulation is:
   a. the outward movement of cells at the dorsal lip of the blastopore.
   b. the displacement of the archenteron by the blastocoel.
   c. the formation of the coelom from the endoderm.
   d. the extension of ectoderm and endoderm to form the yolk sac.
   e. the development of ectoderm to form epidermal and neural tissues.
5. To contribute to the formation of a nervous system:
   a. the neural crest develops into motor neurons.
   b. the neural tube is converted into a neural plate.
   c. the notochord induces the overlying ectoderm to become a neural plate.
   d. the roof of the archenteron induces the formation of the neural tube.
   e. somites give rise to the autonomic nervous system.

6. In mammalian development:
   a. the morula develops into a trophoblast.
   b. the chorionic villi allow the blastocyst to move down the oviduct.
   c. the allantois takes over the work of the amnion.
   d. the pharyngeal arches transform into the pharynx, larynx, and nasal cavities.
   e. prolactin stimulates parturition.
7. In the development of the female sex organs:
   a. all ducts in the 7-week embryo become Wolffian ducts.
   b. the *SRY* gene is activated.
   c. anti-Mullerian hormone is secreted.
   d. the Mullerian ducts develop into oviducts.
   e. the mother secretes oxytocin.
8. In the embryonic development of the eye:
   a. the optic vesicle cells permanently adhere to each other to prevent movement, whereas the optic cup cells are very motile.
   b. signals from the optic cup trigger surface receptors on the lens placode.
   c. gradients determine that the ectoderm overlying the lens vesicle develops into the optic vesicle.
   d. microtubules powered by myosins and microfilaments powered by dyneins move the eye components around in the head region.
   e. cadherins function in the presence of calcium to allow the lens placode and optic cup to break apart.
9. In mammals, the nose is located on the anterior end and the heart in the center. These positions are the result of activation of:
   a. *Hox* genes arranged along a number of chromosomes in the same order as they are expressed along the anterior–posterior axis.
   b. maternal-effect genes after somites differentiate into muscle.
   c. *Hox* genes scattered randomly among different chromosomes.
   d. a transcription factor called the homeobox.
   e. a homeodomain that binds ribosomes.
10. During embryonic development in many humans, the cells of the lower earlobe die resulting in an unattached earlobe. For this to occur one would deduce that:
   a. *CED-3* genes are inhibited.
   b. *CED-4* genes are inhibited.
   c. caspase-9 is deactivated.
   d. *CED-9* is actively expressed.
   e. the proteins encoded by *Bcl* genes are inactivated.

Questions for Discussion

1. Experimentally, it is possible to divide an amphibian egg so that the gray crescent is wholly within one of the two cells formed. If the two cells are separated, only the cell with the gray crescent will form an embryo with a long axis, notochord, nerve cord, and back musculature. The other forms a shapeless mass of immature gut and blood cells. Propose an explanation of these outcomes.
2. The renowned developmental biologist Lewis Wolpert once observed that birth, marriage, and death are not the most important events in human life; rather, gastrulation is. In what sense was he correct?
3. Arguably, in sexually reproducing animals development begins when eggs and sperm form in the parents. In a paragraph, explain the rationale for this idea.
4. Investigators discovered a *Drosophila* protein that triggers development of the nerve cord on the ventral side of the embryos. When an mRNA encoding the protein was injected into cells on the ventral side of *Xenopus* embryos, dorsal structures were formed on the ventral side, including incomplete heads. What do these findings suggest about the evolution of embryonic development?

Experimental Analysis

As you have learned in this chapter, embryogenesis in *Drosophila* has been well described. In Chapter 18 you also learned that the genome of *Drosophila* has been completely sequenced, allowing each gene in the genome to be cataloged. Design an experiment to identify all the genes that are activated during embryogenesis, and when they are activated.

Evolution Link

Every one of more than a million species of insects has six legs, a pair on each of the three thoracic segments. By contrast, other arthropods, such as crustaceans, have a variable number of limbs; some species have limbs on every segment in both the thorax and abdomen. Propose a molecular mechanism by which limbs might have been lost during the evolution of the insects.

How Would You Vote?

Sanitation and medical advances have greatly extended the average human life span, especially in developed countries. Some researchers are now looking for ways to extend the human life span even further. Do you think research into life extension should be supported by federal research funding? Go to www.thomsonedu.com/login to investigate both sides of the issue and then vote.