

47

Animal Development



▲ **Figure 47.1** How did a single cell develop into this intricately detailed embryo?

KEY CONCEPTS

- 47.1** Fertilization and cleavage initiate embryonic development
- 47.2** Morphogenesis in animals involves specific changes in cell shape, position, and survival
- 47.3** Cytoplasmic determinants and inductive signals contribute to cell fate specification

OVERVIEW

A Body-Building Plan

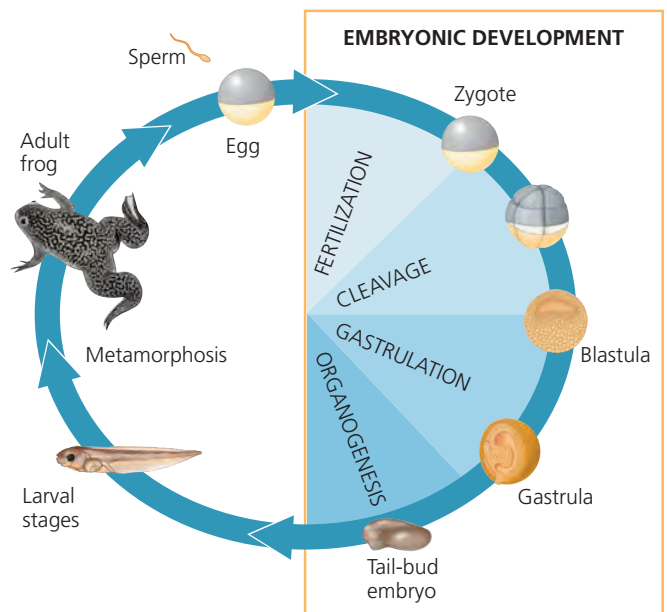
The 7-week-old human embryo in **Figure 47.1** has already achieved a remarkable number of milestones in its development. Its heart—the red spot in the center—is beating, and a digestive tract traverses the length of its body. Its

brain is forming (at the upper left in the photo), while the blocks of tissue that will give rise to the vertebrae are lined up along its back.

Development occurs at many points in the life cycle of an animal (**Figure 47.2**). In a frog, for example, a major developmental period is metamorphosis, when the larva (tadpole) is transformed into an adult. Other developmental events in the adult gonads produce sperm and eggs (gametes). In this chapter, our focus is on embryonic development.

Across a range of animal species, embryonic development involves common stages that occur in a set order. As shown in **Figure 47.2**, the first is fertilization, the fusion of sperm and egg, which forms a zygote. Development proceeds with the cleavage stage, during which a series of cell divisions divide, or cleave, the zygote into a many-celled embryo. These cleavage divisions, which typically are rapid and lack accompanying cell growth, convert the embryo to a hollow ball of cells called a blastula. Next, the blastula folds in on itself, rearranging into a three-layered embryo, the gastrula, in a process called gastrulation. During organogenesis, the last major stage of embryonic development, local changes in cell shape and large-scale changes in cell location generate the rudimentary organs from which adult structures grow.

By combining molecular genetics with classical embryology, developmental biologists have learned a great deal about the transformation of a fertilized egg into an adult. As an embryo develops, specific patterns of gene expression direct cells to adopt distinct fates. Although animals display



▲ **Figure 47.2** Developmental events in the life cycle of a frog.

widely differing body plans, they share many basic mechanisms of development and use a common set of regulatory genes. For example, the gene that specifies heart location in a human embryo (such as the one in Figure 47.1) has a close counterpart with a nearly identical function in the fruit fly, *Drosophila*. (Noting that the fly heart fails to develop when this gene is defective, researchers named the *Drosophila* gene *tinman*, after the similarly affected character from *The Wizard of Oz*.)

In studying development, biologists frequently make use of **model organisms**, species chosen for the ease with which they can be studied in the laboratory. *Drosophila* is a useful model organism: Its life cycle is short, and mutants can be readily identified and studied (see Chapters 15 and 18). In this chapter, we will concentrate on four other model organisms: the sea urchin, the frog, the chick, and the nematode (roundworm). We will also explore some aspects of human embryonic development. Even though humans are not model organisms, we are, of course, intensely interested in our own species.

Our exploration of embryonic development will begin with a description of the basic stages common to most animals. We will then look at some of the cellular mechanisms that generate body form. Finally, we will consider how a cell becomes committed to a particular specialized role.

CONCEPT 47.1

Fertilization and cleavage initiate embryonic development

With the preceding overview of embryonic development in mind, let's take a closer look at the events surrounding **fertilization**, the formation of a diploid zygote from a haploid egg and sperm.

Fertilization

Molecules and events at the egg surface play a crucial role in each step of fertilization. First, sperm dissolve or penetrate any protective layer surrounding the egg to reach the plasma membrane. Next, molecules on the sperm surface bind to receptors on the egg surface, helping ensure that a sperm of the same species fertilizes the egg. Finally, changes at the surface of the egg prevent *polyspermy*, the entry of multiple sperm nuclei into the egg. If polyspermy were to occur, the resulting abnormal number of chromosomes in the embryo would be lethal.

The cell surface events that take place during fertilization have been studied most extensively in sea urchins (members of the phylum Echinodermata; see Figure 33.43). Sea urchin gametes are easy to collect, and fertilization is external.

As a result, researchers can observe fertilization and subsequent events simply by combining eggs and sperm in seawater in the laboratory. Furthermore, fertilization in sea urchins provides a good general model for the same process in vertebrates.

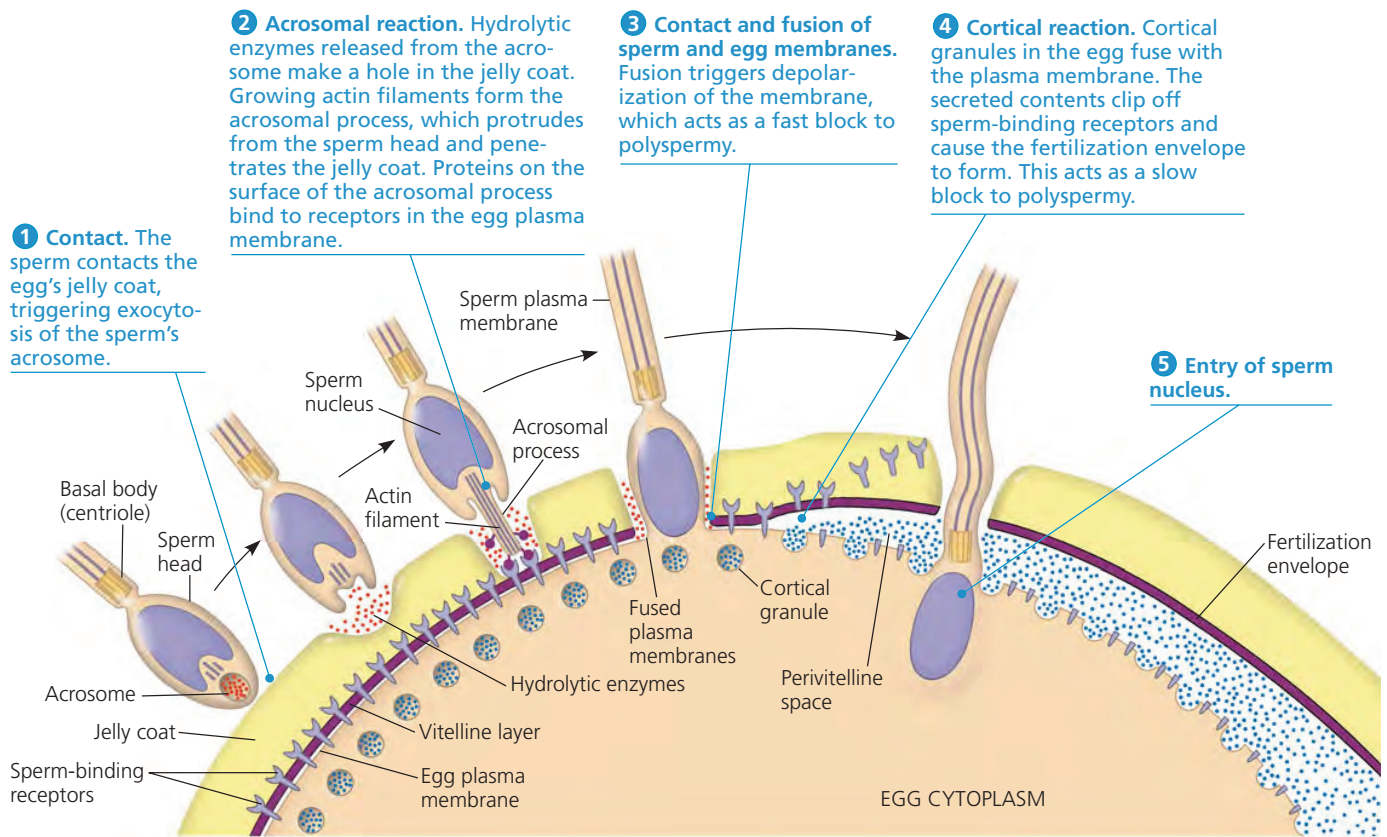
The Acrosomal Reaction

When sea urchins release their gametes into the water, the jelly coat that surrounds the egg exudes soluble molecules that attract the sperm, which swim toward the egg. As soon as the head of a sea urchin sperm contacts the jelly coat of a sea urchin egg, molecules in the jelly coat trigger the **acrosomal reaction** in the sperm (Figure 47.3). This reaction begins with the discharge of hydrolytic enzymes from the **acrosome**, a specialized vesicle at the tip of the sperm. These enzymes partially digest the jelly coat, enabling a sperm structure called the *acrosomal process* to elongate and penetrate the coat. Protein molecules on the tip of the extended acrosomal process bind to specific receptor proteins that jut out from the egg plasma membrane. This “lock-and-key” recognition is especially important for sea urchins and other species with external fertilization because the surrounding water may contain gametes of other species.

Contact between the tip of the acrosomal process and the receptors on the egg leads to the fusion of the sperm and egg plasma membranes. The sperm nucleus then enters the egg cytoplasm as ion channels open in the egg's plasma membrane. Sodium ions diffuse into the egg and cause *depolarization*, a decrease in the membrane potential (see Chapter 7). The depolarization occurs within about 1–3 seconds after a sperm binds to an egg. By preventing additional sperm from fusing with the egg's plasma membrane, this depolarization acts as a **fast block to polyspermy**.

The Cortical Reaction

Membrane depolarization lasts for only a minute or so. A longer-lasting block to polyspermy is established by vesicles that lie just beneath the egg plasma membrane, in the rim of cytoplasm known as the *cortex*. Within seconds after a sperm binds to the egg, these vesicles, called cortical granules, fuse with the egg plasma membrane (see Figure 47.3, 4). The contents of the cortical granules are released into the space between the plasma membrane and the surrounding *vitelline layer*, a structure formed by the extracellular matrix of the egg. Enzymes and other macromolecules from the granules trigger a cortical reaction, which lifts the vitelline layer away from the egg and hardens the layer into a protective fertilization envelope. Additional enzymes clip off and release the external portions of the remaining receptor proteins, along with any attached sperm. Together, the fertilization envelope



▲ Figure 47.3 The acrosomal and cortical reactions during sea urchin fertilization. The events following contact of a single sperm and egg ensure that the nucleus of only one sperm enters the egg cytoplasm.

The icon above is a simplified drawing of an adult sea urchin. Throughout the chapter, this and other icons of an adult frog, chicken, nematode, and human indicate the animals whose embryos are featured in certain figures.

and other changes in the egg's surface impede the entry of additional sperm nuclei and thus act as a longer-term **slow block to polyspermy**.

Formation of the fertilization envelope requires a high concentration of calcium ions (Ca^{2+}) in the egg. Does a change in the Ca^{2+} concentration trigger the cortical reaction? To answer this question, researchers at the University of California, Berkeley, used a calcium-sensitive dye to assess the amount and distribution of Ca^{2+} in the egg during fertilization. As described in **Figure 47.4**, on the next page, they found that Ca^{2+} spread across the egg in a wave that correlated with the appearance of the fertilization envelope. Further studies demonstrated that release of Ca^{2+} into the cytosol from the endoplasmic reticulum is controlled by a signal transduction pathway activated by sperm binding. The resulting increase in Ca^{2+} levels causes cortical granules to fuse with the plasma membrane. Although understood in greatest detail in sea urchins, the cortical reaction triggered by Ca^{2+} also occurs in vertebrates such as fishes and mammals.

Egg Activation

A major function of fertilization is the combining of haploid sets of chromosomes from two individuals into a single diploid cell, the zygote. However, the events of fertilization also initiate metabolic reactions that trigger the onset of embryonic development, thus "activating" the egg. There is, for example, a marked increase in the rates of cellular respiration and protein synthesis in the egg following fertilization.

What triggers egg activation? Studies show that injecting Ca^{2+} into an unfertilized egg activates egg metabolism in many species, despite the absence of sperm. Researchers therefore conclude that the rise in Ca^{2+} concentration that causes the cortical reaction also causes egg activation. Further experiments have revealed that artificial activation is possible even if the nucleus has been removed from the egg. This finding indicates that egg activation requires only the proteins and mRNAs already present in the egg cytoplasm.

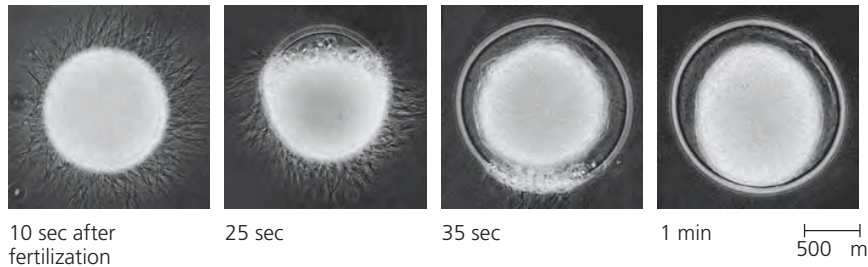
▼ Figure 47.4

INQUIRY

Does the distribution of Ca^{2+} in an egg correlate with formation of the fertilization envelope?

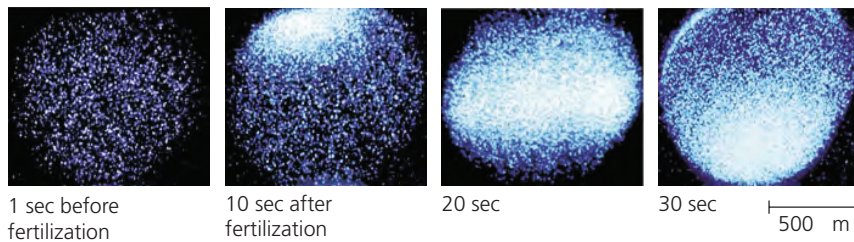


EXPERIMENT During fertilization, fusion of cortical granules with the egg plasma membrane causes the fertilization envelope to rise and spread around the egg from the point of sperm binding.

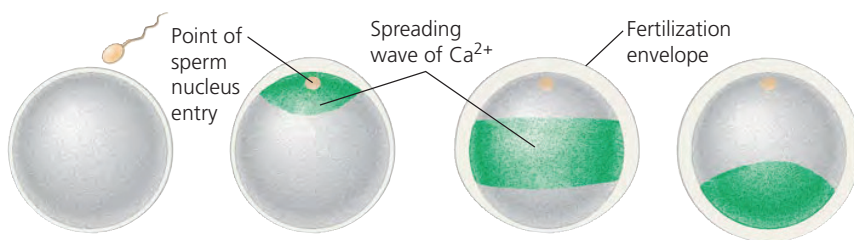


Calcium ion (Ca^{2+}) signaling is involved in fusion of vesicles with the plasma membrane during neurotransmitter release, insulin secretion, and plant pollen tube formation. Rick Steinhardt, Gerald Schatten, and colleagues, then at the University of California at Berkeley, hypothesized that an increase in Ca^{2+} levels similarly triggers cortical granule fusion. To test this hypothesis, they tracked the release of free Ca^{2+} in sea urchin eggs after sperm binding to see if it correlated with formation of the fertilization envelope. A fluorescent dye that glows when it binds free Ca^{2+} was injected into unfertilized eggs. The researchers then added sea urchin sperm and observed the eggs with a fluorescence microscope. Schatten and colleagues later repeated the experiment using a more sensitive dye, producing the results shown here.

RESULTS A rise in cytosolic Ca^{2+} concentration began at the point of sperm entry and spread in a wave to the other side of the egg. Soon after the wave passed, the fertilization envelope rose.



CONCLUSION The researchers concluded that Ca^{2+} release is correlated with the cortical reaction and formation of the fertilization envelope, supporting their hypothesis that an increase in Ca^{2+} levels triggers cortical granule fusion.



SOURCE R. Steinhardt et al., Intracellular calcium release at fertilization in the sea urchin egg, *Developmental Biology* 58:185–197 (1977). M. Hafner et al., Wave of free calcium at fertilization in the sea urchin egg visualized with Fura-2, *Cell Motility and the Cytoskeleton* 9:271–277 (1988).

See the related Experimental Inquiry Tutorial in MasteringBiology.

WHAT IF? Suppose you were given a chemical compound that could enter the egg and bind to Ca^{2+} , blocking its function. How would you use this compound to further test the hypothesis that a rise in Ca^{2+} levels triggers cortical granule fusion?

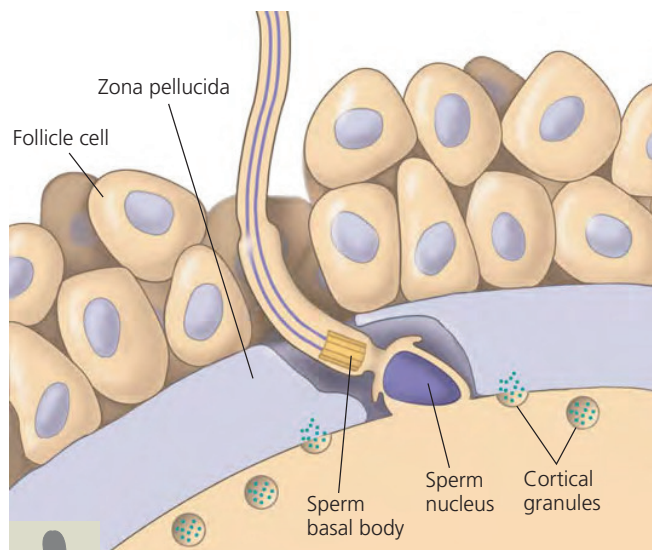
About 20 minutes after the sperm nucleus enters the sea urchin egg, the sperm and egg nuclei fuse. DNA synthesis begins, and the first cell division occurs after about 90 minutes, marking the end of the fertilization stage.

Fertilization in other species shares many features with the process in sea urchins. However, the timing of events differs, as does the stage of meiosis the egg has reached by the time it is fertilized. Sea urchin eggs have already completed meiosis when they are released from the female. In other species, eggs are arrested at a specific stage of meiosis and do not complete the meiotic divisions until fertilization occurs. Human eggs, for example, are arrested at metaphase of meiosis II prior to fertilization (see Figure 46.12).

Fertilization in Mammals

Unlike sea urchins and most other marine invertebrates, terrestrial animals, including mammals, fertilize eggs internally. Secretions in the mammalian female reproductive tract not only provide a moist environment for the sperm, but also bring about changes in sperm motility and structure. Only after these changes occur do sperm have the capacity to fertilize an egg. In humans, this process of *capacitation* occurs during the first 6 hours after the sperm enter the female reproductive tract.

Support cells of the developing follicle surround the mammalian egg and remain with it during and after ovulation (see Figure 46.12). A sperm must travel through this layer of follicle cells before it reaches the **zona pellucida**, the extracellular matrix of the egg. Within the zona pellucida is a component that functions as a receptor for sperm. Binding of a sperm to this receptor induces an acrosomal reaction, facilitating sperm passage through the zona pellucida to the egg. This binding also exposes a protein on the sperm that binds with the egg plasma membrane. At this point, the two cells fuse (**Figure 47.5**).



▲ **Figure 47.5 Fertilization in mammals.** The sperm shown here has traveled through the follicle cells and zona pellucida and has fused with the egg. The cortical reaction has begun, initiating events that ensure that only one sperm nucleus enters the egg.

As in sea urchin fertilization, sperm binding triggers changes within the mammalian egg that lead to a cortical reaction, the release of enzymes from cortical granules to the outside of the cell. These enzymes catalyze changes in the zona pellucida, which then functions as the slow block to polyspermy. (No fast block to polyspermy has been identified in mammals.)

After the egg and sperm membranes fuse, the whole sperm is taken into the egg. Once the envelopes of both haploid

nuclei have dispersed, the sperm and egg chromosomes are organized onto a single mitotic spindle. Only after the first division is there a true diploid nucleus with a nuclear membrane.

Overall, fertilization is much slower in mammals than in sea urchins: The first cell division occurs 12–36 hours after sperm binding in mammals, compared with about 90 minutes in sea urchins. This cell division marks the end of fertilization and the beginning of the next stage, cleavage.

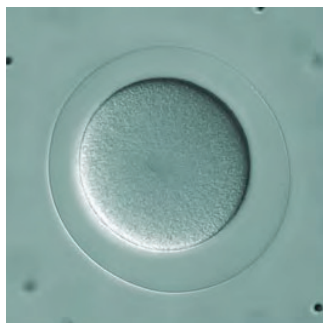
Cleavage

Once fertilization is complete, many animal species undergo a succession of rapid cell divisions that characterize the **cleavage** stage of early development. During cleavage, the cell cycle consists primarily of the S (DNA synthesis) and M (mitosis) phases. Cells essentially skip the G₁ and G₂ (gap) phases, and little or no protein synthesis occurs (see Figure 12.6 for a review of the cell cycle). As a result, cleavage partitions the cytoplasm of the large fertilized egg into many smaller cells called **blastomeres**, as shown in **Figure 47.6**.

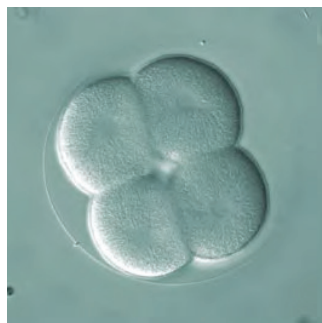
The first five to seven cleavage divisions produce a hollow ball of cells, the **blastula**, surrounding a fluid-filled cavity called the **blastocoel** (see Figure 47.6).

Cleavage Patterns

In frogs and many other animals, the distribution of **yolk** (stored nutrients) is a key factor influencing the pattern of cleavage. Yolk is often concentrated toward one pole of the egg, called the **vegetal pole**. The yolk concentration decreases significantly toward the opposite pole, the **animal pole**. This difference in yolk distribution results



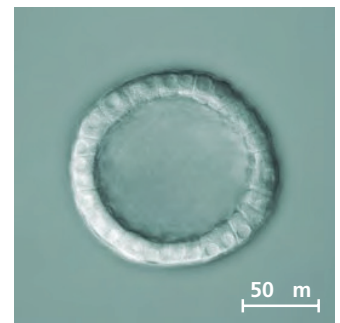
(a) **Fertilized egg.** Shown here is the zygote shortly before the first cleavage division, surrounded by the fertilization envelope.



(b) **Four-cell stage.** Remnants of the mitotic spindle can be seen between the two pairs of cells that have just completed the second cleavage division.

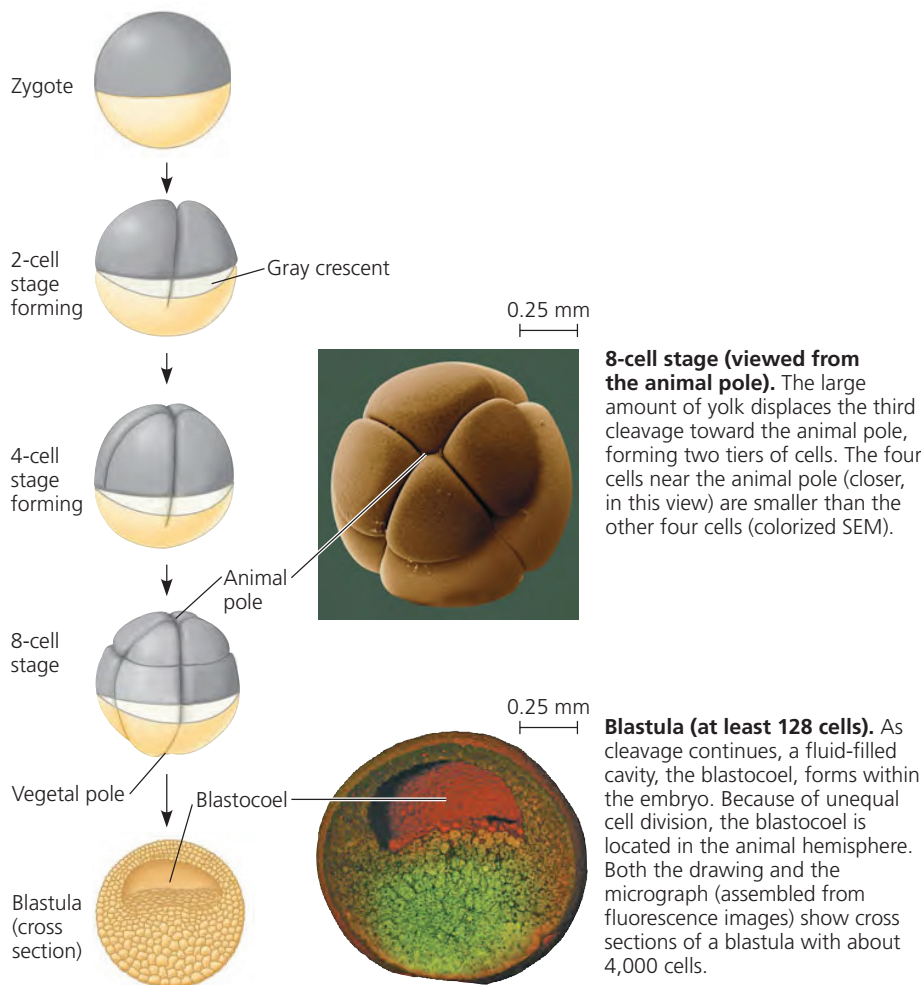


(c) **Early blastula.** After further cleavage divisions, the embryo is a multicellular ball that is still surrounded by the fertilization envelope. The blastocoel has begun to form in the center.



(d) **Later blastula.** A single layer of cells surrounds a large blastocoel. Although not visible here, the fertilization envelope is still present; the embryo will soon hatch from it and begin swimming.

▲ **Figure 47.6 Cleavage in an echinoderm embryo.** Cleavage is a series of mitotic cell divisions that transform the fertilized egg into a blastula, a hollow ball composed of cells called blastomeres. These light micrographs show the cleavage stages of a sand dollar embryo, which are virtually identical to those of a sea urchin.



▲ Figure 47.7 Cleavage in a frog embryo. The cleavage planes in the first and second divisions extend from the animal pole to the vegetal pole, but the third cleavage is perpendicular to the polar axis. In some species, the first division bisects the gray crescent, a lighter-colored region that appears opposite the site of sperm entry.

in animal and vegetal hemispheres that differ in appearance (**Figure 47.7**).

During cell division, an indentation called a *cleavage furrow* forms in the cell surface as cytokinesis divides the cell in half. As shown in **Figure 47.7**, the first two cleavage furrows in the frog lie parallel to the line (or meridian) connecting the two poles. The second cell division begins before the first is complete, so the second cleavage furrow further divides the animal hemisphere while the first furrow is dividing the yolk cytoplasm of the vegetal hemisphere. Nevertheless, the first two divisions eventually produce four blastomeres of equal size, each extending from the animal pole to the vegetal pole.

During the third division of the frog egg, the asymmetric distribution of yolk in the embryo affects the relative size of cells produced in the two hemispheres. This division is equatorial (perpendicular to the line connecting the poles) and produces an eight-celled embryo. However, as each of the four blastomeres begins this division, the high concentration of yolk around the

vegetal pole displaces the mitotic apparatus toward the animal pole. Consequently, the cleavage furrow is also displaced from the egg equator toward the animal pole, yielding smaller blastomeres in the animal hemisphere than in the vegetal hemisphere. The displacing effect of the yolk persists in the subsequent divisions that produce a blastula. In frogs, these unequal cell divisions cause the blastocoel to form entirely in the animal hemisphere (see **Figure 47.7**).

Although yolk affects where division occurs in the eggs of frogs and other amphibians, the cleavage furrow still passes entirely through the egg. Cleavage in amphibian development is thus said to be **holoblastic** (from the Greek *holos*, complete). Holoblastic cleavage is also seen in many other groups of animals, including echinoderms, mammals, and annelids. The orientation of the cleavage furrows varies within these groups, resulting in blastulas that vary considerably in appearance. In those animals whose eggs contain relatively little yolk, the blastocoel forms centrally and the blastomeres are often of similar size, particularly during the first few divisions (see **Figure 47.6**). This is the case for humans, whose embryos complete three divisions in the first 3 days after fertilization.

Yolk is most plentiful and has its most pronounced effect on cleavage in the eggs of birds, other reptiles, many

fishes, and insects. In these animals, the volume of yolk is so great that cleavage furrows cannot pass through it, and only the region of the egg lacking yolk undergoes cleavage. This incomplete cleavage of a yolk-rich egg is said to be **meroblastic** (from the Greek *meros*, partial).

In birds, the part of the egg commonly called the yolk is actually the entire egg cell, swollen with yolk nutrients. Cell divisions are limited to a small whitish area at the animal pole. These divisions produce a cap of cells that sort into upper and lower layers. The cavity between these two layers is the avian version of the blastocoel.

In the eggs of *Drosophila* and most other insects, the sperm and egg nuclei fuse *within* a mass of yolk. Multiple rounds of mitosis occur without cytokinesis. In other words, no cell membranes form around the early nuclei. The first several hundred nuclei spread throughout the yolk and later migrate to the outer edge of the embryo. After several more rounds of mitosis, a plasma membrane forms around each nucleus, and

the embryo, now the equivalent of a blastula, consists of a single layer of about 6,000 cells surrounding a mass of yolk (see Figure 18.22).

Regulation of Cleavage

The number of cleavage divisions varies among animals but appears to be controlled by a shared mechanism. Experimental results support the hypothesis that an animal embryo finishes the cleavage stage when the ratio of material in each nucleus to that in the cytoplasm is sufficiently large. One line of evidence comes from experiments in which researchers changed the starting amount of cytoplasm and then counted the cleavage divisions that occurred. For example, when half the normal amount of cytoplasm surrounds the newly formed zygotic nucleus, one fewer cleavage division occurs, consistent with the nuclear-cytoplasmic ratio reaching the threshold after one fewer cell cycle.

What is the adaptive advantage of linking the duration of the cleavage stage to the ratio of material in the nucleus and cytoplasm? The single nucleus in a newly fertilized egg has too little DNA to produce the amount of messenger RNA required to meet the cell's need for new proteins. Instead, the initial stages of development are carried out by RNA and proteins deposited in the egg during oogenesis. After cleavage, the egg cytoplasm has been divided among the many blastomeres, each with its own nucleus. Because each blastomere is much smaller than the entire egg or embryo, its nucleus can make enough RNA to program the cell's metabolism and its further development. The increase in the number of cells also sets the stage for morphogenesis, the transformation of embryo organization and shape.

CONCEPT CHECK 47.1

1. How does the fertilization envelope form in sea urchins? What is its function?
2. **WHAT IF?** Predict what would happen if you injected Ca^{2+} into an unfertilized sea urchin egg.
3. **MAKE CONNECTIONS** Review Figure 12.17 on page 240. Would you expect MPF activity to fluctuate or remain steady during cleavage? Explain your logic.

For suggested answers, see Appendix A.

CONCEPT 47.2

Morphogenesis in animals involves specific changes in cell shape, position, and survival

After cleavage, the rate of cell division slows considerably as the normal cell cycle is restored. The last two stages of embryonic

development are responsible for **morphogenesis**, the cellular and tissue-based processes by which the animal body takes shape. During **gastrulation**, a set of cells at or near the surface of the blastula moves to an interior location, cell layers are established, and a primitive digestive tube is formed. Further transformation occurs during **organogenesis**, the formation of organs. We will discuss these two stages in turn, focusing in each case on the development of a few model organisms.

Gastrulation

Gastrulation is a dramatic reorganization of the hollow blastula into a two-layered or three-layered embryo called a **gastrula**. The cell layers produced by gastrulation are collectively called the embryonic **germ layers** (from the Latin *germen*, to sprout or germinate). In the late gastrula, **ectoderm** forms the outer layer and **endoderm** lines the embryonic digestive compartment or tract. In cnidarians and a few other radially symmetrical animals, only these two germ layers form during gastrulation. Such animals are called diploblasts (see Chapter 32). In contrast, animals with bilateral symmetry are triploblasts, having a third germ layer, the **mesoderm**, between the ectoderm and the endoderm.

Each germ layer contributes to a distinct set of structures in the adult animal (Figure 47.8). Note that some organs and many organ systems of the adult derive from more than one germ layer. For example, the adrenal gland has both ectodermal and mesoderm tissue, and many other endocrine glands contain endodermal tissue.

ECTODERM (outer layer of embryo)

- Epidermis of skin and its derivatives (including sweat glands, hair follicles)
- Nervous and sensory systems
- Pituitary gland, adrenal medulla
- Jaws and teeth
- Germ cells

MESODERM (middle layer of embryo)

- Skeletal and muscular systems
- Circulatory and lymphatic systems
- Excretory and reproductive systems (except germ cells)
- Dermis of skin
- Adrenal cortex

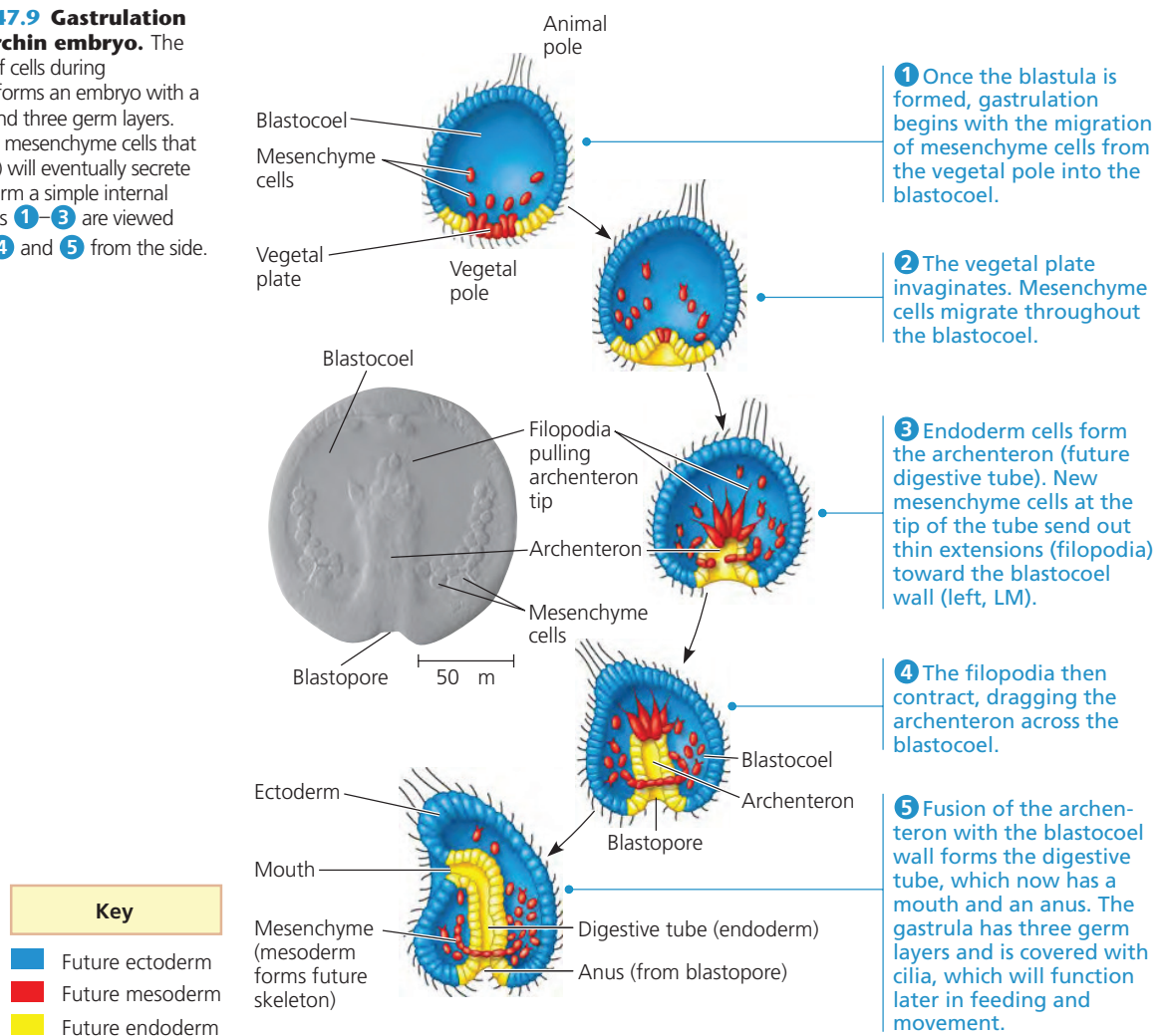
ENDODERM (inner layer of embryo)

- Epithelial lining of digestive tract and associated organs (liver, pancreas)
- Epithelial lining of respiratory, excretory, and reproductive tracts and ducts
- Thymus, thyroid, and parathyroid glands

▲ **Figure 47.8** Major derivatives of the three embryonic germ layers in vertebrates.



► **Figure 47.9 Gastrulation in a sea urchin embryo.** The movement of cells during gastrulation forms an embryo with a primitive digestive tube and three germ layers. Some of the mesodermal mesenchyme cells that migrate inward (step 1) will eventually secrete calcium carbonate and form a simple internal skeleton. Embryos in steps 1–3 are viewed from the front, those in 4 and 5 from the side.



Gastrulation in Sea Urchins

Gastrulation in the sea urchin begins at the vegetal pole of the blastula (Figure 47.9). There, cells called *mesenchyme cells* individually detach from the blastocoel wall and enter the blastocoel. The remaining cells near the vegetal pole flatten slightly and cause that end of the embryo to buckle inward as a result of cell shape changes we will discuss later. This process—the infolding of a sheet of cells into the embryo—is called *invagination*. Extensive rearrangement of cells transforms the shallow depression into a deeper, narrower, blind-ended tube called the **archenteron**. The open end of the archenteron, which will become the anus, is called the **blastopore**. A second opening, which will become the mouth, forms when the opposite end of the archenteron touches the inside of the ectoderm and the two layers fuse, producing a rudimentary digestive tube.

As you learned in Chapter 32, animals can be categorized by whether the mouth develops from the first opening that forms in the embryo (protostomes) or the second (deuterostomes).

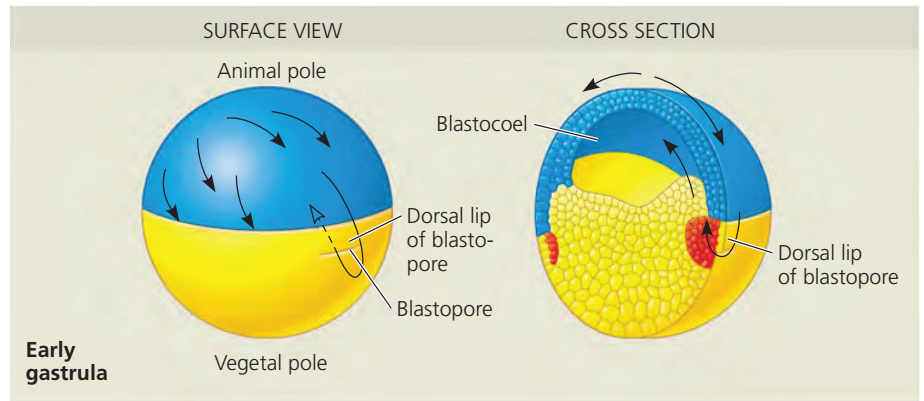
Sea urchins and other echinoderms are deuterostomes, as are chordates like ourselves and other vertebrates.

Upon completing gastrulation, sea urchin embryos develop into ciliated larvae that drift in ocean surface waters as zooplankton, feeding on bacteria and unicellular algae. Eventually, each larva metamorphoses into the adult form of the sea urchin, which takes up residence on the ocean floor.

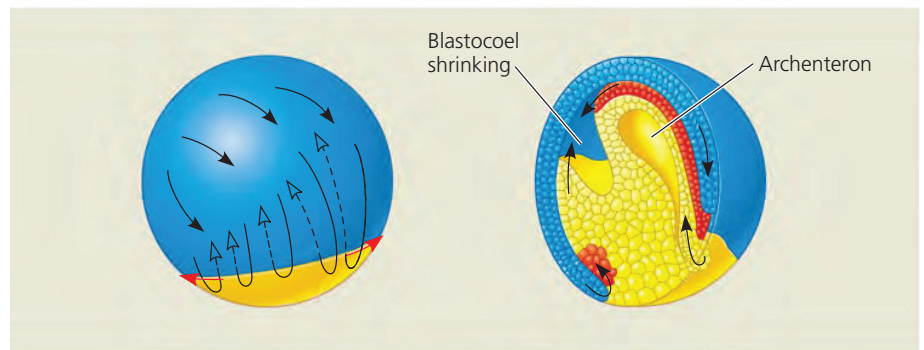
Gastrulation in Frogs

The frog blastula contains large, yolk-laden cells in the vegetal hemisphere and a blastocoel wall that in most species is more than one cell thick. Recall from Chapter 32 that frogs and other bilaterally symmetrical animals have a dorsal (top) side and a ventral (bottom) side, a left side and a right side, and an anterior (front) end and a posterior (back) end. As shown in Figure 47.10, frog gastrulation begins when a group of cells on the dorsal side of the blastula begins to invaginate. This process forms a crease along the region where the gray crescent formed (see Figure 47.7). It may help to

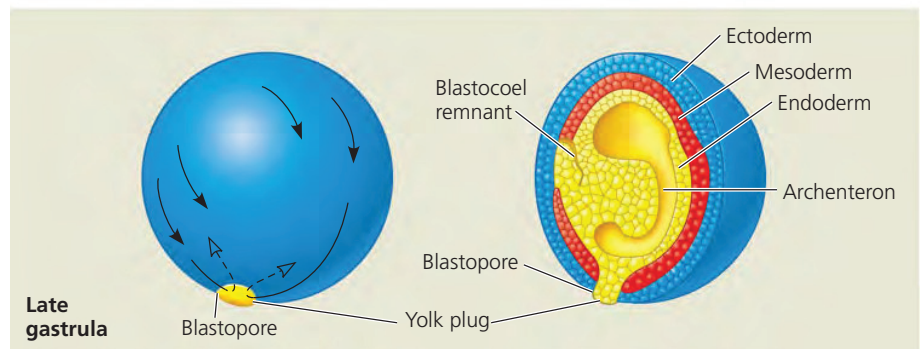
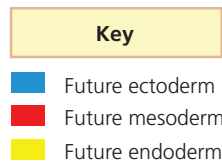
- 1 Gastrulation begins when a small indented crease, the blastopore, appears on the dorsal side of the late blastula. The crease is formed by cells changing shape and invaginating. Sheets of outer cells then roll inward over the dorsal lip (involution) and move into the interior (shown by the dashed arrow), where they will form endoderm and mesoderm. Meanwhile, cells at the animal pole change shape and begin spreading over the outer surface.



- 2 The blastopore extends around both sides of the embryo (red arrows) as more cells invaginate. When the ends meet, the blastopore forms a circle that becomes smaller as ectoderm spreads downward over the surface. Internally, continued involution expands the endoderm and mesoderm, and the archenteron begins to form; as a result, the blastocoel becomes smaller.



- 3 Late in gastrulation, the endoderm-lined archenteron has completely replaced the blastocoel and the three germ layers are in place. The circular blastopore surrounds a plug of yolk-filled cells.



▲ **Figure 47.10 Gastrulation in a frog embryo.** In the frog blastula, the blastocoel is displaced toward the animal pole and is surrounded by a wall several cells thick. The cell movements that begin gastrulation occur on the dorsal side of the blastula, opposite where the sperm entered the egg.

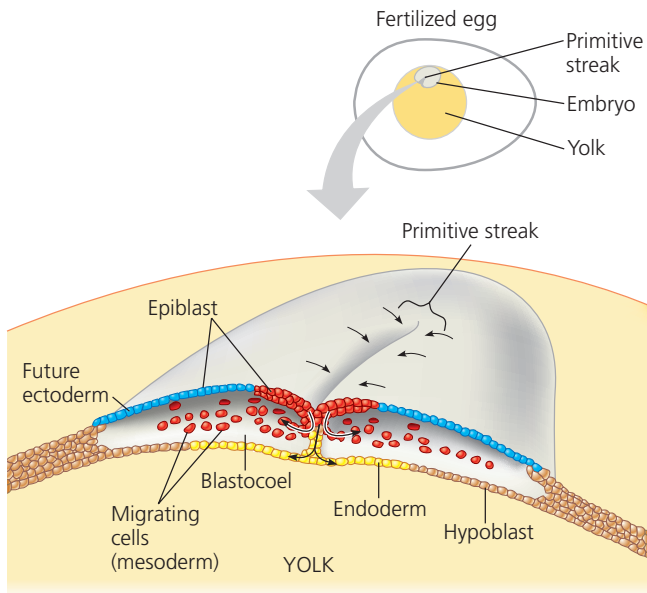
think of this crease as the site where two thin lips are pressed together. The part above the crease becomes the dorsal side of the blastopore, called the **dorsal lip**.

As the blastopore is forming, a sheet of cells begins to spread out of the animal hemisphere. Some of these cells roll over the edge of the lip into the interior of the embryo, a process called *involution*. Once inside the embryo, these cells move away from the blastopore toward the animal pole and become organized into layers of endoderm and mesoderm, with the endoderm on the inside. Cells continue to spread over the gastrula surface, shifting and shrinking the blastopore. In the interior of the embryo, an archenteron forms and grows as the blastocoel shrinks and eventually disappears.

At the end of gastrulation, the cells remaining on the surface make up the ectoderm, the tube of endoderm is the innermost layer, and the mesoderm lies between them. As in the sea urchin, the frog's anus develops from the blastopore, and the mouth eventually breaks through at the opposite end of the archenteron.

Gastrulation in Chicks

The starting point for gastrulation in chicks is an embryo consisting of upper and lower layers—the *epiblast* and *hypoblast*—lying atop a yolk mass. All the cells that will form the embryo come from the epiblast. During gastrulation, some epiblast cells move toward the midline of the blastoderm, detach, and



▲ **Figure 47.11 Gastrulation in a chick embryo.**

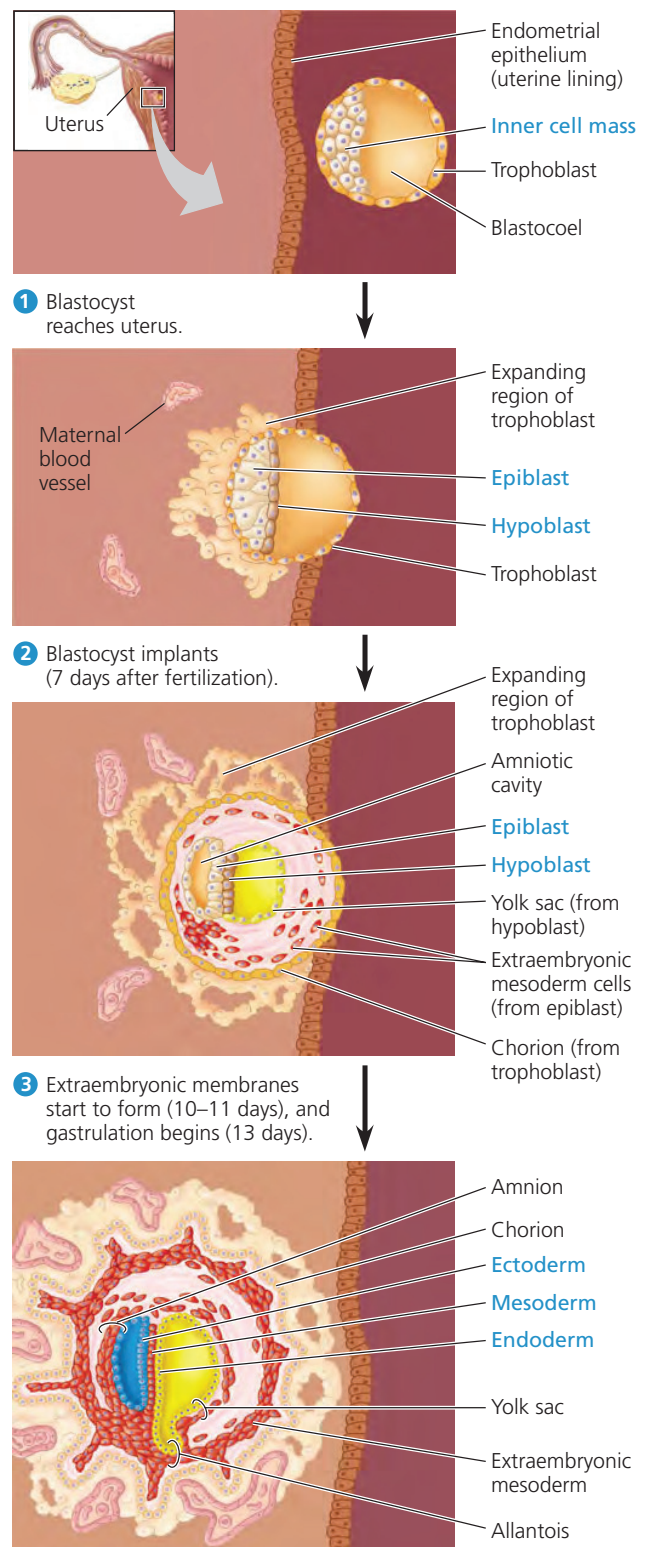
The chick blastula consists of an upper layer of cells, the epiblast, and a lower layer, the hypoblast, with a space (the blastocoel) between them. This is a cross section at a right angle to the primitive streak, looking toward the anterior end of a gastrulating embryo. During gastrulation, some cells of the epiblast migrate (arrows) into the interior of the embryo through the primitive streak. Some of these cells move downward and form endoderm, pushing aside the hypoblast cells, while others migrate laterally and form mesoderm. The cells left behind on the surface of the embryo at the end of gastrulation will become ectoderm.

move inward toward the yolk (**Figure 47.11**). The pileup of cells moving inward at the blastoderm's midline produces a thickening called the **primitive streak**. Although the hypoblast contributes no cells to the embryo, it is required for normal development and seems to help direct the formation of the primitive streak before the onset of gastrulation. The hypoblast cells later segregate from the endoderm and eventually form part of the sac that surrounds the yolk and also part of the stalk that connects the yolk mass to the embryo.

Gastrulation in Humans

Unlike the large, yolky eggs of many vertebrates, human eggs are quite small, storing little in the way of food reserves. Fertilization takes place in the oviduct, and the earliest stages of development occur while the embryo completes its journey down the oviduct to the uterus (see Figure 46.15). Knowledge about gastrulation in humans is therefore largely based on what we can extrapolate from other mammals, such as the mouse, and on observation of very early human development following *in vitro* fertilization.

Figure 47.12 depicts development of the human embryo starting about 6 days after fertilization. The description on page 1031 follows the numbered stages in the figure.



4 Gastrulation has produced a three-layered embryo with four extraembryonic membranes.

▲ **Figure 47.12 Four stages in the early embryonic development of a human.**



- 1 At the end of cleavage, the embryo has more than 100 cells arranged around a central cavity and has traveled down the oviduct to the uterus. At this stage of development, the embryo is called a **blastocyst**, the mammalian version of a blastula. Clustered at one end of the blastocyst cavity is a group of cells called the **inner cell mass**, which will develop into the embryo proper. It is the cells of the very early blastocyst stage that are the source of embryonic stem cell lines.
- 2 The **trophoblast**, the outer epithelium of the blastocyst, does not contribute to the embryo itself but instead supports embryo growth in a number of ways. It initiates implantation by secreting enzymes that break down molecules of the endometrium, the lining of the uterus. This allows the blastocyst to invade the endometrium. As the trophoblast thickens through cell division, it extends finger-like projections into the surrounding maternal tissue. Invasion by the trophoblast leads to erosion of capillaries in the endometrium, causing blood to spill out and bathe trophoblast tissues. Around the time of implantation, the inner cell mass of the blastocyst forms a flat disk with an upper layer of cells, the *epiblast*, and a lower layer, the *hypoblast*. As in birds, the human embryo develops almost entirely from epiblast cells.
- 3 Following implantation, the trophoblast continues to expand into the endometrium, and four new membranes appear. Although these **extraembryonic membranes** are formed by the embryo, they enclose specialized structures located outside the embryo. As implantation is completed, gastrulation begins. Cells move inward from the epiblast through a primitive streak and form mesoderm and endoderm, just as in the chick (see Figure 47.11).
- 4 By the end of gastrulation, the embryonic germ layers have formed. Extraembryonic mesoderm and the four extraembryonic membranes now surround the embryo. As development proceeds, the invading trophoblast, cells from the epiblast, and adjacent endometrial tissue will all contribute to formation of the placenta. This vital organ mediates exchange of nutrients, gases, and nitrogenous wastes between the embryo and the mother (see Figure 46.16).

Developmental Adaptations of Amniotes

EVOLUTION As you read in Chapter 34, birds and other reptiles, like mammals, form four extraembryonic membranes. In all these groups, such membranes provide a “life-support system” for further embryonic development. Why, then, did this adaptation appear in the evolutionary

history of reptiles and mammals but not other vertebrates, such as fishes and amphibians? We can formulate a reasonable hypothesis by considering a few basic facts about embryonic development. All vertebrate embryos require an aqueous environment for their development. The embryos of fishes and amphibians usually develop in the surrounding sea or pond and need no specialized water-filled enclosure. However, the extensive colonization of land by vertebrates was possible only after the evolution of structures that would allow reproduction in dry environments. Two such structures exist today: (1) the shelled egg of birds and other reptiles as well as a few mammals (the monotremes) and (2) the uterus of marsupial and eutherian mammals. Inside the shell or uterus, the embryos of these animals are surrounded by fluid within a sac formed by one of the extraembryonic membranes, the amnion. Mammals and reptiles, including birds, are therefore called **amniotes** (see Chapter 34).

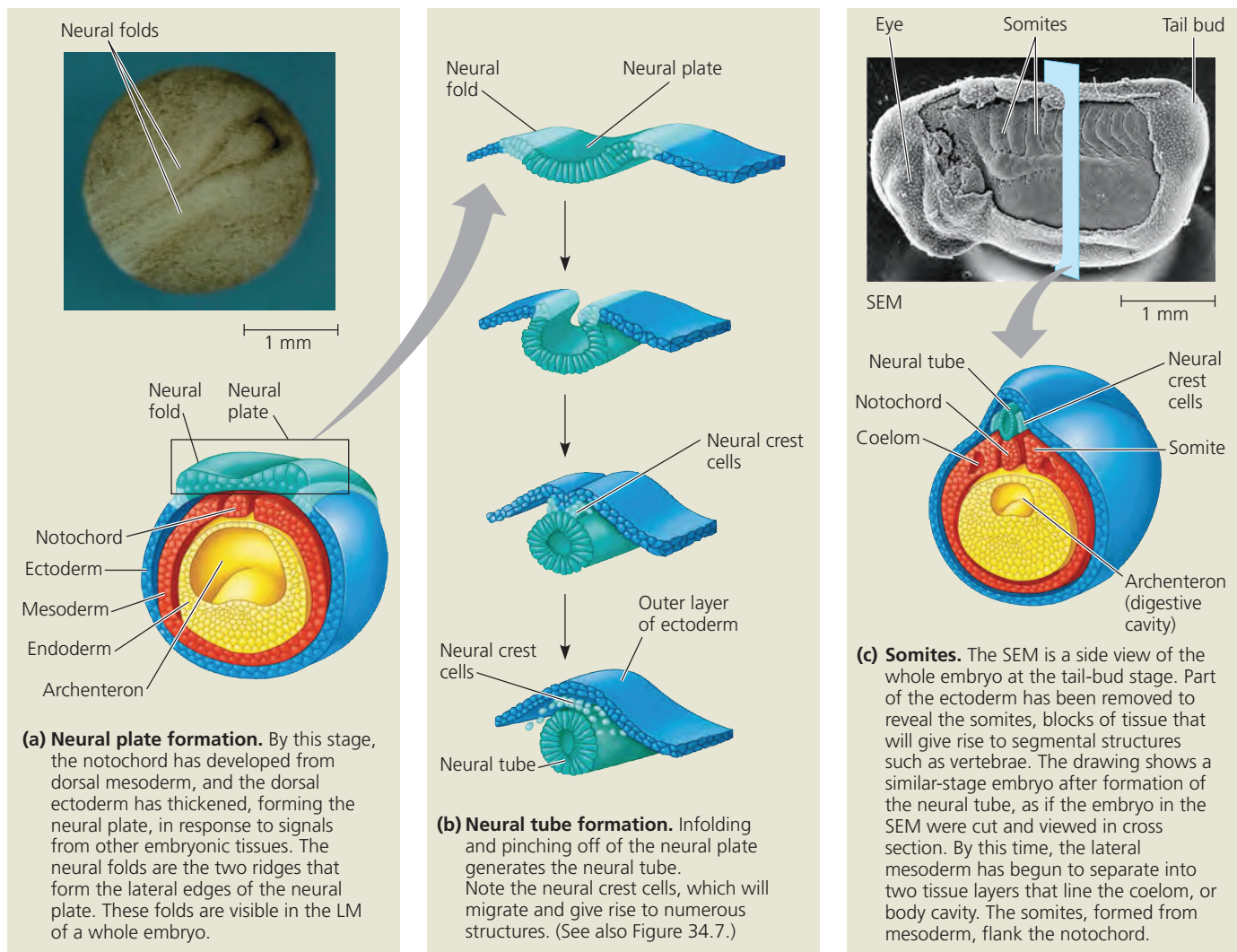
We can explore the evolution of extraembryonic membranes by comparing their functions in different groups of amniotes. For the purposes of this discussion, you may find it useful to refer to Figure 34.26, which describes the functions of the extraembryonic membranes in the egg of a reptile.

For the most part, the extraembryonic membranes have similar functions in mammals and reptiles, consistent with a common evolutionary origin. The chorion is the site of gas exchange, and the fluid within the amnion physically protects the developing embryo. (This amniotic fluid is released from the vagina when a pregnant woman’s “water breaks” just before childbirth.) The allantois, which disposes of wastes in the reptilian egg, is incorporated into the umbilical cord in mammals. There it forms blood vessels that transport oxygen and nutrients from the placenta to the embryo and rid the embryo of carbon dioxide and nitrogenous wastes. The fourth extraembryonic membrane, the yolk sac, encloses yolk in the eggs of reptiles. In mammals it is a site of early formation of blood cells, which later migrate into the embryo proper. Thus, although the extraembryonic membranes of reptiles were conserved in mammals in the course of evolution, modifications appeared that were adapted to development within the uterus of the mother.

After gastrulation is complete and any extraembryonic membranes are formed, the next stage of embryonic development begins: organ formation.

Organogenesis

During organogenesis, regions of the three embryonic germ layers develop into the rudiments of organs. Whereas gastrulation involves mass movements of cells, organogenesis involves



▲ **Figure 47.13** Neurulation in a frog embryo.



more localized changes. To illustrate the basic principles of this process, we'll focus on *neurulation*, the first steps in the formation of the brain and spinal cord in vertebrates.

Neurulation begins as cells from the dorsal mesoderm come together to form the **notochord**, the rod that extends along the dorsal side of the chordate embryo, seen in **Figure 47.13a** for the frog. Signaling molecules secreted by these mesodermal cells and other tissues induce the ectoderm above the notochord to become the *neural plate*. Next, the cells of the neural plate change shape, curving the neural plate inward. In this way, the neural plate rolls itself into the **neural tube**, which runs along the anterior-posterior axis of the embryo (**Figure 47.13b**). The neural tube will become the brain in the head and the spinal cord along the rest of the body.

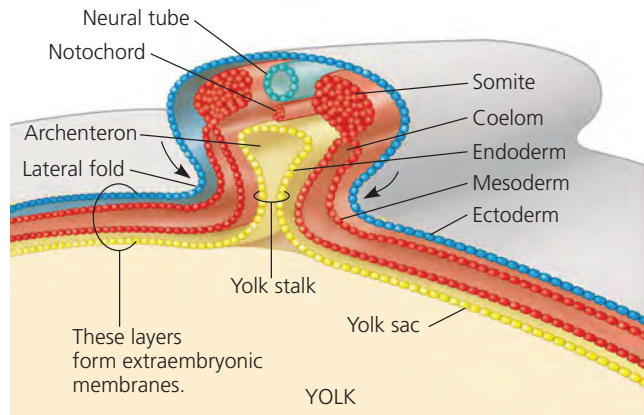
In vertebrate embryos, two sets of cells develop near the neural tube and then migrate elsewhere in the body. The first

set is a band of cells called the **neural crest**, which develops along the borders where the neural tube pinches off from the ectoderm. Neural crest cells subsequently migrate to many parts of the embryo, forming a variety of tissues that include peripheral nerves as well as parts of the teeth and skull bones. The second set of migratory cells is formed when groups of cells located in strips of mesoderm lateral to the notochord separate into blocks called **somites** (**Figure 47.13c**). The somites are arranged serially on both sides along the length of the notochord. Parts of the somites dissociate into mesenchyme cells, which migrate individually to new locations.

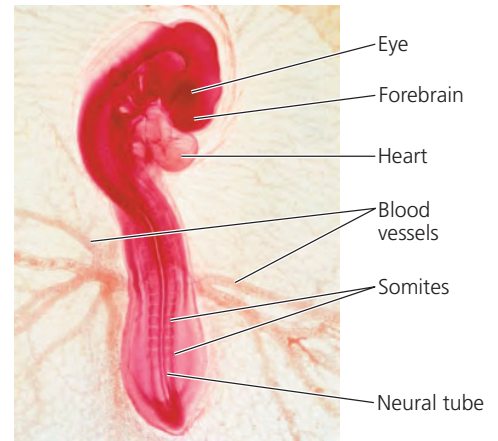
Somites play a major role in organizing the segmented structure of the vertebrate body. One of the major functions of the mesenchyme cells that leave the somites is formation of the vertebrae. Although the notochord disappears before birth, parts of the notochord persist as the inner portions of



► **Figure 47.14**
Organogenesis in a chick embryo.



(a) Early organogenesis. The archenteron forms when lateral folds pinch the embryo away from the yolk. The embryo remains open to the yolk, attached by the yolk stalk, about midway along its length, as shown in this cross section. The notochord, neural tube, and somites subsequently develop much as they do in the frog. The germ layers lateral to the embryo itself form extraembryonic membranes.



(b) Late organogenesis. Rudiments of most major organs have already formed in this chick embryo, which is 3 days old and about 2–3 mm long. The extraembryonic membranes eventually are supplied by blood vessels extending from the embryo; several major blood vessels are seen here (LM).

the vertebral disks in adults. (These are the disks that can herniate or rupture, causing back pain.) Somite cells that become mesenchymal later form the muscles associated with the vertebral column and the ribs. Through these processes, serially repeating structures of the embryo (somites) form repeated structures in the adult. Chordates can thus be described as segmented animals, although the segmentation becomes less obvious later in development. Lateral to the somites, the mesoderm splits into two layers that form the lining of the body cavity, or coelom (see Figure 32.8).

Early organogenesis in other vertebrates is quite similar to that in the frog. In the chick, for example, the borders of the blastoderm fold downward and come together, pinching the embryo into a three-layered tube joined under the middle of the body to the yolk (**Figure 47.14a**). By the time the chick embryo is 3 days old, rudiments of the major organs, including the brain, eyes, and heart, are readily apparent (**Figure 47.14b**).

In humans, an error in neural tube formation results in *spina bifida*, the most common disabling birth defect in the United States. In *spina bifida*, a portion of the neural tube fails to develop or close properly, leaving an opening in the spinal column and causing nerve damage. Although the opening can be surgically repaired shortly after birth, the nerve damage is permanent, resulting in varying degrees of leg paralysis.

Organogenesis is somewhat different in invertebrates, which is not surprising, given that their body plans diverge significantly from those of vertebrates. The underlying mechanisms, however, involve many of the same cellular activities: cell migration, cell signaling between different tissues, and cell shape changes generating new organs. In insects, for example, tissues of the nervous system form when ectoderm

along the anterior-posterior axis rolls into a tube inside the embryo, similar to the vertebrate neural tube. Interestingly, the tube is on the ventral side of the insect embryo rather than the dorsal side, where it is in vertebrates. In spite of the different locations, the molecular signaling pathways that bring about the events in the two groups are very similar, underscoring their ancient shared evolutionary history.

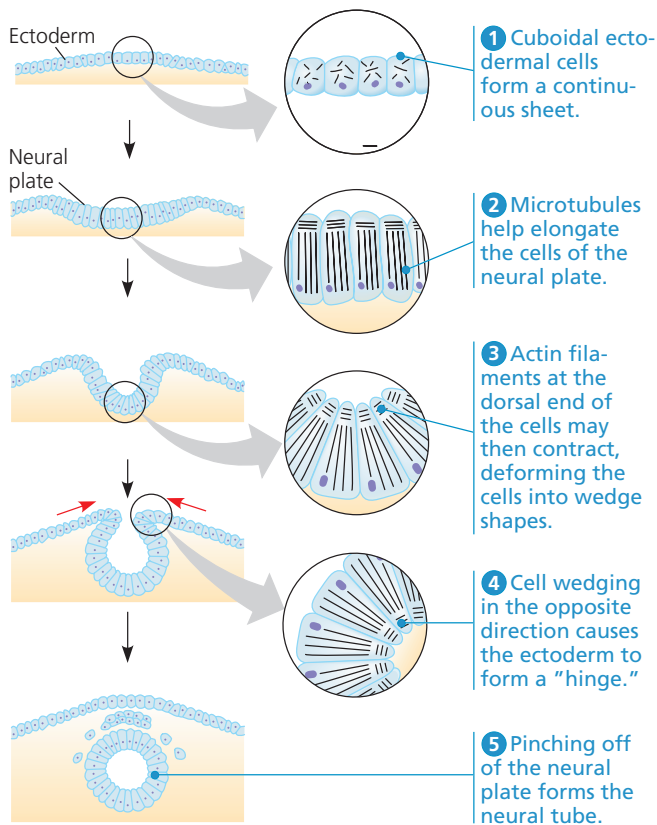
As we have seen in our consideration of gastrulation and organogenesis, changes in cell shape and location are essential to early development. We will turn now to an exploration of how these changes take place.

Mechanisms of Morphogenesis

Morphogenesis is a major stage of development in both animals and plants, but only in animals does it involve the *movement* of cells. The rigid cell wall that surrounds plant cells prevents complex movements like those that occur during gastrulation and organogenesis. In animals, movement of parts of a cell can bring about changes in cell shape or enable a cell to migrate from one place to another within the embryo. Here we will consider some of the cellular components that contribute to these events. We'll begin with the roles of the microtubules and microfilaments that make up the cytoskeleton (see Table 6.1).

The Cytoskeleton in Morphogenesis

Reorganization of the cytoskeleton is a major force in changing cell shape during development. As an example, let's return to the topic of neurulation. At the onset of neural tube formation, microtubules oriented from dorsal to ventral in a sheet of ectodermal cells help lengthen the cells along that



▲ **Figure 47.15 Change in cell shape during morphogenesis.** Reorganization of the cytoskeleton is associated with morphogenetic changes in embryonic tissues, as shown here for the formation of the neural tube in vertebrates.

axis (**Figure 47.15**). At the dorsal end of each cell is a bundle of actin filaments (microfilaments) oriented crosswise. These actin filaments contract, giving the cells a wedge shape that bends the ectoderm layer inward. Similar changes in cell shape occur at the hinge regions where the neural tube is pinching off from the ectoderm. However, the generation of wedge-shaped cells is not limited to neurulation or even to vertebrates. In *Drosophila* gastrulation, for instance, the formation of wedge-shaped cells along the ventral surface is responsible for invagination of a tube of cells that form the mesoderm.

The cytoskeleton directs a different type of morphogenetic movement in promoting elongation of the archenteron in the sea urchin embryo (see Figure 47.9). In this case, cytoskeletal changes direct **convergent extension**, a rearrangement of the cells of a tissue layer that causes the sheet to become narrower (converge) while it becomes longer (extends). It's as if a crowd of people waiting to

enter a theater for a concert began to form a single-file line; the line would become much longer as it narrowed. In the embryo, the cells elongate, with their ends pointing in the direction they will move, and they wedge between each other into fewer columns of cells (**Figure 47.16**). Convergent extension is also important in other developmental settings, such as involution in the frog gastrula. There, convergent extension changes the gastrulating embryo from a spherical shape to the rounded rectangular shape seen in Figure 47.13c.

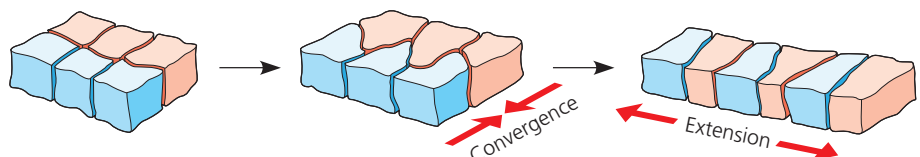
The cytoskeleton is responsible not only for cell shape changes but also for cell migration. During organogenesis in vertebrates, cells from the neural crest and from somites migrate to locations throughout the embryo. Cells "crawl" within the embryo by using cytoskeletal fibers to extend and retract cellular protrusions. This type of motility is akin to the amoeboid movement described in Figure 6.27b. Transmembrane glycoproteins called *cell adhesion molecules* play a key role in cell migration by promoting interaction between pairs of cells. Cell migration also involves the *extracellular matrix (ECM)*, the meshwork of secreted glycoproteins and other macromolecules lying outside the plasma membranes of cells (see Figure 6.30). The ECM helps to guide cells in many types of movements, such as migration of individual cells and shape changes of cell sheets. Cells that line migration pathways regulate movement of migrating cells by secreting specific molecules into the ECM.

Programmed Cell Death

Just as certain cells of the embryo are programmed to change shape or location, others are programmed to die. A type of *programmed cell death* called **apoptosis** is in fact a common feature of animal development. At various times in development, individual cells, sets of cells, or whole tissues cease to develop and are engulfed by neighboring cells. In some cases, a structure functions in a larval or other immature form of the organism and then is eliminated during later development. One familiar example is provided by the cells in the tail of a tadpole, which undergo apoptosis during frog metamorphosis (see Figure 45.19). Apoptosis can also occur when cells compete with one another for survival. For instance, many more neurons are produced during development of the vertebrate nervous system than exist in the adult. In general, neurons survive if they make functional connections with other neurons and die if they do not.

Some cells that undergo apoptosis don't seem to have any function in the developing embryo. Why do such cells form? The answer can be found by considering the evolution of

► **Figure 47.16 Convergent extension of a sheet of cells.** In this simplified diagram, the cells elongate in a particular direction and crawl between each other (convergence) as the sheet becomes longer and narrower (extension).



amphibians, birds, and mammals. When these groups began to diverge during evolution, the developmental program for making a vertebrate body was already in place. The differences in present-day body forms arose through modification of that common developmental program (which is why the early embryos of all vertebrates look so similar). As these groups evolved, many structures produced by the ancestral program that no longer offered a selective advantage were targeted for cell death. For example, the shared developmental program generates webbing between the embryonic digits, but in many birds and mammals the webbing is eliminated by apoptosis (see Figure 11.22).

As you have seen, cell behavior and the molecular mechanisms underlying it are crucial to the morphogenesis of the embryo. In the next section, you'll learn that a shared set of cellular and genetic processes ensure that the various types of cells end up in the right places in each embryo.

CONCEPT CHECK 47.2

1. In the frog embryo, convergent extension elongates the notochord. Explain how the words *convergent* and *extension* apply to this process.
2. **WHAT IF?** Predict what would happen if, just before neural tube formation, you treated embryos with a drug that blocks the function of microfilaments.
3. **MAKE CONNECTIONS** Unlike some other types of birth defects, neural tube defects are largely preventable. Explain (see Figure 41.4, p. 879).

For suggested answers, see Appendix A.

CONCEPT 47.3

Cytoplasmic determinants and inductive signals contribute to cell fate specification

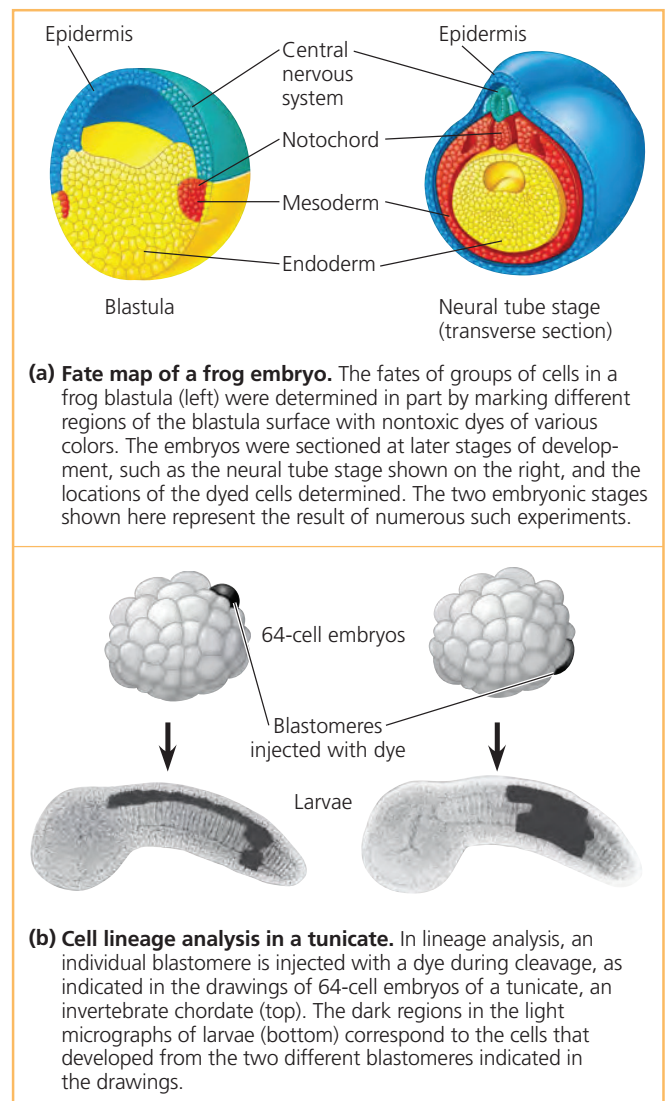
During embryonic development, cells arise by division, take up particular locations in the body, and become specialized in structure and function. Where a cell resides, how it appears, and what it does define its development fate. Developmental biologists use the terms **determination** to refer to the process by which a cell or group of cells becomes committed to a particular fate and **differentiation** to refer to the resulting specialization in structure and function.

Every diploid cell formed during an animal's development has the same genome. With the exception of certain mature immune cells, the collection of genes present is the same throughout the cell's life. How, then, do cells acquire different fates? As discussed in Concept 18.4, particular tissues, and often cells within a tissue, differ from one another by expressing distinct sets of genes from their shared genome.

A major focus of developmental biology is to uncover the mechanisms that direct the differences in gene expression underlying developmental fates. As one step toward this goal, scientists often seek to trace tissues and cell types back to their origins in the early embryo.

Fate Mapping

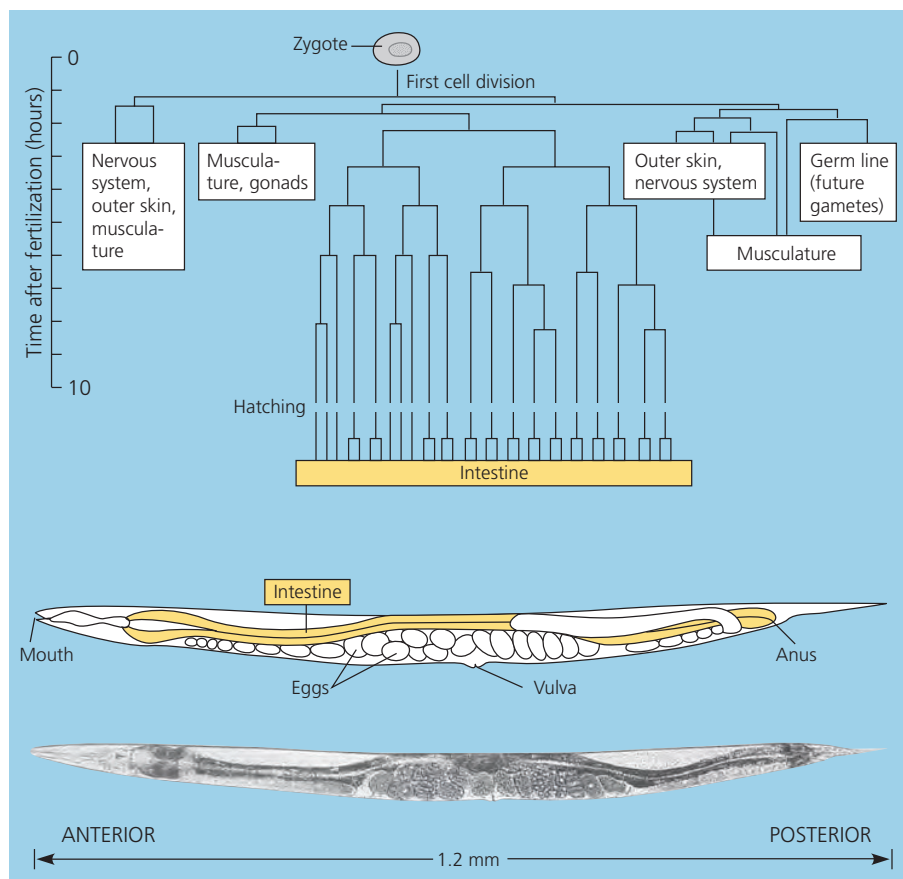
One way to trace the ancestry of embryonic cells is direct observation through the microscope. Such studies produced the first **fate maps**, diagrams showing the structures arising from each region of an embryo. In the 1920s, German embryologist Walther Vogt used this approach to determine where groups of cells from the blastula end up in the gastrula (Figure 47.17a). Later researchers developed techniques that allowed them to mark an individual blastomere during cleavage and then follow the marker as it was distributed to all the mitotic descendants of that cell (Figure 47.17b).



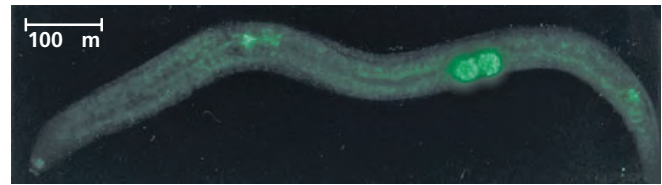
▲ **Figure 47.17** Fate mapping for two chordates.

A much more comprehensive approach to fate mapping has been carried out on the soil-dwelling nematode *Caenorhabditis elegans*. This roundworm is about 1 mm long, has a simple, transparent body with only a few types of cells, and develops into a mature adult hermaphrodite in only 3½ days in the laboratory. These attributes allowed Sydney Brenner, Robert Horvitz, and John Sulston to determine the complete cell lineage of *C. elegans*. They found that every adult hermaphrodite has exactly 959 somatic cells, which arise from the fertilized egg in virtually the same way for every individual. Careful microscopic observations of worms at all stages of development, coupled with experiments in which particular cells or groups of cells were destroyed by a laser beam or through mutations, resulted in the cell lineage diagram shown in **Figure 47.18**.

As an example of a particular cell fate, we'll consider *germ cells*, the specialized cells that give rise to eggs or sperm. In all animals studied, complexes of RNA and protein are involved in the specification of germ cell fate. In *C. elegans*, such complexes, called *P granules*, persist throughout development and can be detected in the germ cells of the adult gonad (**Figure 47.19**).



▲ Figure 47.18 Cell lineage in *Caenorhabditis elegans*. The *C. elegans* embryo is transparent, making it possible for researchers to trace the lineage of every cell, from the zygote to the adult worm (LM). The diagram shows a detailed lineage only for the intestine, which is derived exclusively from one of the first four cells formed from the zygote. The eggs will be fertilized internally and released through the vulva.



▲ Figure 47.19 Determination of germ cell fate in *C. elegans*. Labeling with an antibody specific for a *C. elegans* P granule protein (green) reveals the specific incorporation of P granules into the cells of the adult worm that will produce sperm or eggs.

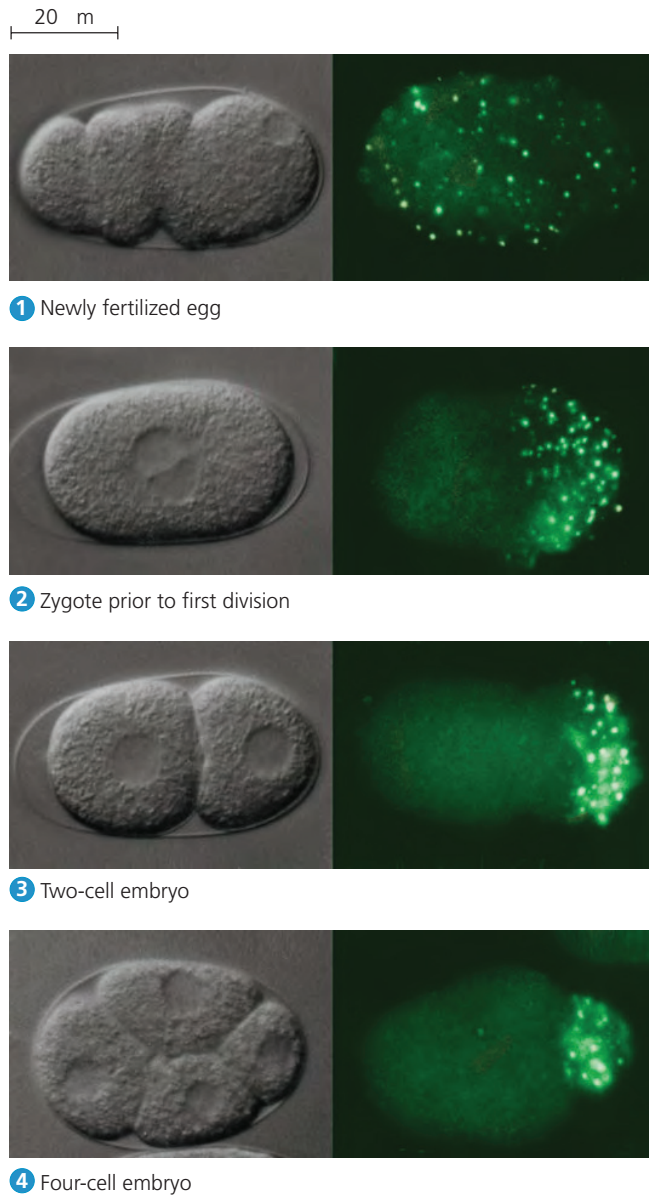
Tracing the position of the P granules provides a dramatic illustration of cell fate specification during development. The P granules are distributed throughout the newly fertilized egg but move to the posterior end of the zygote before the first cleavage division (**Figure 47.20 1** and **2**). As a result, only the posterior of the two cells formed by the first division contains P granules (**Figure 47.20 3**). The P granules continue to be asymmetrically partitioned during subsequent divisions (**Figure 47.20 4**). Thus, the P granules act as cytoplasmic determinants (see Concept 18.4), fixing germ cell fate at the earliest stage of *C. elegans* development.

Fate mapping in *C. elegans* paved the way for major discoveries about programmed cell death. Lineage analysis demonstrated that exactly 131 cells die during normal *C. elegans* development. In the 1980s, researchers found that a mutation inactivating a single gene allows all 131 cells to live. Further research revealed that this gene is part of a pathway that controls and carries out apoptosis in a wide range of animals, including humans. In 2002, Brenner, Horvitz, and Sulston shared a Nobel Prize for their use of the *C. elegans* fate map in studies of programmed cell death and organogenesis.

Having established fate maps for early development, scientists were positioned to answer questions about underlying mechanisms, such as how the basic axes of the embryo are established, a process known as axis formation.

Axis Formation

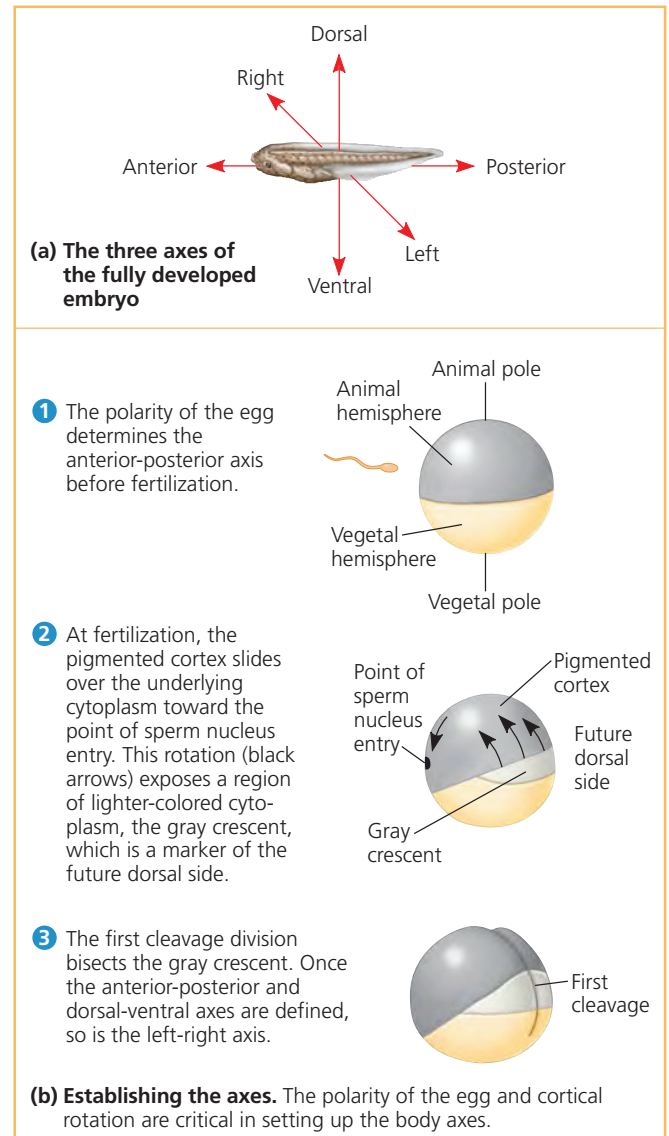
A body plan with bilateral symmetry is found across a range of animals, including nematodes, echinoderms, and vertebrates (see Chapter 32). As shown for a frog tadpole in **Figure 47.21a**, this body plan exhibits asymmetry along the dorsal-ventral and anterior-posterior axes. The right-left axis is largely symmetrical, as



▲ Figure 47.20 Partitioning of P granules during *C. elegans* development. The differential interference contrast micrographs (left) highlight the boundaries of nuclei and cells through the first two cell divisions. The immunofluorescence micrographs (right) show identically staged embryos stained with a labeled antibody specific for a P granule protein.

the two sides are roughly mirror images of each other. These three body axes are established early in development.

The anterior-posterior axis of the frog embryo is determined during oogenesis. Asymmetry is apparent in the formation of two distinct hemispheres: Dark melanin granules are embedded in the cortex of the animal hemisphere, whereas a yellow yolk fills the vegetal hemisphere. This animal-vegetal asymmetry dictates where the anterior-posterior axis forms in the embryo. Note, however, that the anterior-posterior and



▲ Figure 47.21 The body axes and their establishment in an amphibian. All three axes are established before the zygote begins to undergo cleavage.

WHAT IF? To study axis establishment, researchers can block cortical rotation or force it to occur in a specific direction. One such study resulted in a two-headed embryo because the “back” developed on both sides. What do you think the researchers did to obtain such an embryo?

animal-vegetal axes are not the same; that is, the head of the embryo does not form at the animal pole.

The dorsal-ventral axis of the frog embryo is not determined until fertilization. Upon fusion of the egg and the sperm, the egg surface—the plasma membrane and associated cortex—rotates with respect to the inner cytoplasm, a movement called *cortical rotation*. From the perspective of the animal pole, this rotation is always toward the point of sperm entry (**Figure 47.21b**).

How does cortical rotation establish the dorsal-ventral axis? Cortical rotation allows molecules in one portion of the vegetal cortex to interact with molecules in the inner cytoplasm of the animal hemisphere. These inductive interactions activate regulatory factors in specific portions of the vegetal cortex, leading to expression of different sets of genes in dorsal and ventral regions of the embryo.

In chicks, gravity is apparently involved in establishing the anterior-posterior axis as the egg travels down the hen's oviduct before being laid. Later, pH differences between the two sides of the blastoderm cells establish the dorsal-ventral axis. If the pH is artificially reversed above and below the blastoderm, the cells' fates will be reversed: The side facing the egg white will become the ventral part of the embryo, whereas the side facing the yolk will become the dorsal part.

In mammals, no polarity is obvious until after cleavage. However, the results of recent experiments suggest that the orientation of the egg and sperm nuclei before they fuse influences the location of the first cleavage plane and thus may play a role in establishing the embryonic axes. In insects, morphogen gradients establish both the anterior-posterior and dorsal-ventral axes (see Chapter 18).

Once the anterior-posterior and dorsal-ventral axes are established, the position of the left-right axis is fixed. Nevertheless, specific molecular mechanisms must establish which side is left and which is right. In vertebrates, there are marked left-right differences in the location of internal organs as well as in the organization and structure of the heart and brain. Recent research has revealed that cilia are involved in setting up this left-right asymmetry. We will discuss this and other developmental roles of cilia at the end of this chapter.

Restricting Developmental Potential

Earlier we described determination in terms of commitment to a particular cell fate. Is cell fate commitment immediately irreversible, or is there a period of time during which cell fate can be modified? The German zoologist Hans Spemann addressed this question in 1938. By manipulating embryos to perturb normal development and then examining cell fate after the manipulation, he was able to assay a

cell's *developmental potential*, the range of structures to which it can give rise (Figure 47.22). Spemann found that the fates of embryonic cells are affected by both the distribution of determinants and the pattern of cleavage relative to this distribution. Furthermore, the work of Spemann and others demonstrated that the first two blastomeres of the frog embryo are **totipotent**, meaning that they can each develop into all the different cell types of that species.

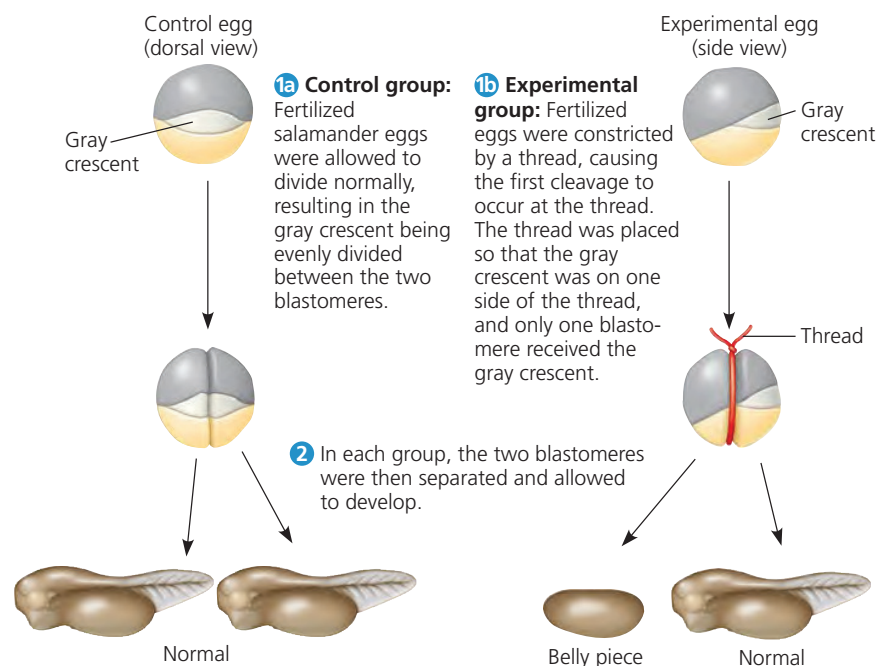
In mammals, embryonic cells remain totipotent through the eight-cell stage, much longer than in many other animals. Recent work, however, indicates that the very early cells (even the first two) are not actually equivalent in a normal embryo. Rather, their totipotency when isolated likely

▼ Figure 47.22

INQUIRY

How does distribution of the gray crescent affect the developmental potential of the first two daughter cells?

EXPERIMENT Hans Spemann, at the University of Freiburg-im-Breisgau, in Germany, carried out the following experiment in 1938 to test whether substances were located asymmetrically in the gray crescent.



RESULTS Blastomeres that received half or all of the material in the gray crescent developed into normal embryos, but a blastomere that received none of the gray crescent gave rise to an abnormal embryo without dorsal structures. Spemann called it a "belly piece."

CONCLUSION The developmental potential of the two blastomeres normally formed during the first cleavage division depends on their acquisition of cytoplasmic determinants localized in the gray crescent.

SOURCE H. Spemann, *Embryonic Development and Induction*, Yale University Press, New Haven, CT (1938).

WHAT IF? In a similar experiment 40 years earlier, embryologist Hans Roux allowed the first cleavage to occur and then used a needle to kill just one blastomere. The embryo that developed from the remaining blastomere (plus remnants of the dead cell) was abnormal, resembling a half-embryo. Propose a hypothesis to explain why Roux's result differed from the control result in Spemann's experiment.

means that the cells can regulate their fate in response to their embryonic environment. Once the 16-cell stage is reached, mammalian cells are determined to form the trophoblast or the inner cell mass. Although the cells have a limited developmental potential from this point onward, their nuclei remain totipotent, as demonstrated in cloning experiments like that described in Figure 20.19.

As you learned in Chapter 46, identical (monozygotic) twins can develop when embryonic cells become separated. If the separation occurs before the trophoblast and inner cell mass become differentiated, two embryos grow, each with its own chorion and amnion. This is the case for about a third of identical twins. For the rest, the two embryos that develop share a chorion and, in very rare cases where separation is particularly late, an amnion as well.

Regardless of how uniform or varied early embryonic cells are in a particular species, the progressive restriction of developmental potential is a general feature of development in all animals. In general, the tissue-specific fates of cells are fixed in a late gastrula, but not always so in an early gastrula. For example, if the dorsal ectoderm of an early amphibian gastrula is experimentally replaced with ectoderm from some other location in the same gastrula, the transplanted tissue forms a neural plate. But if the same experiment is performed on a late-stage gastrula, the transplanted ectoderm does not respond to its new environment and does not form a neural plate.

Cell Fate Determination and Pattern Formation by Inductive Signals

As embryonic cells acquire distinct fates, the cells begin to influence each other's fates by induction. At the molecular level, the response to an inductive signal is usually to switch on a set of genes that make the receiving cells differentiate into a specific tissue. Here we will examine two examples of induction, an essential process in the development of many tissues in most animals.

The "Organizer" of Spemann and Mangold

Before his studies of totipotency in the fertilized frog egg, Spemann had investigated cell fate determination during gastrulation. In these experiments, he and his student Hilde Mangold transplanted tissues between early gastrulas. In their most famous such experiment, summarized in Figure 47.23, they made a remarkable discovery. Not only did a transplanted dorsal lip of the blastopore continue to be a blastopore lip, but it also triggered gastrulation of the surrounding tissue. They concluded that the dorsal lip of the blastopore in the early gastrula functions as an "organizer" of the embryo's body plan, inducing changes in surrounding tissue that direct formation of the notochord, the neural tube, and other organs.

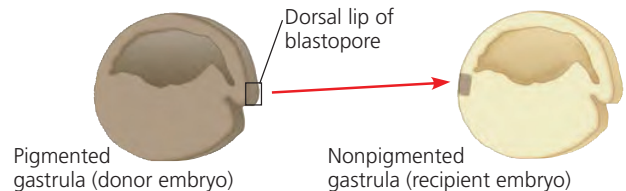
Nearly a century later, developmental biologists are still actively studying the basis of induction by *Spemann's organizer*. An important clue has come from studies of a growth factor called

▼ Figure 47.23

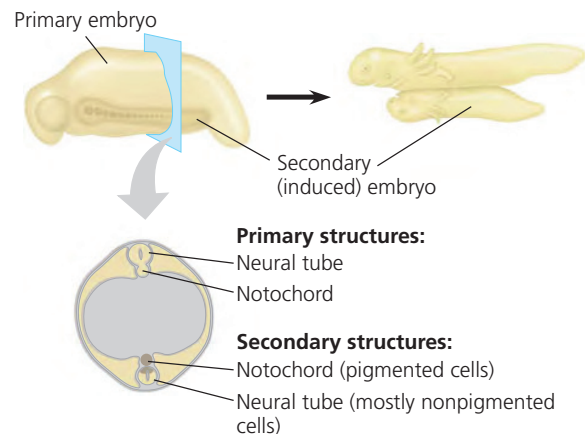
INQUIRY

Can the dorsal lip of the blastopore induce cells in another part of the amphibian embryo to change their developmental fate?

EXPERIMENT In 1924, Hans Spemann and Hilde Mangold, at the University of Freiburg-im-Breisgau, in Germany, transplanted a piece of the dorsal lip from a pigmented newt gastrula to the ventral side of a nonpigmented newt gastrula to investigate the inductive ability of the dorsal lip. Cross sections of the gastrulas are shown here.



RESULTS The recipient embryo formed a second notochord and neural tube in the region of the transplant, and eventually most of a second embryo developed. Examination of the interior of the double embryo revealed that the secondary structures were formed partly, but not wholly, from recipient tissue.



CONCLUSION The transplanted dorsal lip was able to induce cells in a different region of the recipient to form structures different from their normal fate. In effect, the transplanted dorsal lip "organized" the later development of an entire extra embryo.

SOURCE H. Spemann and H. Mangold, Induction of embryonic primordia by implantation of organizers from a different species, *Trans. V. Hamburger* (1924). Reprinted in *International Journal of Developmental Biology* 45:13–38 (2001).

WHAT IF? Because the transplanted dorsal lip caused the recipient tissue to become something it would not otherwise have become, a signal of some sort must have passed from the dorsal lip. If you identified a protein candidate for the signaling molecule, how could you test whether it actually functions in signaling?

bone morphogenetic protein 4 (BMP-4). (Bone morphogenetic proteins, a family of related proteins with a variety of developmental roles, derive their name from members of the family that are important in bone formation.) One major function of the cells of the organizer seems to be to *inactivate* BMP-4 on the dorsal side of the embryo. Inactivation of BMP-4 allows cells on

the dorsal side to make dorsal structures, such as the notochord and neural tube. Proteins related to BMP-4 and its inhibitors are also found in other animals, including invertebrates such as the fruit fly, where they also regulate the dorsal-ventral axis.

Formation of the Vertebrate Limb

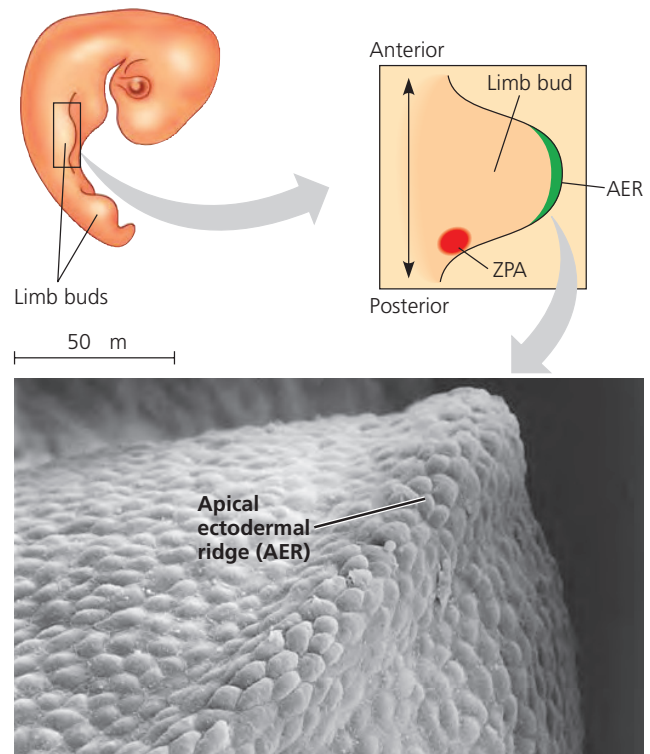
Inductive signals play a major role in **pattern formation**, the development of an animal's spatial organization, the arrangement of organs and tissues in their characteristic places in three-dimensional space. The molecular cues that control pattern formation, called **positional information**, tell a cell where it is with respect to the animal's body axes and help to determine how the cell and its descendants will respond to molecular signaling.

In Chapter 18, we discussed pattern formation in the development of *Drosophila*. For the study of pattern formation in vertebrates, a classic model system has been limb development in the chick. The wings and legs of chicks, like all vertebrate limbs, begin as limb buds, bumps of mesodermal tissue covered by a layer of ectoderm (**Figure 47.24a**). Each component of a chick limb, such as a specific bone or muscle, develops with a precise location and orientation relative to three axes: the proximal-distal axis (the “shoulder-to-fingertip” axis), the anterior-posterior axis (the “thumb-to-little finger” axis), and the dorsal-ventral axis (the “knuckle-to-palm” axis). The embryonic cells within a limb bud respond to positional information indicating location along these three axes (**Figure 47.24b**).

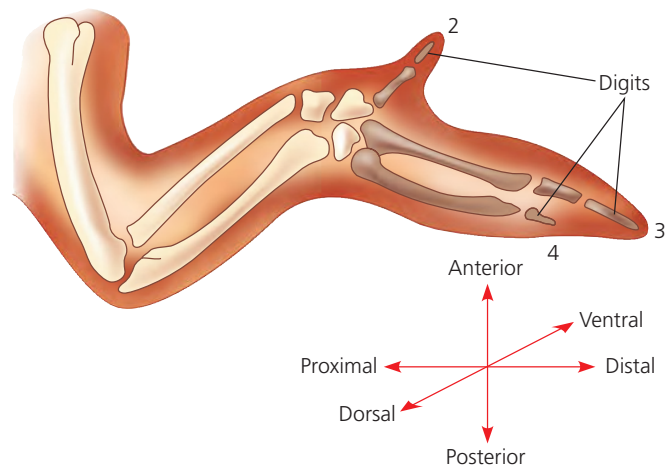
Two regions in a limb bud have profound effects on the limb's development. These regions are present in all vertebrate limb buds, including those that will develop into forelimbs (such as wings or arms) and those destined to become hind limbs. The cells of these regions secrete proteins that provide key positional information to the other cells of the bud.

One region regulating limb-bud development is the **apical ectodermal ridge (AER)**, a thickened area of ectoderm at the tip of the bud (see **Figure 47.24a**). Removing the AER blocks outgrowth of the limb along the proximal-distal axis. The cells of the AER secrete several protein signals in the fibroblast growth factor (FGF) family that promote limb-bud outgrowth. If the AER is surgically removed and beads soaked with FGF are put in its place, a nearly normal limb will develop. In 2006, researchers identified an FGF-secreting AER that appears to be responsible for building a shark's unpaired (median) fins. This finding suggests that the specific function of the AER predated the appearance of paired limbs in the vertebrate lineage.

The second major limb-bud regulatory region is the **zone of polarizing activity (ZPA)**, a block of mesodermal tissue located underneath the ectoderm where the posterior side of the bud is attached to the body (see **Figure 47.24a**). The ZPA is necessary for proper pattern formation along the anterior-posterior axis of the limb. Cells nearest the ZPA give rise to the posterior structures, such as the most posterior of the chick's three digits (positioned like our little finger); cells



(a) Organizer regions. Vertebrate limbs develop from protrusions called limb buds, each consisting of mesoderm cells covered by a layer of ectoderm. Two regions in each limb bud, the apical ectodermal ridge (AER, shown in this SEM) and the zone of polarizing activity (ZPA), play key roles as organizers in limb pattern formation.



(b) Wing of chick embryo. As the bud develops into a limb, a specific pattern of tissues emerges. In the chick wing, for example, the digits are always present in the arrangement shown here. Pattern formation requires each embryonic cell to receive some kind of positional information indicating location along the three axes of the limb. The AER and ZPA secrete molecules that help provide this information. (Numbers are assigned to the digits based on a convention established for vertebrate limbs. The chicken wing has only four digits; the first digit points backward and is not shown in the diagram.)

▲ Figure 47.24 Vertebrate limb development.

farthest from the ZPA form anterior structures, including the most anterior digit (like our thumb).

The tissue transplantation experiment outlined in **Figure 47.25** supports the hypothesis that the inductive signal produced by the ZPA conveys positional information indicating “posterior.” Indeed, researchers have discovered that the cells of the ZPA secrete a growth factor called Sonic hedgehog. (Sonic hedgehog gets its name from two sources: its similarity to a *Drosophila* protein called Hedgehog, which is involved in segmentation of the fly embryo, and a video game character.) If cells genetically engineered to produce large amounts of Sonic hedgehog are implanted in the anterior region of a normal limb bud, a mirror-image limb results—just as if a ZPA had been grafted there. Studies of the mouse version of Sonic hedgehog suggest that extra toes in mice—and perhaps also in humans—can result when this protein is produced in part of the limb bud where it is normally absent. Sonic hedgehog and other similar Hedgehog proteins function in many developmental settings and organisms, including pattern formation in *Drosophila* and regulation of cell fate and number in the vertebrate nervous system.

Signaling by Sonic hedgehog plays a vital role in limb-bud development, but what determines whether a limb bud develops into a forelimb or a hind limb? It turns out that the cells receiving the Hedgehog signals from the AER and ZPA respond according to their developmental histories. Before the AER or ZPA issues its signals, earlier developmental signaling sets up specific spatial patterns of *Hox* gene expression (see Figure 21.18). Differences in *Hox* gene expression cause cells of the forelimb and hind limb buds—and cells in different parts of each limb bud—to react differently to the same positional cues.

Hedgehog, FGF, and BMP-4 are examples of a much larger set of signaling molecules that govern cell fates in animals. Having mapped out many of the basic functions of these molecules in embryonic development, researchers are now addressing their role in organogenesis, focusing in particular on the development of the brain.

Cilia and Cell Fate

For many years, developmental biologists largely ignored the cellular organelles known as cilia. That is no longer the case. There is now good experimental evidence that ciliary function is essential for proper specification of cell fate in the human embryo.

Like other mammals, humans have stationary and motile cilia (see Figure 6.24). Stationary primary cilia, or *monocilia*, exist as a single projection on the surface of nearly all cells. Motile cilia are found on cells that propel fluid over their surface, such as the epithelial cells of airways, and on sperm (in the form of flagella that propel sperm movement). Both stationary and motile cilia play vital roles in development.

In 2003, geneticists discovered that certain mutations disrupting development of the mouse nervous system affect

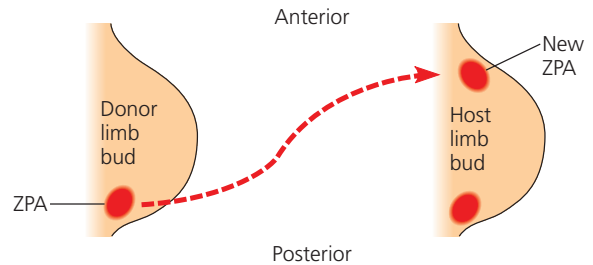
▼ **Figure 47.25**

INQUIRY

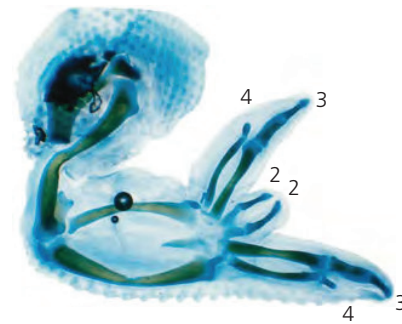
What role does the zone of polarizing activity (ZPA) play in limb pattern formation in vertebrates?



EXPERIMENT In 1985, Dennis Summerbell and Lawrence Honig, then at the National Institute for Medical Research in Mill Hill, near London, were eager to investigate the nature of the zone of polarizing activity. They transplanted ZPA tissue from a donor chick embryo under the ectoderm in the anterior margin of a limb bud in another chick (the host).



RESULTS The host limb bud developed extra digits from host tissue in a mirror-image arrangement to the normal digits, which also formed (compare with Figure 47.24b, which shows a normal chick wing).



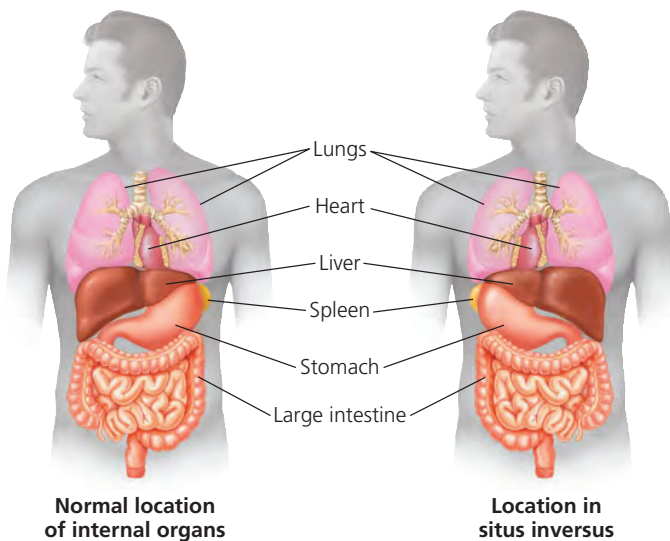
CONCLUSION The mirror-image duplication observed in this experiment suggests that ZPA cells secrete a signal that diffuses from its source and conveys positional information indicating “posterior.” As the distance from the ZPA increases, the signal concentration decreases, and hence more anterior digits develop.

SOURCE L. S. Honig and D. Summerbell, Maps of strength of positional signaling activity in the developing chick wing bud, *Journal of Embryology and Experimental Morphology* 87:163–174 (1985).

WHAT IF? Suppose you learned that the ZPA forms after the AER, leading you to develop the hypothesis that the AER is necessary for formation of the ZPA. Given what you know about molecules expressed in the AER and ZPA (see the text), how could you test your hypothesis?

genes that function in the assembly of monocilia. Other researchers found that mutations responsible for a severe kidney disease in mice alter a gene important for the transport of materials up and down monocilia. Mutations that block the function of monocilia have also been linked to cystic kidney disease in humans.

Given that monocilia are stationary, how do they function in development? The answer is that each acts as an antenna on the cell surface, receiving signals from multiple signaling



▲ **Figure 47.26** *Situs inversus*, a reversal of normal left-right asymmetry in the chest and abdomen.

proteins, including Sonic hedgehog. When the monocilia are defective, signaling is disrupted.

Research on the role of motile cilia in development grew from the observation that certain individuals share a particular set of medical conditions, later named Kartagener's syndrome. Such individuals are prone to infections of the nasal sinuses and bronchi. Males with Kartagener's syndrome also produce immotile sperm. But the most intriguing feature of this syndrome is *situs inversus*, a reversal of the normal left-right asymmetry of the organs in the chest and abdomen (**Figure 47.26**). For example, in *situs inversus*, the heart is on the right side rather than the left. (About one in 10,000 individuals have *situs inversus*, which causes no significant medical problems by itself.)

The conditions associated with Kartagener's syndrome all result from a defect that makes cilia immotile. Without motility, sperm tails cannot beat and airway cells cannot sweep

mucus and microbes out of the airway. But what causes *situs inversus* in these individuals? The current model proposes that ciliary motion in a particular part of the embryo is essential for normal development. Evidence indicates that movement of the cilia generates a leftward fluid flow, breaking the symmetry between left and right sides. Without that flow, asymmetry along the left-right axis arises randomly, and half of the affected embryos develop *situs inversus*.

If we step back from the specification of particular cell fates to consider development as a whole, we see a sequence of events marked by cycles of signaling and differentiation. Initial cell asymmetries allow different types of cells to influence each other, resulting in the expression of specific sets of genes. The products of these genes then direct cells to differentiate into specific types. Through pattern formation and morphogenesis, differentiated cells ultimately produce a complex arrangement of tissues and organs, each functioning in its appropriate location and in coordination with other cells, and organs throughout the organism.

CONCEPT CHECK 47.3

1. How do axis formation and pattern formation differ?
2. **MAKE CONNECTIONS** How does a morphogen gradient differ from cytoplasmic determinants and inductive interactions with regard to the set of cells it affects (see Concept 18.4, p. 367)?
3. **WHAT IF?** If the ventral cells of an early frog gastrula are experimentally induced to express large amounts of a protein that inhibits BMP-4, could a second embryo develop? Explain.
4. **WHAT IF?** If you removed the ZPA from a limb bud and then placed a bead soaked in Sonic hedgehog in the middle of the limb bud, what would be the most likely result?

For suggested answers, see Appendix A.

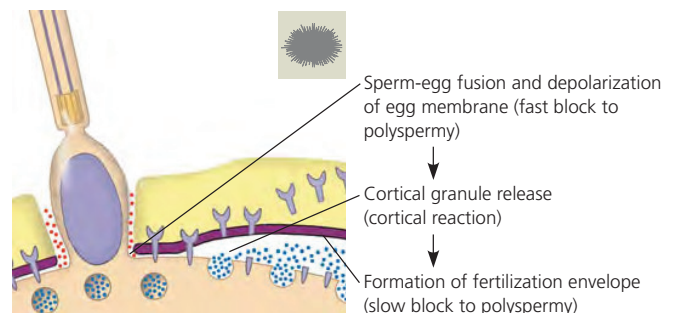
47 CHAPTER REVIEW

SUMMARY OF KEY CONCEPTS

CONCEPT 47.1

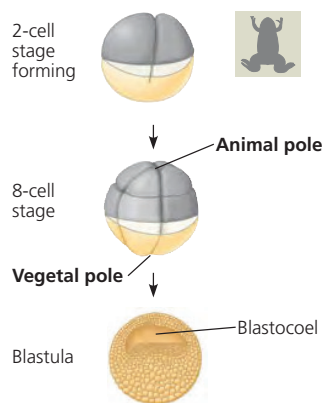
Fertilization and cleavage initiate embryonic development (pp. 1022–1027)

- **Fertilization** brings together the nuclei of sperm and egg, forming a diploid zygote, and activates the egg, initiating embryonic development. The **acrosomal reaction**, which is triggered when the sperm meets the egg, releases hydrolytic enzymes that digest material surrounding the egg. Gamete contact and/or fusion depolarizes the egg cell membrane and sets up a **fast block to polyspermy** in many animals. Sperm-egg fusion also initiates the cortical reaction.



In mammalian fertilization, the cortical reaction modifies the zona pellucida as a **slow block to polyspermy**.

- Fertilization is followed by **cleavage**, a period of rapid cell division without growth, which results in the production of a large number of cells called **blastomeres**. In many species, cleavage creates a multicellular ball called the **blastula**, which contains a fluid-filled cavity, the **blastocoel**. **Holoblastic** cleavage (division of the entire egg) occurs in species whose eggs have little or moderate amounts of **yolk** (as in sea urchins, frogs, and mammals). **Meroblastic** cleavage (incomplete division of the egg) occurs in species with yolk-rich eggs (as in birds and other reptiles).

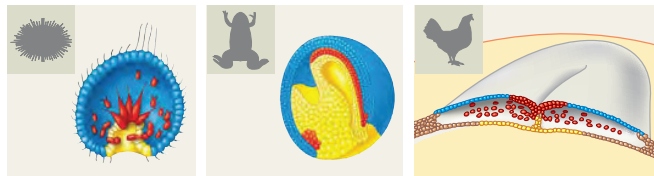


? What cell-surface barrier prevents fertilization of an egg by a sperm of a different species?

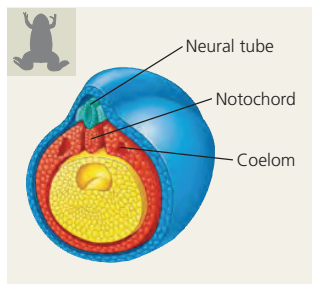
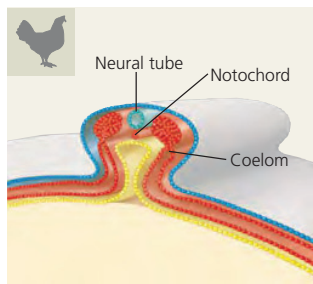
CONCEPT 47.2

Morphogenesis in animals involves specific changes in cell shape, position, and survival (pp. 1027–1035)

- Gastrulation** converts the blastula to a **gastrula**, which has a primitive digestive cavity and three **germ layers**: **ectoderm** (blue), **mesoderm** (red), and **endoderm** (yellow).



- Mammalian eggs are small, store few nutrients, exhibit holoblastic cleavage, and show no obvious polarity. However, gastrulation and organogenesis in mammals resemble the processes in birds and other reptiles. After fertilization and early cleavage in the oviduct, the **blastocyst** implants in the uterus. The **trophoblast** initiates formation of the fetal portion of the placenta, and the embryo proper develops from a single layer of cells, the epiblast, within the blastocyst.
- The embryos of birds, other reptiles, and mammals develop within a fluid-filled sac that is contained within a shell or the uterus. In these organisms, the three germ layers give rise not only to embryonic tissue but also to the four **extraembryonic membranes**: the amnion, chorion, yolk sac, and allantois.
- The organs of the animal body develop from specific portions of the three embryonic germ layers. Early events in **organogenesis** in vertebrates include neurulation: formation of the **notochord** by cells of the dorsal mesoderm and development of the **neural tube** from infolding of the ectodermal neural plate.



- Cytoskeletal rearrangements are responsible for changes in the shape of cells that underlie cell movements in gastrulation and organogenesis, including invaginations and **convergent extension**. The cytoskeleton is also involved in cell migration, which relies on cell adhesion molecules and the extracellular matrix to help cells reach specific destinations.

? How does the neural tube form? How do neural crest cells arise?

CONCEPT 47.3

Cytoplasmic determinants and inductive signals contribute to cell fate specification (pp. 1035–1042)

- Experimentally derived **fate maps** of embryos show that specific regions of the zygote or blastula develop into specific parts of older embryos. The complete cell lineage has been worked out for *C. elegans*. Mechanisms for establishing cellular asymmetries include morphogen gradients, localized determinants, and inductive interactions. As embryonic development proceeds, the developmental potential of cells becomes progressively more limited in all species.
- Cells in a developing embryo receive and respond to **positional information** that varies with location. This information is often in the form of signaling molecules secreted by cells in specific regions of the embryo, such as the dorsal lip of the blastopore in the amphibian gastrula and the **apical ectodermal ridge** and **zone of polarizing activity** of the vertebrate limb bud. The signaling molecules influence gene expression in the cells that receive them, leading to **differentiation** and the development of particular structures.

? Suppose you found two classes of mouse mutations, one that affected limb development only and one that affected both limb and kidney development. Which class would be more likely to alter the function of monocilia? Explain.

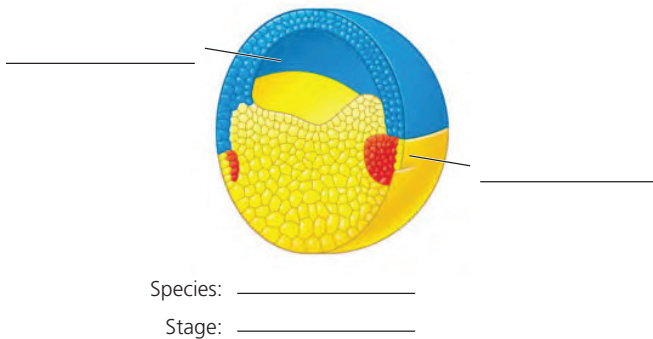
TEST YOUR UNDERSTANDING

LEVEL 1: KNOWLEDGE/COMPREHENSION

- The cortical reaction of sea urchin eggs functions directly in
 - the formation of a fertilization envelope.
 - the production of a fast block to polyspermy.
 - the release of hydrolytic enzymes from the sperm.
 - the generation of an electrical impulse by the egg.
 - the fusion of egg and sperm nuclei.
- Which of the following is common to the development of both birds and mammals?
 - holoblastic cleavage
 - epiblast and hypoblast
 - trophoblast
 - yolk plug
 - gray crescent
- The archenteron develops into
 - the mesoderm.
 - the blastocoel.
 - the endoderm.
 - the placenta.
 - the lumen of the digestive tract.
- What structural adaptation in chickens allows them to lay their eggs in arid environments rather than in water?
 - extraembryonic membranes
 - yolk
 - cleavage
 - gastrulation
 - development of the brain from ectoderm

LEVEL 2: APPLICATION/ANALYSIS

5. In an egg cell treated with EDTA, a chemical that binds calcium and magnesium ions,
 - a. the acrosomal reaction would be blocked.
 - b. the fusion of sperm and egg nuclei would be blocked.
 - c. the fast block to polyspermy would not occur.
 - d. the fertilization envelope would not form.
 - e. the zygote would not contain maternal and paternal chromosomes.
6. In humans, identical twins are possible because
 - a. cytoplasmic determinants are distributed unevenly in unfertilized eggs.
 - b. extraembryonic cells interact with the zygote nucleus.
 - c. convergent extension occurs.
 - d. early blastomeres can form a complete embryo if isolated.
 - e. the gray crescent divides the dorsal-ventral axis into new cells.
7. Cells transplanted from the neural tube of a frog embryo to the ventral part of another embryo develop into nervous system tissues. This result indicates that the transplanted cells were
 - a. totipotent.
 - b. determined.
 - c. differentiated.
 - d. mesenchymal.
 - e. apoptotic.
8. **DRAW IT** Fill in the blanks in the figure below, and draw arrows showing the movement of ectoderm, mesoderm, and endoderm.



LEVEL 3: SYNTHESIS/EVALUATION

9. **EVOLUTION CONNECTION**

Evolution in insects and vertebrates has involved the repeated duplication of body segments, followed by fusion of some segments and specialization of their structure and function. What parts of vertebrate anatomy reflect the vertebrate segmentation pattern?

10. SCIENTIFIC INQUIRY

The “snout” of a frog tadpole bears a sucker. A salamander tadpole has a mustache-shaped structure called a balancer in the same area. Suppose that you perform an experiment in which you transplant ectoderm from the side of a young salamander embryo to the snout of a frog embryo. The tadpole that develops has a balancer. When you transplant ectoderm from the side of a slightly older salamander embryo to the snout of a frog embryo, the frog tadpole ends up with a patch of salamander skin on its snout. Suggest a hypothesis to explain these results in terms of developmental mechanisms. How might you test your hypothesis?

11. SCIENCE, TECHNOLOGY, AND SOCIETY

Many scientists think that fetal tissue transplants offer great potential for treating Parkinson’s disease, epilepsy, diabetes, Alzheimer’s disease, and spinal cord injuries. Why might tissues from a fetus be particularly useful for replacing diseased or damaged cells in patients with such conditions? Some people would allow only tissues from miscarriages to be used in fetal transplant research. However, most researchers prefer to use tissues from surgically aborted fetuses. Why? Explain your position on this controversial issue.

12. WRITE ABOUT A THEME

Emergent Properties In a short essay (100–150 words), describe how the emergent properties of the cells of the gastrula direct embryonic development.

For selected answers, see Appendix A.

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