Chapter 47

Animal Development

Lecture Outline

Overview: A Body-Building Plan for Animals

- The question of how a zygote becomes an animal has been asked for centuries.
- As recently as the 18th century, the prevailing idea was *preformation,* the notion that an egg or sperm contains an embryo that is a preformed miniature adult.
- The competing theory is *epigenesis,* proposed 2,000 years earlier by Aristotle, which states that the form of an animal emerges from a relatively formless egg.
- As microscopy improved in the 19th century, biologists could see that embryos took shape in a series of gradual progressive steps.
- Epigenesis thus displaced preformation as the favored explanation among embryologists.
- A genetic program including the genome of the zygote and molecules placed into the egg by the mother determines an organism's development.
- These molecules, including proteins and RNAs, are called **cytoplasmic determinants.**
- As the zygote divides, early embryonic cells become different due to the uneven distribution of cytoplasmic determinants and signals from neighboring cells.
    - These differences set the stage for distinct programs of gene expression to be carried out in each cell and its descendents.
- As cell division continues, the specific pattern of gene expression in each cell sends the cells on unique paths to their ultimate fate in the developing organism.
- This process of cell specialization in structure and function is called **cell differentiation**.
- Along with cell division and differentiation, development involves **morphogenesis**, the process by which an organism takes shape and differentiated cells occupy their appropriate locations.
- Molecular genetics and classical embryology have both contributed to our understanding of animal development.
- Because animals display a wide variety of body plans, it is not surprising that embryonic development occurs by different schemes.
- Despite these differences, animals share many basic mechanisms of development and use a common genetic toolkit.
- **Model organisms** are species that lend themselves to the study of a particular question, are representative of a larger group, and are easy to grow in the lab.
- Several model organisms have been the subject of classical embryological studies as well as more recent molecular analyses: the sea urchin, the frog, the chick, and the nematode *Caenorhabditis elegans.*

Concept 47.1 After fertilization, embryonic development proceeds through cleavage, gastrulation, and organogenesis.

- Important processes regulating development occur during fertilization and the three stages that begin to build the body of most animals.
    1. During cleavage, cell division creates a hollow ball of cells, the blastula, from the zygote.
    2. Then gastrulation rearranges the blastula into a three-layered embryo, the gastrula.
    3. During organogenesis, interactions and movements of the three layers generate rudimentary organs from which adult structures grow.

Fertilization activates the egg and brings together the nuclei of sperm and egg.

- The gametes (egg and sperm) are highly specialized cell types.

- Fertilization combines haploid sets of chromosomes from two individuals into a single diploid cell, the zygote.

- Another key function of fertilization is to activate the egg.
    ○ Contact of the sperm with the egg's surface initiates metabolic reactions within the egg that trigger the onset of embryonic development.

- Sea urchin fertilization has been extensively studied.
    ○ Sea urchins are deuterostomes, like chordates (including vertebrates).
    ○ Despite differences in details, fertilization and early development in sea urchins provide good general models for similar events in vertebrates.

- Sea urchin egg and sperm encounter each other after the animals release their gametes into seawater.

- The jelly coat of the egg attracts the sperm, which swims toward the egg.

- When the head of the sperm comes into contact with the jelly coat, the **acrosomal reaction** is triggered, and the **acrosome**, a specialized vesicle at the tip of the sperm, discharges its contents by exocytosis.

- The hydrolytic enzymes that are released enable the *acrosomal process* to elongate through the egg's jelly coat.

- Molecules on the tip of the acrosomal process adhere to special receptor proteins that extend from the egg's plasma membrane.

- These receptors extend through the *vitelline layer,* just external to the egg's plasma membrane.
    ○ This lock-and-key recognition ensures that eggs will be fertilized only by sperm of the same species.

- The sperm and egg plasma membranes fuse, and the sperm nucleus enters the egg's cytoplasm.

- In response to contact or fusion of the membranes, Na^+ channels in the egg's plasma membrane open.

- Na^+ flows into the egg, and the membrane depolarizes, changing the membrane potential of the egg.
    ○ Such depolarization is common in animals.

- Occurring within 1–3 seconds after the sperm binds to the egg, depolarization prevents additional sperm from fusing with the egg's plasma membrane.
    ○ This **fast block to polyspermy** prevents polyspermy, the fertilization of the egg by multiple sperm.

- Fusion of egg and sperm plasma membranes triggers a series of changes in the egg that cause a longer-lasting block.
  - Numerous vesicles lie just beneath the egg plasma membrane, in the *cortex* of the cell.
  - These vesicles, called **cortical granules**, fuse with the plasma membrane of the egg and release their contents into the *perivitelline space,* between the plasma membrane and the vitelline layer.
  - This is the **cortical reaction**.
- The vitelline layer separates from the plasma membrane and hardens to form a protective **fertilization envelope**, which resists the entry of additional sperm.
  - Another enzyme clips off and releases the external portions of the remaining receptor proteins, along with any attached sperm.
- The fertilization envelope and other changes in the egg's surface function together as a longer-term **slow block to polyspermy**.
- A high calcium (Ca^{2+}) concentration in the egg is essential for the cortical reaction to occur.
  - Sperm binding activates a signal transduction pathway that causes Ca^{2+} to be released from the egg's endoplasmic reticulum into the cytosol.
  - The elevated Ca^{2+} levels cause cortical granule fusion.
  - A cortical reaction triggered by Ca^{2+} also occurs in vertebrates such as fishes and mammals.
- High concentrations of Ca^{2+} in the egg stimulate an increase in the rates of cellular respiration and protein synthesis, known as *egg activation*.
  - Unfertilized eggs can be activated artificially by the injection of Ca^{2+} or by a variety of mildly injurious treatments, such as temperature shock.
  - It is even possible to activate an egg that has had its nucleus removed.
  - Evidently, proteins and mRNAs present in the cytoplasm of the unfertilized egg are sufficient for egg activation.
- About 20 minutes after the sperm enters the egg, the sperm nucleus and egg nucleus join to create the diploid nucleus of the zygote.
- DNA synthesis begins and the first cell division occurs about 90 minutes after fertilization.
- Species vary in the stage of meiosis the egg has reached by the time it is fertilized.
  - When they are released from the female, sea urchin eggs have completed meiosis.
  - In other species, the eggs are arrested at a specific stage of meiosis. Upon fertilization, meiosis is quickly completed along with many of the events already described.
  - Human eggs are arrested at metaphase of meiosis II until they are fertilized in the female reproductive tract.
- Fertilization in terrestrial animals, including mammals, is generally internal, which ensures a moist environment through which the sperm can move toward the egg.
  - Secretions in the mammalian female reproductive tract act to increase sperm motility.
  - In humans, this enhancement of sperm function requires about 6 hours of exposure to the female reproductive tract.
- The mammalian egg is surrounded by follicle cells released with the egg during ovulation.
- A sperm must migrate through a layer of follicle cells before it reaches the **zona pellucida**, the extracellular matrix of the egg.

- Binding of the sperm cell to a sperm receptor on the zona pellucida induces an acrosomal reaction similar to the reaction seen in the sea urchin.
- Enzymes from the acrosome enable the sperm cell to penetrate the zona pellucida; newly exposed proteins on the sperm bind to the egg's plasma membrane.
  - The binding of the sperm cell to the egg triggers changes within the egg, leading to a cortical reaction, the release of enzymes from cortical granules to the outside via exocytosis.
  - The released enzymes catalyze alteration of the zona pellucida, which functions as a slow block to polyspermy. There is no known fast block to polyspermy in mammals.
- After the egg and sperm membranes fuse, the entire sperm, tail and all, is taken into the egg.
  - The centriole that acted as the basal body of the sperm's flagellum generates the mitotic spindle for the first cell division.
  - This centrosome duplicates to form the two centrosomes of the zygote.
  - The centrosomes will generate the mitotic spindle for the first cell division.
- The haploid nuclei of mammalian sperm and egg do not fuse immediately as they do in sea urchin fertilization. Instead, the envelopes of both egg and sperm nuclei disperse.
- The chromosomes from the two gametes share a common spindle apparatus during the first mitotic division of the zygote.
- Only after the first division, as diploid nuclei form in the two daughter cells, do the chromosomes from the two parents come together in a common nucleus.
- Fertilization is much slower in mammals than in the sea urchin.
  - The first cell division occurs 12–36 hours after sperm binding in mammals.

Cleavage partitions the zygote into many smaller cells.

- A succession of rapid cell divisions called **cleavage** follows fertilization.
- During this period, cells go through the S (DNA synthesis) and M (mitosis) phases of the cell cycle but may skip the G_1 and G_2 phases.
- As a result, little or no protein synthesis occurs.
- The embryo does not enlarge significantly during cleavage.
- Cleavage simply partitions the cytoplasm of one large cell, the zygote, into many smaller cells called **blastomeres**, each with its own nucleus.
- The first five to seven divisions form a cluster of cells, within which a fluid-filled cavity called the **blastocoel** begins to form.
- The blastocoel is fully formed within the **blastula**, which is a hollow ball of cells.
  - During cleavage, different regions of cytoplasm present in the original undivided egg end up in separate blastomeres.
  - Because the regions may contain different cytoplasmic determinants, such as specific mRNAs and proteins, this partitioning can set the stage for subsequent developmental events.
- Most animals, with the possible exception of mammals, have eggs and zygotes with a definite polarity.
  - Thus, the planes of division follow a specific pattern relative to the poles of the zygote.
- Polarity is defined by the heterogeneous distribution of substances such as mRNA, proteins, and **yolk.**
- Yolk is most concentrated at the **vegetal pole** and least concentrated at the **animal pole**.

- The animal pole is also the site where the polar bodies of oogenesis budded from the cell.
- In amphibians, a rearrangement of the egg cytoplasm occurs at the time of fertilization.
- The plasma membrane and cortex rotate with respect to the inner cytoplasm, a movement called *cortical rotation*.
- The animal hemisphere cortex moves toward the vegetal inner cytoplasm on the side where the sperm entered, which is always in the animal hemisphere.
- The vegetal hemisphere cortex across from the side of sperm entry moves toward the animal hemisphere inner cytoplasm.
- Molecules in the vegetal cortex on the side opposite sperm entry are now able to interact with inner cytoplasmic molecules in the animal hemisphere, leading to the formation of cytoplasmic determinants that will later affect gene expression in the cells that inherit them, initiating development of dorsal structures.
- Thus, cortical rotation establishes the dorsal-ventral (back-belly) axis of the zygote.
- In some species, cortical rotation also exposes a light gray region of cytoplasm, the **gray crescent**, that had previously been covered by the pigmented animal cortex near the equator of the egg.
  - Located on the side opposite sperm entry, the gray crescent can be used as a marker for the future dorsal side of the embryo. The lighter pigment of the gray crescent persists through many rounds of cell division.
- In frogs, the first two cleavages are vertical and result in four blastomeres of equal size, each extending from the animal pole to the vegetal pole.
- The third division is equatorial (horizontal), producing an eight-celled embryo with two tiers of four cells.
  - The unequal division of yolk displaces the mitotic apparatus and cytokinesis toward the animal end of the dividing cells in equatorial divisions.
  - As a result, the blastomeres in the animal hemisphere are smaller than the blastomeres in the vegetal hemisphere.
- Continued cleavage produces a blastula. Because of unequal cell division, the blastocoel is located in the animal hemisphere.
- Animals with less yolk (such as the sea urchin) also have an animal-vegetal axis.
  - The blastomeres are similar in size, however.
- In animals whose eggs contain relatively little yolk, the blastocoel is centrally located, and the cleavage furrow passes all the way through the cells, a pattern called **holoblastic cleavage**.
- Yolk has its most pronounced effect on cleavage in the eggs of birds, other reptiles, many fishes, and insects.
  - In these species, 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 division of a yolk-rich egg is known as **meroblastic cleavage**.
- The yolk of a chicken egg is actually an egg cell, swollen with yolk nutrients.
- Cleavage of a fertilized bird's egg is restricted to a small disk of yolk-free cytoplasm, while the yolk remains uncleaved.
- Early cleavage in a bird embryo produces a cap of cells that rest on undivided egg yolk.

- The blastomeres sort into upper and lower layers. The cavity between these two layers is the avian version of the blastocoel.
- This embryonic stage is the avian equivalent of the blastula.
- In insects such as *Drosophila*, the zygote's nucleus is located *within* the mass of yolk.
- Cleavage begins with the nucleus undergoing mitotic divisions, unaccompanied by cytokinesis.
- These mitotic divisions produce several hundred nuclei, which migrate to the outer edge of the embryo.
- After several more rounds of mitosis, plasma membranes form around each nucleus, and the embryo, the equivalent of a blastula, consists of a single layer of 6,000 cells surrounding a mass of yolk.

Gastrulation rearranges the blastula to form a three-layered embryo with a primitive gut.

- After cleavage, the rate of cell division slows dramatically.
- **Gastrulation** rearranges the embryo into a three-layered **gastrula** with a primitive digestive tube.
- Gastrulation is driven by the same general mechanisms in all species: changes in cell motility, changes in cell shape, and changes in cellular adhesion to other cells and to molecules of the extracellular matrix.
- The result of gastrulation is that some cells at or near the surface of the blastula move to an interior location, and three cell layers are established.
- The positioning of the three cell layers in the fully developed gastrula allows cells to interact with each other in new ways, which leads to the generation of the body's organs.
- The embryonic **germ layers** are the **ectoderm,** the outer layer of the gastrula; the **mesoderm,** which partly fills the space between the ectoderm and the endoderm; and the **endoderm,** which lines the embryonic gut.
- Sea urchin gastrulation begins at the vegetal pole, where individual cells detach from the blastula wall and enter the blastocoel as migratory *mesenchyme cells.*
- The remaining cells flatten to form a *vegetal plate* that buckles inward in a process called **invagination**.
- The buckled vegetal plate undergoes extensive rearrangement of its cells, transforming the shallow invagination into a primitive gut, or **archenteron**.
  - The open end, the **blastopore**, will become the anus.
  - An opening at the other end of the archenteron will form the mouth of the digestive tube.
- The three-layered body plan produced by gastrulation is characteristic of most animal phyla and is established very early in development.
  - In the sea urchin, the gastrula eventually develops into a ciliated larva that drifts in ocean surface waters as plankton, feeding on bacteria and unicellular algae.
  - After some time, the larva metamorphoses into the adult form of the sea urchin, which lives on the bottom of the ocean.
- Frog gastrulation also produces a three-layered embryo with an archenteron.
  - Gastrulation begins on the dorsal side of the blastula, when a group of cells begins to invaginate, or change shape and push inward, forming a crease along the region where the gray crescent formed in the zygote.
  - The part above this crease becomes the dorsal side of the blastopore, called the **dorsal lip**.
  - The blastopore extends at each end of the crease as new cells push inward.

- ○ Finally, the two ends of the blastopore meet on the ventral side to form a complete circle.
- During frog gastrulation, future endoderm and mesoderm cell layers on the surface of the embryo roll over the edge of the dorsal lip and 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 endoderm on the inside.
- As the process is completed, the lip of the blastopore encircles a **yolk plug**.
- As in the sea urchin, the amphibian anus develops from the blastopore, and the mouth eventually breaks through at the opposite end of the archenteron.
- Gastrulation in the chick is similar to frog gastrulation: It involves cells moving from the surface of the embryo to an interior location.
  - ○ In birds, the inward movement of cells is affected by the large mass of yolk.
  - ○ Cleavage in the chick results in a stage equivalent to the blastula. This stage, called the *blastoderm*, consists of upper and lower layers—the *epiblast* and *hypoblast*—lying on top of the 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, and then they detach and move inward toward the yolk.
- These cells produce a thickening called the **primitive streak**, which runs along what will become the bird's anterior-posterior axis.
  - ○ The primitive steak is the functional equivalent of the frog blastopore.
  - ○ Some of the inward-moving epiblast cells displace hypoblast cells and form the endoderm.
  - ○ Other epiblast cells move laterally into the blastocoel, forming the mesoderm.
  - ○ The epiblast cells that remain on the surface form ectoderm.
- The hypoblast is required for normal development and seems to help direct the formation of the primitive streak.
  - ○ Some hypoblast cells later form portions of the yolk sac.

In organogenesis, the organs of the animal body form from the three embryonic germ layers.

- Various regions of the three embryonic germ layers develop into the rudiments of organs during the process of **organogenesis**.
- Whereas gastrulation involves mass cell movements, organogenesis involves more localized morphogenetic changes in tissue and cell shape.
- The first evidence of organ building is the appearance of folds, splits, and dense clustering (condensation) of cells.
- The first organs to form in the frog are the neural tube and the **notochord**.
  - ○ The notochord is formed from dorsal mesoderm that condenses above the archenteron.
- Signals sent from the mesoderm and other tissues to the overlying ectoderm cause the ectoderm above the notochord to become the *neural plate*.
- The neural plate curves inward, rolling itself into a **neural tube** that runs along the anterior-posterior axis of the embryo.
- The neural tube becomes the central nervous system—the brain and spinal cord.

- The process often seen in organogenesis is one germ layer signaling another, thereby affecting gene expression and determining the fate of the second layer.
- Unique to vertebrate embryos is a band of cells called the **neural crest**, which develops along the border where the neural tube pinches off from the ectoderm.
  - Neural crest cells migrate throughout the embryo, forming peripheral nerves, parts of teeth, skull bones, and so many other different cell types that some developmental biologists have proposed considering the neural crest as a fourth germ layer.
- **Somites** form in strips of mesoderm lateral to the notochord.
  - The somites are arranged serially on both sides along the length of the notochord.
- Mesenchyme cells migrate from the somites to new locations.
- Some mesenchyme cells gather around the notochord and form the vertebrae.
  - Parts of the notochord persist into adulthood as the inner portions of vertebral disks.
- Somite cells also form the muscles associated with the axial skeleton.
- Lateral to the somites, the mesoderm splits into two layers that form the lining of the coelom.
- As organogenesis progresses, morphogenesis and cell differentiation refine the organs that form from the three germ layers.
- Embryonic development leads to an aquatic, herbivorous tadpole larva, which later metamorphoses into a terrestrial, carnivorous adult frog.
- Organogenesis in the chick is similar to the process in the frog.
  - After the three germ layers are formed, 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.
  - Neural tube formation, development of the notochord and somites, and other events in organogenesis occur much as in the frog embryo.
  - The rudiments of the major organs are already formed in a 2- to 3-day-old chick embryo.
- In invertebrates, organogenesis is a somewhat different process, although the underlying mechanisms involve many of the same cellular activities: cell migration, cell condensations, cell signaling between different tissues, and cell shape changes generating new organs.
- In flies and other insects, tissues of the nervous system form when ectoderm along the anterior-posterior axis rolls into a tube that then lies under the exterior ectoderm.
  - The tube is on the ventral side of the fly embryo rather than the dorsal side, however, as in vertebrates.
  - In spite of the differences, the molecular signaling pathways that bring about the events in the two groups are very similar, underscoring their ancient shared evolutionary history.
- Specifying the location of the three germ layers in a gastrula is quite straightforward, but during organogenesis, the layers move and change shape, defying generalization.
- The germ layers produce the major organs and tissues in frogs, chicks, and other vertebrates.
- The derivatives of the ectoderm germ layer include the epidermis of skin and its derivatives, the epithelial lining of the mouth and rectum, the cornea and lens of the eyes, the nervous system, the adrenal medulla, tooth enamel, and the epithelium of the pineal and pituitary glands.
- The endoderm germ layer contributes to the epithelial linings of the digestive tract (except the mouth and rectum), respiratory system, pancreas, thyroid, parathyroids, thymus, urethra, urinary bladder, and reproductive system.

- Derivatives of the mesoderm germ layer are the notochord, the skeletal and muscular systems, the circulatory and lymphatic systems, the excretory system, the reproductive system (except germ cells), the dermis of skin, the lining of the body cavity, and the adrenal cortex.

Amniote embryos develop in a fluid-filled sac within a shell or uterus.

- The **amniote** embryo is the solution to reproduction in a dry environment.
- The shelled eggs of birds and other reptiles, as well as monotreme mammals, and the uterus of marsupial and eutherian (placental) mammals are aqueous environments for development.
  - Inside the shell or uterus, the embryos of these animals are surrounded by fluid within a sac formed by a membrane called the amnion.
  - Reptiles (including birds) and mammals are thus **amniotes**.
- Amniote development includes the formation of four **extraembryonic membranes**: yolk sac, amnion, chorion, and allantois.
  - In amniotes, only part of each germ layer contributes to the embryo itself.
  - The other parts of the germ layers develop into the four extraembryonic membranes, each a sheet of cells derived from two germ layers.
- In the chick, the **chorion** functions in gas exchange.
- The cells of the **yolk sac** digest yolk, providing nutrients to the embryo.
- The **amnion** encloses the embryo in a fluid-filled amniotic sac that protects the embryo from drying out.
- The **allantois** disposes of waste products and functions with the chorion as a respiratory organ.
- These four extraembryonic membranes provide a "life-support system" for further embryonic development within the shelled egg or the uterus of an amniote.

Mammalian development has some unique features.

- The eggs of most mammals are very small and store little food.
- In most mammalian species, fertilization takes place in the oviduct, and the earliest stages of development occur while the embryo is completing its journey down the oviduct to the uterus.
- The mammalian egg and zygote do not exhibit obvious polarity with respect to cytoplasmic contents, and cleavage of the zygote, which lacks yolk, is holoblastic.
  - Despite the lack of yolk, mammalian gastrulation and early organogenesis follow a pattern similar to that of birds and other reptiles.
- Early cleavage is relatively slow in mammals.
  - In humans, the first division is complete after 36 hours, the second division after 60 hours, and the third division after 72 hours.
  - Relatively slow cleavage produces equal-sized blastomeres.
- At the eight-cell stage, the blastomeres become tightly adhered to one another, causing the outer surface to appear smooth.
- At the completion of cleavage, the embryo has more than 100 cells arranged around a central cavity.
- The **blastocyst** travels down the oviduct to reach the uterus.
  - Clustered at one end of the blastocyst is a group of cells called the **inner cell mass** that develops into the embryo and forms or contributes to all the extraembryonic membranes.

- ○ It is the cells of the very early blastocyst stage that are the source of embryonic stem cell lines.
- The **trophoblast,** the outer epithelium of the blastocyst, does not contribute to the embryo.
- The trophoblast initiates implantation by secreting enzymes that break down the endometrium to allow invasion by the blastocyst.
- The trophoblast thickens, pushing fingerlike projections into the surrounding maternal tissue, which is rich in blood vessels.
- Invasion by the trophoblast leads to erosion of the capillaries in the surrounding endometrium, causing the blood to spill out and bathe trophoblast tissue.
- At the time of implantation, the inner cell mass 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 the epiblast.
- As implantation is completed, gastrulation begins.
- Cells move inward from the epiblast through the primitive streak to form mesoderm and endoderm.
- At the same time, extraembryonic membranes develop.
- The trophoblast continues to expand into the endometrium.
- The invading trophoblast, mesodermal cells derived from the epiblast, and adjacent endometrial tissue all contribute to the formation of the placenta.
  - ○ The placenta is a vital organ that mediates the exchange of nutrients, gases, and the products of excretion between the embryo and the mother.
  - ○ The placenta also produces hormones and protects the embryo from a maternal immune response.
- By the end of gastrulation, the embryonic germ layers have formed.
- The three-layered embryo is now surrounded by proliferating extraembryonic mesoderm and the four extraembryonic membranes.
- The embryonic membranes of mammals are homologous with those of birds and other reptiles.
  - ○ Gas exchange occurs across the chorion.
  - ○ The amnion cushions the embryo in a fluid-filled amniotic cavity.
  - ○ The yolk sac encloses another fluid-filled cavity, which contains no yolk. The yolk sac membrane of mammals is the site of the early formation of blood cells, which later migrate to the embryo.
  - ○ The allantois in mammals is incorporated into the umbilical cord, where 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 extraembryonic membranes of reptiles, where embryos are nourished with yolk, were conserved as mammals diverged in the course of evolution but with modifications adapted to development within the reproductive tract of the mother.
- Identical or monozygotic twins can arise when embryonic cells become separated.
- The timing of the separation determines the nature of the twins' arrangement in the uterus with regard to their extraembryonic membranes.

- ○ If the separation occurs before the trophoblast and the inner cell mass become differentiated, then two embryos will grow, each with its own chorion and amnion. This is true of about a third of twin births.
- ○ In most of the remaining twins, the separation occurs after the chorion forms but before amnion formation. The two embryos that develop share a chorion but have separate amnions.
- ○ In very rare cases, two groups of cells become separated even later, and the two embryos share a common chorion and amnion.

Concept 47.2 Morphogenesis in animals involves specific changes in cell shape, position, and adhesion.

- Morphogenesis is a major aspect of development in plants and animals, but only in animals does it involve *cell movement.*
- Movement of parts of a cell can bring about changes in cell shape and can also enable a cell to migrate from one place to another within the embryo.
- Changes in cell shape and cell position are involved in cleavage, gastrulation, and organogenesis.
- Changes in the shape of a cell usually involve the reorganization of the cytoskeleton.
- Consider how the cells of the neural plate form the neural tube.
  - ○ First, the microtubules oriented parallel to the dorsal-ventral axis of the embryo help to lengthen the cells in that direction.
  - ○ At the dorsal end of each cell is a parallel array of microfilaments oriented crosswise.
  - ○ These microfilaments contract, giving the cells a wedge shape that bends the ectoderm inward.
  - ○ Similar changes in cell shape occur during other invaginations and evaginations of tissue layers throughout development.
- The cytoskeleton also drives cell migration.
  - ○ Cells "crawl" within the embryo by extending cytoplasmic fibers to form cellular protrusions, in a manner akin to amoeboid movement.
  - ○ The cellular protrusions of migrating embryonic cells are usually flat sheets (lamellipodia) or spikes (filopodia).
- During gastrulation, invagination is initiated by the wedging of cells from the surface of the blastula, but the movement of cells deeper into the embryo involves the extension of filopodia by cells at the leading edge of the migrating tissue.
- The cells that first move through the blastopore and along the inside of the blastocoel drag others along behind them as a sheet of cells.
- This involuted sheet of cells forms the endoderm and mesoderm of the embryo.
- Many cells migrate individually, as when the cells of a somite or the neural crest disperse to various parts of the embryo.
  - ○ In many species, the mesoderm moves into the embryo as individual cells during gastrulation.
- Cell crawling is also involved in **convergent extension**, a type of morphogenetic movement in which the cells of a tissue layer rearrange themselves so the sheet converges and extends, becoming narrower but longer.

- In the embryo, the cells become elongated, with their ends pointing in the direction they will move, and they wedge between each other into fewer columns of cells.
- Convergent extension allows the archenteron to elongate in the sea urchin and frog and is responsible for the change in the shape of a frog embryo from spherical to submarine-like.

- A group of glycoproteins called **cell adhesion molecules (CAMs)** contribute to cell migration and stable tissue structure.
  - CAMs are located on cell surfaces and bind to CAMs on other cells.
  - CAMs vary in amount and chemical identity with cell type.
  - These differences help to regulate morphogenetic movement and tissue binding.

- One important class of CAMs is the **cadherins**, which require the presence of calcium for proper function.
  - There are many cadherins, and the gene for each cadherin is expressed in specific locations at specific times during embryonic development.
  - Cadherins are involved in the tight adhesion of cells in the mammalian embryo that first occurs at the eight-cell stage, when cadherin production begins.

- The processes of cell migration and tissue organization also involve the extracellular matrix (ECM), a meshwork of secreted glycoproteins lying outside the plasma membrane.
  - The ECM helps to guide cells in many types of morphogenetic movements, such as migration of individual cells or shape changes of cell sheets.
  - On the cells that undergo movement, membrane proteins act as receptors that bind specific ECM molecules.
  - ECM fibers may direct cell movement by functioning as tracks, directing migrating cells along particular routes.
  - Other ECM substances may *inhibit* migration in certain directions.
  - Cells along migration pathways may promote or inhibit the movement of other cells, depending on the specific molecules they secrete into the ECM.

- Several kinds of extracellular glycoproteins, including fibronectin, promote cell migration by providing anchorage for crawling cells.
  - Fibronectin provides anchorage at the leading edge of the involuting tissue during frog gastrulation.
  - Fibronectin fibers line the roof of the blastocoel.
  - As the future mesoderm moves into the interior of the embryo, cells at the free edge of the mesodermal sheet migrate along these fibers.
  - Researchers can prevent the attachment of cells to fibronectin (and prevent the crawling movement of mesoderm) by injecting embryos with antifibronectin antibodies or fibronectin receptors.

- As migrating cells move along specific paths through the embryo, receptor proteins on their surfaces pick up directional cues from the immediate environment.
  - Such signals, which may be ECM molecules or small soluble factors, can direct the orientation of cytoskeletal elements to propel the cell in the proper direction.
  - These signals may also affect the expression or function of other proteins involved in the migration.

- Mungo Marsden and Doug DeSimone investigated whether cell-ECM and cell-cell binding systems affected each other during convergent extension.

- Their experiments support a model in which fibronectin binding to its receptor provides a molecular signal to the cell that ultimately affects the function of cadherins.
- These two classes of molecules are linked in a single pathway driving convergent extension.

Concept 47.3 The developmental fate of cells depends on their history and on inductive signals.

- Development requires the timely differentiation of cells in specific locations.
- The principle of genomic equivalence states that each cell in an organism has the same genome.
- Different cell types make different sets of proteins because they end up expressing different sets of genes.
- Therefore, during differentiation, some mechanism must send cells down different pathways of gene expression.
- Two general principles integrate the current understanding of the genetic and cellular mechanisms that underlie differentiation during embryonic development.
- First, *during early cleavage divisions, embryonic cells must somehow become different from one another.*
- In many animal species, initial differences result from uneven partitioning of cytoplasmic determinants (mRNAs, proteins, and other molecules) to blastomeres during cleavage.
  - These cytoplasmic determinants may function as transcription factors, DNA-binding proteins that activate one set of genes rather than another.
  - The resulting differences in the cytoplasmic composition of cells help specify body axes and influence the expression of genes that affect the developmental fate of cells.
- In amniotes, local environmental differences play the major role in establishing early differences between embryonic cells.
  - For example, the cells of the inner cell mass are internal in the early human embryo, whereas trophoblast cells are on the outer surface of the blastocyst.
  - The difference in cell environment determines the fate of these cells.
- Second, *once initial cell asymmetries are set up, subsequent interactions among the embryonic cells influence their fate, usually by causing changes in gene expression.*
- This mechanism, **induction**, brings about the differentiation of many specialized cell types.
  - Induction may be mediated by diffusible chemical signals or by cell-surface interactions between cells in contact.

Fate mapping can reveal cell genealogies in chordate embryos.

- **Fate maps** illustrate the developmental history of cells.
- In classic experiments in the 1920s, German embryologist Walter Vogt charted fate maps for different regions of early amphibian embryos.
- Vogt's work provided evidence that the lineage of cells making up the three germ layers created by gastrulation is traceable to cells in the blastula, before gastrulation begins.
- Careful microscopic observations during the entire period of development, coupled with experiments in which particular cells or groups of cells were destroyed by a laser beam or through mutations, resulted in determination of the complete cell lineage of *C. elegans,* a soil-dwelling nematode.

- *C. elegans* is about 1 mm long, has a simple transparent body with only a few types of cells, and grows from zygote to mature adult in only three and a half days.
- Most individuals are hermaphrodites, producing both eggs and sperm, which has advantages for genetic studies.
- Every adult hermaphrodite has exactly 959 somatic cells, which arise from the zygote in virtually the same way for every individual.

- In 2002, Sydney Brenner, Robert Horvitz, and Jonathan Sulston shared a Nobel Prize for their notable accomplishment, which laid the groundwork for *C. elegans* to become a model organism of choice among many developmental biologists.

- Developmental biologists have combined fate-mapping studies with experimental manipulation of parts of embryos to ascertain whether a cell's fate can be changed.

- Two important conclusions have emerged.
  1. In most animals, specific tissues of the older embryo are the products of certain early "founder cells" that contain unique factors as the result of asymmetrical cell divisions.
  2. As development proceeds, a cell's *developmental potential*—the range of structures that it can give rise to—becomes restricted.

The eggs of most vertebrates have cytoplasmic determinants that help establish the body axes.

- A bilaterally symmetrical animal has an anterior-posterior axis, a dorsal-ventral axis, and left and right sides.
- Establishing this basic body plan is a first step in morphogenesis and a prerequisite for the development of tissues and organs.
- In nonamniotic vertebrates, basic instructions for establishing the body axes are set down early, during oogenesis or fertilization.
  - In many frogs, the locations of melanin and yolk define the animal and vegetal hemispheres, respectively.
  - The animal-vegetal axis indirectly determines the anterior-posterior body axis.
  - Fertilization in frogs triggers cortical rotation, which establishes the dorsal-ventral axis and leads to the appearance of the gray crescent, whose position marks the dorsal side.
  - Once any two axes are established, the third (right-left) is specified by default.
  - Molecular mechanisms then carry out the program associated with that axis.

- In amniotes, body axes are not fully established until later.
  - In chicks, gravity is involved in establishing the anterior-posterior axis as the egg travels down the oviduct before being laid.
  - Later, pH differences between the two sides of the blastoderm establish the dorsal-ventral axis.

- In mammals, no polarity is obvious until after cleavage, although recent research suggests that the orientation of the egg and sperm nuclei before fusion may play a role in determining the axes because this orientation determines where the first cleavage plane will occur.

The developmental potential of embryonic cells becomes increasingly restricted.

- In many species with cytoplasmic determinants, only the zygote is **totipotent**, capable of developing into all cell types found in the adult.
  - The fate of embryonic cells is affected by both the distribution of cytoplasmic determinants and the cleavage pattern.

- In frogs, the first cleavage occurs along an axis that produces two identical blastomeres with identical developmental potential.

- The cells of the mammalian embryo remain totipotent until the 16-cell stage, when they become arranged into the precursors of the trophoblast and the inner cell mass of the blastocyst. At that time, location determines cell fate.
  - At the 8-cell stage, each of the blastomeres of the mammalian embryo can form a complete embryo if isolated.
  - This was taken as evidence that the early blastomeres of mammals receive equivalent cytoplasmic components from the egg.
  - Recent work, however, suggests that the very early cells (even the first two) are not actually equivalent in a normal embryo, and their ability to form a complete embryo if isolated shows how able they may be to regulate their fate, depending on their environment.
  - The jury is still out on this matter, which is an area of intense interest to researchers.

- The progressive restriction of potency is a general feature of development in animals.
  - In some species, the cells of the early gastrula retain the capacity to give rise to more than one kind of cell, although they are no longer totipotent. If transplanted, the cells respond to their new location and develop appropriately.
  - In general, the tissue-specific fates of cells in the late gastrula are fixed.
  - Even if manipulated experimentally, late gastrula cells give rise to the same type of cells they would have become in their original position.

Inductive signals play an important role in cell fate determination and pattern formation.

- Once embryonic cell division creates cells that are different from one another, the cells begin to influence each other's fates by induction.

- At the molecular level, the effect of induction is usually the switching on of a set of genes that make the receiving cells differentiate into a specific tissue.

- In the 1920s, Hans Spemann and Hilde Mangold carried out transplantation experiments that showed that the dorsal lip of the blastopore in an early gastrula serves as an "organizer" of the embryo's body plan by initiating a chain of inductions that results in the formation of the notochord, neural tube, and other organs.

- Developmental biologists are working to identify the molecular basis of induction by *Spemann's organizer* (also called the *gastrula organizer* or simply the *organizer*).

- A high concentration of a growth factor called bone morphogenetic protein 4 (BMP-4) is present on the ventral side of the gastrula.

- BMP-4 induces those cells to form ventral structures.

- Organizer cells inactivate BMP-4 on the dorsal side of the embryo by producing proteins that bind to BMP-4, rendering it unable to signal.

- The inactivation of BMP-4 promotes the formation of dorsal structures such as the notochord and neural tube.

- In tissues between the dorsal and ventral sides, a lower concentration of BMP-4 results in lateral structures appropriate for the specific location along the dorsal-ventral axis.
  - The varying concentration of BMP-4 along this axis is an example of a morphogen gradient.

- Proteins related to BMP-4 and its inhibitors are found in other animals, including the fruit fly, suggesting that they evolved long ago and may participate in development in many different organisms.

- Many inductions involve a sequence of inductive steps that progressively determine the fate of cells.
  - In late gastrula of the frog, ectoderm cells destined to form the lenses of the eyes receive precisely timed inductive signals from ectodermal, mesodermal, and endodermal cells.
- Inductive signals play a major role in **pattern formation**, the development of an animal's spatial information, the arrangement of organs and tissues in three-dimensional space.
- **Positional information**, supplied by molecular cues, tells a cell where it is relative to the animal's body axes.
- Limb development in chicks serves as a model of pattern formation.
  - The wings and legs of chicks, like all vertebrate limbs, begin as bumps of tissue called limb buds.
  - A limb bud consists of a core of mesodermal tissue covered by a layer of ectoderm.
  - Each component of a chick limb develops with a precise location and orientation relative to three axes: the proximal-distal axis (shoulder to fingertip), the anterior-posterior axis (thumb to little finger), and the dorsal-ventral axis (knuckle to palm).
- Two critical organizer regions are present in all vertebrate limb buds.
- The cells of these regions secrete proteins that provide key positional information to the other cells of the bud.
- One limb-bud organizer region is the **apical ectodermal ridge (AER)**, a thickened area of ectoderm at the tip of the bud.
  - The AER is required for the outgrowth of the limb along the proximal-distal axis and for patterning along this axis.
  - The cells of the AER produce several secreted protein signals belonging to the fibroblast growth factor (FGF) family. These signals promote limb-bud outgrowth.
  - If the AER is surgically removed and beads soaked in 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 the median fin in a shark.
  - This finding suggests that the AER may have predated the appearance of paired limbs in the vertebrate lineage, giving the AER a longer evolutionary history than previously thought.
- The second major limb-bud organizer 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.
  - The ZPA is necessary for proper pattern formation along the anterior-posterior axis of the limb.
  - Cells nearest the ZPA give rise to posterior structures (such as our little finger); cells farthest from the ZPA form anterior structures (such as our thumb).
  - Tissue transplantation experiments support the hypothesis that the ZPA produces an inductive signal that conveys positional information indicating "posterior."
  - The cells of the ZPA secrete a protein growth factor called Sonic hedgehog.
  - If cells genetically engineered to produce large amounts of Sonic hedgehog are implanted into the anterior region of a normal limb bud, a mirror-image limb bud results.
  - Extra toes and fingers in mice (and maybe humans) result from the production of Sonic hedgehog in the wrong part of the limb bud.

- *Hox* genes also play different roles at several distinct points during limb pattern formation.
    - A human condition called polysyndactyly ("many fingers joined together") is caused by a specific mutation in the *Hox* gene *HoxD13*.
- These experiments lead to the conclusion that pattern formation requires cells to receive and interpret environmental cues that vary with location.
    - These environmental cues tell cells where they are in the three-dimensional realm of a developing organ.
    - Organizers such as the AER and the ZPA function as signaling centers.
    - The AER and the ZPA also interact with each other via signaling molecules and signaling pathways that influence each other's developmental fates.
    - Mutual signaling interactions between the mesoderm and ectoderm also occur during the formation of the neural tube and many other tissues and organs.
- What determines whether a limb bud develops into a forelimb or a hindlimb?
    - The cells that receive signals from the AER and the ZPA respond according to their own developmental histories.
    - Earlier developmental signals have set up patterns of *Hox* gene expression that distinguish future forelimbs from future hindlimbs.
- Construction of a fully formed animal involves a sequence of events that includes many steps of signaling and differentiation.
    - Initial cell asymmetries allow different types of cells to influence each other to express specific sets of genes.
    - The products of these genes direct cells to differentiate into specific types.
    - Coordinated with morphogenesis, various pathways of pattern formation occur in all the different parts of the developing embryo.
- These processes produce a complex arrangement of multiple tissues and organs, each functioning in the appropriate location, coordinated with the others, to form a whole organism.