Chapter 47

Animal Development

Lecture Outline

Overview: A Body-Building Plan

- Embryonic development of most animals involves common stages that occur in a set order: fertilization, cleavage, gastrulation, and organogenesis.
- As an embryo develops, specific patterns of gene expression direct cells to adopt distinct fates.
- Although animals display widely differing body plans, they share many basic mechanisms of development and use a common set of regulatory genes.
  - The gene that specifies heart location in a human embryo has a close counterpart with a nearly identical function in the fruit fly, *Drosophila*.
- Biologists study development in model organisms, species chosen for the ease with which they can be studied in the laboratory.
  - The sea urchin, the frog, the chick, and the nematode have been important model organisms for the study of development.

Concept 47.1 Fertilization and cleavage initiate embryonic development

- Molecules and events at the egg surface play a crucial role in each step of fertilization.
  - Sperm dissolve or penetrate protective layers surrounding the egg to reach the plasma membrane.
  - Receptors on the egg surface bind to complementary molecules on the sperm surface, guaranteeing that sperm of the same species fertilize the egg.
  - Changes at the surface of the egg prevent *polyspermy*, the entry of multiple sperm nuclei into the egg that leads to abnormal number of chromosomes in the embryo.
- Fertilization is studied most extensively in sea urchins.

Contact between egg and sperm triggers the acrosomal reaction in the sperm.

- The jelly coat surrounding the egg exudes soluble molecules that attract the sperm, which swim toward the egg.
- When 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.
  - Hydrolytic enzymes discharged from the **acrosome**, a specialized vesicle at the tip of the sperm, partially digest the jelly coat.
  - This enables an *acrosomal process* to elongate and penetrate the jelly 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 guarantees that sperm and egg are from one 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 enters the egg 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, within about 1–3 seconds.
- By preventing additional sperm from fusing with the egg's plasma membrane, this depolarization acts as a **fast block to polyspermy**.

The cortical reaction forms a protective fertilization envelope.

- Vesicles called **cortical granules** lie beneath the egg plasma membrane in an area cytoplasm called the *cortex*.
  - Within seconds of sperm binding, these vesicles fuse with the egg plasma membrane and release their contents into the space between the plasma membrane and a surrounding *vitelline layer* formed by the extracellular matrix of the egg.
- Enzymes 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**.
  - The remaining receptor proteins and any attached sperm are released as the envelope forms.
  - Together, the fertilization envelope 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?
  - 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.
  - Ca^{2+} spreads across the egg in a wave that correlates with the appearance of the fertilization envelope.
- 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 in sea urchins and in vertebrates such as fishes and mammals.

Fertilization leads to activation of the egg.

- Fertilization initiates metabolic reactions that trigger the onset of embryonic development, thus "activating" the egg.
  - Rates of cellular respiration and protein synthesis increase markedly in the egg following fertilization.
- What triggers egg activation?
  - Injecting Ca^{2+} into an unfertilized egg activates egg metabolism in many species, despite the absence of sperm.
  - Researchers conclude that the rise in Ca^{2+} concentration that causes the cortical reaction also causes egg activation.
- Further experiments show that artificial activation is possible even if the nucleus has been removed from the egg.

- Egg activation requires only the proteins and mRNAs already present in the egg cytoplasm.

- 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, although the timing of events may differ.
  - Sea urchin eggs have already completed meiosis when they are released from the female.
  - Human eggs, however, are arrested at metaphase of meiosis II prior to fertilization.

Fertilization in mammals is internal.

- Terrestrial animals, including mammals, fertilize eggs internally.
- Secretions in the mammalian female reproductive tract provide a moist environment for the sperm, and alter 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.
- Follicle cells surround the mammalian egg and remain with it during and after ovulation.
  - A sperm must penetrate this layer to reach the **zona pellucida**, the extracellular matrix of the egg.
- The zona pellucida contains a sperm receptor.
  - Binding of a sperm to this receptor induces an acrosomal reaction, facilitating sperm passage through the zona pellucida to the egg and exposing a protein on the sperm that binds with the egg plasma membrane.
  - At this point, the two cells fuse.
- Sperm binding triggers changes in 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.
- In mammalian fertilization, the whole sperm is taken into the egg.
- The envelopes of both haploid nuclei disperse and the sperm and egg chromosomes are organized onto a single mitotic spindle.
  - A true diploid nucleus with a nuclear membrane forms only after the first division.
- 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.

Cleavage is the next developmental stage.

- A succession of rapid cell divisions characterizes the **cleavage** stage of early development.
- During cleavage, the cell cycle consists primarily of the S (DNA synthesis) and M (mitosis) phases.
  - Cells skip the G_1 and G_2 (gap) phases, and little or no protein synthesis occurs.

- Cleavage partitions the cytoplasm of a large fertilized egg into many smaller cells or **blastomeres**.
  - The first five to seven cleavage divisions produce a hollow ball of cells, the **blastula**, surrounding a fluid-filled cavity called the **blastocoel**.
- The distribution of **yolk** is a key factor influencing the pattern of cleavage in many animals.
  - Yolk is often concentrated toward one pole of the egg, called the **vegetal pole**, and decreases significantly toward the opposite pole, the **animal pole**.
  - This difference in yolk distribution results in animal and vegetal hemispheres that differ in appearance.
- During cell division, an indentation or groove called a *cleavage furrow* forms in the cell surface as cytokinesis divides the cell in half.
- The first two cleavage furrows in the frog lie parallel to the meridian connecting the two poles.
- Near the vegetal pole, the presence of yolk slows the progress of the cleavage furrow.
  - As a result, the second cell division begins before the first cleavage furrow reaches the vegetal pole.
- 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, yolk distribution affects not only cleavage furrow movement but also the relative size of cells produced.
  - This division is equatorial (perpendicular to the line connecting the poles) and produces an eight-celled embryo.
  - During this division, the high concentration of yolk around the vegetal pole displaces the mitotic apparatus toward the animal pole and also displaces the cleavage furrow from the egg equator toward the animal pole.
- The result is the formation of smaller blastomeres in the animal hemisphere than in the vegetal hemisphere.
  - In frogs, these unequal cell divisions cause the blastocoel to form entirely in the animal hemisphere.
- Cleavage in amphibian development is **holoblastic**, because the cleavage furrow passes entirely through the egg.
  - Cleavage in echinoderms, mammals, and annelids is also holoblastic.
  - In animals with little yolk, the blastocoel forms centrally and the blastomeres are of similar size, particularly during the first few divisions.
  - This is the case for humans, whose embryos complete three divisions in the first three 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.
  - The incomplete cleavage of a yolk-rich egg is said to be **meroblastic.**
- In birds, the yolk is the entire egg cell, swollen with yolk nutrients. Cell divisions are limited to a small 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 blastocoel.

- In the eggs of *Drosophila* and other insects, the sperm and egg nuclei fuse *within* a mass of yolk.
  - Multiple rounds of mitosis occur without cytokinesis.
  - 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.
- The number of cleavage divisions varies among animals but is controlled by a shared mechanism.
  - Experimental results suggest that an animal embryo finishes the cleavage stage when the nucleus: cytoplasm ratio is sufficiently large.
  - Researchers changed the starting amount of cytoplasm and then counted the cleavage divisions that occurred.
  - 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 messenger RNA required to meet the cell's need for new proteins.
  - 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 further development.

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

- Cleavage is followed by **gastrulation**, in which 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.
  - These two stages of embryonic development are responsible for **morphogenesis**, the process by which cells occupy their appropriate locations in the embryo and the animal body takes shape.

Gastrulation reorganizes the hollow blastula and produces embryonic germ layers.

- Gastrulation is a dramatic reorganization of the hollow blastula into a two- or three-layered embryo called a **gastrula**.
- The cell layers produced by gastrulation are collectively called the embryonic **germ layers**.
- In the late gastrula, **ectoderm** forms the outer layer and **endoderm** lines the embryonic digestive tract.

- In cnidarians and a few other radially symmetrical animals, only these two germ layers form during gastrulation. Such animals are called diploblasts.
- In contrast, animals with bilateral symmetry are triploblasts, having a third germ layer, the **mesoderm**, between the ectoderm and the endoderm.
- Although each germ layer contributes to a distinct set of structures in the adult animal, 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, while other endocrine glands contain endodermal tissue.
- Gastrulation in the sea urchin begins at the vegetal pole of the blastula as 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.
    - This process—the infolding of a sheet of cells into the embryo—is called *invagination*.
- The shallow depression develops into a deep, narrow, 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 to produce a rudimentary digestive tube.
- In protostomes, the mouth develops from the first opening that forms in the embryo; in deuterostomes, the mouth forms from the second opening.
    - Echinoderms and chordates are both deuterostomes.
- After completing gastrulation, the sea urchin embryo develops into a ciliated larva that drifts in ocean surface waters as zooplankton, feeding on bacteria and unicellular algae.
    - Eventually, the larva metamorphoses into the adult form of the sea urchin, which lives on the ocean floor.
- A frog blastula has large, yolky cells in the vegetal hemisphere and a blastocoel wall that is more than one cell thick.
    - 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.
- Frog gastrulation begins when a group of cells on the dorsal side of the blastula invaginates, forming a crease along the region where the gray crescent formed.
    - 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, the cells move away from the blastopore toward the animal pole and become organized into layers of endoderm and mesoderm, with endoderm on the inside.
- Cells continue to spread over the gastrula surface, shrinking the blastopore.
- In the interior of the embryo, an archenteron forms and grows as the blastocoel shrinks and then disappears.
- The cells remaining on the surface at the end of gastrulation form the ectoderm.

- 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 breaks through at the opposite end of the archenteron.
- 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 of the embryo come from the epiblast.
- During gastrulation, epiblast cells move toward the midline of the blastoderm, detach, and move inward toward the yolk.
  - The pileup of cells moving inward at the blastoderm's midline produces a thickening called the **primitive streak**.
- The hypoblast contributes no cells to the embryo, but it is required for normal development and helps direct the formation of the primitive streak before the onset of gastrulation.
  - Hypoblast cells later segregate from the endoderm and eventually form portions of a sac that surrounds the yolk and of a stalk that connects the yolk mass to the embryo.
- Human eggs are quite small, storing few 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.
- At the end of cleavage, the embryo has over 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.
  - The cells of the early blastocyst stage are the source of embryonic stem cell lines.
- The **trophoblast**, the outer epithelium of the blastocyst, does not contribute to the embryo but supports embryo growth.
  - It initiates implantation by secreting enzymes that break down molecules of the endometrium, allowing the blastocyst to invade the endometrium.
- As the trophoblast thickens through cell division, it extends fingerlike projections into the surrounding maternal tissue that lead 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.
- Following implantation, the trophoblast expands into the endometrium and the embryo forms four **extraembryonic membranes**.
- 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.
- 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 contribute to formation of the placenta, a vital organ that mediates exchange of nutrients, gases, and nitrogenous wastes between the embryo and the mother.

What is the adaptive significance of the extraembryonic membranes of amniotes?

- Birds and other reptiles, like mammals, form four extraembryonic membranes.
  - These membranes provide a "life-support system" for further embryonic development.
- Why did this adaptation appear only in the evolutionary history of reptiles and mammals?
  - All vertebrate embryos require an aqueous environment for their development.
  - The embryos of fishes and amphibians develop in the surrounding sea or pond and need no specialized water-filled enclosure.
- The extensive colonization of land by vertebrates was possible only after the evolution of structures to 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 the amnion.
  - Mammals and reptiles, including birds, are therefore called **amniotes**.
- 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.
- 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 yolk sac encloses yolk in the eggs of reptiles.
  - In mammals, it is the site of early formation of blood cells.
- The extraembryonic membranes of reptiles were conserved – but modified – in mammals during the course of evolution.

During organogenesis, various regions of the three embryonic germ layers develop into the rudiments of organs.

- The first step of vertebrate organogenesis is *neurulation*, the formation of the brain and spinal cord.
- Neurulation begins as cells from the dorsal mesoderm come together to form the **notochord**, a rod that extends along the dorsal side of chordate embryos.
  - 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.
- The neural plate rolls into the **neural tube**, which runs along the anterior-posterior axis of the embryo.
  - The neural tube becomes the brain in the head and the spinal cord in 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 migrate throughout the embryo, forming a variety of tissues that include peripheral nerves as well as parts of the teeth and skull bones.
- Groups of cells located in strips of mesoderm lateral to the notochord separate into blocks called **somites**.
    - Somites are arranged serially on both sides 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.
    - Mesenchyme cells that leave the somites form the vertebrae.
    - The notochord disappears before birth, but parts persist as the inner portions of the vertebral disks in adults.
    - Somite cells that become mesenchymal later form the muscles associated with the vertebral column and the ribs.
- Serially repeating structures of the embryo (somites) form repeated structures in the adult.
    - Chordates are thus segmented animals, although the segmentation is 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.
- Early organogenesis in other vertebrates is quite similar to that in the frog.
- In the chick, 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.
    - By the time the chick embryo is 2–3 days old, the rudiments of the major organs, including the brain, eyes, and heart, are readily apparent.
- An error in neural tube formation in humans 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.
    - The opening can be surgically repaired shortly after birth, but the nerve damage is permanent and results in varying degrees of paralysis.
- In invertebrates, organogenesis is somewhat different, but the underlying mechanisms involve many of the same cellular activities: cell migration, cell signaling between different tissues, and cell shape changes generating new organs.
    - For example, in insects, tissues of the nervous system form when ectoderm along the anterior-posterior axis rolls into a tube inside the embryo, like the vertebrate neural tube.
    - The tube is on the ventral side of the insect embryo rather than on the dorsal side as 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.

A number of cellular mechanisms contribute to morphogenesis.

- In animals, morphogenesis involves the *movement* of cells.
- Reorganization of the cytoskeleton changes cell shape during development.
- Consider neurulation: At the onset of neural tube formation, microtubules oriented from dorsal to ventral in a sheet of ectodermal cells lengthen the cells along that axis.
    - 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 in hinge regions where the neural tube pinches off from ectoderm.
- In *Drosophila* gastrulation, the formation of wedge-shaped cells along the ventral surface is responsible for invagination of a tube of cells that form the mesoderm.
- In the sea urchin embryo, cytoskeletal changes direct **convergent extension**, a rearrangement of the cells of a tissue layer that causes the sheet to become narrower and longer.
    - 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.
- Convergent extension also causes involution in a frog gastrula, changing the embryo from a spherical shape to a rounded rectangular shape.
- The cytoskeleton is also responsible for cell migration.
- During organogenesis in vertebrates, cells from the neural crest and somites migrate 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.
- 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.
    - 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.

Some cells are programmed to die.

- *Programmed cell death* or **apoptosis** is a common feature of animal development.
    - During development, individual cells, sets of cells, or whole tissues cease to develop and are engulfed by neighboring cells.
- In some cases, a structure has a function in a larval form but is eliminated during later development.
    - One familiar example is the cells in the tail of a tadpole, which undergo apoptosis during frog metamorphosis.
- Programmed cell death can also occur when cells compete with one another for survival.

- Many more neurons are produced during development of the vertebrate nervous system than exist in the adult.
- Neurons survive if they make functional connections with other neurons and die if they do not.

- Some cells that undergo programmed cell death appear to lack any function in the developing embryo. Why do such cells form?
  - Differences in present-day vertebrates arose through modification of a common developmental program.
  - 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.

Concept 47.3 The developmental fate of cells depends on maternal factors and inductive signals.

- Developmental biologists use the term **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 do cells acquire different fates?
  - 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 differences in gene expression underlying developmental fates.

Fate maps show the organs and other structures that arise from each region of a developing embryo.

- **Fate maps** are diagrams showing which organs and other structures arise 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.
  - Later researchers developed techniques that allowed them to mark an individual blastomere during cleavage and then follow the marker as it is distributed to all the mitotic descendants of that cell.
- A comprehensive fate map has been developed for the soil-dwelling nematode *Caenorhabditis elegans*.
  - This roundworm is 1 mm long with a simple, transparent body with only a few types of cells. It develops into a mature adult hermaphrodite in only $3\frac{1}{2}$ days in the laboratory.
- Sydney Brenner, Robert Horvitz, and John Sulston determined the complete cell lineage of *C. elegans* by 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.

- Every adult hermaphrodite has exactly 959 somatic cells, which arise from the fertilized egg in virtually the same way for every individual.

- *Germ cells* are 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*, complexes called *P granules* persist throughout development and can be detected in the germ cells of the adult gonad.
  - P granules are distributed throughout the newly fertilized egg but move to the posterior end of the zygote before the first cleavage division.
  - As a result, only the posterior of the two cells formed by the first division contains P granules.
  - The P granules, which continue to be asymmetrically partitioned during subsequent divisions, act as cytoplasmic determinants and fix germ cell fate at the earliest stage of *C. elegans* development.
- 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.
  - 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.
- A body plan with bilateral symmetry is found across a range of animals, including nematodes, echinoderms, and vertebrates.
  - This body plan exhibits asymmetry along the dorsal-ventral and anterior-posterior axes.
  - The right-left axis is largely symmetrical, as 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 that the two axes are not coincident; 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*. This rotation is always toward the point of sperm entry.
- 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 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, and the side facing the yolk will become the dorsal part.
- In mammals, no polarity is obvious until after cleavage.
  - Recent experiments suggest that the orientation of the egg and sperm nuclei before they fuse influences the location of the first cleavage plane and may play a role in establishing the embryonic axes.
- In insects, morphogen gradients establish both the anterior-posterior and dorsal-ventral axes.
- Once the anterior-posterior and dorsal-ventral axes are established, the position of the left-right axis is fixed.
  - Specific molecular mechanisms must establish which side is left and which is right.

How do cells become committed to a particular developmental fate?

- Is cell fate commitment immediately irreversible, or is there a period of time during which cell fate can be modified?
- In 1938, the German zoologist Hans Spemann manipulated embryos to perturb normal development and then examined cell fate after manipulation.
  - This allowed Spemann to assay a cell's *developmental potential*, the range of structures that it can form.
- Spemann found that the fates of embryonic cells are affected by the distribution of determinants and the pattern of cleavage relative to this distribution.
- The first two blastomeres of the frog embryo are **totipotent** and can each develop into all the different cell types of that species.
- In mammals, embryonic cells remain totipotent through the 8-cell stage, much longer than in many other animals.
  - The totipotency of very early cells is not fully equivalent in a normal embryo.
  - Their totipotency when isolated likely 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.
- 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.

- 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 in an early gastrula.
  - If the dorsal ectoderm of an early amphibian gastrula is experimentally replaced with ectoderm from another location in the gastrula, the transplanted tissue forms a neural plate.
  - 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.
- 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.

Spemann and Mangold discovered an "organizer."

- Spemann and his student Hilde Mangold investigated cell fate determination during gastrulation by transplanting tissues between early gastrulas.
- In their most famous such experiment, they made a remarkable discovery: The transplanted dorsal lip of the blastopore continued as a blastopore lip and triggered gastrulation of the surrounding tissue.
  - 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.
- Developmental biologists are still actively studying the basis of induction by *Spemann's organizer*.
- A growth factor called bone morphogenetic protein 4 (BMP-4) belongs to a family of related proteins with a variety of developmental roles.
  - One major function of the cells of the organizer is 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 the fruit fly, where they also regulate the dorsal-ventral axis.
- 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.
- A classic model system for the study of pattern formation in vertebrates is limb development in the chick.
  - The wings and legs of chicks begin as limb buds, bumps of mesodermal tissue covered by a layer of ectoderm.

- 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.

- Two regions in a limb bud 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.
- 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 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.
  - 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 farthest from the ZPA form anterior structures, including the most anterior digit (like our thumb).
- The tissue transplantation experiment supports the hypothesis that the inductive signal produced by the ZPA conveys positional information indicating "posterior."
  - The cells of the ZPA secrete a growth factor called Sonic hedgehog.
  - 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.
- What determines whether a limb bud develops into a forelimb or a hind limb?
  - 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.

- 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.

Ciliary function helps specify cell fate in the human embryo.

- Like other mammals, humans have both motile and stationary cilia. Both types of cilia have vital functions in development.
  - Motile cilia are found on cells that move through their environment, such as sperm cells, and on cells that propel fluid over their surface, such as the epithelial cells of airways.
  - Stationary primary cilia, or *monocilia*, exist as a single projection on the surface of nearly all cells.
- Individuals with Kartagener's syndrome are prone to infections of the nasal sinuses and bronchi, while males with Kartagener's syndrome produce immotile sperm.
  - One feature of this syndrome is *situs inversus*, a reversal of the normal left-right asymmetry of the organs in the chest and abdomen.
- 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.
- 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*.
- In 2003, geneticists discovered that mutations disrupting development of the mouse nervous system affect 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.
- How do stationary monocilia function in development?
  - Each acts as an antenna on the cell surface, receiving signals from multiple signaling proteins, including Sonic hedgehog.
  - When monocilia are defective, signaling is disrupted.