Chapter 21

The Genetic Basis of Development

Lecture Outline

Overview

- The application of genetic analysis and DNA technology to the study of development has brought about a revolution in our understanding of how a complex multicellular organism develops from a single cell.
  - In 1995, Swiss researchers identified a gene that functions as a master switch to trigger the development of the eye in Drosophila.
    - A similar gene triggers eye development in mammals.
  - Developmental biologists are discovering remarkable similarities in the mechanisms that shape diverse organisms.
- While geneticists were advancing from Mendel's laws to an understanding of the molecular basis of inheritance, developmental biologists were focusing on embryology.
  - Embryology is the study of the stages of development leading from fertilized egg to fully formed organism.
- In recent years, the concepts and tools of molecular genetics have reached a point where a real synthesis of genetics and developmental biology has been possible.
- When the primary research goal is to understand broad biological principles, the organism chosen for study is called a **model organism.**
  - Researchers select model organisms that are representative of a larger group, suitable for the questions under investigation, and easy to grow in the lab.
- For study of the connections between genes and development, suitable model organisms have short generation times and small genomes that are suitable for genetic analysis.
  - Model organisms used in developmental genetics include the fruit fly *Drosophila melanogaster*, the nematode *Caenorhabditis elegans*, the mouse *Mus musculus*, the zebra fish *Danio rerio*, and the plant *Arabidopsis thaliana*.
- The fruit fly *Drosophila melanogaster* was first chosen as a model organism by geneticist T. H. Morgan and intensively studied by generations of geneticists after him.
  - The fruit fly is small and easily grown in the laboratory.
  - It has a generation time of only two weeks and produces many offspring.
  - Embryos develop outside the mother's body.
  - There are vast amounts of information on its genes and other aspects of its biology.
  - However, because first rounds of mitosis occur without cytokinesis, parts of its development are superficially quite different from that of other organisms.
  - Sequencing of the *Drosophila* genome was completed in 2000.
    - It has 180×10^6 base pairs (180 Mb) and contains about 13,700 genes.

- The nematode *Caenorhabditis elegans* normally lives in the soil but is easily grown in petri dishes.
  - Only a millimeter long, it has a simple, transparent body with only a few cell types and grows from zygote to mature adult in only three and a half days.
  - Its genome has been sequenced. It is 97 Mb long and contains an estimated 19,000 genes.
  - Because individuals are hermaphrodites, it is easy to detect recessive mutations.
    - Self-fertilization of heterozygotes produces some homozygous recessive offspring with mutant phenotypes.
  - Every adult *C. elegans* has exactly 959 somatic cells.
    - These arise from the zygote in virtually the same way for every individual.
    - By following all cell divisions with a microscope, biologists have constructed the organism's complete cell lineage, showing the ancestry of every cell in the adult body.
- The mouse *Mus musculus* has a long history as a mammalian model of development.
  - Much is known about its biology.
  - The mouse genome is about 2,600 Mb long with about 25,000 genes, about the same as the human genome.
  - Researchers are adept at manipulating mouse genes to make transgenic mice and mice in which particular genes are "knocked out" by mutation.
  - Mice are complex animals with a genome as large as ours.
    - Their embryos develop in the mother's uterus, hidden from view.
- A second vertebrate model, the zebra fish *Danio rerio*, has some unique advantages.
  - These small fish (2–4 cm long) are easy to breed in the laboratory in large numbers.
  - The transparent embryos develop outside the mother's body.
  - Although generation time is two to four months, the early stages of development proceed quickly.
    - By 24 hours after fertilization, most tissues and early versions of the organs have formed.
    - After two days, the fish hatches out of the egg case.
    - The zebra fish genome is estimated to be 1,700 Mb, and is still being mapped and sequenced.
- For studying the molecular genetics of plant development, researchers are focusing on a small weed, *Arabidopsis thaliana* (a member of the mustard family).
  - One plant can grow and produce thousands of progeny after eight to ten weeks.
  - A hermaphrodite, each flower makes eggs and sperm.
  - For gene manipulation research, scientists can induce cultured cells to take up foreign DNA (genetic transformation).
  - Its relatively small genome, about 118 Mb, contains an estimated 25,500 genes.

A. From Single Cell to Multicellular Organism

- In the development of most multicellular organisms, a single-celled zygote gives rise to cells of many different types.
  - Each type has a different structure and corresponding function.

- Cells of similar types are organized into tissues, tissues into organs, organs into organ systems, and organ systems into the whole organism.
- Thus, the process of embryonic development must give rise not only to cells of different types, but also to higher-level structures arranged in a particular way in three dimensions.

1. Embryonic development involves cell division, cell differentiation, and morphogenesis.

- An organism arises from a fertilized egg cell as the result of three interrelated processes: cell division, cell differentiation, and morphogenesis.
- Through a succession of mitotic cell divisions, the zygote gives rise to a large number of cells.
  - Cell division alone would produce only a great ball of identical cells.
- During development, cells become specialized in structure and function, undergoing **cell differentiation.**
- Different kinds of cells are organized into tissues and organs.
- The physical processes that give an organism its shape constitute **morphogenesis,** the "creation of form."
- The processes of cell division, differentiation, and morphogenesis overlap during development.
- Early events of morphogenesis lay out the basic body plan very early in embryonic development.
  - These include establishing the head of an animal embryo or the roots of a plant embryo.
  - Later morphogenetic events establish relative locations within smaller regions of the embryo, such as the digits on a vertebrate limb.
- The overall schemes of morphogenesis in animals and plants are very different.
  - In animals, but not in plants, *movements* of cells and tissues are necessary to transform the embryo into the characteristic 3-D form of the organism.
  - In plants, morphogenesis and growth in overall size are not limited to embryonic and juvenile periods but occur throughout the life of the plant.
- **Apical meristems,** perpetually embryonic regions in the tips of shoots and roots, are responsible for the plant's continual growth and formation of new organs, such as leaves and roots.
- In animals, ongoing development in adults is restricted to the generation of cells, such as blood cells, that must be continually replenished.

B. Differential Gene Expression

- During differentiation and morphogenesis, embryonic cells behave and function in ways different from one another, even though all of them have arisen from the same zygote.
- The differences between cells in a multicellular organism come almost entirely from differences in *gene expression*, not differences in the cell's genomes.
- These differences arise during development, as regulatory mechanisms turn specific genes off and on.

1. Different types of cells in an organism have the same DNA.

- Much evidence supports the conclusion that nearly all the cells of an organism have *genomic equivalence*—that is, they all have the same genes.

- An important question that emerges is whether genes are irreversibly inactivated during differentiation.
- One experimental approach to the question of genomic equivalence is to try to generate a whole organism from differentiated cells of a single type.
    - In many plants, whole new organisms *can* develop from differentiated somatic cells.
    - During the 1950s, F. C. Steward and his students found that differentiated root cells removed from the root could grow into normal adult plants when placed in a medium culture.
- These **cloning** experiments produced genetically identical individuals, popularly called **clones.**
- The fact that a mature plant cell can dedifferentiate (reverse its function) and give rise to all the different kinds of specialized cells of a new plant shows that differentiation does not necessarily involve irreversible changes in the DNA.
- In plants, at least, cells can remain **totipotent.**
    - They retain the zygote's potential to form all parts of the mature organism.
- Plant cloning is now used extensively in agriculture.
- Differentiated cells from animals often fail to divide in culture, much less develop into a new organism.
- Animal researchers have approached the genomic equivalence question by replacing the nucleus of an unfertilized egg or zygote with the nucleus of a differentiated cell.
    - The pioneering experiments in *nuclear transplantation* were carried out by Robert Briggs and Thomas King in the 1950s and extended later by John Gordon in the 1980s.
    - They destroyed or removed the nucleus of a frog egg and transplanted a nucleus from an embryonic or tadpole cell from the same species into an enucleated egg.
- The ability of the transplanted nucleus to support normal development is inversely related to the donor's age.
    - Transplanted nuclei from relatively undifferentiated cells from an early embryo lead to the development of most eggs into tadpoles.
    - Transplanted nuclei from fully differentiated intestinal cells lead to fewer than 2% of the cells developing into normal tadpoles.
        - Most of the embryos failed to make it through even the earliest stages of development.
- Developmental biologists agree on several conclusions about these results.
    - First, nuclei *do* change in some ways as cells differentiate.
        - While the DNA sequences do not change, histones may be modified or DNA may be methylated.
    - In frogs and most other animals, nuclear "potency" tends to be restricted more and more as embryonic development and cell differentiation progress.
        - However, chromatin changes are sometimes reversible, and the nuclei of most differentiated animal cells probably have all the genes required for making an entire organism.
- The ability to clone mammals using nuclei or cells from early embryos has long been possible.
- In 1997, Scottish researchers announced the birth of Dolly, a lamb cloned from an adult sheep by nuclear transplantation from a differentiated mammary cell.

- The mammary cells were fused with sheep egg cells whose nuclei had been removed.
  - The resulting cells divided to form early embryos, which were implanted into surrogate mothers.
- One of several hundred implanted embryos completed normal development.
- In 2003, Dolly developed a lung disease usually seen in much older sheep and was euthanized.
  - Dolly's premature death as well as her arthritis led to speculation that her cells were older than those of a normal sheep, possibly reflecting incomplete reprogramming of the original transplanted nucleus.
- Since 1997, cloning has been demonstrated in numerous mammals, including mice, cats, cows, horses, and pigs.
- The possibility of cloning humans raises unprecedented ethical issues.
  - In most cases, the goal is to produce new individuals.
  - This is known as *reproductive cloning.*
- These experiments have led to some interesting results.
  - Cloned animals in the same species do not look or behave identically.
  - Clearly, environmental influences and random phenomena can play a significant role during development.
- The successful cloning of various mammals raised interest in human cloning.
  - In early 2004, South Korean researchers reported success in the first step of reproductive cloning of humans.
  - Nuclei from differentiated human cells were transplanted into unfertilized enucleated eggs.
    - The eggs divided, and some embryos reached the *blastocyst stage* before development was halted.
- In most nuclear transplantation studies, only a small percentage of cloned embryos develop normally to birth.
  - Like Dolly, many cloned animals have various defects, such as obesity, pneumonia, liver failure, and premature death.
- In the nuclei of fully differentiated cells, a small subset of genes is turned on and the expression of the rest is repressed.
  - This regulation is often the result of epigenetic changes in chromatin, such as the acetylation of histones or the methylation of DNA.
  - Many of these changes must be reversed in the nucleus of the donor animal in order for genes to be expressed or repressed appropriately for early stages of development.
  - Researchers have found that the DNA in embryonic cells from cloned embryos, like that of differentiated cells, often has more methyl groups than does the DNA in equivalent cells from uncloned embryos of the same species.
  - Because DNA methylation helps regulate gene expression, methylated DNA of donor nuclei may interfere with the pattern of gene expression necessary for normal embryonic development.
- Another hot research area involves **stem cells.**
  - A stem cell is a relatively unspecialized cell that can reproduce itself and, under appropriate conditions, differentiate into specialized cell types.

- In addition to contributing to the study of differentiation, stem cell research has enormous potential in medicine.
  - The ultimate goal is to supply cells for the repair of damaged or diseased organs.
  - For example, providing insulin-producing pancreatic cells to diabetics or certain brain cells to individuals with Parkinson's disease could cure these diseases.
- Many early animal embryos contain **totipotent** stem cells, which can give rise to differentiated cells of any type.
  - In culture, these *embryonic stem cells* reproduce indefinitely and can differentiate into various specialized cells.
- The adult body has various kinds of stem cells, which replace nonreproducing specialized cells.
  - Adult stem cells are said to be **pluripotent,** able to give rise to many, but not all, cell types.
    - For example, stem cells in the bone marrow give rise to all the different kinds of blood cells.
  - The adult brain contains stem cells that continue to produce certain kinds of nerve cells.
  - Although adult animals have only tiny numbers of stem cells, scientists are learning to identify, isolate, and culture these cells from various tissues.
    - Under some culture conditions, with the addition of specific growth factors, cultured adult stem cells can differentiate into multiple types of specialized cells.
  - Stem cells from early embryos are somewhat easier to culture than those from adults and can produce differentiated cells of any type.
    - Embryonic stem cells are currently obtained from embryos donated by parents undergoing fertility treatments, or from long-term cell cultures originally established with cells isolated from donated embryos.
    - Because the cells are derived from human embryos, their use raises ethical and political issues.
    - With the recent cloning of human embryos to the blastocyst stage, scientists might be able to use these clones as the source of embryonic stem cells in the future.
    - When the major aim of cloning is to produce embryonic stem cells to treat disease, the process is called *therapeutic cloning.*
      - Opinions vary about the morality of therapeutic cloning.

2. Different cell types make different proteins, usually as a result of transcriptional regulation.

- During embryonic development, cells become visibly different in structure and function as they differentiate.
- The earliest changes that set a cell on a path to specialization show up only at the molecular level.
- Molecular changes in the embryo drive the process, termed **determination,** which leads up to observable differentiation of a cell.
  - At the end of this process, an embryonic cell is irreversibly *committed* to its final fate.
  - If a determined cell is experimentally placed in another location in the embryo, it will differentiate as if it were in its original position.
- The outcome of determination—cell differentiation—is caused by the expression of genes that encode *tissue-specific proteins.*

- These give a cell its characteristic structure and function.
- Differentiation begins with the appearance of mRNA and is finally observable in the microscope as changes in cellular structure.

- In most cases, the pattern of gene expression in a differentiated cell is controlled at the level of transcription.
- Cells produce the proteins that allow them to carry out their specialized roles in the organism.
  - For example, lens cells, and only lens cells, devote 80% of their capacity for protein synthesis to making just one type of protein, crystallin proteins.
    - These form transparent fibers that allow the lens to transmit and focus light.
  - Similarly, skeletal muscle cells have high concentrations of proteins specific to muscle tissues, such as a muscle-specific version of the contractile protein myosin and the structural protein actin.
    - They also have membrane receptor proteins that detect signals from nerve cells.
- Muscle cells develop from embryonic precursors that have the potential to develop into a number of alternative cell types, including cartilage cells, fat cells, or multinucleate muscle cells.
  - As the muscle cells differentiate, they become *myoblasts* and begin to synthesize muscle-specific proteins.
  - They fuse to form mature, elongated, multinucleate skeletal muscle cells.
- Researchers developed the hypothesis that certain muscle-specific regulatory genes are active in myoblasts, leading to muscle cell determination.
  - To test this, researchers isolated mRNA from cultured myoblasts and used reverse transcriptase to prepare a cDNA library containing all the genes that are expressed in cultured myoblasts.
  - Transplanting these cloned genes into embryonic precursor cells led to the identification of several "master regulatory genes" that, when transcribed and translated, commit the cells to become skeletal muscle.
- One of these master regulatory genes is called *myoD*, a transcription factor.
  - *myoD* encodes MyoD protein, which binds to specific control elements and stimulates the transcription of various genes, including some that encode for other muscle-specific transcription factors.
    - These secondary transcription factors activate the muscle protein genes.
    - MyoD also stimulates expression of the *myoD* gene itself, perpetuating its effect in maintaining the cell's differentiated state.
- MyoD protein is capable of changing fully differentiated nonmuscle cells into muscle cells.
- However, not *all* cells will transform.
  - Nontransforming cells may lack a *combination* of regulatory proteins, in addition to MyoD.

3. Transcriptional regulation is directed by maternal molecules in the cytoplasm and signals from other cells.

- Two sources of information "tell" a cell, such as a myoblast or even the zygote, which genes to express at any given time.
- One source of information is the cytoplasm of the unfertilized egg cell, which contains RNA and protein molecules encoded by the mother's DNA.

- Messenger RNA, proteins, other substances, and organelles are distributed unevenly in the unfertilized egg.
- This impacts embryonic development in many species.

- Maternal substances that influence the course of early development are called **cytoplasmic determinants.**
  - These substances regulate the expression of genes that affect the developmental fate of the cell.
  - After fertilization, the cell nuclei resulting from mitotic division of the zygote are exposed to different cytoplasmic environments.
    - The set of cytoplasmic determinants a particular cell receives helps determine its developmental fate by regulating expression of the cell's genes during the course of cell differentiation.
- The other important source of developmental information is the environment around the cell, especially signals impinging on an embryonic cell from other nearby embryonic cells.
  - In animals, these include contact with cell-surface molecules on neighboring cells and the binding of growth factors secreted by neighboring cells.
  - In plants, the cell-cell junctions known as plasmodesmata allow signal molecules to pass from one cell to another.
    - The synthesis of these signals is controlled by the embryo's own genes.
- These signal molecules cause **induction,** triggering observable cellular changes by causing a change in gene expression in the target cell.

C. Genetic and Cellular Mechanisms of Pattern Formation

- Before morphogenesis can shape an animal or plant, the organism's *body plan* must be established.
- Cytoplasmic determinants and inductive signals contribute to **pattern formation,** the development of *spatial organization* in which the tissues and organs of an organism are all in their characteristic places.
  - Pattern formation continues throughout the life of a plant in the apical meristems.
  - In animals, pattern formation is mostly limited to embryos and juveniles.
- Pattern formation begins in the early embryo, when the major axes of an animal and the root-shoot axis of the plant are established.
  - The molecular cues that control pattern formation, **positional information,** tell a cell its location relative to the body axes and to neighboring cells.
  - They also determine how the cells and their progeny will respond to future molecular signals.

1. Drosophila development is controlled by a cascade of gene activations.

- Pattern formation has been most extensively studied in *Drosophila melanogaster*, where genetic approaches have had spectacular success.
  - These studies have established that genes control development and have identified the key roles that specific molecules play in defining position and directing differentiation.
  - Combining anatomical, genetic, and biochemical approaches in the study of *Drosophila* development, researchers have discovered developmental principles common to many other species, including humans.

- Fruit flies and other arthropods have a modular construction, an ordered series of segments.
  - These segments make up the three major body parts: the head, thorax (with wings and legs), and abdomen.
  - Like other bilaterally symmetrical animals, *Drosophila* has an anterior-posterior axis and a dorsal-ventral axis.
    - Cytoplasmic determinants in the unfertilized egg provide positional information for the two developmental axes before fertilization.
  - After fertilization, positional information establishes a specific number of correctly oriented segments and finally triggers the formation of each segment's characteristic structures.
  - The *Drosophila* egg cell develops in the female's ovary, surrounded by ovarian cells called nurse cells and follicle cells that supply the egg cell with nutrients, mRNAs, and other substances needed for development.
- Development of the fruit fly from egg cell to adult fly occurs in a series of discrete stages.
  1. Mitosis follows fertilization and egg laying.
  - Early mitosis occurs without growth of the cytoplasm and without cytokinesis, producing one big multinucleate cell.
  2. At the tenth nuclear division, the nuclei begin to migrate to the periphery of the embryo.
  3. At division 13, the cytoplasm partitions the 6,000 or so nuclei into separate cells.
  - The basic body plan—including body axes and segment boundaries—has already been determined by this time.
  - A central yolk nourishes the embryo, and the eggshell continues to protect it.
  4. Subsequent events in the embryo create clearly visible segments, which at first look very much alike.
  5. Some cells move to new positions, organs form, and a wormlike larva hatches from the shell.
  - During three larval stages, the larva eats, grows, and molts.
  6. During the third larval stage, the larva transforms into the pupa enclosed in a case.
  7. Metamorphosis, the change from larva to adult fly, occurs in the pupal case, and the fly emerges.
  - Each segment is anatomically distinct, with characteristic appendages.
- The results of detailed anatomical observations of development in several species and experimental manipulations of embryonic tissues laid the groundwork for understanding the mechanisms of development.
- In the 1940s, Edward B. Lewis demonstrated that the study of mutants could be used to investigate *Drosophila* development.
- He studied bizarre developmental mutations and located the mutations on the fly's genetic map.
- This research provided the first concrete evidence that genes somehow direct the developmental process.
- In the late 1970s, Christiane Nüsslein-Volhard and Eric Weischaus pushed the understanding of early pattern formation to the molecular level.
- Their goal was to identify *all* the genes that affect segmentation in *Drosophila*, but they faced three problems.

- Because *Drosophila* has about 13,700 genes, there could be only a few genes affecting segmentation or so many that the pattern would be impossible to discern.
- Mutations that affect segmentation are likely to be **embryonic lethals,** leading to death at the embryonic or larval stage.
  - Because flies with embryonic lethal mutations never reproduce, they cannot be bred for study.
- Because of maternal effects on axis formation in the egg, researchers also need to study maternal genes.

- Nüsslein-Volhard and Wieschaus focused on recessive mutations that could be propagated in heterozygous flies.
  - After mutating flies, they looked for dead embryos and larvae with abnormal segmentation among the fly's descendents.
  - Through appropriate crosses, they could identify living heterozygotes carrying embryonic lethal mutations.
  - They hoped that the segmental abnormalities would suggest how the affected genes normally functioned.
- Nüsslein-Volhard and Wieschaus identified 1,200 genes essential for embryonic development.
  - About 120 of these were essential for pattern formation leading to normal segmentation.
  - After several years, they were able to group the genes by general function, map them, and clone many of them.
- Their results, combined with Lewis's early work, created a coherent picture of *Drosophila* development.
  - In 1995, Nüsslein-Volhard, Wieschaus, and Lewis were awarded the Nobel Prize.

2. Gradients of maternal molecules in the early embryo control axis formation.

- Cytoplasmic determinants establish the axes of the *Drosophila* body.
  - Substances are produced under the direction of **maternal effect genes** that are deposited in the unfertilized egg.
    - When a maternal effect gene is mutated, the offspring has an abnormal mutant phenotype.
- In fruit fly development, maternal effect genes encode proteins or mRNA that are placed in the egg while it is still in the ovary.
  - When the mother has a mutated gene, she makes a defective gene product (or none at all), and her eggs will not develop properly when fertilized.
- These maternal effect genes are also called **egg-polarity genes,** because they control the orientation of the egg and consequently the fly.
  - One group of genes sets up the anterior-posterior axis, while a second group establishes the dorsal-ventral axis.
- One of these, the *bicoid* gene, affects the front half of the body.
- An embryo whose mother has a mutant *bicoid* gene lacks the front half of its body and has duplicate posterior structures at both ends.
  - This suggests that the product of the mother's *bicoid* gene is essential for setting up the anterior end of the fly.
  - It also suggests that the gene's products are concentrated at the future anterior end.

- This is a specific version of a *general gradient hypothesis*, in which gradients of **morphogens** establish an embryo's axes and other features.
- Using DNA technology and biochemical methods, researchers were able to clone the *bicoid* gene and use it as a probe for *bicoid* mRNA in the egg.
    - As predicted, the *bicoid* mRNA is concentrated at the extreme anterior end of the egg cell.
- After the egg is fertilized, *bicoid* mRNA is transcribed into bicoid protein, which diffuses from the anterior end toward the posterior, resulting in a gradient of proteins in the early embryo.
- Injections of pure *bicoid* mRNA into various regions of early embryos results in the formation of anterior structures at the injection sites as the mRNA is translated into protein.
- The *bicoid* research is important for three reasons.
    1. It identified a specific protein required for some of the earliest steps in pattern formation.
    2. It increased our understanding of the mother's role in development of an embryo.
        - As one developmental biologist put it, "Mom tells Junior which way is up."
    3. It demonstrated a key developmental principle that a gradient of molecules can determine polarity and position in the embryo.
- Gradients of specific proteins determine the posterior end as well as the anterior and also are responsible for establishing the dorsal-ventral axis.

3. A cascade of gene activations sets up the segmentation pattern in Drosophila.

- The *bicoid* protein and other morphogens are transcription factors that regulate the activity of some of the embryo's own genes.
- Gradients of these morphogens bring about regional differences in the expression of **segmentation genes,** the genes that direct the actual formation of segments after the embryo's major axes are defined.
- In a cascade of gene activations, sequential activation of three sets of segmentation genes provides the positional information for increasingly fine details of the body plan.
    - The three sets are called gap genes, pair-rule genes, and segment polarity genes.
- The products of many segmentation genes are transcription factors that directly activate the next set of genes in the hierarchical scheme of pattern formation.
- Other segmentation proteins operate more indirectly.
    - Some are components of cell-signaling pathways, including signal molecules used in cell-cell communication and the membrane receptors that recognize them.
- Working together, the products of egg-polarity genes such as *bicoid* regulate the regional expression of gap genes, which control the localized expression of pair-rule genes, which in turn activate specific segment polarity genes in different parts of each segment.
- The boundaries and axes of segments are set by this hierarchy of genes (and their products).

4. Homeotic genes direct the identity of body parts.

- In a normal fly, structures such as antennae, legs, and wings develop on the appropriate segments.
- The anatomical identity of the segments is controlled by master regulatory genes, the **homeotic genes.**

- Discovered by Edward Lewis, these genes specify the types of appendages and other structures that each segment will form.
- Mutations to homeotic genes produce flies with such strange traits as legs growing from the head in place of antennae.
    - Structures characteristic of a particular part of the animal arise in the wrong place.
- Like other developmental genes, the homeotic genes encode transcription factors that control the expression of genes responsible for specific anatomical structures.
    - For example, a homeotic protein made in a thoracic segment may activate genes that bring about leg development, while a homeotic protein in a certain head segment activates genes for antennal development.
    - A mutant version of this protein may label a segment as "thoracic" instead of "head," causing legs to develop in place of antennae.
- Scientists are now working to identify the genes activated by the homeotic proteins—the genes specifying the proteins that actually build the fly structures.
- Amazingly, many of the molecules and mechanisms that regulate development in the *Drosophila* embryo have close counterparts throughout the animal kingdom.

5. Neighboring cells instruct other cells to form particular structures: cell signaling and induction in the nematode.

- The development of a multicellular organism requires close communication among cells.
    - Signals generated by neighboring nurse cells trigger the localization of *bicoid* mRNA in the egg of the *Drosophila*.
- Once the embryo is truly multicellular, cells signal nearby cells to change in a specific way, in a process called induction.
    - Induction brings about cell differentiation through transcriptional regulation of specific genes.
- The nematode *C. elegans* has proved to be a very useful model organism for investigating the roles of cell signaling, induction, and programmed cell death in development.
- Researchers know the entire ancestry of every cell in the body of an adult *C. elegans*—the organism's complete **cell lineage.**
- As early as the four-cell stage in *C. elegans*, cell signaling helps direct daughter cells down appropriate pathways.
- Researchers have combined genetic, biochemical, and embryological approaches to study the development of the *vulva*, through which the worm lays its eggs.
- The pathway from fertilized egg to adult nematode involves four larval stages (during which the larvae look much like smaller versions of the adult) during which this structure develops.
    - Already present on the ventral surface of the second-stage larva are six cells from which the vulva will arise.
    - A single cell in the embryonic gonad, the *anchor cell*, initiates a cascade of signals that establishes the fate of the six vulval precursor cells.
    - If an experimenter destroys the anchor cell with a laser beam, the vulva fails to form and the precursor cells simply become part of the worm's epidermis.
- Secreted factors or cell-surface proteins bind to receptors on the recipient cell, initiating intracellular signal transduction pathways.

- This example illustrates a number of important concepts that apply to development of *C. elegans* and many other animals.
  - In the developing embryo, sequential inductions drive organ formation.
  - The effect of an inducer can depend on its concentration.
  - Inducers produce their effects via signal transduction pathways similar to those operating in adult cells.
  - The induced cell's response is often the activation of genes—transcriptional regulation—that, in turn, establishes a pattern of gene activity characteristic of a particular kind of differentiated cell.
- Lineage analysis of *C. elegans* highlights another outcome of cell signaling, programmed cell death, or **apoptosis.**
  - The timely suicide of cells occurs exactly 131 times in the course of *C. elegans*'s normal development.
  - At precisely the same points in development, signals trigger the activation of a cascade of "suicide" proteins in the cells destined to die.
- During apoptosis, a cell shrinks and becomes lobed (called "blebbing"), the nucleus condenses, and the DNA is fragmented.
  - Neighboring cells quickly engulf and digest the membrane-bound remains, leaving no trace.
- Genetic screening of *C. elegans* has revealed two key apoptosis genes, *ced-3* and *ced-4* (*ced* stands for cell death), which encode proteins (Ced-3 and Ced-4) that are essential for apoptosis.
- In *C. elegans*, a protein in the outer mitochondrial membrane called Ced-9 (the product of *ced-9*) is a master regulator of apoptosis.
  - *ced-9* acts as a brake in the absence of a signal promoting apoptosis.
- When the cell receives an external death signal, Ced-9 is inactivated, allowing both Ced-4 and Ced-3 to be active.
  - The apoptosis pathway activates proteases and nucleases to cut up the proteins and DNA of the cell.
- The main proteases of apoptosis are called *caspases.*
  - In nematodes, Ced-3 is the chief caspase—the main protease of apoptosis.
- Apoptosis is regulated not at the level of transcription or translation, but through changes in the *activity* of proteins that are continually present in the cell.
- Apoptosis pathways in humans and other mammals are more complicated.
- Research on mammals has revealed a prominent role for mitochondria in apoptosis.
  - Signals from apoptosis pathways or others somehow cause the outer mitochondrial membrane to leak, releasing proteins that promote apoptosis.
    - Surprisingly, these proteins include cytochrome *c*, which functions in mitochondrial electron transport in healthy cells but acts as a cell death factor when released from mitochondria.
  - Still controversial is whether mitochondria play a central role in apoptosis or only a subsidiary role.
- A cell must make a life-or-death "decision" by somehow integrating both the "death" and "life" (growth factor) signals that it receives.

- A built-in cell suicide mechanism is essential to development in all animals.
  - Similarities between the apoptosis genes in mammals and nematodes, as well as the observation that apoptosis occurs in multicellular fungi and unicellular yeast, indicate that the basic mechanism evolved early in animal evolution.
  - The timely activation of apoptosis proteins in some cells functions during normal development and growth in both embryos and adults.
    - It is part of the normal development of the nervous system, normal operation of the immune system, and normal morphogenesis of human hands and feet.
- A low level of apoptosis in developing limbs accounts for the webbed feet of ducks.
- Problems with the cell suicide mechanism may have health consequences, ranging from minor to serious.
  - Failure of normal cell death during morphogenesis of the hands and feet can result in webbed fingers and toes.
  - Researchers are also investigating the possibility that certain degenerative diseases of the nervous system result from inappropriate activation of the apoptosis genes.
  - Others are investigating the possibility that some cancers result from a failure of cell suicide that normally occurs if the cell has suffered irreparable damage, especially DNA damage.
    - Damaged cells normally generate *internal* signals that trigger apoptosis.

6. Plant development depends on cell signaling and transcriptional regulation.

- The genetic analysis of plant development, using model organisms such as *Arabidopsis*, has lagged behind that of animal models.
  - Biologists are just beginning to understand the molecular basis of plant development.
- In general, cell linage is less important for pattern formation in plants than in animals.
  - Many plant cells are totipotent, and their fates depend more on positional information than on cell lineage.
- Plant development, like that of animals, depends on cell signaling (induction) and transcriptional regulation.
- The embryonic development of most plants occurs in seeds that are relatively inaccessible to study.
- However, other important aspects of plant development are observable in plant meristems, particularly the apical meristems at the tips of shoots.
  - These give rise to new organs, such as leaves or the petals of flowers.
- Environmental signals (such as day length or temperature) trigger signal transduction pathways that convert ordinary shoot meristems to floral meristems.
  - A floral meristem is a “bump” with three cell layers, all of which participate in the formation of a flower with four types of organs: *carpels* (containing egg cells), *petals*, *stamens* (containing sperm-bearing pollen), and *sepals* (leaflike structures outside the petals).
- To examine induction of the floral meristem, researchers grafted stems from a mutant tomato plant onto a wild-type plant and then grew new plants from the shoots at the graft sites.
  - Plants homozygous for the mutant allele *fasciated* (*f*) produce flowers with an abnormally large number of organs.

- The new plants were **chimeras,** organisms with a mixture of genetically different cells.
- Some of the chimeras produced floral meristems in which the three cell layers did not all come from the same "parent."
- The number of organs per flower depends on genes of the L3 (innermost) cell layer.
  - This induces the L2 and L1 layers to form that number of organs.
- In contrast to genes controlling organ *number* in flowers, genes controlling organ *identity* **(organ identity genes)** determine the types of structure that will grow from a meristem.
- In *Arabidopsis* and other plants, organ identity genes are analogous to homeotic genes in animals and are often referred to as plant homeotic genes.
  - Mutations cause plant structures to grow in unusual places, such as carpels in the place of sepals.
- Researchers have identified and cloned a number of floral identity genes, and they are beginning to determine how they act.
  - In plants with a "homeotic" mutation, specific organs are missing or repeated.
  - Like the homeotic genes of animals, the organ identity genes of plants encode transcription factors that regulate specific target genes by binding to their enhancers in the DNA.

7. Homeobox genes have been highly conserved in evolution.

- Biologists in the field of evolutionary developmental biology, or "evo-devo," compare developmental processes of different multicellular organisms.
  - Their aim is to understand how developmental processes have evolved and how changes in the processes can modify existing organismal features or lead to new ones.
  - Biologists are finding that the genomes of related species with strikingly different forms may have only minor differences in gene sequence or regulation.
- All homeotic genes of *Drosophila* include a 180-nucleotide sequence called the **homeobox,** which specifies a 60-amino-acid *homeodomain.*
  - An identical, or very similar, sequence of nucleotides (often called *Hox* genes) is found in many other animals, including humans.
  - The vertebrate genes homologous to the homeotic genes of fruit flies have even kept their chromosomal arrangement.
  - Related sequences have been found in the regulatory genes of plants, yeasts, and even prokaryotes.
- The homeobox DNA sequence must have evolved very early in the history of life and is sufficiently valuable that it has been conserved virtually unchanged in animals and plants for hundreds of millions of years.
- Most, but not all, homeobox-containing genes are homeotic genes that are associated with development.
  - For example, in *Drosophila*, homeoboxes are present not only in the homeotic genes, but also in the egg-polarity gene *bicoid*, in several segmentation genes, and in the master regulatory gene for eye development.
- The homeobox-encoded homeodomain is part of a protein that binds to DNA when the protein functions as a transcriptional regulator.
  - However, the shape of the homeodomain allows it to bind to any DNA segment.

- Other, more variable, domains of the overall protein determine which genes it will regulate.
- Interaction of these latter domains with still other transcription factors helps a homeodomain-protein recognize specific enhancers in the DNA.

- Proteins with homeodomains probably regulate development by coordinating the transcription of batteries of developmental genes.
  - In *Drosophila*, different combinations of homeobox genes are active in different parts of the embryo and at different times, leading to pattern formation.
- Many other genes involved in development are highly conserved from species to species.
  - These include numerous genes encoding components of signaling pathways.
- How can the same genes be involved in the development of so many different animals?
  - In some cases, small changes in regulatory sequences of particular genes can lead to major changes in body form.
  - For example, varying expression of the *Hox* genes along the body axis produce different numbers of leg-bearing segments in insects and crustaceans.
- Plants also have homeobox-containing genes.
  - However, they do not appear to function as master regulatory switches in plants.
  - Other genes appear to be responsible for pattern formation in plants.

8. There are some basic similarities—and many differences—in the development of plants and animals.

- The last common ancestor of plants and animals was a single-celled microbe living hundreds of millions of years ago, so the processes of development evolved independently in the two lineages.
  - Plants have rigid cell walls that prevent cell movement, while morphogenetic movements are very important in animals.
  - Morphogenesis in plants is dependent on differing planes of cell division and selective cell enlargement.
- Nevertheless, there are some basic similarities of development.
  - In both plants and animals, development relies on a cascade of transcriptional regulators turning on or off genes in a finely tuned series.
- The genes that direct these processes are very different in plants and animals.
  - Quite a few of the master regulatory switches in *Drosophila* are homeobox-containing *Hox* genes.
  - Those in *Arabidopsis* belong to the *Mads-box* family of genes.
- Although homeobox-containing genes can be found in plants and *Mads-box* genes can be found in animals, they do not play the same major roles in development in plants and animals.
- The unity of life is reflected in the similarity of biological mechanisms used to establish body pattern, although the exact genes directing develop may differ.
- The similarities reflect the common ancestry of life on Earth, while the differences have created the diversity of living organisms.