Development of Multicellular Organisms

22

An animal or plant starts its life as a single cell—a fertilized egg. During development, this cell divides repeatedly to produce many different cells in a final pattern of spectacular complexity and precision. Ultimately, the genome determines the pattern, and the puzzle of developmental biology is to understand how it does so.

The genome is normally identical in every cell; the cells differ not because they contain different genetic information, but because they express different sets of genes. This selective gene expression controls the four essential processes by which the embryo is constructed: (1) *cell proliferation*, producing many cells from one, (2) *cell specialization,* creating cells with different characteristics at different positions, (3) *cell interactions,* coordinating the behavior of one cell with that of its neighbors, and (4) *cell movement,* rearranging the cells to form structured tissues and organs (**Figure 22–1**).

In a developing embryo, all these processes are happening at once, in a kaleidoscopic variety of different ways in different parts of the organism. To understand the basic strategies of development, we have to narrow our focus. In particular, we must understand the course of events from the standpoint of the individual cell and the way the genome acts within it. There is no commanding officer standing above the fray to direct the troops; each of the millions of cells in the embryo has to make its own decisions, according to its own copy of the genetic instructions and its own particular circumstances.

The complexity of animals and plants depends on a remarkable feature of the genetic control system. Cells have a memory: the genes a cell expresses and the way it behaves depend on the cell's past as well as its present environment. The cells of your body—the muscle cells, the neurons, the skin cells, the gut cells, and so on—maintain their specialized characters not because they continually receive the same instructions from their surroundings, but because they retain a record of signals their ancestors received in early embryonic development. The molecular mechanisms of cell memory have been introduced in Chapter 7. In this chapter we shall encounter its consequences.

In This Chapter

UNIVERSAL MECHANISMS OF ANIMAL DEVELOPMENT

There are about ten million species of animals, and they are fantastically varied. One would no more expect the worm, the flea, the eagle and the giant squid all to be generated by the same developmental mechanisms, than one would suppose that the same methods were used to make a shoe and an airplane. Some similar abstract principles might be involved, perhaps, but surely not the same specific molecules?

One of the most astonishing revelations of the past 10 or 20 years has been that our initial suspicions are wrong. In fact, much of the basic machinery of development is essentially the same, not just in all vertebrates but in all the major phyla of invertebrates too. Recognizably similar, evolutionarily related molecules define our specialized cell types, mark the differences between body regions, and help create the body's pattern. Homologous proteins are often

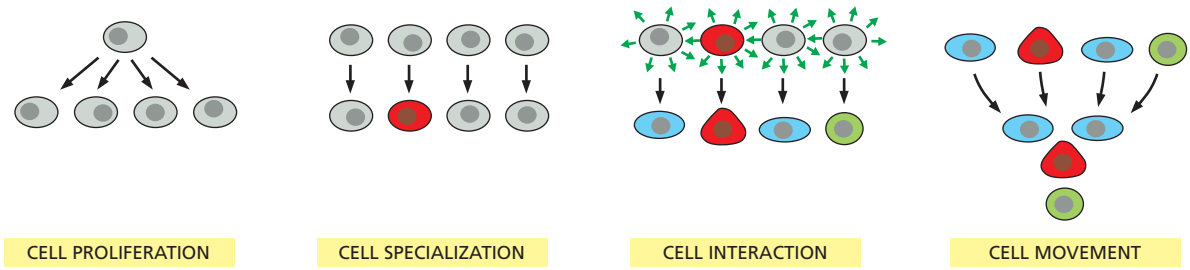


Figure 22–1 The four essential processes by which a multicellular organism is made: cell proliferation, cell specialization, cell interaction, and cell movement.

functionally interchangeable between very different species. A mouse protein produced artificially in a fly can often perform the same function as the fly's own version of that protein, and vice versa, successfully controlling the development of an eye, for example, or the architecture of the brain (**Figure 22–2**). Thanks to this underlying unity of mechanism, as we shall see, developmental biologists are now well on their way toward a coherent understanding of animal development.

Plants are a separate kingdom: they have evolved their multicellular organization independently of animals. For their development too, a unified account can be given, but it is different from that for animals. Animals will be our main concern in this chapter, but we shall return to plants briefly at the end.

We begin by reviewing some of the basic general principles of animal development and by introducing the seven animal species that developmental biologists have adopted as their chief model organisms.

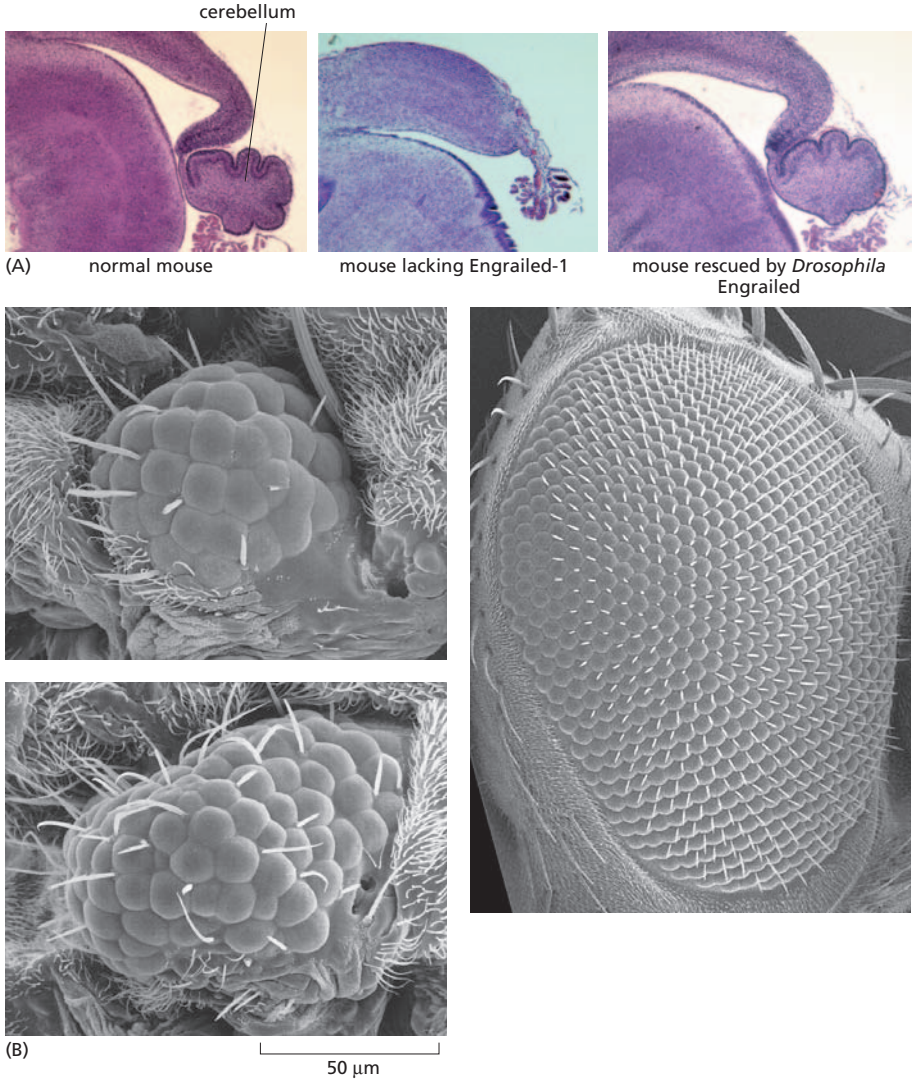


Figure 22–2 Homologous proteins functioning interchangeably in the development of mice and flies. (A) A fly protein used in a mouse. The DNA sequence from *Drosophila* coding for the Engrailed protein (a gene regulatory protein) can be substituted for the corresponding sequence coding for the Engrailed-1 protein of the mouse. Loss of Engrailed-1 in the mouse causes a defect in its brain (the cerebellum fails to develop); the *Drosophila* protein acts as an efficient substitute, rescuing the transgenic mouse from this deformity. (B) A mollusk protein used in a fly. The Eyeless protein controls eye development in *Drosophila,* and when misexpressed can cause an eye to develop in an abnormal site, such as a leg. The homologous protein, Pax6, from a mouse, a squid, or practically any animal possessing eyes, when similarly misexpressed in a transgenic fly, has the same effect. The scanning electron micrographs show a patch of eye tissue on the leg of a fly resulting from misexpression of *Drosophila Eyeless* (top) and of squid *Pax6* (bottom). The right panel shows, at lower magnification, the entire eye of a normal *Drosophila,* for comparison. (A, from M.C. Hanks et al., *Development* 125:4521–4530, 1998. With permission from The Company of Biologists; B, from S.I. Tomarev et al., *Proc. Natl Acad. Sci. U.S.A.* 94:2421–2426, 1997. With permission from National Academy of Sciences and courtesy of Kevin Moses.)

Animals Share Some Basic Anatomical Features

The similarities between animal species in the genes that control development reflect the evolution of animals from a common ancestor in which these genes were already present. Although we do not know what it looked like, the common ancestor of worms, mollusks, insects, vertebrates, and other complex animals must have had many differentiated cell types that would be recognizable to us: epidermal cells, for example, forming a protective outer layer; gut cells to absorb nutrients from ingested food; muscle cells to move; neurons and sensory cells to control the movements. The body must have been organized with a sheet of skin covering the exterior, a mouth for feeding and a gut tube to contain and process the food—with muscles, nerves and other tissues arranged in the space between the external sheet of skin and the internal gut tube.

These features are common to almost all animals, and they correspond to a common basic anatomical scheme of development. The egg cell—a giant storehouse of materials—divides, or **cleaves**, to form many smaller cells. <ATTT> These cohere to create an epithelial sheet facing the external medium. Much of this sheet remains external, constituting the **ectoderm**—the precursor of the epidermis and of the nervous system. A part of the sheet becomes tucked into the interior to form **endoderm**—the precursor of the gut and its appendages, such as lung and liver. Another group of cells move into the space between ectoderm and endoderm, and form the **mesoderm**—the precursor of muscles, connective tissues, and various other components. This transformation of a simple ball or hollow sphere of cells into a structure with a gut is called **gastrulation** (from the Greek word for a belly), and in one form or another it is an almost universal feature of animal development. **Figure 22–3** illustrates the process as it is seen in the sea urchin.

Evolution has diversified upon the molecular and anatomical fundamentals that we describe in this chapter to produce the wonderful variety of present-day species. But the underlying conservation of genes and mechanisms means that studying the development of one animal very often leads to general insights into

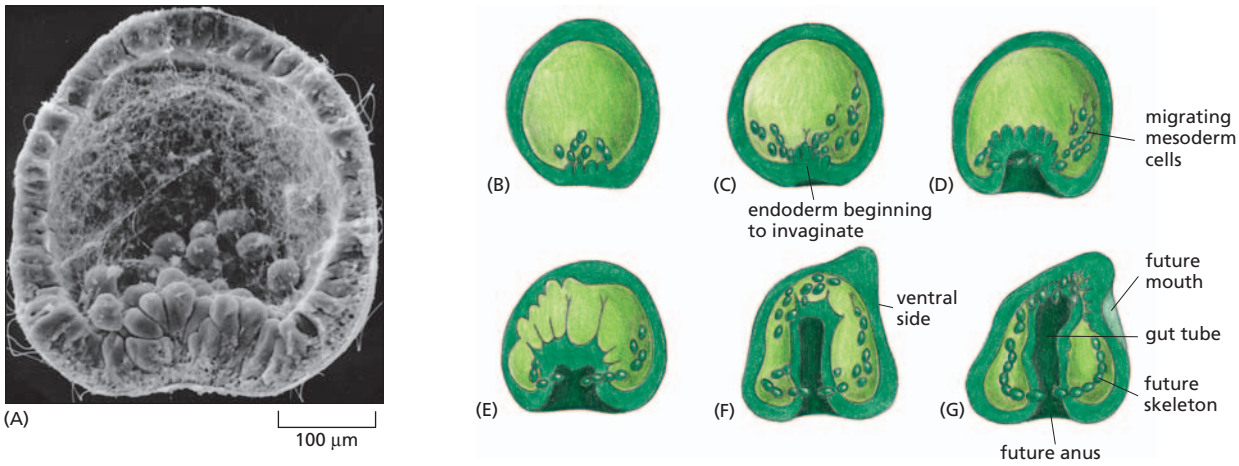
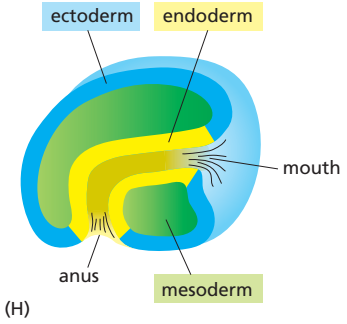


Figure 22–3 Sea urchin gastrulation. A fertilized egg divides to produce a blastula—a hollow sphere of epithelial cells surrounding a cavity. Then, in the process of gastrulation, some of the cells tuck into the interior to form the gut and other internal tissues. (A) Scanning electron micrograph showing the initial intucking of the epithelium. (B) Drawing showing how a group of cells break loose from the epithelium to become mesoderm. (C) These cells then crawl over the inner face of the wall of the blastula. (D) Meanwhile, epithelium is continuing to tuck inward to become endoderm. (E and F) The invaginating endoderm extends into a long gut tube. (G) The end of the gut tube makes contact with the wall of the blastula at the site of the future mouth opening. Here the ectoderm and endoderm will fuse and a hole will form. (H) The basic animal body plan, with a sheet of ectoderm on the outside, a tube of endoderm on the inside, and mesoderm sandwiched between them. (A, from R.D. Burke et al., *Dev. Biol.* 146:542–557, 1991. With permission from Academic Press; B-G, after L. Wolpert and T. Gustafson, *Endeavour* 26:85–90, 1967. With permission from Elsevier.)



the development of many other types of animals. As a result, developmental biologists today, like cell biologists, have the luxury of addressing fundamental questions in whatever species offers the easiest path to an answer.

Multicellular Animals Are Enriched in Proteins Mediating Cell Interactions and Gene Regulation

Genome sequencing reveals the extent of molecular similarities between species. The nematode worm *Caenorhabditis elegans*, the fly *Drosophila melanogaster*, and the vertebrate *Homo sapiens* are the first three animals for which a complete genome sequence was obtained. In the family tree of animal evolution, they are very distant from one another: the lineage leading to the vertebrates is thought to have diverged from that leading to the nematodes, insects and mollusks more than 600 million years ago. Nevertheless, when the 20,000 genes of *C. elegans,* the 14,000 genes of *Drosophila*, and the 25,000 genes of the human are systematically compared with one another, it is found that about 50% of the genes in each of these species have clearly recognizable homologs in one or both of the other two species. In other words, recognizable versions of at least 50% of all human genes were already present in the common ancestor of worms, flies, and humans.

Of course, not everything is conserved: there are some genes with key roles in vertebrate development that have no homologs in the genome of *C. elegans* or *Drosophila*, and vice versa. However, a large proportion of the 50% of genes that lack identifiable homologs in other phyla may do so simply because their functions are of minor importance. Although these nonconserved genes are transcribed and well represented in cDNA libraries, studies of DNA and amino acid sequence variability in and between natural populations indicate that these genes are unusually free to mutate without seriously harming fitness; when they are artificially inactivated, the consequences are not so often severe as for genes with homologs in distantly related species. Because they are free to evolve so rapidly, a few tens of millions of years may be enough to obliterate any family resemblance or to permit loss from the genome.

The genomes of different classes of animals differ also because, as discussed in Chapter 1, there are substantial variations in the extent of gene duplication: the amount of gene duplication in the evolution of the vertebrates has been particularly large, with the result that a mammal or a fish often has several homologs corresponding to a single gene in a worm or a fly.

Despite such differences, to a first approximation we can say that all these animals have a similar set of proteins at their disposal for their key functions. In other words, they construct their bodies using roughly the same molecular kit of parts.

What genes, then, are needed to produce a multicellular animal, beyond those necessary for a solitary cell? Comparison of animal genomes with that of budding yeast—a unicellular eucaryote—suggests that two classes of proteins are especially important for multicellular organization. The first class is that of the transmembrane molecules used for cell adhesion and cell signaling. As many as 2000 *C. elegans* genes encode cell surface receptors, cell adhesion proteins, and ion channels that are either not present in yeast or present in much smaller numbers. The second class is that of gene regulatory proteins: these DNA-binding proteins are much more numerous in the *C. elegans* genome than in yeast. For example, the basic helix–loop–helix family has 41 members in *C. elegans*, 84 in *Drosophila*, 131 in humans and only 7 in yeast, and other families of regulators of gene expression are also dramatically overrepresented in animals as compared to yeast. Not surprisingly, these two classes of proteins are central to developmental biology: as we shall see, the development of multicellular animals is dominated by cell–cell interactions and by differential gene expression.

As discussed in Chapter 7, micro-RNAs also play a significant part in controlling gene expression during development, but they seem to be of secondary importance by comparison with proteins. Thus a mutant zebrafish embryo that completely lacks the Dicer enzyme, which is required for production of functional miRNAs, will still begin its development almost normally, creating

specialized cell types and a more-or-less correctly organized body plan, before abnormalities become severe.

Regulatory DNA Defines the Program of Development

A worm, a fly, a mollusc and a mammal share many of the same essential cell types, and they do all have a mouth, a gut, a nervous system and a skin; but beyond a few such basic features they seem radically different in their body structure. If the genome determines the structure of the body and these animals all have such a similar collection of genes, how can they be so different?

The proteins encoded in the genome can be viewed as the components of a construction kit. Many things can be built with this kit, just as a child's construction kit can be used to make trucks, houses, bridges, cranes, and so on by assembling the components in different combinations. Some components necessarily go together—nuts with bolts, wheels with tires and axles—but the large-scale organization of the final object is not defined by these substructures. Rather, it is defined by the instructions that accompany the components and prescribe how they are to be assembled.

To a large extent, the instructions needed to produce a multicellular animal are contained in the noncoding, regulatory DNA that is associated with each gene. As discussed in Chapter 4, each gene in a multicellular organism is associated with thousands or tens of thousands of nucleotides of noncoding DNA. This DNA may contain, scattered within it, dozens of separate regulatory elements or *enhancers*—short DNA segments that serve as binding sites for specific complexes of gene regulatory proteins. Roughly speaking, as explained in Chapter 7, the presence of a given regulatory module of this sort leads to expression of the gene whenever the complex of proteins recognizing that segment of DNA is appropriately assembled in the cell (in some cases, an inhibition or a more complicated effect on gene expression is produced instead). If we could decipher the full set of regulatory modules associated with a gene, we would understand all the different molecular conditions under which the product of that gene is to be made. This regulatory DNA can therefore be said to define the sequential program of development: the rules for stepping from one state to the next, as the cells proliferate and read their positions in the embryo by reference to their surroundings, switching on new sets of genes according to the activities of the proteins that they currently contain (**Figure 22–4**). Variations in the proteins themselves do, of course, also contribute to the differences between species. But even if the set of proteins encoded in the genome remained completely unchanged, the variation in the regulatory DNA would be enough to generate radically different tissues and body structures.

When we compare animal species with similar body plans—different vertebrates such as a fish, a bird and a mammal, for example—we find that corresponding genes usually have similar sets of regulatory modules: the DNA sequences of many of the individual modules have been well conserved and are recognizably homologous in the different animals. The same is true if we compare different species of nematode worm, or different species of insect. But when we compare vertebrate regulatory regions with those of worm or fly, it is

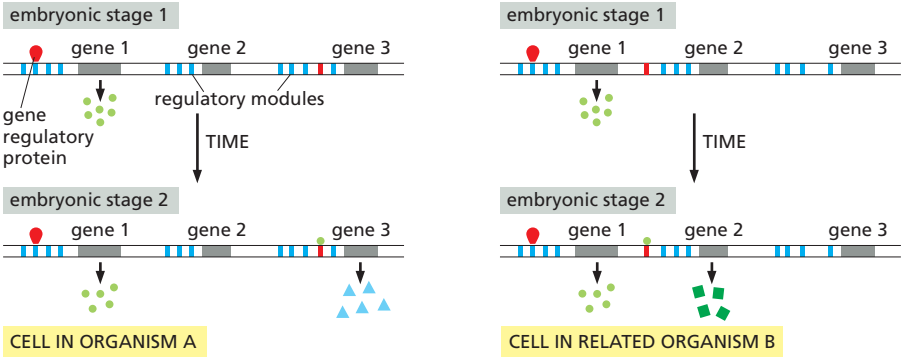


Figure 22–4 How regulatory DNA defines the succession of gene expression patterns in development. The genomes of organisms A and B code for the same set of proteins but have different regulatory DNA. The two cells in the cartoon start in the same state, expressing the same proteins at stage 1, but step to quite different states at stage 2 because of their different arrangements of regulatory modules.

hard to see any such resemblance. The protein-coding sequences are unmistakably similar, but the corresponding regulatory DNA sequences appear very different. This is the expected result if different body plans are produced mainly by changing the program embodied in the regulatory DNA, while retaining most of the same kit of proteins.

Manipulation of the Embryo Reveals the Interactions Between Its Cells

Confronted with an adult animal, in all its complexity, how does one begin to analyze the process that brought it into being? The first essential step is to describe the anatomical changes—the patterns of cell division, growth, and movement—that convert the egg into the mature organism. This is the job of *descriptive embryology*, and it is harder than one might think. To explain development in terms of cell behavior, we need to be able to track the individual cells through all their divisions, transformations, and migrations in the embryo. The foundations of descriptive embryology were laid in the nineteenth century, but the fine-grained task of *cell lineage tracing* continues to tax the ingenuity of developmental biologists (**Figure 22–5**)

Given a description, how can one go on to discover the causal mechanisms? Traditionally, *experimental embryologists* have tried to understand development in terms of the ways in which cells and tissues interact to generate the multicellular structure. *Developmental geneticists*, meanwhile, have tried to analyze development in terms of the actions of genes. These two approaches are complementary, and they have converged to produce our present understanding.

In experimental embryology, cells and tissues from developing animals are removed, rearranged, transplanted, or grown in isolation, in order to discover how they influence one another. The results are often startling: an early embryo cut in half, for example, may yield two complete and perfectly formed animals, or a small piece of tissue transplanted to a new site may reorganize the whole structure of the developing body (**Figure 22–6**). Observations of this type can be

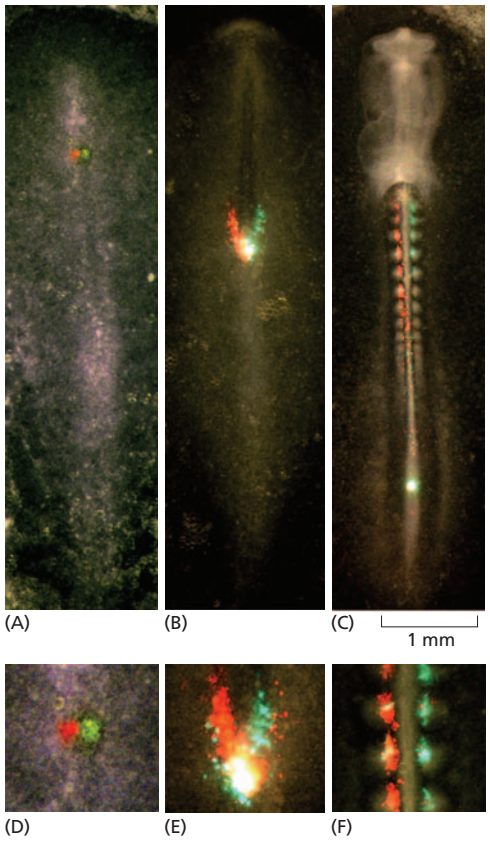


Figure 22–5 Cell lineage tracing in the early chick embryo. The pictures in the top row are at low magnification and show the whole embryo; the pictures below are details, showing the distribution of labeled cells. The tracing experiment reveals complex and dramatic cell rearrangements. (A,D) Two tiny dots of fluorescent dye, one *red,* the other *green,* have been used to stain small groups of cells in an embryo at 20 hours of incubation. Though the embryo still appears as an almost featureless sheet of tissue, there is already some specialization. The dots have been placed on each side of a structure called the node. (B,E) Six hours later, some of the labeled cells have remained at the node (which has moved backwards), giving a bright spot of fluorescence there, while other cells have begun to move forwards relative to the node. (C,F) After a further 8 hours, the body plan is clearly visible, with a head at the front end *(top),* a central axis, and rows of embryonic body segments, called somites, on either side of this. The node has regressed still further tailwards; some of the originally labeled cells have stayed in the node, forming a bright spot of fluorescence, while others have migrated to positions far anterior to this and become parts of somites. (Courtesy of Raquel Mendes and Leonor Saúde.)

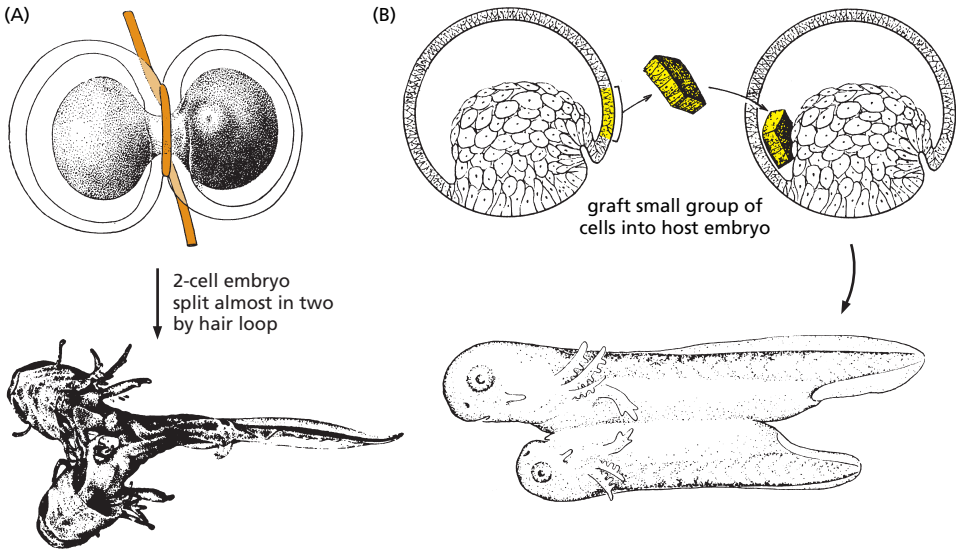


Figure 22–6 Some striking results obtained by experimental embryology. <ATTG> In (A), an early amphibian embryo is split almost into two parts with a hair loop. In (B), an amphibian embryo at a somewhat later stage receives a graft of a small cluster of cells from another embryo at that stage. The two quite different operations both cause a single embryo to develop into a pair of conjoined (Siamese) twins. It is also possible in experiment (A) to split the early embryo into two completely separate halves; two entire separate well-formed tadpoles are then produced. (A, after H. Spemann, Embryonic Development and Induction. New Haven: Yale University Press, 1938; B, after J. Holtfreter and V. Hamburger, in Analysis of Development [B.H. Willier, P.A. Weiss and V. Hamburger, eds.], pp. 230–296. Philadelphia: Saunders, 1955.)

extended and refined to decipher the underlying cell–cell interactions and rules of cell behavior. The experiments are easiest to perform in large embryos that are readily accessible for microsurgery. Thus, the most widely used species have been birds—especially the chick—and amphibians—particularly the African frog *Xenopus laevis*.

Studies of Mutant Animals Identify the Genes That Control Developmental Processes

Developmental genetics begins with the isolation of mutant animals whose development is abnormal. This typically involves a *genetic screen*, as described in Chapter 8. Parent animals are treated with a chemical mutagen or ionizing radiation to induce mutations in their germ cells, and large numbers of their progeny are examined. Those rare mutant individuals that show some interesting developmental abnormality—altered development of the eye, for example—are picked out for further study. In this way, it is possible to discover genes that are required specifically for the normal development of any chosen feature. By cloning and sequencing a gene found in this way, it is possible to identify its protein product, to investigate how it works, and to begin an analysis of the regulatory DNA that controls its expression.

The genetic approach is easiest in small animals with short generation times that can be grown in the laboratory. The first animal to be studied in this way was the fruit fly *Drosophila melanogaster,* which will be discussed at length below. But the same approach has been successful in the nematode worm, *Caenorhabditis elegans,* the zebrafish, *Danio rerio,* and the mouse, *Mus musculus*. Although humans are not intentionally mutagenized, they get screened for abnormalities in enormous numbers through the medical care system. Many mutations have arisen in humans that cause abnormalities compatible with life, and analyses of the affected individuals and of their cells have provided important insights into developmental processes.

A Cell Makes Developmental Decisions Long Before It Shows a Visible Change

By simply watching closely, or with the help of tracer dyes and other cell-marking techniques, one can discover what the fate of a given cell in an embryo will be if that embryo is left to develop normally. The cell may be fated to die, for example, or to become a neuron, to form part of an organ such as the foot, or to give progeny cells scattered all over the body. To know the **cell fate**, in this sense, however, is to know next to nothing about the cell's intrinsic character. At one

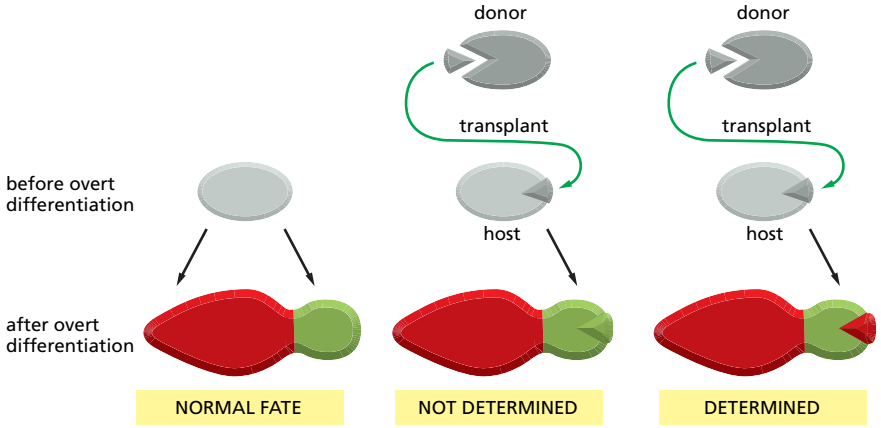


Figure 22–7 The standard test for cell determination.

extreme, the cell that is fated to become, say, a neuron may be already specialized in a way that guarantees that it will become a neuron no matter how its surroundings are disturbed; such a cell is said to be **determined** for its fate. At the opposite extreme, the cell may be biochemically identical to other cells destined for other fates, the only difference between them being the accident of position, which exposes the cells to different future influences.

A cell's state of determination can be tested by transplanting it to altered environments (**Figure 22–7**). One of the key conclusions of experimental embryology has been that, thanks to cell memory, a cell can become determined long before it shows any obvious outward sign of differentiation.

Between the extremes of the fully determined and the completely undetermined cell, there is a whole spectrum of possibilities. A cell may, for example, be already somewhat specialized for its normal fate, with a strong tendency to develop in that direction, but still able to change and undergo a different fate if it is put in a sufficiently coercive environment. (Some developmental biologists would describe such a cell as *specified* or *committed*, but not yet determined.) Or the cell may be determined, say, as a brain cell, but not yet determined as to whether it is to be a neuronal or a glial component of the brain. And often, it seems, adjacent cells of the same type interact and depend on mutual support to maintain their specialized character, so that they will behave as determined if kept together in a cluster, but not if taken singly and isolated from their usual companions.

Cells Have Remembered Positional Values That Reflect Their Location in the Body

In many systems, long before cells become committed to differentiating as a specific cell type, they become *regionally determined*: that is, they switch on and maintain expression of genes that can best be regarded as markers of position or region in the body. This position-specific character of a cell is called its **positional value**, and it shows its effects in the way the cell behaves in subsequent steps of pattern formation.

The development of the chick leg and wing provides a striking example. The leg and the wing of the adult both consist of muscle, bone, skin, and so on—almost exactly the same range of differentiated tissues. The difference between the two limbs lies not in the types of tissues, but in the way in which those tissues are arranged in space. So how does the difference come about?

In the chick embryo the leg and the wing originate at about the same time in the form of small tongue-shaped buds projecting from the flank. The cells in the two pairs of limb buds appear similar and uniformly undifferentiated at first. But a simple experiment shows that this appearance of similarity is deceptive. A small block of undifferentiated tissue at the base of the leg bud, from the region that would normally give rise to part of the thigh, can be cut out and grafted into the tip of the wing bud. Remarkably, the graft forms not the appropriate part of the wing tip, nor a misplaced piece of thigh tissue, but a toe (**Figure 22–8**). This

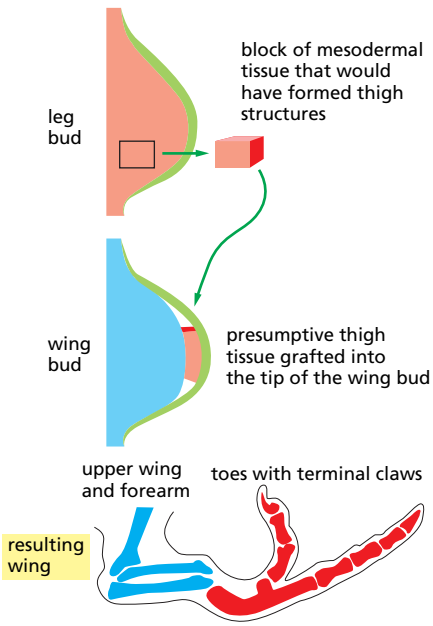


Figure 22–8 Prospective thigh tissue grafted into the tip of a chick wing bud forms toes. (After J.W. Saunders et al., *Dev. Biol.* 1:281–301, 1959. With permission from Academic Press.)

Figure 22–9 Chick embryos at 6 days of incubation, showing the limb buds stained by *in situ* hybridization with probes to detect expression of the *Tbx4, Tbx5,* and *Pitx1* genes, all coding for related gene regulatory proteins. The cells expressing *Tbx5* will form a wing; those expressing *Tbx4* and *Pitx1* will form a leg. *Pitx1*, when artificially misexpressed in the wing bud, causes the limb to develop with leg-like characteristics. (Courtesy of Malcolm Logan.)

experiment shows that the early leg-bud cells are already determined as leg but are not yet irrevocably committed to form a particular part of the leg: they can still respond to cues in the wing bud so that they form structures appropriate to the tip of the limb rather than the base. The signaling system that controls the differences between the parts of the limb is apparently the same for leg and wing. The difference between the two limbs results from a difference in the internal states of their cells at the outset of limb development.

The difference of positional value between vertebrate forelimb cells and hindlimb cells corresponds to expression of different sets of genes, coding for gene regulatory proteins that are thought to make the cells in the two limb buds behave differently (**Figure 22–9**).Later in this chapter we shall explain how the next, more detailed level of patterning is set up inside an individual limb bud.

Inductive Signals Can Create Orderly Differences Between Initially Identical Cells

At each stage in its development, a cell in an embryo is presented with a limited set of options according to the state it has attained: the cell travels along a developmental pathway that branches repeatedly. At each branch in the pathway it has to make a choice, and its sequence of choices determines its final destiny. In this way, a complicated array of different cell types is produced.

To understand development, we need to know how each choice between options is controlled, and how those options depend on the choices made previously. To reduce the question to its simplest form: how do two cells with the same genome, but separated in space, come to be different?

The most straightforward way to make cells different is by exposing them to different environments, and the most important environmental cues acting on cells in an embryo are signals from neighboring cells. Thus, in what is probably the commonest mode of pattern formation, a group of cells start out all having the same developmental potential, and a signal from cells outside the group then drives one or more of the members of the group into a different developmental pathway, leading to a changed character. This process is called an **inductive interaction**. Generally, the signal is limited in time and space so that only a subset of the competent cells—those closest to the source of the signal—take on the induced character (**Figure 22–10**).

Some inductive signals are short-range—notably those transmitted via cell–cell contacts; others are long-range, mediated by molecules that can diffuse through the extracellular medium. The group of initially similar cells competent to respond to the signal is sometimes called an *equivalence group* or a *morphogenetic field*. It can consist of as few as two cells or as many as thousands, and any number of the total can be induced depending on the amount and distribution of the signal.

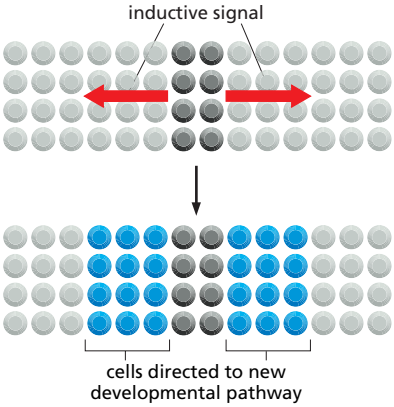


Figure 22–10 Inductive signaling.

Sister Cells Can Be Born Different by an Asymmetric Cell Division

Cell diversification does not always have to depend on extracellular signals: in some cases, sister cells are born different as a result of an **asymmetric cell division**, in which some significant set of molecules is divided unequally between

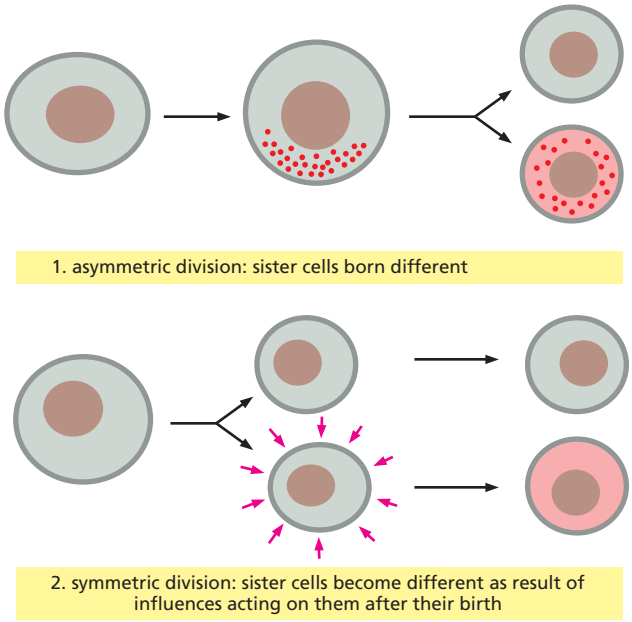


Figure 22–11 Two ways of making sister cells different.

the two of them at the time of division. This asymmetrically segregated molecule (or set of molecules) then acts as a *determinant* for one of the cell fates by directly or indirectly altering the pattern of gene expression within the daughter cell that receives it (**Figure 22–11**).

Asymmetric divisions often occur at the beginning of development, when the fertilized egg divides to give daughter cells with different fates, but they are also encountered at some later stages—in the genesis of nerve cells, for example.

Positive Feedback Can Create Asymmetry Where There Was None Before

Inductive signaling and asymmetric cell division represent two distinct strategies for creating differences between cells. Both of them, however, presuppose some prior asymmetry in the system: the source of inductive signal must be localized so that some cells receive the signal strongly and others do not; or the mother cell must already have an internal asymmetry before she divides. Very often, the history of the system ensures that some such asymmetry will be present. But what if it is not, or if the initial asymmetry is only very slight?

The answer lies in **positive feedback**: through positive feedback, a system that starts off homogeneous and symmetrical can pattern itself spontaneously, even where there is no organized external signal at all. And where, as very often happens, the environment or the starting conditions impose some weak but definite initial asymmetry, positive feedback provides the means to magnify the effect and create a full-blown pattern.

To illustrate the idea, consider a pair of adjacent cells that start off in a similar state and can exchange signals to influence one another's behavior (**Figure 22–12**). The more that either cell produces of some product X, the more it signals to its neighbor to inhibit production of X by the neighbor. This type of cell–cell interaction is called *lateral inhibition*, and it gives rise to a positive feedback loop that tends to amplify any initial difference between the two cells. Such a difference may arise from a bias imposed by some external or prior factor, or it may simply originate from spontaneous random fluctuations, or "noise"—an inevitable feature of the genetic control circuitry in cells, as discussed in Chapter 7. In either case, lateral inhibition means that if cell #1 makes a little more of X, it will thereby cause cell #2 to make less; and because cell #2 makes less X, it delivers less inhibition to cell #1 and so allows the amount of X in cell #1 to rise higher still; and so on, until a steady state is reached where cell #1 contains a lot of X and cell #2 contains very little.

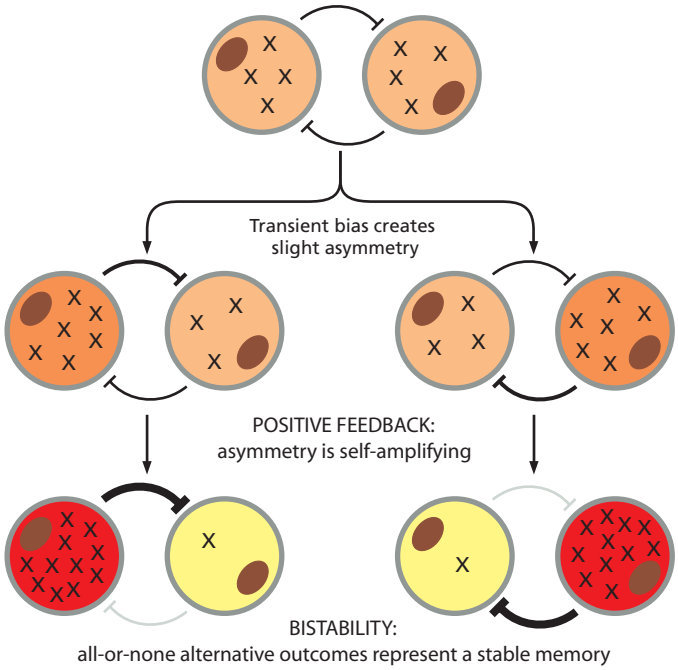


Figure 22–12 Genesis of asymmetry through positive feedback. In this example, two cells interact, each producing a substance X that acts on the other cell to inhibit its production of X, an effect known as lateral inhibition. An increase of X in one of the cells leads to a positive feedback that tends to increase X in that cell still further, while decreasing X in its neighbor. This can create a runaway instability, making the two cells become radically different. Ultimately the system comes to rest in one or the other of two opposite stable states. The final choice of state represents a form of memory: the small influence that initially directed the choice is no longer required to maintain it.

Mathematical analysis shows that this phenomenon depends on the strength of the lateral inhibition effect: if it is too weak, fluctuations will fade and have no lasting effect; but if it is strong enough and steep enough, they will be self-amplifying in a runaway fashion, breaking the initial symmetry between the two cells. Lateral inhibition, often mediated by exchange of signals at cell–cell contacts via the Notch signaling pathway (as discussed in Chapter 15), is a common mechanism for cell diversification in animal tissues, driving neighboring cells to specialize in different ways.

Positive Feedback Generates Patterns, Creates All-or-none Outcomes, and Provides Memory

Somewhat similar positive feedback processes can operate over larger arrays of cells to create many types of spatial patterns. For example, a substance A (a short-range activator) may stimulate its own production in the cells that contain it and their immediate neighbors, while also causing them to produce a signal H (a long-range inhibitor) that diffuses widely and inhibits production of A in the cells at larger distances. If the cells all start out on an equal footing, but one group of cells gains a slight advantage by making a little more A than the rest, the asymmetry can be self-amplifying. Short-range activation combined with long-range inhibition in this way may account for the formation of clusters of cells within an initially homogeneous tissue that become specialized as localized *signaling centers*.

At the opposite end of the size spectrum, positive feedback can also be the means by which an individual cell becomes spontaneously polarized and internally asymmetrical, through systems of intracellular signals that make a weak initial asymmetry self-amplifying.

Through all these and many other variations on the theme of positive feedback, certain general principles apply. In each of the above examples, the positive feedback leads to *broken symmetry*, and the symmetry-breaking is an *all-or-none* phenomenon. If the feedback is below a certain threshold strength, the cells remain essentially the same; if the feedback is above the threshold, they become sharply different. Above this threshold, the system is *bistable* or *multistable*—it lurches toward one or other of two or more sharply different outcomes, according to which of the cells (or which of the ends of the single cell) gains the initial advantage.

The choice between the alternative outcomes can be dictated by an external signal that gives one of the cells a small initial advantage. But once the positive

feedback has done its work, this external signal becomes irrelevant. The broken symmetry, once established, is very hard to reverse: positive feedback makes the chosen asymmetric state self-sustaining, even after the biasing signal has disappeared. In this way, positive feedback provides the system with a *memory* of past signals.

All these effects of positive feedback—symmetry-breaking, all-or-none outcomes, bistability, and memory—go hand in hand and are encountered again and again in developing organisms. They are fundamental to the production of sharply delineated, stable patterns of cells in different states.

A Small Set of Signaling Pathways, Used Repeatedly, Controls Developmental Patterning

What, then, are the molecules that act as signals to coordinate spatial patterning in an embryo, either to create asymmetry *de novo,* or as inducers from established signaling centers to control the diversification of neighboring cells? In principle, any kind of extracellular molecule could serve. In practice, most of the known inductive events in animal development are governed by just a handful of highly conserved families of signal proteins, which are used over and over again in different contexts. The discovery of this limited vocabulary that cells use for developmental communications has emerged over the past 10 or 20 years as one of the great simplifying discoveries of developmental biology. In **Table 22–1**, we briefly review six major families of signal proteins that serve repeatedly as inducers in animal development. Details of the intracellular mechanisms through which these molecules act are given in Chapter 15.

The ultimate result of most inductive events is a change in DNA transcription in the responding cell: some genes are turned on and others are turned off. Different signaling molecules activate different kinds of gene regulatory proteins. Moreover, the effect of activating a given gene regulatory protein will depend on which other gene regulatory proteins are also present in the cell, since these generally function in combinations. As a result, different types of cells will generally respond differently to the same signal, and the same cells will often respond differently to the same signal given at a different time. The response will depend both on the other gene regulatory proteins that are present before the signal arrives—reflecting the cell's memory of signals received previously—and on the other signals that the cell is receiving concurrently.

Morphogens Are Long-Range Inducers That Exert Graded Effects

Signal molecules often seem to govern a simple yes–no choice: one outcome when their concentration is high, another when it is low. Positive feedback can

Table 22–1 Some Signal Proteins That Are Used Over and Over Again as Inducers in Animal Development

SIGNALING PATHWAY	LIGAND FAMIILY	RECEPTOR FAMILY	EXTRACELLULAR INHIBITORS/MODULATORS
Receptor tyrosine kinase (RTK)	EGF	EGF receptors	Argos
	FGF (Branchless)	FGF receptors (Breathless)	
	Ephrins	Eph receptors	
TGFβ superfamily	TGFβ	TGFβ receptors	chordin (Sog), noggin
	BMP (Dpp)	BMP receptors	
	Nodal		
Wnt	Wnt (Wingless)	Frizzled	Dickkopf, Cerberus
Hedgehog	Hedgehog	Patched, Smoothened	
Notch	Delta	Notch	Fringe

Only a few representatives of each class of proteins are listed—mainly those mentioned in this chapter. Names peculiar to *Drosophila* are shown in parentheses. Many of the listed components have several homologs distinguished by numbers (FGF1, FGF2, etc.) or by forenames (Sonic hedgehog, Lunatic fringe). Other signaling pathways, including the JAK/STAT, nuclear hormone receptor, and G-protein-coupled receptor pathways, also play important parts in some developmental processes.

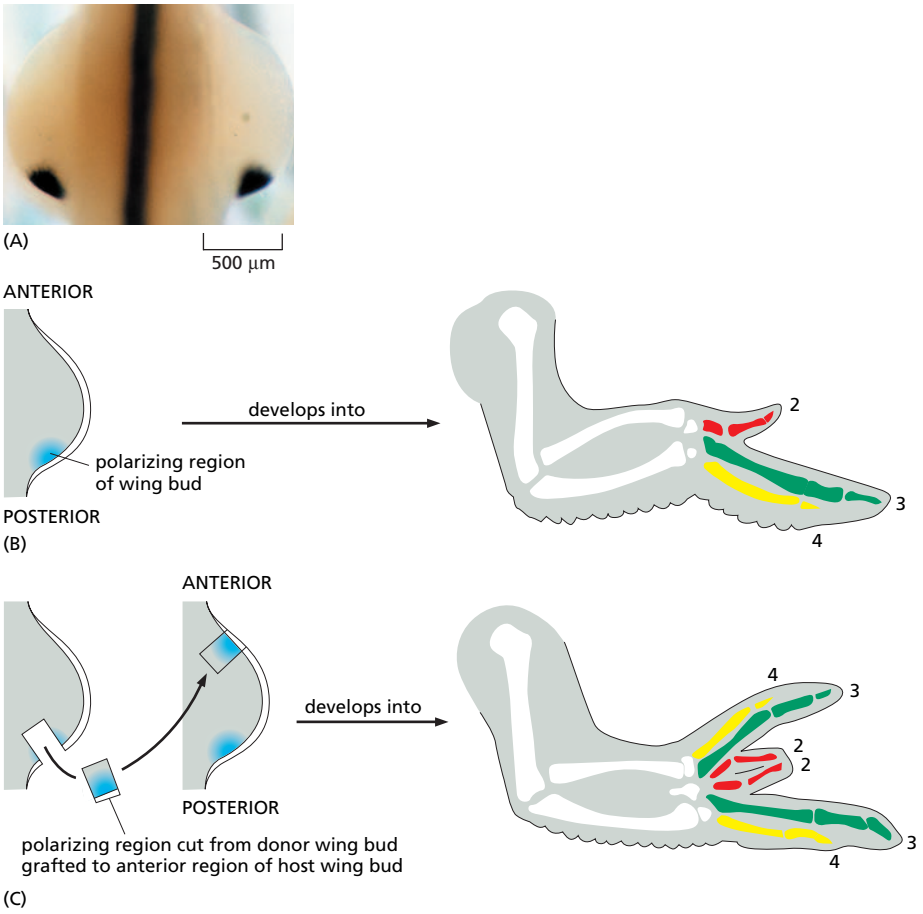


Figure 22–13 Sonic hedgehog as a morphogen in chick limb development. (A) Expression of the Sonic hedgehog gene in a 4-day chick embryo, shown by *in situ* hybridization (dorsal view of the trunk at the level of the wing buds). The gene is expressed in the midline of the body and at the posterior border (the polarizing region) of each of the two wing buds. Sonic hedgehog protein spreads out from these sources. (B) Normal wing development. (C) A graft of tissue from the polarizing region causes a mirror-image duplication of the pattern of the host wing. The type of digit that develops is thought to be dictated by the local concentration of Sonic hedgehog protein; different types of digit (labeled 2, 3, and 4) therefore form according to their distance from a source of Sonic hedgehog. (A, courtesy of Randall S. Johnson and Robert D. Riddle.)

make the cellular responses all-or-none, so that one result is obtained when the signal is below a certain critical strength, and another result when it is above that strength. In many cases, however, responses are more finely graded: a high concentration may, for example, direct target cells into one developmental pathway, an intermediate concentration into another, and a low concentration into yet another. An important case is that in which the signal molecule diffuses out from a localized signaling center, creating a signal concentration gradient. Cells at different distances from the source are driven to behave in a variety of different ways, according to the signal concentration that they experience.

A signal molecule that imposes a pattern on a whole field of cells in this way is called a **morphogen**. Vertebrate limbs provide a striking example: a group of cells at one side of the embryonic limb bud become specialized as a signaling center and secrete Sonic hedgehog protein—a member of the Hedgehog family of signal molecules. This protein spreads out from its source, forming a *morphogen gradient* that controls the characters of the cells along the thumb-to-little-finger axis of the limb bud. If an additional group of signaling cells is grafted into the opposite side of the bud, a mirror duplication of the pattern of digits is produced (**Figure 22–13**).

Extracellular Inhibitors of Signal Molecules Shape the Response to the Inducer

Especially for molecules that can act at a distance, it is important to limit the action of the signal, as well as to produce it. Most developmental signal proteins have extracellular antagonists that can inhibit their function. These antagonists are generally proteins that bind to the signal or its receptor, preventing a productive interaction from taking place.

A surprisingly large number of developmental decisions are actually regulated by inhibitors rather than by the primary signal molecule. The nervous system in a

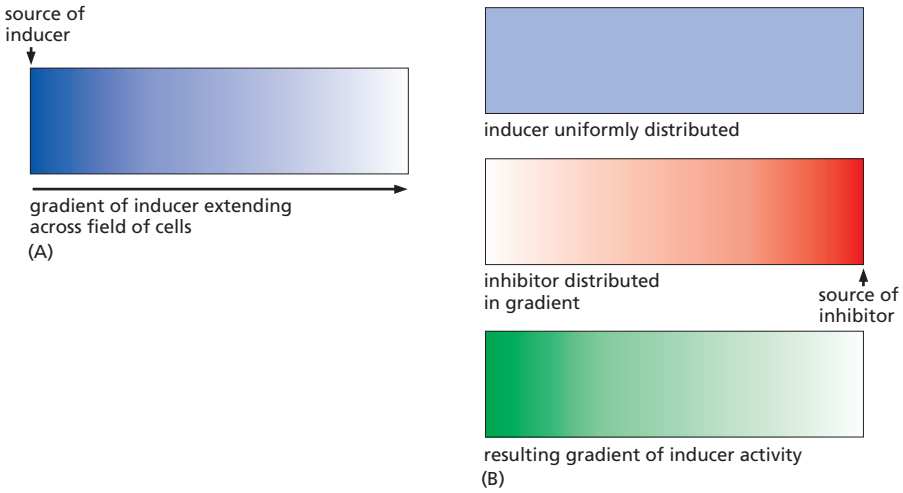


Figure 22–14 Two ways to create a morphogen gradient. (A) By localized production of an inducer—a morphogen—that diffuses away from its source. (B) By localized production of an inhibitor that diffuses away from its source and blocks the action of a uniformly distributed inducer.

frog embryo arises from a field of cells that is competent to form either neural or epidermal tissue. An inducing tissue releases the protein chordin, which favors the formation of neural tissue. Chordin does not have its own receptor. Instead it is an inhibitor of signal proteins of the BMP/TGFβ family, which induce epidermal development and are present throughout the neuroepithelial region where neurons and epidermis form. The induction of neural tissue is thus due to an inhibitory gradient of an antagonistic signal (**Figure 22–14**).

Developmental Signals Can Spread Through Tissue in Several Different Ways

Many developmental signals are thought to spread through tissues by simple diffusion through the spaces between cells. If some specialized group of cells produces a signal molecule at a steady rate, and this morphogen is then degraded as it diffuses away from this source, a smooth gradient will be set up, with its maximum at the source. The speed of diffusion and the half-life of the morphogen will together determine the steepness of the gradient (**Figure 22–15**).

This simple mechanism can be modified in many ways to adjust the shape and steepness of the gradient. Receptors on the surfaces of cells along the way may trap the diffusing morphogen and cause it to be endocytosed and degraded, shortening its effective halflife. Or it may bind to molecules in the extracellular matrix, reducing its effective diffusion rate. In some cases, it seems

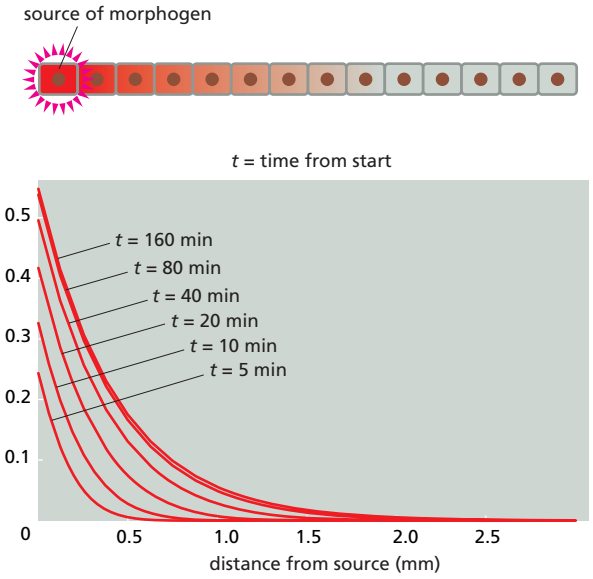


Figure 22–15 Setting up a signal gradient by diffusion. The graphs show successive stages in the build-up of the concentration of a signal molecule that is produced at a steady rate at the origin, with production starting at time 0. The molecule undergoes degradation as it diffuses away from the source, creating a concentration gradient with its peak at the source. The graphs are calculated on the assumption that diffusion is occurring along one axis in space, that the molecule has a half-life $t_{1/2}$ of 20 minutes, and that it diffuses with a diffusion constant $D = 0.4\ mm^2\ hr^{-1}$, typical of a small (30 kilodalton) protein molecule in water. Note that the gradient is already close to its steady-state form within an hour, and that the concentration at steady state (large times) falls off exponentially with distance.

that a morphogen is taken up into cells by endocytosis and then disgorged, only to be taken up and then disgorged by other cells in turn, so that the signal spreads through a largely intracellular route.

Yet another mechanism for signal distribution depends on long thin filopodia or *cytonemes* that extend over several cell diameters from cells in some epithelial tissues. A cell may send out cytonemes to make contact with distant cells, either to deliver or to receive signals from them. In this way, for example, a cell can deliver lateral inhibition via the Notch pathway to an extended set of neighbors.

Programs That Are Intrinsic to a Cell Often Define the Time-Course of its Development

Signals such as those we have just discussed play a large part in controlling the timing of events in development, but it would be wrong to imagine that every developmental change needs an inductive signal to trigger it. Many of the mechanisms that alter cell character are intrinsic to the cell and require no cue from the cell's surroundings: the cell will step through its developmental program even when kept in a constant environment. There are numerous cases where one might suspect that something of this sort is occurring to control the duration of a developmental process. For example, in a mouse, the neural progenitor cells in the cerebral cortex of the brain carry on dividing and generating neurons for just 11 cell cycles, and in a monkey for approximately 28 cycles, after which they stop. Different kinds of neurons are generated at different stages in this program, suggesting that as the progenitor cell ages, it changes the specifications that it supplies to the differentiating progeny cells.

It is difficult to prove in the context of the intact embryo that such a course of events is strictly the result of a cell-autonomous timekeeping process, since the cell environment is changing. Experiments on cells in culture, however, give clear-cut evidence. For example, glial progenitor cells isolated from the optic nerve of a 7-day postnatal rat and cultured under constant conditions in an appropriate medium will carry on proliferating for a strictly limited time (corresponding to a maximum of about eight cell division cycles) and then differentiate into oligodendrocytes (the glial cells that form myelin sheaths around axons in the brain), obeying a timetable similar to the one that they would have followed if they had been left in place in the embryo.

The molecular mechanisms underlying such slow changes in the internal states of cells, played out over days, weeks, months or even years, are still unknown. One possibility is that they reflect progressive changes in the state of the chromatin (discussed in Chapter 4).

The mechanisms that control the timing of more rapid processes, though still poorly understood, are not quite such a mystery. Later, we shall discuss an example—the gene expression oscillator, known as the *segmentation clock*, that governs formation of the somites in vertebrate embryos—the rudiments of the series of vertebrae, ribs, and associated muscles.

Initial Patterns Are Established in Small Fields of Cells and Refined by Sequential Induction as the Embryo Grows

The signals that organize the spatial pattern of an embryo generally act over short distances and govern relatively simple choices. A morphogen, for example, typically acts over a distance of less than 1 mm—an effective range for diffusion (see Figure 22–15)—and directs choices between no more than a handful of developmental options for the cells on which it acts. But the organs that eventually develop are much larger and more complex than this.

The cell proliferation that follows the initial specification accounts for the size increase, while the refinement of the initial pattern is explained by a series of local inductions that embroider successive levels of detail on an initially simple sketch. As soon as two sorts of cells are present, one of them can produce a

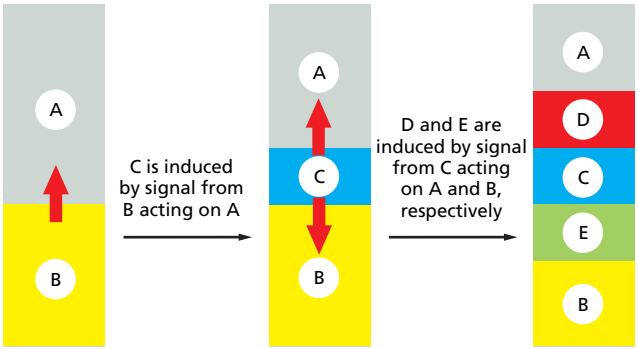


Figure 22–16 Patterning by sequential induction. A series of inductive interactions can generate many types of cells, starting from only a few.

factor that induces a subset of the neighboring cells to specialize in a third way. The third cell type can in turn signal back to the other two cell types nearby, generating a fourth and a fifth cell type, and so on (**Figure 22–16**).

This strategy for generating a progressively more complicated pattern is called **sequential induction**. It is chiefly through sequential inductions that the body plan of a developing animal, after being first roughed out in miniature, becomes elaborated with finer and finer details as development proceeds.

In the sections that follow, we focus on a small selection of model organisms to see how the principles that we have outlined in this first section operate in practice. We begin with the nematode worm, *Caenorhabditis elegans*.

Summary

The obvious changes of cell behavior that we see as a multicellular organism develops are the outward signs of a complex molecular computation, dependent on cell memory, that is taking place inside the cells as they receive and process signals from their neighbors and emit signals in return. The final pattern of differentiated cell types is thus the outcome of a more hidden program of cell specialization—a program played out in the changing patterns of expression of gene regulatory proteins, giving one cell different potentialities from another long before terminal differentiation begins. Developmental biologists seek to decipher the hidden program and to relate it, through genetic and microsurgical experiments, to the signals the cells exchange as they proliferate, interact, and move.

Animals as different as worms, flies, and humans use remarkably similar sets of proteins to control their development, so that what we discover in one organism very often gives insight into the others. A handful of evolutionarily conserved cell–cell signaling pathways are used repeatedly, in different organisms and at different times, to regulate the creation of an organized multicellular pattern. Differences of body plan seem to arise to a large extent from differences in the regulatory DNA associated with each gene. This DNA has a central role in defining the sequential program of development, calling genes into action at specific times and places according to the pattern of gene expression that was present in each cell at the previous developmental stage.

Differences between cells in an embryo arise in various ways. Positive feedback can lead to broken symmetry, creating a radical and permanent difference between cells that are initially almost identical. Sister cells can be born different as a result of an asymmetric cell division. Or a group of initially similar cells may receive different exposures to inductive signals from cells outside the group; long-range inducers with graded effects, called morphogens, can organize a complex pattern. Through cell memory, such transient signals can have a lasting effect on the internal state of a cell, causing it, for example, to become determined for a specific fate. In these ways, sequences of simple signals acting at different times and places in growing cell arrays give rise to the intricate and varied multicellular organisms that fill the world around us.

CAENORHABDITIS ELEGANS: DEVELOPMENT FROM THE PERSPECTIVE OF THE INDIVIDUAL CELL

The nematode worm *Caenorhabditis elegans* is a small, relatively simple, and precisely structured organism. The anatomy of its development has been described in extraordinary detail, and one can map out the exact lineage of every cell in the body. Its complete genome sequence is also known, and large numbers of mutant phenotypes have been analyzed to determine gene functions. If there is any multicellular animal whose development we should be able to understand in terms of genetic control, this is it.

DNA sequence comparisons indicate that, while the lineages leading to nematodes, insects, and vertebrates diverged from one another at about the same time, the rate of evolutionary change in the nematode lineage has been substantially greater: its genes, its body structure, and its developmental strategies are more divergent from our own than are those of *Drosophila.* Nevertheless, at a molecular level many of its developmental mechanisms are similar to those of insects or vertebrates, and governed by homologous systems of genes. If one wants to know how an eye, a limb, or a heart develops, one must look elsewhere: *C. elegans* lacks these organs. But at a more fundamental level, it is highly instructive: it poses the basic general questions of animal development in a relatively simple form, and it lets us answer them in terms of gene functions and the behavior of individual, identified cells.

Caenorhabditis elegans Is Anatomically Simple

As an adult, *C. elegans* consists of only about 1000 somatic cells and 1000–2000 germ cells (exactly 959 somatic cell nuclei plus about 2000 germ cells in one sex; exactly 1031 somatic cell nuclei plus about 1000 germ cells in the other) (**Figure 22–17**). The anatomy has been reconstructed, cell by cell, by electron microscopy of serial sections. The body plan of the worm is simple: it has a roughly bilaterally symmetrical, elongate body composed of the same basic tissues as in other animals (nerve, muscle, gut, skin), organized with mouth and brain at the anterior end and anus at the posterior. The outer body wall is composed of two layers: the protective epidermis, or "skin," and the underlying muscular layer. A tube of endodermal cells forms the intestine. A second tube, located between the intestine and the body wall, constitutes the gonad; its wall is composed of somatic cells, with the germ cells inside it.

C. elegans has two sexes—a hermaphrodite and a male. The hermaphrodite can be viewed most simply as a female that produces a limited number of sperm: she can reproduce either by self-fertilization, using her own sperm, or by cross-fertilization after transfer of male sperm by mating. Self-fertilization allows a single heterozygous worm to produce homozygous progeny. This is an important feature that helps to make *C. elegans* an exceptionally convenient organism for genetic studies.

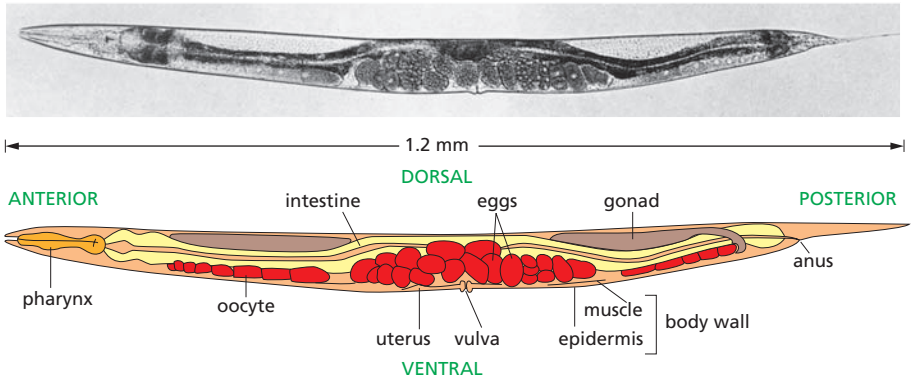
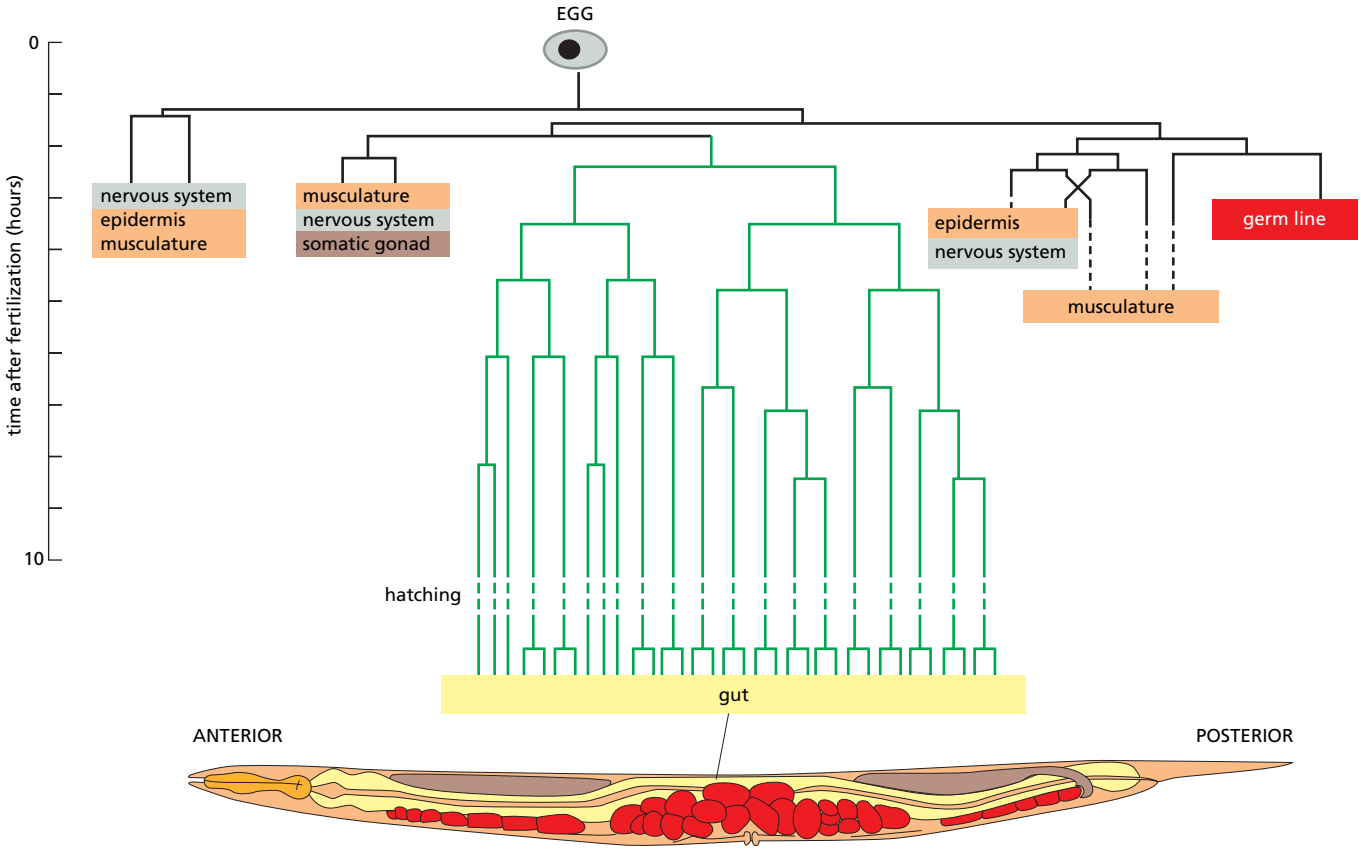


Figure 22–17 ***Caenorhabditis elegans.*** A side view of an adult hermaphrodite is shown. (From J.E. Sulston and H.R. Horvitz, *Dev. Biol.* 56:110–156, 1977. With permission from Academic Press.)



Cell Fates in the Developing Nematode Are Almost Perfectly Predictable

C. elegans begins life as a single cell, the fertilized egg, which gives rise, through repeated cell divisions, to 558 cells that form a small worm inside the egg shell. After hatching, further divisions result in the growth and sexual maturation of the worm as it passes through four successive larval stages separated by molts. After the final molt to the adult stage, the hermaphrodite worm begins to produce its own eggs. The entire developmental sequence, from egg to egg, takes only about three days.

The lineage of all of the cells from the single-cell egg to the multicellular adult was mapped out by direct observation of the developing animal. In the nematode, a given precursor cell follows the same pattern of cell divisions in every individual, and with very few exceptions the fate of each descendant cell can be predicted from its position in the lineage tree (**Figure 22–18**).

This degree of stereotyped precision is not seen in the development of larger animals. At first sight, it might seem to suggest that each cell lineage in the nematode embryo is rigidly and independently programmed to follow a set pattern of cell division and cell specialization, making the worm a woefully unrepresentative model organism for development. We shall see that this is far from true: as in other animals, development depends on cell–cell interactions as well as on processes internal to the individual cells. The outcome in the nematode is almost perfectly predictable simply because the pattern of cell–cell interactions is highly reproducible and is accurately correlated with the sequence of cell divisions.

In the developing worm, as in other animals, most cells do not become restricted to generate progeny cells of a single differentiated type until quite late in development, and cells of a particular type, such as muscle, usually derive from several spatially dispersed precursors that also give rise to other types of cells. The exceptions, in the worm, are the gut and the gonad, each of which forms from a single dedicated *founder cell*, born at the 8-cell stage of development for the gut-cell lineage and at the 16-cell stage for the germ-cell lineage, or

Figure 22–18 The lineage tree for the cells that form the gut (the intestine) of *C. elegans*. Note that although the intestinal cells form a single clone (as do the germ-line cells), the cells of most other tissues do not. Nerve cells (not shown in the drawing of the adult at the bottom) are mainly clustered in ganglia near the anterior and posterior ends of the animal and in a ventral nerve cord that runs the length of the body.

germ line. But in any case, cell diversification starts early, as soon as the egg begins to cleave: long before terminal differentiation, the cells begin to step through a series of intermediate states of specialization, following different programs according to their locations and their interactions with their neighbors. How do these early differences between cells arise?

Products of Maternal-Effect Genes Organize the Asymmetric Division of the Egg

The worm is typical of most animals in the early specification of the cells that will eventually give rise to the germ cells (eggs or sperm). The worm's germ line is produced by a strict series of asymmetric cell divisions of the fertilized egg. The asymmetry originates with a cue from the egg's environment: the sperm entry point defines the future posterior pole of the elongated egg. The proteins in the egg then interact with one another and organize themselves in relation to this point so as to create a more elaborate asymmetry in the interior of the cell. The proteins involved are mainly translated from the accumulated mRNA products of the genes of the mother. Because this RNA is made before the egg is laid, it is only the mother's genotype that dictates what happens in the first steps of development. Genes acting in this way are called **maternal-effect genes.**

A subset of maternal-effect genes are specifically required to organize the asymmetric pattern of the nematode egg. These are called *Par* (*Partitioning-defective*) genes, and at least six have been identified, through genetic screens for mutants where this pattern is disrupted. The *Par* genes have homologs in insects and vertebrates, where they play a fundamental part in the organization of cell polarity, as discussed in Chapter 19. In fact, one of the keys to our present understanding of the general mechanisms of cell polarity was the discovery of these genes through studies of early development in *C. elegans*.

In the nematode egg, as in other cells both in the nematode and other animals, the Par proteins (the products of the *Par* genes) are themselves asymmetrically located, some at one end of the cell and some at the other. They serve in the egg to bring a set of ribonucleoprotein particles called *P granules* to the posterior pole, so that the posterior daughter cell inherits P granules and the anterior daughter cell does not. Throughout the next few cell divisions, the Par proteins operate in a similar way, orienting the mitotic spindle and segregating the P granules to one daughter cell at each mitosis, until, at the 16-cell stage, there is just one cell that contains the P granules (**Figure 22–19**). This one cell gives rise to the germ line.

The specification of the germ-cell precursors as distinct from somatic-cell precursors is a key event in the development of practically every type of animal, and the process has common features even in phyla with very different body plans. Thus, in *Drosophila*, particles similar to P granules are also segregated

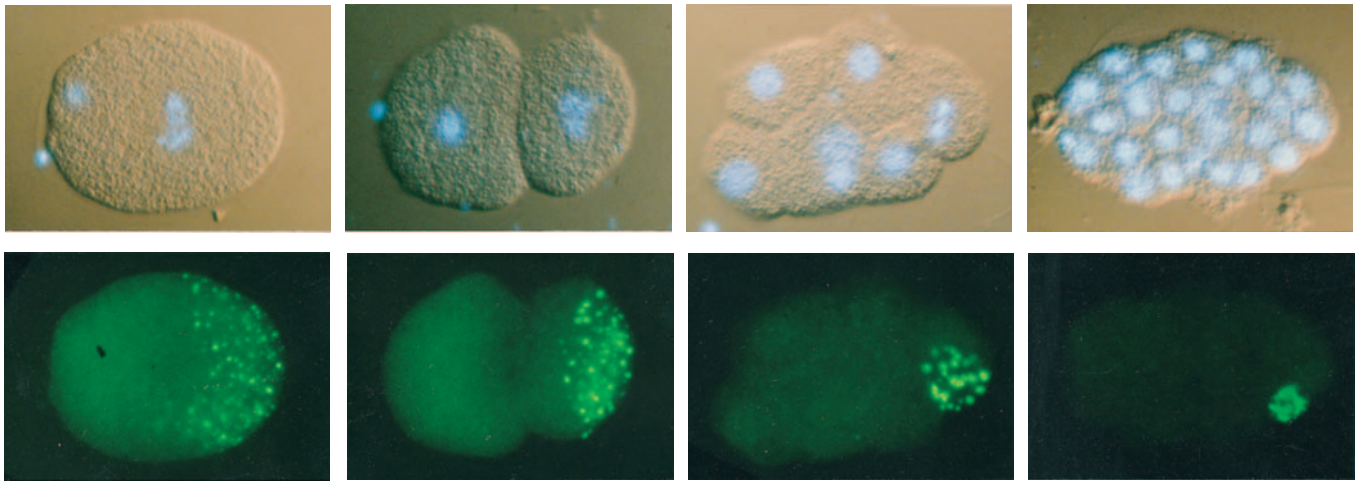


Figure 22–19 Asymmetric divisions segregating P granules into the founder cell of the *C. elegans* germ line. The micrographs in the upper row show the pattern of cell divisions, with cell nuclei stained *blue* with a DNA-specific fluorescent dye; below are the same cells stained with an antibody against P granules. These small granules (0.5–1 μm in diameter) are distributed randomly throughout the cytoplasm in the unfertilized egg (not shown). After fertilization, at each cell division up to the 16-cell stage, both they and the intracellular machinery that regulates their asymmetric localization are segregated into a single daughter cell. (Courtesy of Susan Strome.)

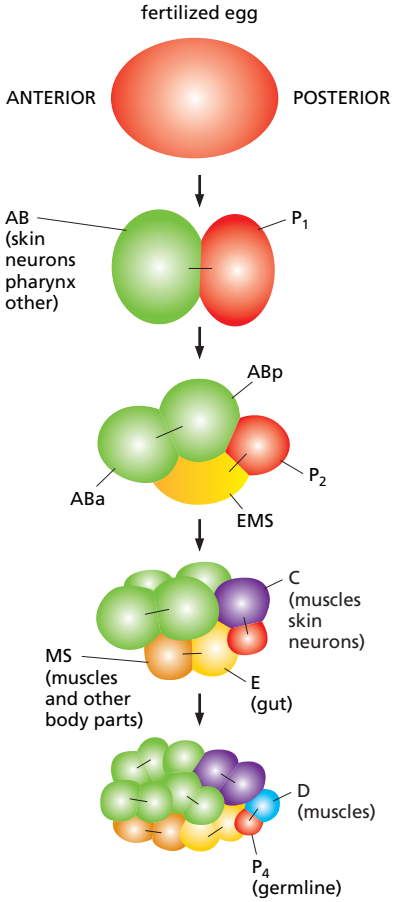


Figure 22–20 The pattern of cell divisions in the early *C. elegans* embryo, indicating the names and fates of the individual cells. Cells that are sisters are shown linked by a *short black line*. (After K. Kemphues, *Cell* 101:345–348, 2000. With permission from Elsevier.)

into one end of the egg, and become incorporated into the germ-line precursor cells to determine their fate. Similar phenomena occur in fish and frogs. In all these species, one can recognize at least some of the same proteins in the germ-cell-determining material, including homologs of an RNA-binding protein called Vasa. How Vasa and its associated proteins and RNA molecules act to define the germ line is still unknown.

Progressively More Complex Patterns Are Created by Cell–Cell Interactions

The egg, in *C. elegans* as in other animals, is an unusually big cell, with room for complex internal patterning. In addition to the P granules, other factors become distributed in an orderly way along its anteroposterior axis under the control of the Par proteins, and thus are allocated to different cells as the egg goes through its first few cell-division cycles. These divisions occur without growth (since feeding cannot begin until a mouth and a gut have formed) and therefore subdivide the egg into progressively smaller cells. Several of the localized factors are gene regulatory proteins, which act directly in the cell that inherits them to either drive or block the expression of specific genes, adding to the differences between that cell and its neighbors and committing it to a specialized fate.

While the first few differences between cells along the anteroposterior axis of *C. elegans* result from asymmetric divisions, further patterning, including the pattern of cell types along the other axes, depends on interactions between one cell and another. The cell lineages in the embryo are so reproducible that individual cells can be assigned names and identified in every animal (**Figure 22–20**); the cells at the four-cell stage, for example, are called ABa and ABp (the two anterior sister cells), and EMS and P_2 (the two posterior sister cells). As a result of the asymmetric divisions we have just described, the P_2 cell expresses a signal protein on its surface—a nematode homolog of the Notch ligand Delta—while the ABa and ABp cells express the corresponding transmembrane receptor—a homolog of Notch. The elongated shape of the eggshell forces these cells into an arrangement such that the most anterior cell, ABa, and the most posterior cell, P_2, are no longer in contact with one another. Thus only the ABp cell receives the signal from P_2, making ABp different from ABa and defining the future dorsal–ventral axis of the worm (**Figure 22–21**).

At the same time, P_2 also expresses another signal molecule, a Wnt protein, which acts on a Wnt receptor (a Frizzled protein) in the membrane of the EMS cell. This signal polarizes the EMS cell in relation to its site of contact with P_2, controlling the orientation of the mitotic spindle. The EMS cell then divides to give two daughters that become committed to different fates as a result of the Wnt signal from P_2. One daughter, the MS cell, will give rise to muscles and various other body parts; the other daughter, the E cell, is the founder cell for the gut, committed to give rise to all the cells of the gut and to no other tissues (see Figure 22–21).

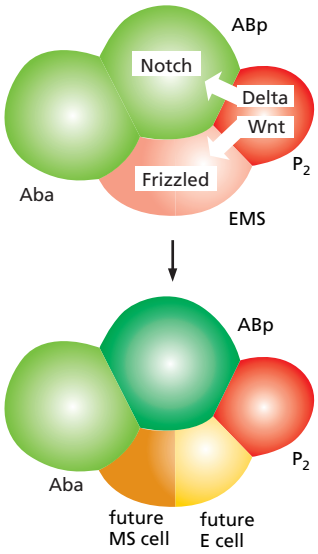


Figure 22–21 Cell signaling pathways controlling assignment of different characters to the cells in a four-cell nematode embryo. The P_2 cell uses the Notch signaling pathway to send an inductive signal to the ABp cell, causing this to adopt a specialized character. The ABa cell has all the molecular apparatus to respond in the same way to the same signal, but it does not do so because it is out of contact with P_2. Meanwhile, a Wnt signal from the P_2 cell causes the EMS cell to orient its mitotic spindle and generate two daughters that become committed to different fates as a result of their different exposure to Wnt protein—the MS cell and the E cell (the founder cell of the gut).

Having sketched the chain of cause and effect in early nematode development, we now examine some of the methods that have been used to decipher it.

Microsurgery and Genetics Reveal the Logic of Developmental Control; Gene Cloning and Sequencing Reveal Its Molecular Mechanisms

To discover the causal mechanisms, we need to know the developmental potential of the individual cells in the embryo. At what points in their lives do they undergo decisive internal changes that determine them for a particular fate, and at what points do they depend on signals from other cells? In the nematode, using laser microbeam microsurgery, one can accurately kill one or more of a cell's neighbors and then observe directly how the cell behaves in the altered circumstances. Alternatively, cells of the early embryo can be pushed around and rearranged inside the eggshell using a fine needle. For example, the relative positions of ABa and ABp can be flipped at the four-cell stage of development. The ABa cell then undergoes what would normally be the fate of the ABp cell, and vice versa, showing that the two cells initially have the same developmental potential and depend on signals from their neighbors to make them different. A third tactic is to remove the eggshell of an early *C. elegans* embryo by digesting it with enzymes, and then to manipulate the cells in culture. The existence of a polarizing signal from P_2 to EMS was demonstrated in this way.

Genetic screens were used to identify the genes involved in the P_2–EMS cell interaction. A search was made for mutant strains of worms in which no gut cells were induced (called *Mom* mutants, because they had *mo*re *m*esoderm—mesoderm being the fate of both of the EMS cell daughters when induction fails). Cloning and sequencing the *Mom* genes revealed that one encodes a Wnt signal protein that is expressed in the P_2 cell, while another encodes a Frizzled protein (a Wnt receptor) that is expressed in the EMS cell. A second genetic screen was conducted for mutant strains of worms with the opposite phenotype, in which extra gut cells are induced (called *Pop* mutants, for *po*sterior *p*harynx defect). One of the *Pop* genes (*Pop1*) turns out to encode a gene regulatory protein (a LEF1/TCF homolog) whose activity is down-regulated by Wnt signaling in *C. elegans*. When Pop1 activity is absent, both daughters of the EMS cell behave as though they have received the Wnt signal from P_2. Similar genetic methods were used to identify the genes whose products mediate the Notch-dependent signaling from P_2 to ABa.

Continuing in this way, it is possible to build up a detailed picture of the decisive events in nematode development, and of the genetically specified machinery that controls them.

Cells Change Over Time in Their Responsiveness to Developmental Signals

The complexity of the adult nematode body is achieved through repeated use of a handful of patterning mechanisms, including those we have just seen in action in the early embryo. For example, cell divisions with a molecular asymmetry dependent on the Pop1 gene regulatory proteins occur throughout *C. elegans* development, creating anterior and posterior sister cells with different characters.

As emphasized earlier, while the same few types of signals act repeatedly at different times and places, the effects they have are different because the cells are programmed to respond differently according to their age and their past history. We have seen, for example, that at the four-cell stage of development, one cell, ABp, changes its developmental potential because of a signal received via the Notch pathway. At the 12-cell stage of development, the granddaughters of the ABp cell and the granddaughters of the ABa cell both encounter another Notch signal, this time from a daughter of the EMS cell. The ABa granddaughter

changes its internal state in response to this signal and begins to form the pharynx. The ABp granddaughter does no such thing—the earlier exposure to a Notch signal has made it unresponsive. Thus, at different times in their history, both ABa lineage cells and ABp lineage cells respond to Notch, but the outcomes are different. Somehow a Notch signal at the 12-cell stage induces pharynx, but a Notch signal at the 4-cell stage has other effects—which include the prevention of pharynx induction by Notch at a later stage. This phenomenon, in which the same signaling mechanism evokes different effects at different stages and in different contexts—is seen in the development of all animals, and in all of them Notch signaling is used repeatedly in this way.

Heterochronic Genes Control the Timing of Development

A cell does not have to receive an external cue in order to change: one set of regulatory molecules inside the cell can provoke the production of another, and the cell can thus step through a series of different states through its own internal mechanisms. These states differ not only in their responsiveness to external signals, but also in other aspects of their internal chemistry, including proteins that stop or start the cell-division cycle. In this way, the internal mechanisms of the cell, together with the past and present signals received, dictate both the sequence of biochemical changes in the cell and the timing of its cell divisions.

The specific molecular details of the mechanisms governing the temporal program of development are still mysterious. Remarkably little is known, even in the nematode embryo with its rigidly predictable pattern of cell divisions, about how the sequence of cell divisions is controlled. However, for the later stages, when the larva feeds and grows and moults to become an adult, it has been possible to identify some of the genes that control the timing of cellular events. Mutations in these genes cause *heterochronic* phenotypes: the cells in a larva of one stage behave as though they belonged to a larva of a different stage, or cells in the adult carry on dividing as though they belonged to a larva (**Figure 22–22**).

Through genetic analyses, one can determine that the products of the heterochronic genes act in series, forming regulatory cascades. Curiously, two genes at the top of their respective cascades, called *Lin4* and *Let7*, do not code for proteins but for microRNAs—short untranslated regulatory RNA molecules, 21 or 22 nucleotides long. These act by binding to complementary sequences in the noncoding regions of mRNA molecules transcribed from other heterochronic genes, thereby inhibiting their translation and promoting their degradation, as discussed in Chapter 7. Increasing levels of *Lin4* RNA govern the progression from larval stage-1 cell behavior to larval stage-3 cell behavior; increasing levels

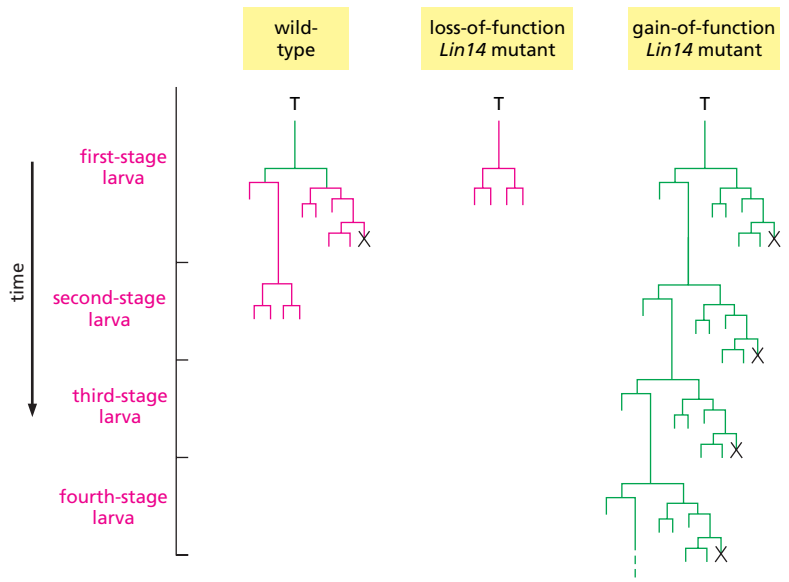


Figure 22–22 Heterochronic mutations in the *Lin14* gene of *C. elegans*. The effects on only one of the many affected lineages are shown. The loss-of-function (recessive) mutation in *Lin14* causes premature occurrence of the pattern of cell division and differentiation characteristic of a late larva, so that the animal reaches its final state prematurely and with an abnormally small number of cells. The gain-of-function (dominant) mutation has the opposite effect, causing cells to reiterate the patterns of cell divisions characteristic of the first larval stage, continuing through as many as five or six molt cycles and persisting in the manufacture of an immature type of cuticle. The cross denotes a programmed cell death. *Green lines* represent cells that contain Lin14 protein (which binds to DNA), *red lines* those that do not. In normal development the disappearance of Lin14 is triggered by the beginning of larval feeding. (After V. Ambros and H.R. Horvitz, *Science* 226:409–416, 1984, with permission from AAAS; and P. Arasu, B. Wightman and G. Ruvkun, *Growth Dev. Aging* 5:1825–1833, 1991, with permission from Growth Publishing Co., Inc.)

of *Let7* RNA govern the progression from late larva to adult. In fact, *Lin4* and *Let7* were the first microRNAs to be described in any animal: it was through developmental genetic studies in *C. elegans* that the importance of this whole class of molecules for gene regulation in animals was discovered.

RNA molecules that are identical or almost identical to the *Let7* RNA are found in many other species, including *Drosophila*, zebrafish, and human. Moreover, these RNAs appear to act in a similar way to regulate the level of their target mRNA molecules, and the targets themselves are homologous to the targets of *Let7* RNA in the nematode. In *Drosophila*, this system of molecules seems to be involved in the metamorphosis of the larva into a fly, hinting at a conserved role in governing the timing of developmental transitions.

Cells Do Not Count Cell Divisions in Timing Their Internal Programs

Since the steps of cell specialization have to be coordinated with cell divisions, it is often suggested that the cell division cycle might serve as a clock to control the tempo of other events in development. In this view, changes of internal state would be locked to passage through each division cycle: the cell would click to the next state as it went through mitosis, so to speak. Although there are indeed some cases where changes of cell state are conditional on cell cycle events, this is far from being the general rule. Cells in developing embryos, whether they be worms, flies, or vertebrates, usually carry on with their standard timetable of determination and differentiation even when progress through the cell-division cycle is artificially blocked. Necessarily, there are some abnormalities, if only because a single undivided cell cannot differentiate in two ways at once. But in most cases that have been studied, it seems that the cell changes its state with time more or less regardless of cell division, and that this changing state controls both the decision to divide and the decision as to when and how to specialize.

Selected Cells Die by Apoptosis as Part of the Program of Development

The control of cell numbers in development depends on cell death as well as cell division. A *C. elegans* hermaphrodite generates 1030 somatic cell nuclei in the course of its development, but 131 of the cells die. These programmed cell deaths occur in an absolutely predictable pattern. In *C. elegans*, they can be chronicled in detail, because one can trace the fate of each individual cell and see which dies, watching as each suicide victim undergoes apoptosis and is rapidly engulfed and digested by neighboring cells (**Figure 22–23**). In other organisms, where close observation is harder, such deaths easily go unnoticed; but cell death by apoptosis is probably the fate of a substantial fraction of the cells produced in most animals, playing an essential part in generating an individual with the right cell types in the right numbers and places, as discussed in Chapter 18.

Genetic screens in *C. elegans* have been crucial in identifying the genes that bring about apoptosis and in highlighting its importance in development. Three genes, called *Ced3*, *Ced4*, and *Egl1* (*Ced* stands for *cell death abnormal*), are found to be required for the 131 normal cell deaths to occur. If these genes are inactivated by mutation, cells that are normally fated to die survive instead, differentiating as recognizable cell types such as neurons. Conversely, over-expression or misplaced expression of the same genes causes many cells to die that

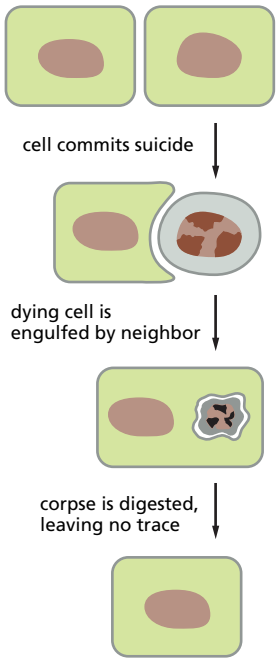


Figure 22–23 Apoptotic cell death in *C. elegans*. Death depends on expression of the *Ced3* and *Ced4* genes in the absence of *Ced9* expression—all in the dying cell itself. The subsequent engulfment and disposal of the remains depend on expression of other genes in the neighboring cells.

would normally survive, and the same effect results from mutations that inactivate another gene, *Ced9*, which normally represses the death program.

All these genes code for conserved components of the cell-death machinery. As described in Chapter 18, *Ced3* codes for a caspase homolog, while *Ced4*, *Ced9*, and *Egl1* are respectively homologs of *Apaf1*, *Bcl2*, and *Bad*. Without the insights that came from detailed analysis of the development of the transparent, genetically tractable nematode worm, it would have been very much harder to discover these genes and understand the cell-death process in vertebrates.

Summary

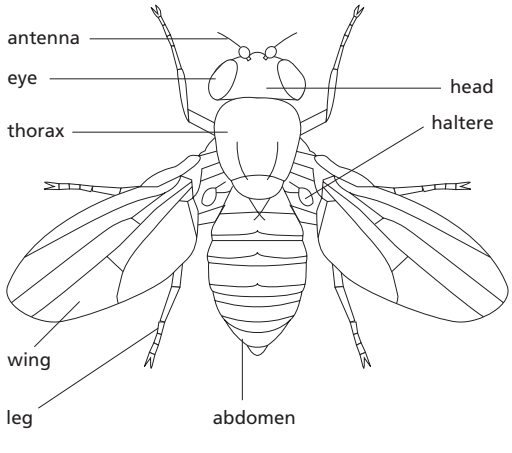
The development of the small, relatively simple, transparent nematode worm Caenorhabditis elegans *is extraordinarily reproducible and has been chronicled in detail, so that a cell at any given position in the body has the same lineage in every individual, and this lineage is fully known. Also, the genome has been completely sequenced. Thus, powerful genetic and microsurgical approaches can be combined to decipher developmental mechanisms. As in other organisms, development depends on an interplay of cell–cell interactions and cell-autonomous processes. Development begins with an asymmetric division of the fertilized egg, dividing it into two smaller cells containing different cell-fate determinants. The daughters of these cells interact via the Notch and Wnt cell signaling pathways to create a more diverse array of cell states. Meanwhile, through further asymmetric divisions one cell inherits materials from the egg that determine it at an early stage as progenitor of the germ line.*

Genetic screens identify the sets of genes responsible for these and later steps in development, including, for example, cell-death genes that control the apoptosis of a specific subset of cells as part of the normal developmental program. Heterochronic genes that govern the timing of developmental events have also been found, although in general our understanding of temporal control of development is still very poor. There is good evidence, however, that the tempo of development is not set by the counting of cell divisions.

DROSOPHILA AND THE MOLECULAR GENETICS OF PATTERN FORMATION: GENESIS OF THE BODY PLAN

It is the fly *Drosophila melanogaster* (**Figure 22–24**), more than any other organism, that has transformed our understanding of how genes govern the patterning of the body. The anatomy of *Drosophila* is more complex than that of *C. elegans*,

(A)



(B)

Figure 22–24 *Drosophila melanogaster.* Dorsal view of a normal adult fly. (A) Photograph. (B) Labeled drawing. (Photograph courtesy of E.B. Lewis.)

with more than 100 times as many cells, and it shows more obvious parallels with our own body structure. Surprisingly, the fly has fewer genes than the worm—about 14,000 as compared with 20,000. On the other hand, it has almost twice as much DNA per gene (about 10,000 nucleotides on average, as compared with about 5000), most of this being noncoding sequence. The molecular construction kit has fewer types of parts, but the assembly instructions—as specified by the regulatory sequences in the non-coding DNA—seem to be more voluminous.

Decades of genetic study, culminating in massive systematic genetic screens, have yielded a catalogue of the developmental control genes that define the spatial pattern of cell types and body structures of the fly, and molecular biology has given us the tools to watch these genes in action. By *in situ* hybridization using DNA or RNA probes on whole embryos, or by staining with labeled antibodies to reveal the distribution of specific proteins, one can observe directly how the internal states of the cells are defined by the sets of regulatory genes that they express at different times of development. Moreover, by analyzing animals that are a patchwork of mutant and nonmutant cells, one can discover how each gene operates as part of a system to specify the organization of the body.

Most of the genes controlling the pattern of the body in *Drosophila* turn out to have close counterparts in higher animals, including ourselves. In fact, many of the basic devices for defining the body plan and patterning individual organs and tissues are astonishingly similar. Thus, quite surprisingly, the fly has provided the key to understanding the molecular genetics of our own development.

Flies, like nematode worms, are ideal for genetic studies: cheap to breed, easy to mutagenize, and rapid in their reproductive cycle. But there is a more fundamental reason why they have been so important for developmental geneticists. As emphasized earlier, as a result of gene duplications, vertebrate genomes often contain two or three homologous genes corresponding to a single gene in the fly. A mutation that disrupts one of these genes very often fails to reveal the gene's core function, because the other homologs share this function and remain active. In the fly, with its more economical gene set, this phenomenon of genetic redundancy is less prevalent. The phenotype of a single mutation in the fly therefore more often directly uncovers the function of the mutant gene.

The Insect Body Is Constructed as a Series of Segmental Units

The timetable of *Drosophila* development, from egg to adult, is summarized in **Figure 22–25**. The period of *embryonic development* begins at fertilization and takes about a day, at the end of which the embryo hatches out of the egg shell to become a *larva.* The larva then passes through three stages, or *instars,* separated by molts in which it sheds its old coat of cuticle and lays down a larger one. At the end of the third instar it pupates. Inside the *pupa,* a radical remodeling of the body takes place—a process called *metamorphosis.* Eventually, about nine days after fertilization, an adult fly, or *imago,* emerges.

The fly consists of a head, with mouth, eyes, and antennae, followed by three thoracic segments (numbered T1 to T3), and eight or nine abdominal segments (numbered A1 to A9). Each segment, although different from the others, is built according to a similar plan. Segment T1, for example, carries a pair of legs, T2 carries a pair of legs plus a pair of wings, and T3 carries a pair of legs plus a pair of halteres—small knob-shaped balancers important in flight, evolved from the second pair of wings that more primitive insects possess. The quasi-repetitive segmentation develops in the early embryo during the first few hours after fertilization (**Figure 22–26**), but it is more obvious in the larva (**Figure 22–27**), where the segments look more similar than in the adult. In the embryo it can be seen that the rudiments of the head, or at least the future adult mouth parts, are likewise segmental. At the two ends of the animal, however, there are highly specialized terminal structures that are not segmentally derived.

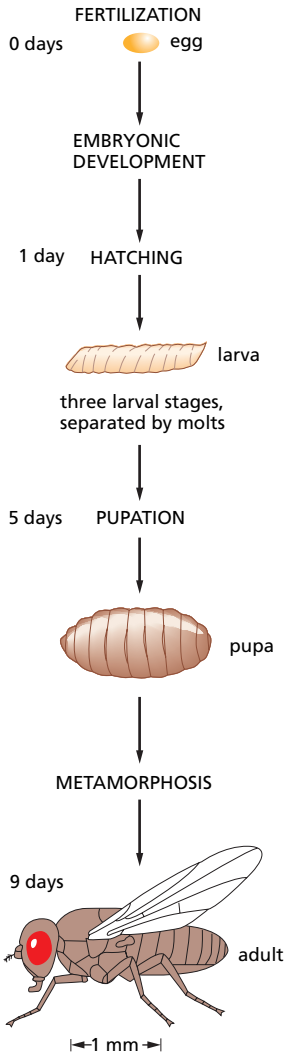


Figure 22–25 Synopsis of *Drosophila* development from egg to adult fly.

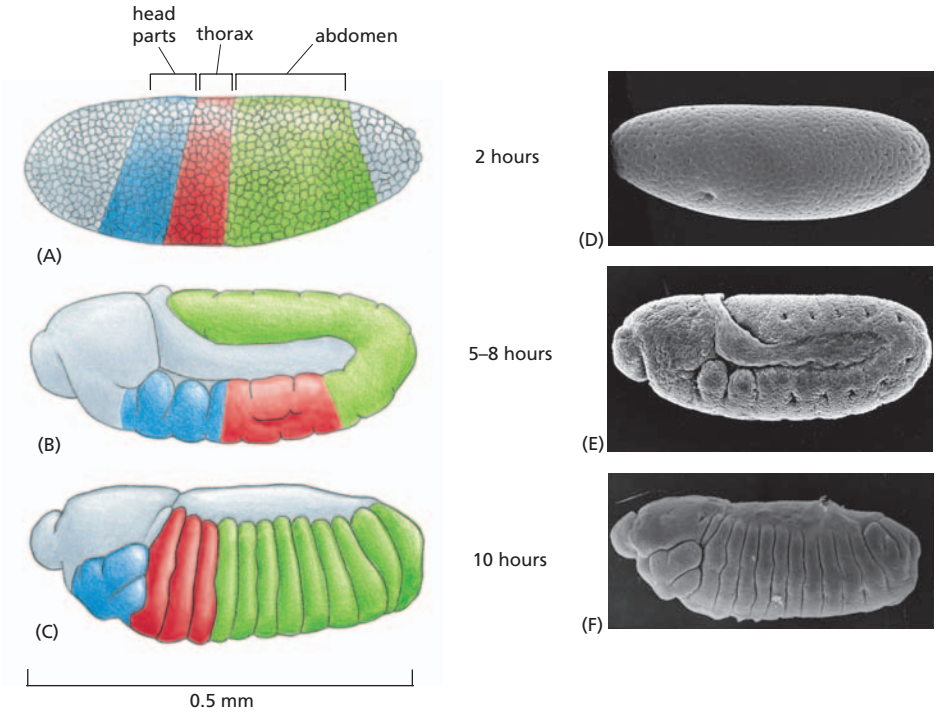


Figure 22–26 The origins of the *Drosophila* body segments during embryonic development. <AACG> The embryos are seen in side view in drawings (A–C) and corresponding scanning electron micrographs (D–F). (A and D) At 2 hours the embryo is at the syncytial blastoderm stage (see Figure 22–28) and no segmentation is visible, although a fate map can be drawn showing the future segmented regions (color in A). (B and E) At 5–8 hours the embryo is at the extended germ band stage: gastrulation has occurred, segmentation has begun to be visible, and the segmented axis of the body has lengthened, curving back on itself at the tail end so as to fit into the egg shell. (C and F) At 10 hours the body axis has contracted and become straight again, and all the segments are clearly defined. The head structures, visible externally at this stage, will subsequently become tucked into the interior of the larva, to emerge again only when the larva goes through pupation to become an adult. (D and E, courtesy of F.R. Turner and A.P. Mahowald, *Dev. Biol.* 50:95–108, 1976; F, from J.P. Petschek, N. Perrimon and A.P. Mahowald, *Dev. Biol.* 119:175–189, 1987. All with permission from Academic Press.)

The boundaries between segments are traditionally defined by visible anatomical markers; but in discussing gene expression patterns it is often convenient to draw a different set of segmental boundaries, defining a series of segmental units called *parasegments*, half a segment out of register with the traditional divisions (see Figure 22–27).

Drosophila Begins Its Development as a Syncytium

The egg of *Drosophila* is about 0.5 mm long and 0.15 mm in diameter, with a clearly defined polarity. Like the eggs of other insects, but unlike most vertebrates, it begins its development in an unusual way: a series of nuclear divisions without cell division creates a syncytium. The early nuclear divisions are synchronous and extremely rapid, occurring about every 8 minutes. The first nine divisions generate a cloud of nuclei, most of which migrate from the middle of the egg toward the surface, where they form a monolayer called the *syncytial blastoderm*. After another four rounds of nuclear division, plasma membranes

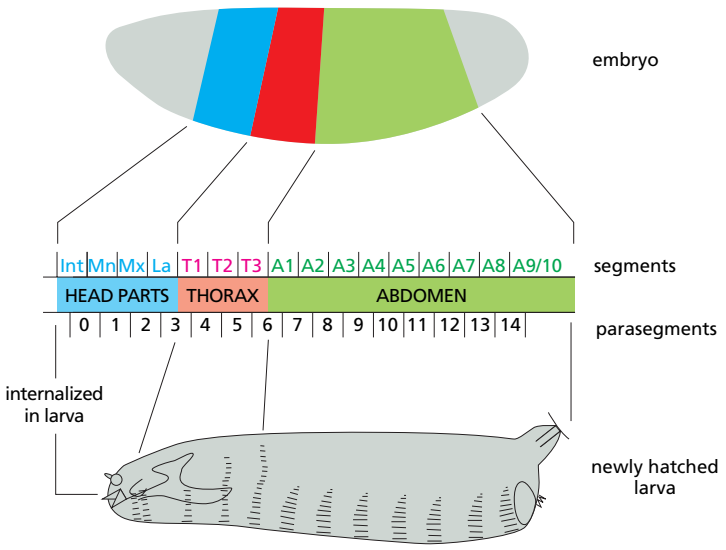
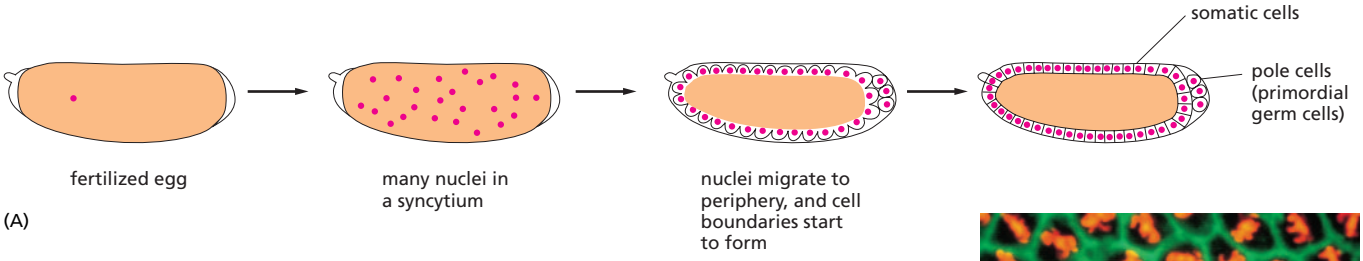


Figure 22–27 The segments of the *Drosophila* larva and their correspondence with regions of the blastoderm. The parts of the embryo that become organized into segments are shown in *color*. The two ends of the embryo, shaded *gray*, are not segmented and become tucked into the interior of the body to form the internal structures of the head and gut. (The future external, segmental structures of the adult head are also transiently tucked into the interior in the larva.) Segmentation in *Drosophila* can be described in terms of either segments or parasegments: the relationship is shown in the middle part of the figure. Parasegments often correspond more simply to patterns of gene expression. The exact number of abdominal segments is debatable: eight are clearly defined, and a ninth is present vestigially in the larva, but absent in the adult.



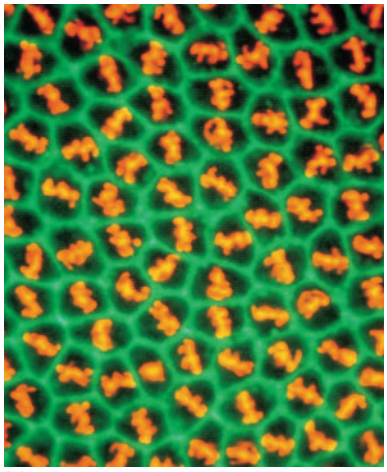


Figure 22–28 Development of the *Drosophila* egg from fertilization to the cellular blastoderm stage. (A) Schematic drawings. (B) Surface view—an optical-section photograph of blastoderm nuclei undergoing mitosis at the transition from the syncytial to the cellular blastoderm stage. Actin is stained *green,* chromosomes *orange.* (A, after H.A. Schneiderman, in Insect Development [P.A. Lawrence, ed.], pp. 3–34. Oxford, UK: Blackwell, 1976; B, courtesy of William Sullivan.)

grow inward from the egg surface to enclose each nucleus, thereby converting the syncytial blastoderm into a *cellular blastoderm* consisting of about 6000 separate cells (**Figure 22–28**). About 15 of the nuclei populating the extreme posterior end of the egg are segregated into cells a few cycles earlier; these *pole cells* are the germ-line precursors (primordial germ cells) that will give rise to eggs or sperm.

Up to the cellular blastoderm stage, development depends largely—although not exclusively—on stocks of maternal mRNA and protein that accumulated in the egg before fertilization. The frantic rate of DNA replication and nuclear division evidently gives little opportunity for transcription. After cellularization, cell division continues in a more conventional way, asynchronously and at a slower rate, and the rate of transcription increases dramatically. Gastrulation begins a little while before cellularization is complete, when parts of the sheet of cells forming the exterior of the embryo start to tuck into the interior to form the gut, the musculature, and associated internal tissues. A little later and in another region of the embryo, a separate set of cells move from the surface epithelium into the interior to form the central nervous system. By marking and following the cells through these various movements, one can draw a fate map for the monolayer of cells on the surface of the blastoderm (**Figure 22–29**).

As gastrulation nears completion, a series of indentations and bulges appear in the surface of the embryo, marking the subdivision of the body into segments along its anteroposterior axis (see Figure 22–26). Soon a fully segmented larva emerges, ready to start eating and growing. Within the body of the larva, small groups of cells remain apparently undifferentiated, forming structures called *imaginal discs.* These will grow as the larva grows, and eventually they will give rise to most of the structures of the adult body, as we shall see later.

A head end and a tail end, a ventral (belly) side and a dorsal (back) side, a gut, a nervous system, a series of body segments—these are all features of the

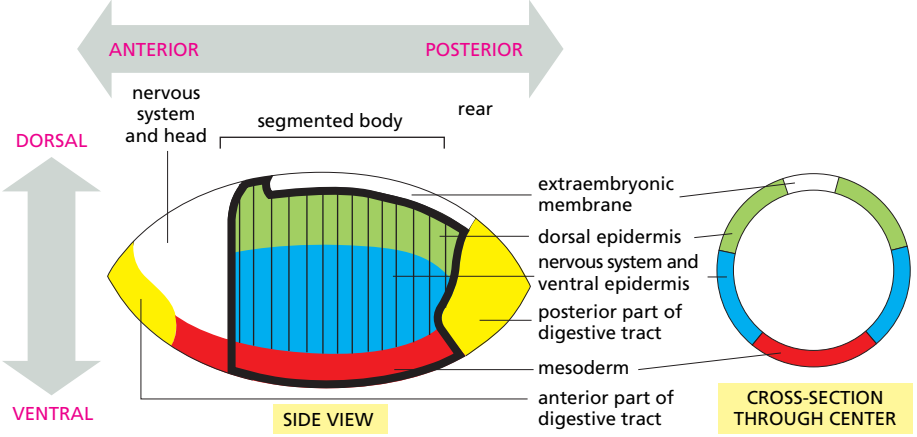


Figure 22–29 Fate map of a *Drosophila* embryo at the cellular blastoderm stage. The embryo is shown in side view and in cross section, displaying the relationship between the dorsoventral subdivision into future major tissue types and the anteroposterior pattern of future segments. A heavy line encloses the region that will form segmental structures. During gastrulation the cells along the ventral midline invaginate to form mesoderm, while the cells fated to form the gut invaginate near each end of the embryo. (After V. Hartenstein, G.M. Technau and J.A. Campos-Ortega, *Wilhelm Roux' Arch. Dev. Biol.* 194:213–216, 1985. With permission from Elsevier.)

basic body plan that *Drosophila* shares with many other animals, including ourselves. We begin our account of the mechanisms of *Drosophila* development by considering how this body plan is set up.

Genetic Screens Define Groups of Genes Required for Specific Aspects of Early Patterning

By carrying out a series of genetic screens based on saturation mutagenesis (discussed in Chapter 8), it has been possible to amass a collection of *Drosophila* mutants that appears to include changes in a large proportion of the genes affecting development. Independent mutations in the same gene can be distinguished from mutations in separate genes by a complementation test (see Panel 8–1, p. 555), leading to a catalog of genes classified according to their mutant phenotypes. In such a catalog, a group of genes with very similar mutant phenotypes will often code for a set of proteins that work together to perform a particular function.

Sometimes the developmental functions revealed by mutant phenotypes are those that one would expect; sometimes they are a surprise. A large-scale genetic screen focusing on early *Drosophila* development revealed that the key genes fall into a relatively small set of functional classes defined by their mutant phenotypes. Some—the *egg-polarity genes* (**Figure 22–30**)—are required to

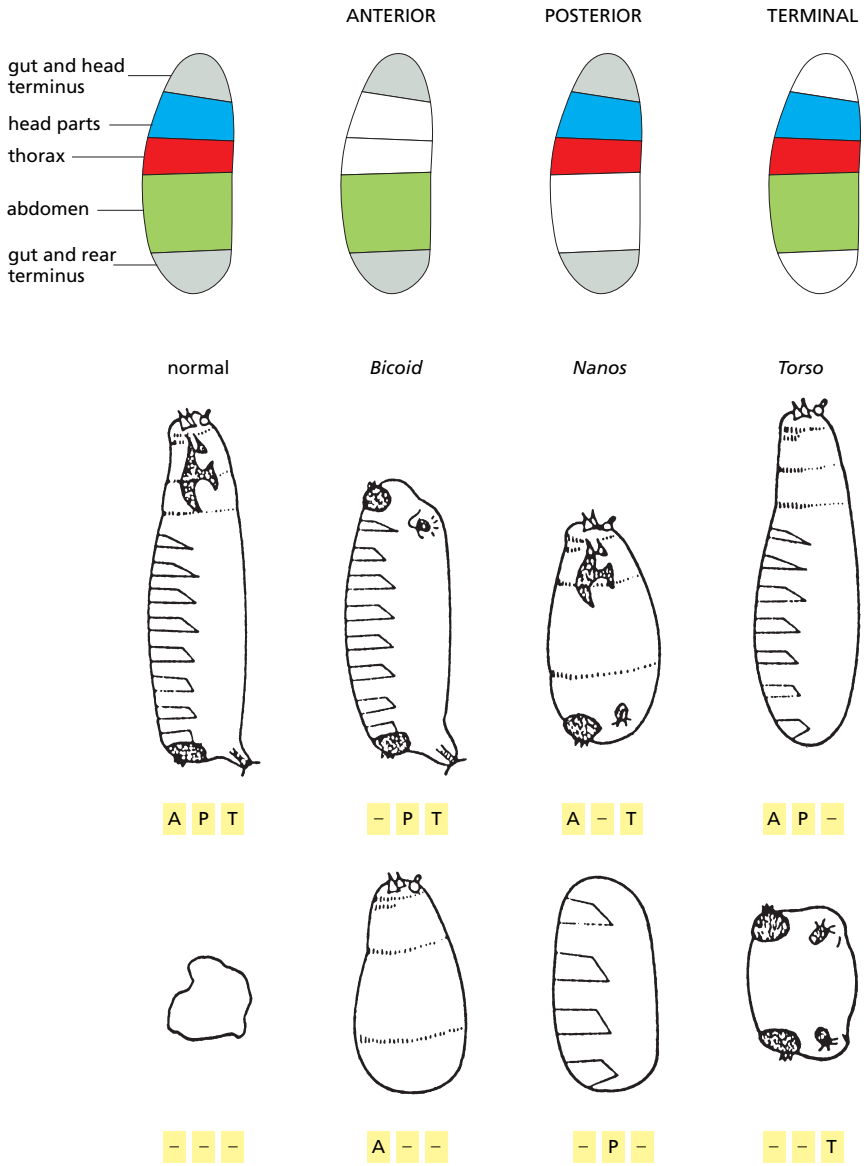


Figure 22–30 The domains of the anterior, posterior, and terminal systems of egg-polarity genes. The upper diagrams show the fates of the different regions of the egg/early embryo and indicate (in *white*) the parts that fail to develop if the anterior, posterior, or terminal system is defective. The middle row shows schematically the appearance of a normal larva and of mutant larvae that are defective in a gene of the anterior system (for example, *Bicoid*), of the posterior system (for example, *Nanos*), or of the terminal system (for example, *Torso*). The bottom row of drawings shows the appearances of larvae in which none or only one of the three gene systems is functional. The lettering beneath each larva specifies which systems are intact (A P T for a normal larva, – P T for a larva where the anterior system is defective but the posterior and terminal systems are intact, and so on). Inactivation of a particular gene system causes loss of the corresponding set of body structures; the body parts that form correspond to the gene systems that remain functional. Note that larvae with a defect in the anterior system can still form terminal structures at their anterior end, but these are of the type normally found at the rear end of the body rather than the front of the head. (Slightly modified from D. St. Johnston and C. Nüsslein-Volhard, *Cell* 68:201–219, 1992. With permission from Elsevier.)

define the anteroposterior and dorsoventral axes of the embryo and mark out its two ends for special fates, by mechanisms involving interactions between the oocyte and surrounding cells in the ovary. Others, the *gap genes*, are required in specific broad regions along the anteroposterior axis of the early embryo to allow their proper development. A third category, the *pair-rule genes*, are required, more surprisingly, for development of alternate body segments. A fourth category, the *segment polarity genes*, are responsible for organizing the anteroposterior pattern of each individual segment.

The discovery of these four systems of genes and the subsequent analysis of their functions (an enterprise that still continues) was a famous tour-de-force of developmental genetics. It had a revolutionary impact on all of developmental biology by showing the way toward a systematic, comprehensive account of the genetic control of embryonic development. In this section, we shall summarize only briefly the conclusions relating to the earliest phases of *Drosophila* development, because these are insect-specific; we dwell at greater length on the parts of the process that illustrate more general principles.

Interactions of the Oocyte With Its Surroundings Define the Axes of the Embryo: the Role of the Egg-Polarity Genes

Surprisingly, the earliest steps of animal development are among the most variable, even within a phylum. A frog, a chicken, and a mammal, for example, even though they develop in similar ways later, make eggs that differ radically in size and structure, and they begin their development with different sequences of cell divisions and cell specialization events.

The style of early development that we have described for *C. elegans* is typical of many classes of animals. In contrast, the early development of *Drosophila* represents a rather extreme variant. The main axes of the future insect body are defined before fertilization by a complex exchange of signals between the unfertilized egg, or oocyte, and the follicle cells that surround it in the ovary (**Figure 22–31**). Then, in the syncytial phase following fertilization, an exceptional amount of patterning occurs in the array of rapidly dividing nuclei, before the first partitioning of the egg into separate cells. Here, there is no need for the usual forms of cell–cell communication involving transmembrane signaling; neighboring regions of the early *Drosophila* embryo can communicate by means of gene regulatory proteins and mRNA molecules that diffuse or are actively transported through the cytoplasm of the giant multinuclear cell.

In the stages before fertilization, the anteroposterior axis of the future embryo becomes defined by three systems of molecules that create landmarks in the oocyte (**Figure 22–32**). Following fertilization, each landmark serves as a beacon, providing a signal, in the form of a morphogen gradient, that organizes the developmental process in its neighborhood. Two of these signals are generated from localized deposits of specific mRNA molecules. The future anterior end of the embryo contains a high concentration of mRNA for a gene regulatory protein called Bicoid; this mRNA is translated to produce Bicoid protein, which

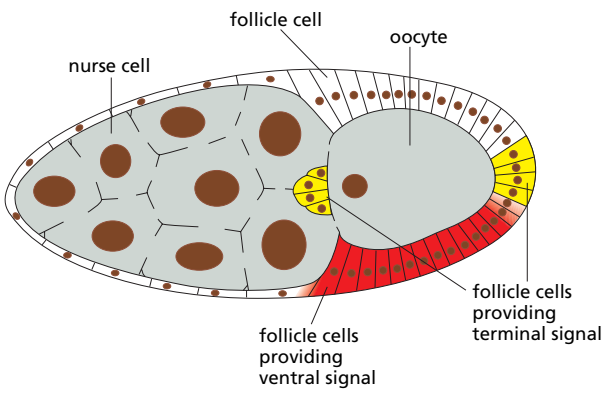


Figure 22–31 A *Drosophila* oocyte in its follicle. The oocyte is derived from a germ cell that divides four times to give a family of 16 cells that remain in communication with one another via cytoplasmic bridges *(gray)*. One member of the family group becomes the oocyte, while the others become nurse cells, which make many of the components required by the oocyte and pass them into it via the cytoplasmic bridges. The follicle cells that partially surround the oocyte have a separate ancestry. As indicated, they are the sources of terminal and ventral egg-polarizing signals.

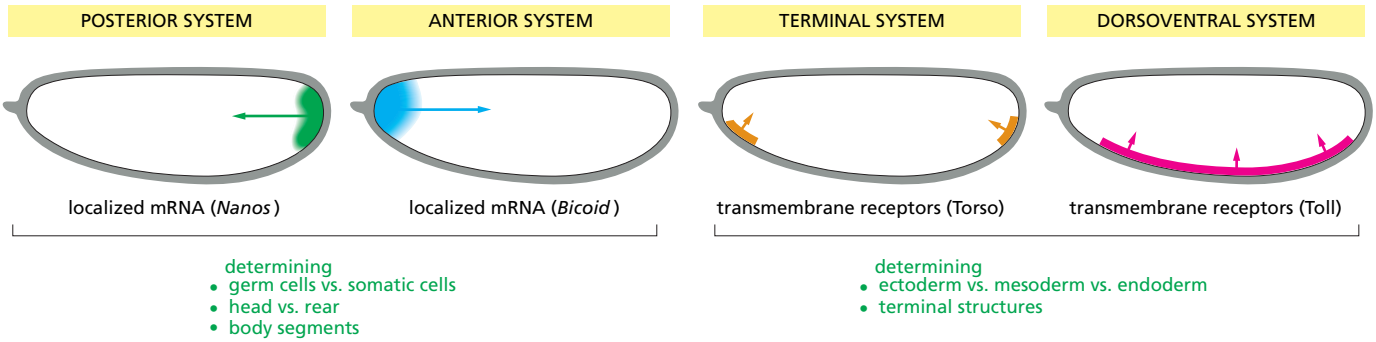


Figure 22–32 The organization of the four egg-polarity gradient systems. The receptors Toll and Torso are distributed all over the membrane; the coloring in the diagrams on the right indicates where they become activated by extracellular ligands.

diffuses away from its source to form a concentration gradient with its maximum at the anterior end of the egg. The future posterior end of the embryo contains a high concentration of mRNA for a regulator of translation called Nanos, which sets up a posterior gradient in the same way. The third signal is generated symmetrically at both ends of the egg, by local activation of a transmembrane tyrosine kinase receptor called Torso. The activated receptor exerts its effects over a shorter range, marking the sites of specialized terminal structures that will form at the head and tail ends of the future larva and also defining the rudiments of the future gut. The three sets of genes responsible for these localized determinants are referred to as the **anterior**, **posterior**, and **terminal** sets of **egg-polarity** genes.

A fourth landmark defines the dorsoventral axis (see Figure 22–32): a protein that is produced by follicle cells underneath the future ventral region of the embryo leads to localized activation of another transmembrane receptor, called Toll, in the oocyte membrane. The genes required for this function are called **dorsoventral** egg-polarity genes.

All the egg-polarity genes in these four classes are maternal-effect genes: it is the mother's genome, not the zygotic genome, that is critical. Thus, a fly whose chromosomes are mutant in both copies of the *Bicoid* gene but who is born from a mother carrying one normal copy of *Bicoid* develops perfectly normally, without any defects in the head pattern. However, if that daughter fly is a female no functional *Bicoid* mRNA can be deposited into the anterior part of her own eggs, and all of these will develop into headless embryos regardless of the father's genotype.

Each of the four egg-polarity signals—provided by Bicoid, Nanos, Torso, and Toll—exerts its effect by regulating (directly or indirectly) the expression of genes in the nuclei of the blastoderm. The use of these particular molecules to organize the egg is not a general feature of early animal development—indeed, only *Drosophila* and closely related insects possess a *Bicoid* gene. And Toll has been coopted here for dorsoventral patterning; its more ancient and universal function is in the innate immune response, as discussed in Chapter 24.

Nevertheless, the egg-polarity system shows some highly conserved features. For example, the localization of *Nanos* mRNA at one end of the egg is linked to, and dependent on, the localization of germ-cell determinants at that site, just as it is in *C. elegans*. Later in development, as the zygotic genome comes into play under the influence of the egg-polarity system, more similarities with other animal species become apparent. We shall use the dorsoventral system to illustrate this point.

The Dorsoventral Signaling Genes Create a Gradient of a Nuclear Gene Regulatory Protein

Localized activation of the Toll receptor on the ventral side of the egg controls the distribution of Dorsal, a gene regulatory protein inside the egg. The Dorsal protein belongs to the same family as the NFκB gene regulatory protein of vertebrates (discussed in Chapter 15). Its Toll-regulated activity, like that of NFκB,

depends on its translocation from the cytoplasm, where it is held in an inactive form, to the nucleus, where it regulates gene expression. In the newly laid egg, both the *Dorsal* mRNA (detected by *in situ* hybridization) and the protein it encodes (detected with antibodies) are distributed uniformly in the cytoplasm. After the nuclei have migrated to the surface of the embryo to form the blastoderm, however, a remarkable redistribution of the Dorsal protein occurs: dorsally the protein remains in the cytoplasm, but ventrally it is concentrated in the nuclei, with a smooth gradient of nuclear localization between these two extremes (**Figure 22–33**). The signal transmitted by the Toll protein controls this redistribution of Dorsal through a signaling pathway that is essentially the same as the Toll-dependent pathway involved in innate immunity.

Once inside the nucleus, the Dorsal protein turns on or off the expression of different sets of genes depending on its concentration. The expression of each responding gene depends on its regulatory DNA—specifically, on the number and affinity of the binding sites that this DNA contains for Dorsal and other regulatory proteins. In this way, the regulatory DNA can be said to *interpret* the positional signal provided by the Dorsal protein gradient, so as to define a dorsoventral series of territories—distinctive bands of cells that run the length of the embryo (**Figure 22–34**A). Most ventrally—where the concentration of Dorsal protein is highest—it switches on, for example, the expression of a gene called *Twist* that is specific for mesoderm (**Figure 22–35**). Most dorsally, where the concentration of Dorsal protein is lowest, the cells switch on *Decapentaplegic (Dpp)*. And in an intermediate region, where the concentration of Dorsal protein is high enough to repress *Dpp* but too low to activate *Twist*, the cells switch on another set of genes, including one called *Short gastrulation (Sog)*.

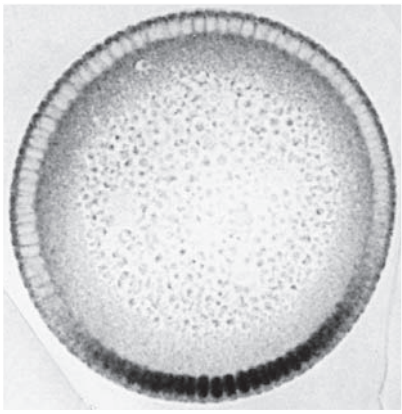


Figure 22–33 The concentration gradient of Dorsal protein in the nuclei of the blastoderm, as revealed by an antibody. Dorsally, the protein is present in the cytoplasm and absent from the nuclei; ventrally, it is depleted in the cytoplasm and concentrated in the nuclei. (From S. Roth, D. Stein and C. Nüsslein-Volhard, *Cell* 59:1189–1202, 1989. With permission from Elsevier.)

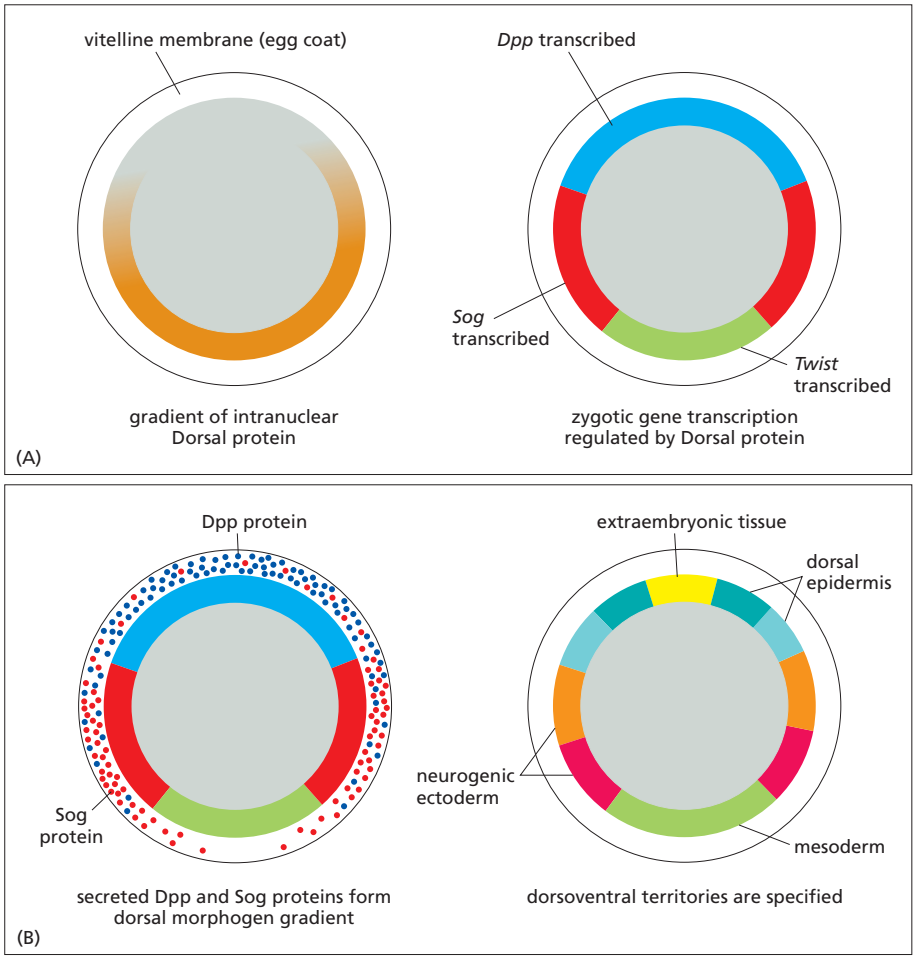


Figure 22–34 Morphogen gradients patterning the dorsoventral axis of the embryo. (A) The gradient of Dorsal protein defines three broad territories of gene expression, marked here by the expression of three representative genes—*Dpp, Sog,* and *Twist.* (B) Slightly later, the cells expressing *Dpp* and *Sog* secrete, respectively, the signal proteins Dpp (a TGFβ family member) and Sog (an antagonist of Dpp). These two proteins diffuse and interact with one another (and with certain other factors) to set up a gradient of Dpp activity that guides a more detailed patterning process.

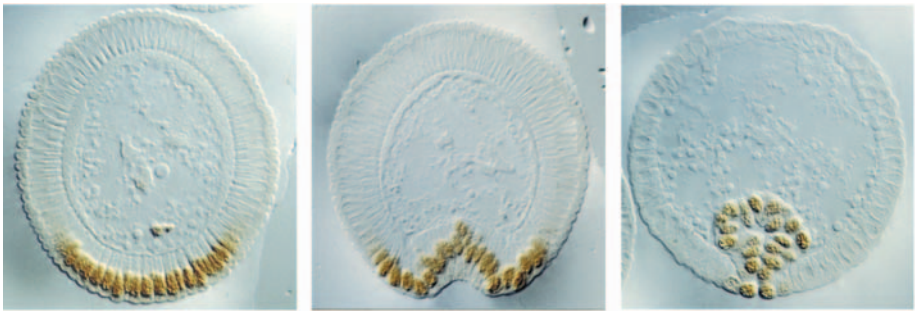


Figure 22–35 Origin of the mesoderm from cells expressing Twist. Embryos were fixed at successive stages, cross-sectioned, and stained with an antibody against the Twist protein, a gene regulatory protein of the bHLH family. The cells that express Twist move into the interior of the embryo to form mesoderm. (From M. Leptin, J. Casal, B. Grunewald and R. Reuter, *Development Suppl.* 23–31, 1992. With permission from The Company of Biologists.)

Dpp and Sog Set Up a Secondary Morphogen Gradient to Refine the Pattern of the Dorsal Part of the Embryo

Products of the genes directly regulated by the Dorsal protein generate in turn more local signals that define finer subdivisions of the dorsoventral axis. These signals act after cellularization, and take the form of conventional extracellular signal molecules. In particular, *Dpp* codes for the secreted Dpp protein, which forms a local morphogen gradient in the dorsal part of the embryo. The gene *Sog*, meanwhile, codes for another secreted protein that is produced in the neurogenic ectoderm and acts as an antagonist of Dpp. The opposing diffusion gradients of these two proteins create a steep gradient of Dpp activity. The highest Dpp activity levels, in combination with certain other factors, cause development of the most dorsal tissue of all—extraembryonic membrane; intermediate levels cause development of dorsal ectoderm; and very low levels allow development of neurogenic ectoderm (Figure 22–34B).

The Insect Dorsoventral Axis Corresponds to the Vertebrate Ventrodorsal Axis

Dpp is a member of the TGFβ superfamily of signal molecules that is also important in vertebrates; Sog is a homolog of the vertebrate protein chordin. It is striking that a Dpp homolog, BMP4, and chordin work together in vertebrates in the same way as do Dpp and Sog in *Drosophila*. These two proteins control the dorsoventral pattern of the ectoderm, with high levels of chordin defining the region that is neurogenic and high levels of BMP4 activity defining the region that is not. This, combined with other molecular parallels, strongly suggests that this part of the body plan has been conserved between insects and vertebrates. However, the axis is inverted, so that dorsal in the fly corresponds to ventral in the vertebrate (**Figure 22–36**). At some point in its evolutionary history, it seems, the ancestor of one of these classes of animals took to living life upside down.

Three Classes of Segmentation Genes Refine the Anterior–Posterior Maternal Pattern and Subdivide the Embryo

After the initial gradients of Bicoid and Nanos are created to define the anteroposterior axis, the **segmentation genes** refine the pattern. Mutations in any one of the segmentation genes alter the number of segments or their basic internal organization without affecting the global polarity of the embryo. Segmentation genes are expressed by subsets of cells in the embryo, so their products are the

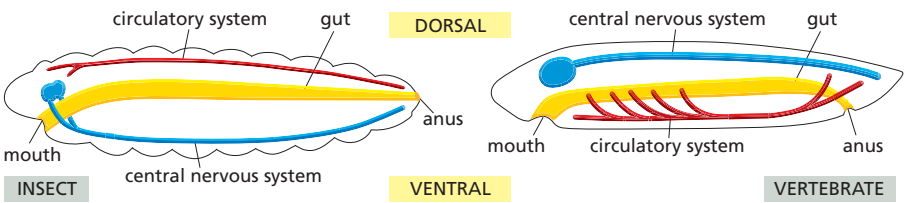


Figure 22–36 The vertebrate body plan as a dorsoventral inversion of the insect body plan. The mechanism of dorsoventral patterning in a vertebrate embryo is discussed in more detail later in this chapter. Note the correspondence with regard to the circulatory system as well as the gut and nervous system. In insects, the circulatory system is represented by a tubular heart and a main dorsal blood vessel, which pumps blood out into the tissue spaces through one set of apertures and receives blood back from the tissues through another set. In contrast with vertebrates, there is no system of capillary vessels to contain the blood as it percolates through the tissues. Nevertheless, heart development depends on homologous genes in vertebrates and insects, reinforcing the relationship between the two body plans. (After E.L. Ferguson, *Curr. Opin. Genet. Dev.* 6:424–431, 1996. With permission from Elsevier.)

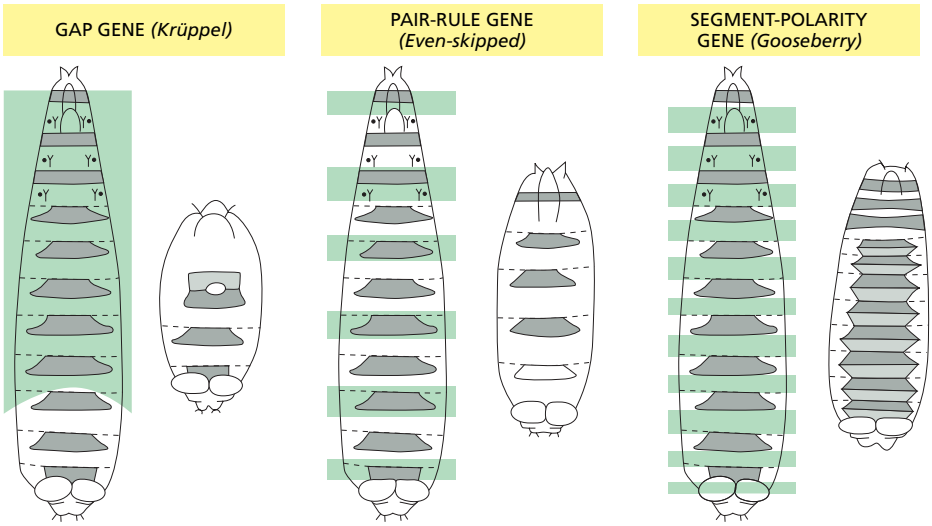


Figure 22–37 Examples of the phenotypes of mutations affecting the three types of segmentation genes. In each case the areas shaded in *green* on the normal larva *(left)* are deleted in the mutant or are replaced by mirror-image duplicates of the unaffected regions. (Modified from C. Nüsslein-Volhard and E. Wieschaus, *Nature* 287:795–801, 1980. With permission from Macmillan Publishers Ltd.)

first components that the embryo's own genome, rather than the maternal genome, contributes to embryonic development. They are therefore called *zygotic-effect genes* to distinguish them from the earlier maternal-effect genes.

The segmentation genes fall into three groups according to their mutant phenotypes (**Figure 22–37**). It is convenient to think of these three groups as acting in sequence, although in reality their functions overlap in time. First come a set of at least six **gap genes**, whose products mark out coarse subdivisions of the embryo. Mutations in a gap gene eliminate one or more groups of adjacent segments, and mutations in different gap genes cause different but partially overlapping defects. In the mutant *Krüppel,* for example, the larva lacks eight segments, from T1 to A5 inclusive.

The next category of segmentation genesis a set of eight **pair-rule genes**. Mutations in these cause a series of deletions affecting alternate segments, leaving the embryo with only half as many segments as usual. While all the pair-rule mutants display this two-segment periodicity, they differ in the precise positioning of the deletions relative to the segmental or parasegmental borders. The pair-rule mutant *Even-skipped (Eve),* for example, which is discussed in Chapter 7, lacks the whole of each odd-numbered parasegment, while the pair-rule mutant *Fushi tarazu (Ftz)* lacks the whole of each even-numbered parasegment, and the pair-rule mutant *Hairy* lacks a series of regions that are of similar width but out of register with the parasegmental units.

Finally, there are at least 10 **segment-polarity genes**. Mutations in these genes produce larvae with a normal number of segments but with a part of each segment deleted and replaced by a mirror-image duplicate of all or part of the rest of the segment. In *Gooseberry* mutants, for example, the posterior half of each segment (that is, the anterior half of each parasegment) is replaced by an approximate mirror image of the adjacent anterior half-segment (see Figure 22–37).

We see later that, in parallel with the segmentation process, a further set of genes, the *homeotic selector genes,* serve to define and preserve the differences between one segment and the next.

The phenotypes of the various segmentation mutants suggest that the segmentation genes form a coordinated system that subdivides the embryo progressively into smaller and smaller domains along the anteroposterior axis, distinguished by different patterns of gene expression. Molecular genetics has helped to reveal how this system works.

The Localized Expression of Segmentation Genes Is Regulated by a Hierarchy of Positional Signals

About three-quarters of the segmentation genes, including all of the gap genes and pair-rule genes, code for gene regulatory proteins. Their actions on one

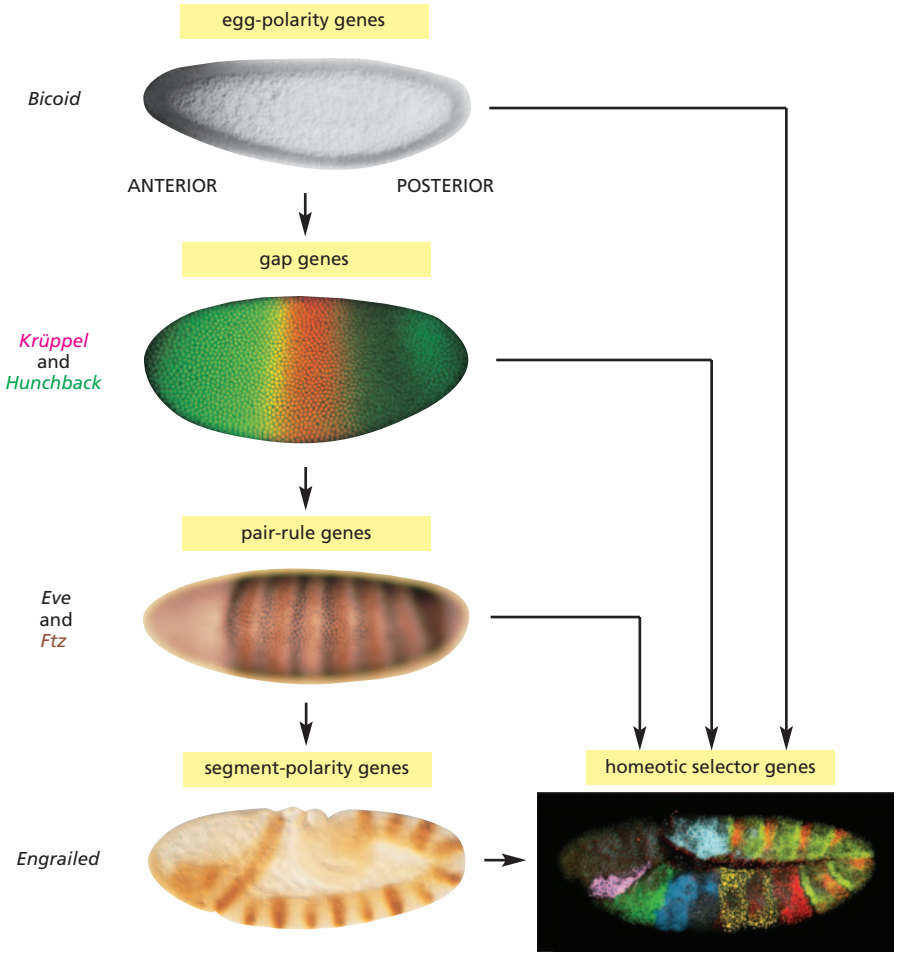


Figure 22–38 The regulatory hierarchy of egg-polarity, gap, segmentation, and homeotic selector genes. The photographs show expression patterns of representative examples of genes in each category, revealed by staining with antibodies against the protein products. The homeotic selector genes, discussed below, define the lasting differences between one segment and the next. (Photographs from top (i) from W. Driever and C. Nüsslein-Volhard, *Cell* 54:83–104, 1988. With permission from Elsevier; (ii) courtesy of Jim Langeland, Steve Paddock, Sean Carroll, and the Howard Hughes Medical Institute; (iii) from P.A. Lawrence, The Making of a Fly. Oxford, UK: Blackwell, 1992; (iv) from C. Hama, Z. Ali and T.B. Kornberg, *Genes Dev.* 4:1079–1093, 1990. With permission from Cold Spring Harbor Laboratory Press; (v) courtesy of William McGinnis, adapted from D. Kosman et al., *Science* 305:846, 2004. With permission from AAAS.)

another and on other genes can therefore be observed by comparing gene expression in normal and mutant embryos. By using appropriate probes to detect the gene transcripts or their protein products, one can, in effect, take snapshots as genes switch on and off in changing patterns. Repeating the process with mutants that lack a particular segmentation gene, one can begin to dissect the logic of the entire gene control system.

The products of the egg-polarity genes provide the global positional signals in the early embryo. These cause particular gap genes to be expressed in particular regions. The products of the gap genes then provide a second tier of positional signals that act more locally to regulate finer details of patterning through the expression of yet other genes, including the pair-rule genes (**Figure 22–38**). The pair-rule genes in turn collaborate with one another and with the gap genes to set up a regular periodic pattern of expression of segment-polarity genes, and the segment-polarity genes collaborate with one another to define the internal pattern of each individual segment. The strategy, therefore, is one of sequential induction (see Figure 22–16). By the end of the process, the global gradients produced by the egg-polarity genes have triggered the creation of a fine-grained pattern through a hierarchy of sequential, progressively more local, positional controls. Because the global positional signals that start the process do not have to directly specify fine details, the individual cell nuclei do not have to be governed with extreme precision by small differences in the concentration of these signals. Instead, at each step in the sequence, new signals come into play, providing substantial localized differences of concentration to define new details. Sequential induction is thus a robust strategy. It works reliably to produce fly embryos that all have the same pattern, despite the essential imprecision of biological control systems, and despite variations in conditions such as the temperature at which the fly develops.

The Modular Nature of Regulatory DNA Allows Genes to Have Multiple Independently Controlled Functions

The elaborate patterning process just described depends on the long stretches of noncoding DNA sequence that control the expression of each of the genes involved. These regulatory regions bind multiple copies of the gene regulatory proteins produced by the patterning genes expressed earlier. Like an input–output logic device, an individual gene is thus turned on and off according to the particular combination of proteins bound to its regulatory regions at each stage of development. In Chapter 7 we describe one particular segmentation gene—the pair-rule gene *Even-skipped (Eve)*—and discuss how the decision whether to transcribe the gene is made on the basis of all these inputs (see Figure 7–55). This example can be taken further to illustrate some important principles of developmental patterning.

Individual stripes of *Eve* expression depend on separate regulatory modules in the *Eve* regulatory DNA. Thus, one regulatory module is responsible for driving *Eve* expression in stripes 1 + 5, another for stripe 2, another for stripes 3 + 7 , and yet another for stripes 4 + 6 (**Figure 22–39**). Each regulatory module defines a different set of requirements for gene expression according to the concentrations of the products of the egg-polarity and gap genes. In this way, the *Eve* regulatory DNA serves to translate the complex nonrepetitive pattern of egg-polarity and gap proteins into the periodic pattern of expression of a pair-rule gene.

The modular organization of the *Eve* regulatory DNA just described is typical of gene regulation in multicellular animals and plants, and it has profound implications. By stringing together sequences of modules that respond to different combinations of regulatory proteins, it is possible to generate almost any pattern of gene expression on the basis of almost any other. Modularity, moreover, allows the regulatory DNA to define patterns of gene expression that are not merely complex, but whose parts are independently adjustable. A change in one of the regulatory modules can alter one part of the expression pattern without affecting the rest, and without requiring changes in regulatory proteins that would have repercussions for the expression of other genes in the genome. As described in Chapter 7, it is such regulatory DNA that contains the key to the complex organization of multicellular plants and animals, and its properties make possible the independent adaptability of each part of an organism's body structure in the course of evolution.

Most of the segmentation genes also have important functions at other times and places in the development of *Drosophila*. The *Eve* gene, for example, is expressed in subsets of neurons, in muscle precursor cells, and in various

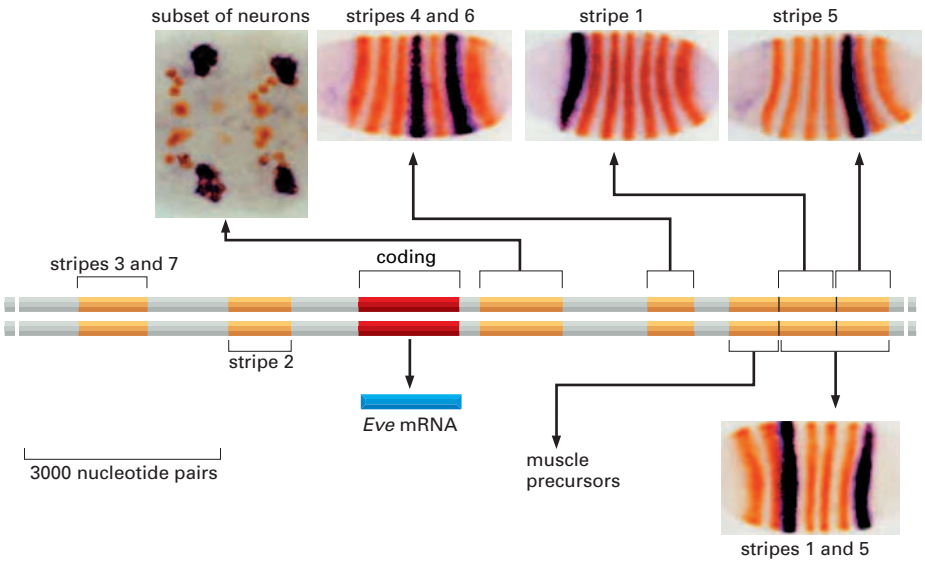


Figure 22–39 Modular organization of the regulatory DNA of the *Eve* gene. In the experiment shown, cloned fragments of the regulatory DNA were linked to a *LacZ* reporter (a bacterial gene). Transgenic embryos containing these constructs were then stained by *in situ* hybridization to reveal the pattern of expression of *LacZ (blue/black),* and counterstained with an anti-Eve antibody *(orange)* to show the positions of the normal *Eve* expression stripes. Different segments of the *Eve* regulatory DNA *(ochre)* are thus found to drive gene expression in regions corresponding to different parts of the normal *Eve* expression pattern. Two segments in tandem drive expression in a pattern that is the sum of the patterns generated by each of them individually. Separate regulatory modules are responsible for different times of gene expression, as well as different locations: the leftmost panel shows the action of a module that comes into play later than the others illustrated and drives expression in a subset of neurons. (From M. Fujioka et al., *Development* 126:2527–538, 1999. With permission from The Company of Biologists.)

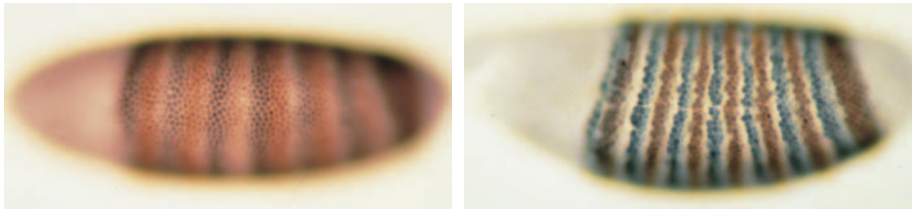


Figure 22–40 The formation of *Ftz* and *Eve* stripes in the *Drosophila* blastoderm. *Ftz* and *Eve* are both pair-rule genes. Their expression patterns (shown in *brown* for *Ftz* and in *gray* for *Eve*) are at first blurred but rapidly resolve into sharply defined stripes. (From P.A. Lawrence, The Making of a Fly. Oxford, UK: Blackwell, 1992.)

other sites, under the control of additional enhancers (see Figure 22–39). By addition of new modules to its regulatory DNA, any gene can be co-opted during evolution for new purposes at new sites in the body, without detriment to its other functions.

Egg-Polarity, Gap, and Pair-Rule Genes Create a Transient Pattern That Is Remembered by Other Genes

Within the first few hours after fertilization, the gap genes and the pair-rule genes are activated. Their mRNA products appear first in patterns that only approximate the final picture; then, within a short time—through a series of interactive adjustments—the fuzzy initial distribution of gene products resolves itself into a regular, crisply defined system of stripes (**Figure 22–40**). But this system itself is unstable and transient. As the embryo proceeds through gastrulation and beyond, the regular segmental pattern of gap and pair-rule gene products disintegrates. Their actions, however, have stamped a permanent set of labels—positional values—on the cells of the blastoderm. These positional labels are recorded in the persistent activation of certain of the segment-polarity genes and of the homeotic selector genes, which serve to maintain the segmental organization of the larva and adult. The segment-polarity gene *Engrailed* provides a good example. Its RNA transcripts are seen in the cellular blastoderm in a series of 14 bands, each approximately one cell wide, corresponding to the anteriormost portions of the future parasegments (**Figure 22–41**).

The segment-polarity genes are expressed in patterns that repeat from one parasegment to the next, and their bands of expression appear in a fixed relationship to the bands of expression of the pair-rule genes that help to induce them. However, the production of this pattern within each parasegment depends on interactions among the segment-polarity genes themselves. These interactions occur at stages when the blastoderm has already become fully partitioned into separate cells, so that cell–cell signaling of the usual sort has to come into play. A large subset of the segment-polarity genes code for components of two signal transduction pathways, the Wnt pathway and the Hedgehog pathway, including the secreted signal proteins Wingless (a Wnt family member) and Hedgehog. These are expressed in different bands of cells that serve as signaling

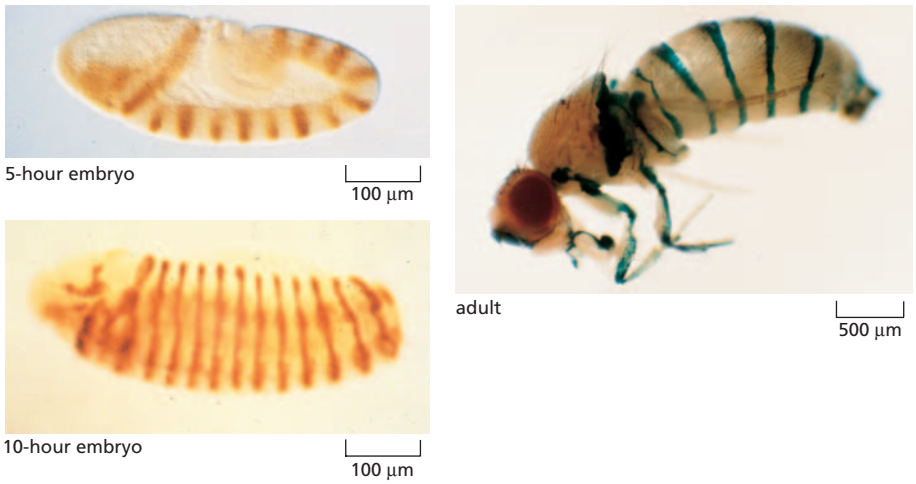


Figure 22–41 The pattern of expression of *Engrailed*, a segment-polarity gene. The *Engrailed* pattern is shown in a 5-hour embryo (at the extended germband stage), a 10-hour embryo, and an adult (whose wings have been removed in this preparation). The pattern is revealed by an antibody *(brown)* against the Engrailed protein (for the 5- and 10-hour embryos) or (for the adult) by constructing a strain of *Drosophila* containing the control sequences of the *Engrailed* gene coupled to the coding sequence of the reporter *LacZ*, whose product is detected histochemically through the blue product of a reaction that it catalyzes. Note that the *Engrailed* pattern, once established, is preserved throughout the animal's life. (From C. Hama, Z. Ali and T.B. Kornberg, *Genes Dev.* 4:1079–1093, 1990. With permission from Cold Spring Harbor Laboratory Press.)

centers within each parasegment, and they act to maintain and refine the expression of other segment-polarity genes. Moreover, although their initial expression is determined by the pair-rule genes, the two signal proteins regulate one another's expression in a mutually supportive way, and they proceed to help trigger expression of genes such as *Engrailed* in precisely the correct sites.

The *Engrailed* expression pattern will persist throughout life, long after the signals that organized its production have disappeared (see Figure 22–41). This example illustrates not only the progressive subdivision of the embryo by means of more and more narrowly localized signals, but also the transition between the transient signaling events of early development and the later stable maintenance of developmental information.

Besides regulating the segment-polarity genes, the products of pair-rule genes collaborate with the products of gap genes to cause the precisely localized activation of a further set of spatial labels—the homeotic selector genes. It is the homeotic selector genes that permanently distinguish one parasegment from another. In the next section we examine these selector genes in detail and consider their role in cell memory.

Summary

The fly Drosophila *has been the foremost model organism for study of the genetics of animal development. Like other insects, it begins its development with a series of nuclear divisions generating a syncytium, and a large amount of early patterning occurs in this single giant multinucleate cell. The pattern originates with asymmetry in the egg, organized both by localized deposits of mRNA inside the egg and by signals from the follicle cells around it. Positional information in the multinucleate embryo is supplied by four intracellular gradients that are set up by the products of four groups of maternal-effect genes called egg-polarity genes. These control four distinctions fundamental to the body plan of animals: dorsal versus ventral, endoderm versus mesoderm and ectoderm, germ cells versus somatic cells, and head versus rear.*

The egg-polarity genes operate by setting up graded distributions of gene regulatory proteins in the egg and early embryo. The gradients along the anteroposterior axis initiate the orderly expression of gap genes, pair-rule genes, segment-polarity genes, and homeotic selector genes. These, through a hierarchy of interactions, become expressed in some regions of the embryo and not others, progressively subdividing the blastoderm into a regular series of repeating modular units called segments. The complex patterns of gene expression reflect the modular organization of the regulatory DNA, with separate enhancers of an individual gene responsible for separate parts of its expression pattern.

The segment-polarity genes come into play toward the end of the segmentation process, soon after the syncytium has become partitioned into separate cells, and they control the internal patterning of each segment through cell–cell signaling via the Wnt (Wingless) and Hedgehog pathways. This leads to persistent localized activation of genes such as Engrailed, *giving cells a remembered record of their anteroposterior address within the segment. Meanwhile, a new cell–cell signaling gradient is also set up along the dorsoventral axis, with the TGFβ family member Decapentaplegic (Dpp) and its antagonist, Short gastrulation, acting as the morphogens. This gradient helps to refine the assignment of different characters to cells at different dorsoventral levels. Homologous proteins are also known to control the patterning of the ventrodorsal axis in vertebrates.*

HOMEOTIC SELECTOR GENES AND THE PATTERNING OF THE ANTEROPOSTERIOR AXIS

As development proceeds, the body becomes more and more complex. In all this growing complexity there is, however, a simplifying feature that puts an understanding of the whole developmental process within our grasp. Again and again, in every species and at every level of organization, we find that complex structures

are made by repeating a few basic themes with variations. Thus, a limited number of basic differentiated cell types, such as muscle cells or fibroblasts, recur with subtle individual variations in different sites. These cell types are organized into a limited variety of tissue types, such as muscle or tendon, which again are repeated with subtle variations in different regions of the body. From the various tissues, organs such as teeth or digits are built—molars and incisors, fingers and thumbs and toes—a few basic kinds of structure, repeated with variations.

Wherever we find this phenomenon of *modulated repetition*, we can break down the developmental biologist's problem into two kinds of question: what is the basic construction mechanism common to all the objects of the given class, and how is this mechanism modified to give the observed variations? The embryo uses a combinatorial strategy to generate its complexity, and we can use a combinatorial strategy to understand it.

The segments of the insect body provide a very clear example. We have already sketched the way in which the rudiment of a single typical segment is constructed. We must now consider how one segment is caused to be different from another.

Figure 22–42 A homeotic mutation. The fly shown here is an *Antennapedia* mutant. Its antennae are converted into leg structures by a mutation in the regulatory region of the *Antennapedia* gene that causes it to be expressed in the head. Compare with the normal fly shown in Figure 22–24. (Courtesy of Matthew Scott.)

The Hox Code Specifies Anterior–Posterior Differences

The first glimpse of a genetic answer to the question of how each segment acquires its individual identity came over 80 years ago, with the discovery of the first of a set of mutations in *Drosophila* that cause bizarre disturbances of the organization of the adult fly. In the *Antennapedia* mutant, for example, legs sprout from the head in place of antennae (**Figure 22–42**), while in the *Bithorax* mutant, portions of an extra pair of wings appear where normally there should be the much smaller appendages called halteres. These mutations transform parts of the body into structures appropriate to other positions and are called *homeotic*. A whole set of **homeotic selector genes** determines the anteroposterior character of the segments of the fly.

The genes of this set—eight of them in the fly—are all related to one another as members of a multigene family, and they all lie in one or the other of two tight gene clusters known as the **Bithorax complex** and the **Antennapedia complex**. The genes in the Bithorax complex control the differences among the abdominal and thoracic segments of the body, while those in the Antennapedia complex control the differences among thoracic and head segments. Comparisons with other species show that the same genes are present in essentially all animals, including humans. These comparisons also reveal that the Antennapedia and Bithorax complexes are the two halves of a single entity, called the **Hox complex**, that has become split in the course of the fly's evolution, and whose members operate in a coordinated way to exert their control over the head-to-tail pattern of the body.

Homeotic Selector Genes Code for DNA-Binding Proteins That Interact with Other Gene Regulatory Proteins

To a first approximation each homeotic selector gene is normally expressed in just those regions that develop abnormally when the gene is mutated or absent. The products of these genes can thus be viewed as molecular address labels possessed by the cells of each parasegment: they are the physical embodiment of the cells' positional value. If the address labels are changed, the parasegment behaves as though it were located somewhere else, and deletion of the entire complex results in a larva whose body segments are all alike (**Figure 22–43**).

A first problem, therefore, is to understand how the homeotic selector gene products act on the basic segment-patterning machinery to give each parasegment its individuality. The products of the homeotic selector genes are gene regulatory proteins, all related to one another by the possession of a highly conserved DNA-binding *homeodomain* (60 amino acids long), discussed in Chapter 7. The corresponding segment in the DNA sequence is called a *homeobox* from which, by abbreviation, the Hox complex takes its name.

Figure 22–43 The effect of deleting most of the genes of the Bithorax complex. (A) A normal *Drosophila* larva shown in dark-field illumination; (B) the mutant larva with the Bithorax complex largely deleted. In the mutant the parasegments posterior to P5 all have the appearance of P5. (From G. Struhl, *Nature* 293:36–41, 1981. With permission from Macmillan Publishers Ltd.)

If the products of the homeotic selector genes are similar in their DNA-binding regions, how do they exert different effects so as to make one parasegment different from the next? The answer seems to lie largely in the parts of the proteins that do not bind directly to DNA but interact with other proteins in DNA-bound complexes. The different partners in these complexes act together with the homeotic selector proteins to dictate which DNA-binding sites will be recognized and whether the effect on transcription at those sites will be activation or repression. In this way, the products of the homeotic selector genes combine with other gene regulatory proteins and modulate their actions so as to give each parasegment its characteristic features.

The Homeotic Selector Genes Are Expressed Sequentially According to Their Order in the Hox Complex

To understand how the Hox complex provides cells with positional values, we also need to consider how the expression of the *Hox* genes themselves is regulated. The coding sequences of the eight homeotic selector genes in the Antennapedia and bithorax complexes are interspersed amid a much larger quantity—a total of about 650,000 nucleotide pairs—of regulatory DNA. This DNA includes binding sites for the products of egg-polarity and segmentation genes. The regulatory DNA in the Hox complex acts as an interpreter of the multiple items of positional information supplied to it by all these gene regulatory proteins. In response, a particular set of homeotic selector genes is transcribed, appropriate to the location.

In the pattern of control there is a remarkable regularity. The sequence in which the genes are ordered along the chromosome, in both the Antennapedia and the Bithorax complexes, corresponds almost exactly to the order in which they are expressed along the axis of the body (**Figure 22–44**). This suggests that the genes are activated serially by some process that is graded—in duration or intensity—along the axis of the body, and whose action spreads gradually along the chromosome. The most "posterior" of the genes expressed in a cell generally dominates, driving down expression of the previously activated "anterior" genes and dictating the character of the segment. The gene regulatory mechanisms underlying these phenomena are still not well understood, but their consequences are profound. We shall see that the serial organization of gene expression in the Hox complex is a fundamental feature that has been highly conserved in the course of evolution.

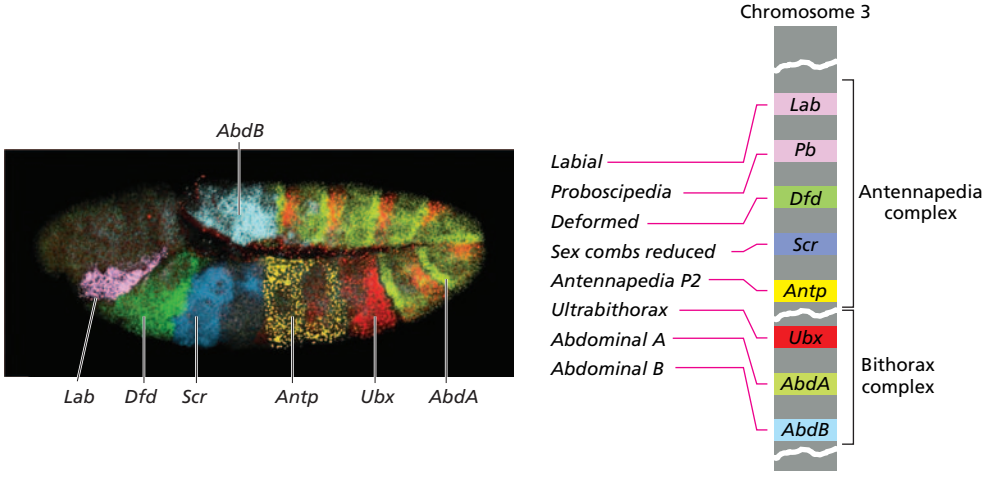


Figure 22–44 The patterns of expression compared to the chromosomal locations of the genes of the Hox complex. The diagram shows the sequence of genes in each of the two subdivisions of the chromosomal complex. This corresponds, with minor deviations, to the spatial sequence in which the genes are expressed, shown in the photograph of an embryo at the extended germ band stage, about 5 hours after fertilization. The embryo has been stained by *in situ* hybridization with differently labeled probes to detect the mRNA products of different *Hox* genes in different colors. (Photograph courtesy of William McGinnis, adapted from D. Kosman et al., *Science* 305:846, 2004. With permission from AAAS.)

There are hundreds of other homeobox-containing genes in the genome of the fly—and of other animal species—but most of them are scattered and not clustered in complexes such as the Hox complex. They have many different gene regulatory functions, but a substantial proportion of them have roles akin to that of the *Hox* genes: they control the variations on a basic developmental theme. Different classes of neurons, for example, are often distinguished from one another by expression of specific genes of this large superfamily.

The Hox Complex Carries a Permanent Record of Positional Information

The spatial pattern of expression of the genes in the Hox complex is set up by signals acting early in development, but the consequences are long-lasting. Although the pattern of expression undergoes complex adjustments as development proceeds, the Hox complex behaves in each cell as though stamped with a permanent record of the anteroposterior position that the cell occupied in the early embryo. In this way, the cells of each segment are equipped with a long-term memory of their location along the anteroposterior axis of the body—in other words, with an anteroposterior positional value. As we shall see in the next section, the memory trace imprinted on the Hox complex governs the segment-specific identity not only of the larval segments, but also of the structures of the adult fly, which are generated at a much later stage from the larval imaginal discs and other nests of imaginal precursor cells in the larva.

The molecular mechanism of the cell memory for this positional information relies on two types of regulatory inputs. One is from the homeotic selector genes themselves: many of the Hox proteins autoactivate the transcription of their own genes. Another crucial input is from two large complementary sets of proteins that control chromatin structure, called the *Polycomb group* and the *Trithorax group*. If these regulators are defective, the pattern of expression of the homeotic selector genes is set up correctly at first but is not correctly maintained as the embryo grows older.

The two sets of regulators act in opposite ways. Trithorax group proteins are needed to maintain the transcription of *Hox* genes in cells where transcription has already been switched on. In contrast, Polycomb group proteins form stable complexes that bind to the chromatin of the Hox complex and maintain the repressed state in cells where *Hox* genes have not been activated at the critical time (**Figure 22–45**). The developmental memory involves specific covalent modifications of histones in nucleosomes in the neighborhood of the *Hox* genes, leading to changes in the state of the chromatin that can be perpetuated from one cell generation to the next, as discussed in Chapters 4 and 7.

The Anteroposterior Axis Is Controlled by *Hox* Selector Genes in Vertebrates Also

Homologs of the *Drosophila* homeotic selector genes have been found in almost every animal species studied, from cnidarians (hydroids) and nematodes to mollusks and mammals. Remarkably, these genes are often grouped in complexes similar to the insect Hox complex. In the mouse there are four such complexes—called the *HoxA, HoxB, HoxC,* and *HoxD* complexes—each on a different chromosome. Individual genes in each complex can be recognized by their sequences as counterparts of specific members of the *Drosophila* set. Indeed, mammalian *Hox* genes can function in *Drosophila* as partial replacements for the corresponding *Drosophila Hox* genes. It appears that each of the four mammalian Hox complexes is, roughly speaking, the equivalent of a complete insect complex (that is, an Antennapedia complex plus a Bithorax complex) (**Figure 22–46**).

The ordering of the genes within each vertebrate Hox complex is essentially the same as in the insect Hox complex, suggesting that all four vertebrate

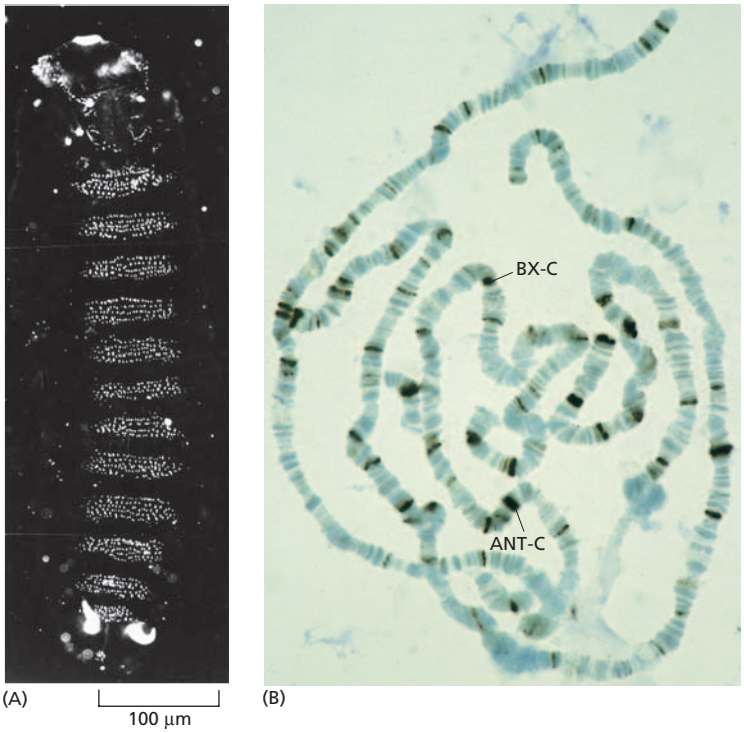


Figure 22–45 Action of genes of the Polycomb group. (A) Photograph of a mutant embryo defective for the gene *Extra sex combs (Esc)* and derived from a mother also lacking this gene. The gene belongs to the Polycomb group. Essentially all segments have been transformed to resemble the most posterior abdominal segment (compare with Figure 22–43). In the mutant the pattern of expression of the homeotic selector genes, which is roughly normal initially, is unstable in such a way that all these genes soon become switched on all along the body axis. (B) The normal pattern of binding of Polycomb protein to *Drosophila* giant chromosomes, visualized with an antibody against Polycomb. The protein is bound to the Antennapedia complex (ANT-C) and the Bithorax complex (BX-C) as well as about 60 other sites. (A, from G. Struhl, *Nature* 293:36–41, 1981. With permission from Macmillan Publishers Ltd. B, courtesy of B. Zink and R. Paro, *Trends Genet.* 6:416–421, 1990. With permission from Elsevier.)

complexes originated by duplications of a single primordial complex and have preserved its basic organization. Most tellingly, when the expression patterns of the *Hox* genes are examined in the vertebrate embryo by *in situ* hybridization, it turns out that the members of each complex are expressed in a head-to-tail series along the axis of the body, just as they are in *Drosophila* (**Figure 22–47**). The pattern is most clearly seen in the neural tube, but is also visible in other tissues, especially the mesoderm. With minor exceptions this anatomical ordering matches the chromosomal ordering of the genes in each complex, and corresponding genes in the four different Hox complexes have almost identical anteroposterior domains of expression.

The gene expression domains define a detailed system of correspondences between insect body regions and vertebrate body regions (see Figure 22–46). The parasegments of the fly correspond to a similarly labeled series of segments in the anterior part of the vertebrate embryo. These are most clearly demarcated in the hindbrain (see Figures 22–46 and 22–47), where they are called *rhombomeres.* In the tissues lateral to the hindbrain the segmentation is seen in the series of *branchial arches,* prominent in all vertebrate embryos—the precursors of the system of gills in fish and of the jaws and structures of the neck in mammals; each pair of rhombomeres in the hindbrain corresponds to one branchial arch. In the hindbrain, as in *Drosophila,* the boundaries of the expression domains of many of the *Hox* genes are aligned with the boundaries of the anatomical segments.

The products of the mammalian *Hox* genes appear to specify positional values that control the anteroposterior pattern of parts in the hindbrain, neck, and trunk (as well as some other parts of the body). As in *Drosophila,* when a posterior *Hox* gene is artificially expressed in an anterior region, it can convert the anterior tissue to a posterior character. Conversely, loss of posterior *Hox* genes allows the posterior tissue where they are normally expressed to adopt an anterior character (**Figure 22–48**). The transformations observed in mouse *Hox* mutants are not always so straightforward and are often incomplete, because of a redundancy between genes in the four *Hox* gene clusters. But it seems clear that the fly and the mouse use essentially the same molecular machinery to give individual characters to successive regions along at least a part of their anteroposterior axis.

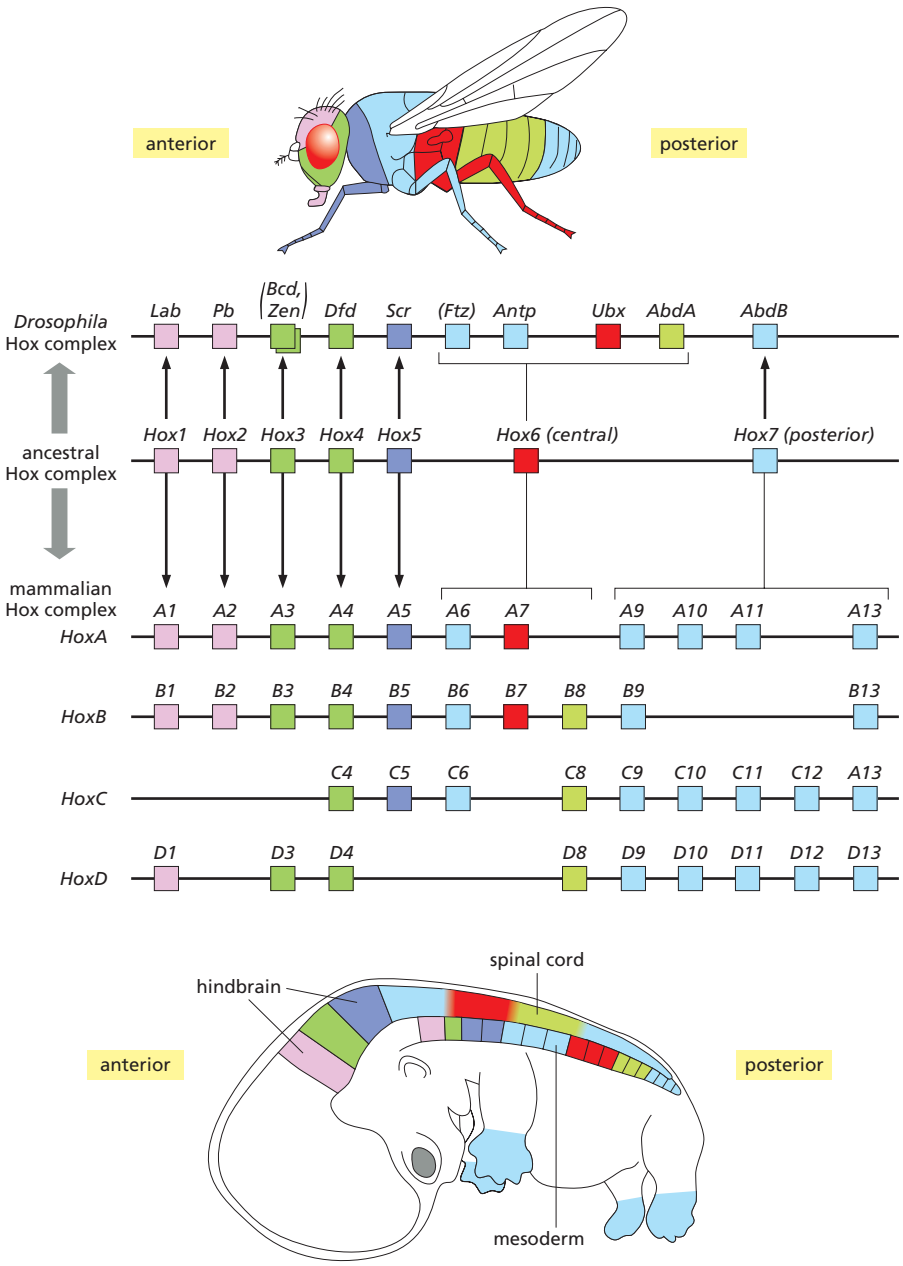


Figure 22–46 The Hox complex of an insect and the Hox complexes of a mammal compared and related to body regions. The genes of the Antennapedia and Bithorax complexes of *Drosophila* are shown in their chromosomal order in the top line; the corresponding genes of the four mammalian Hox complexes are shown below, also in chromosomal order. The gene expression domains in fly and mammal are indicated in a simplified form by *color* in the cartoons of animals above and below. However, the details of the patterns depend on developmental stage and vary somewhat from one mammalian Hox complex to another. Also, in many cases, genes shown here as expressed in an anterior domain are also expressed more posteriorly, overlapping the domains of more posterior *Hox* genes (see, for example, Figure 22–47). The complexes are thought to have evolved as follows: first, in some common ancestor of worms, flies, and vertebrates, a single primordial homeotic selector gene underwent repeated duplication to form a series of such genes in tandem—the ancestral Hox complex. In the *Drosophila* sublineage this single complex became split into separate Antennapedia and Bithorax complexes. Meanwhile, in the lineage leading to the mammals the whole complex was repeatedly duplicated to give four Hox complexes. The parallelism is not perfect because apparently some individual genes have been duplicated, others lost, and still others co-opted for different purposes (genes in parentheses in the top line) since the complexes diverged. (Based on a diagram courtesy of William McGinnis.)

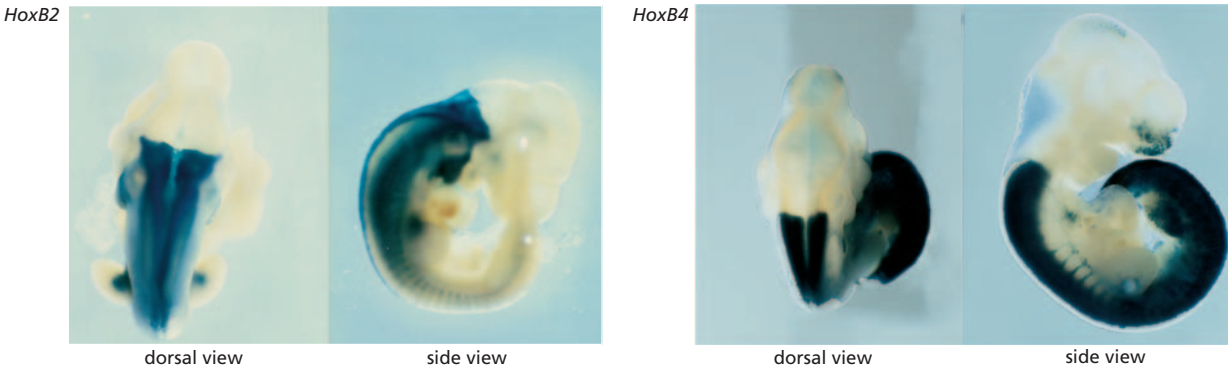
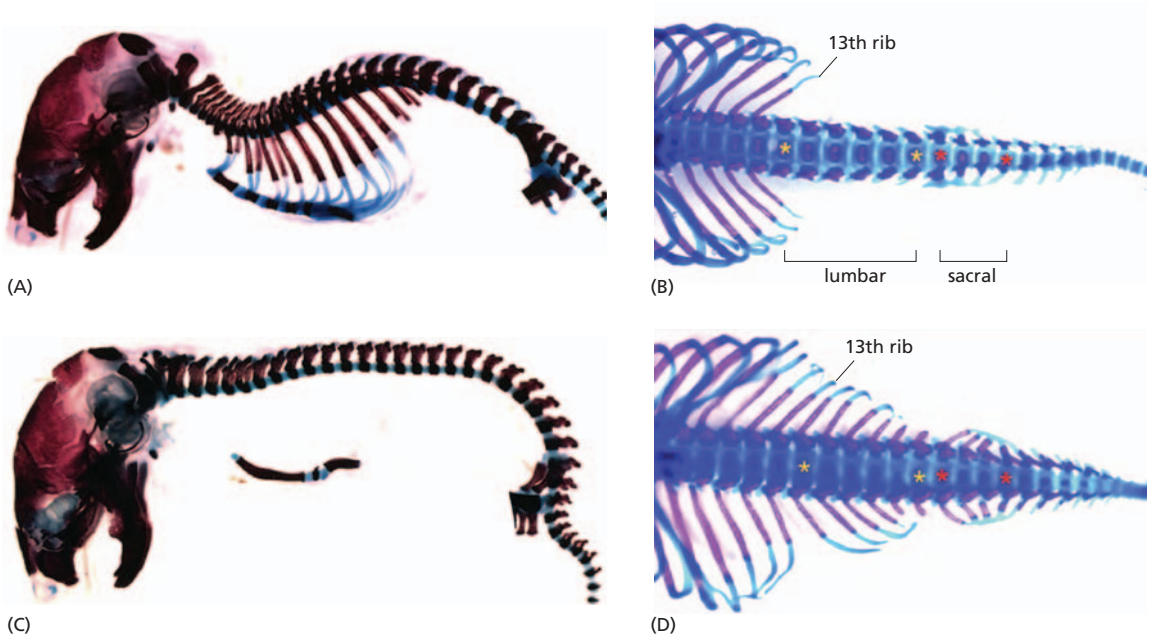


Figure 22–47 Expression domains of *Hox* genes in a mouse. The photographs show whole embryos displaying the expression domains of two genes of the HoxB complex *(blue stain)*. These domains can be revealed by *in situ* hybridization or, as in these examples, by constructing transgenic mice containing the control sequence of a *Hox* gene coupled to a *LacZ* reporter gene, whose product is detected histochemically. Each gene is expressed in a long expanse of tissue with a sharply defined anterior limit. The earlier the position of the gene in its chromosomal complex, the more anterior the anatomical limit of its expression. Thus, with minor exceptions, the anatomical domains of the successive genes form a nested set, ordered according to the ordering of the genes in the chromosomal complex. (Courtesy of Robb Krumlauf.)



Summary

The complexity of the adult body of an animal is built up by modulated repetition of a few basic types of structure. Thus, superimposed on the pattern of gene expression that repeats itself in every segment, there is a serial pattern of expression of homeotic selector genes that confer on each segment a different identity. The homeotic selector genes code for DNA-binding proteins of the homeodomain family. They are grouped in the Drosophila *genome in two clusters, called the Antennapedia and bithorax complexes, believed to be the two parts of a single primordial Hox complex that became split during evolution of the fly. In each complex, the genes are arranged in a sequence that matches their sequence of expression along the axis of the body. Hox gene expression is initiated in the embryo. It is maintained subsequently by the action of DNA-binding proteins of the Polycomb and Trithorax group, which stamp the chromatin of the Hox complex with a heritable record of its embryonic state of activation. Hox complexes homologous to that of* Drosophila *are found in virtually every type of animal that has been examined, from cnidarians to humans, and they appear to have an evolutionarily conserved role in patterning the anteroposterior axis of the body. Mammals have four Hox complexes, each showing a similar relationship between a serial arrangement of the genes in the chromosome and their serial pattern of expression along the body axis.*

Figure 22–48 Control of anteroposterior pattern by *Hox* genes in the mouse. (A,B) A normal mouse has about 65 vertebrae, differing in structure according to their position along the body axis: 7 cervical (neck), 13 thoracic (with ribs), 6 lumbar (bracketed by *yellow* asterisks in (B)), 4 sacral (bracketed by *red* asterisks in (B)), and about 35 caudal (tail). (A) shows a side view; (B) shows a dorsal view; for clarity, the limbs have been removed in each picture. (C) The *HoxA10* gene is normally expressed in the lumbar region (together with its paralogs *HoxC10* and *HoxD10*); here it has been artificially expressed in the developing vertebral tissue all along the body axis. As a result, the cervical and thoracic vertebrae are all converted to a lumbar character. (D) Conversely, when *HoxA10* is knocked out along with *HoxC10* and *HoxD10*, vertebrae that should normally have a lumbar or sacral character take on a thoracic character instead. (A and C, from M. Carapuço et al., *Genes Dev.* 19:2116–2121, 2005. With permission from Cold Spring Harbor Laboratory Press; B and D, from D.M. Wellik and M.R. Capecchi, *Science* 301:363–367, 2003. With permission from AAAS.)

ORGANOGENESIS AND THE PATTERNING OF APPENDAGES

We have seen that the segments of the insect larva are all variations on the same basic theme, with segmentation genes defining the basic repetitive module and homeotic selector genes giving each segment its individual character. The same applies to the major appendages of the adult insect body—legs, wings, antennae, mouthparts and external genitalia: they too are variations on a common basic theme. At a finer level of detail, we encounter the same wonderful simplification: the appendages—and many other parts of the body—consist of substructures that are themselves variations on a small number of basic evolutionarily conserved themes.

In this section we follow the course of development in *Drosophila* through to its end, narrowing our focus at each step to examine one example of the many related structures that are developing in parallel. As we go along, we shall point

out parallels with vertebrate structures that develop similarly, using not only the same general strategies but many of the same specific molecular mechanisms. But to avoid interrupting the narrative later, we must first briefly explain some key experimental methods, required to cope with a special problem that arises when we try to discover how genes control the later stages of development.

Conditional and Induced Somatic Mutations Make it Possible to Analyze Gene Functions Late in Development

As emphasized earlier, the same gene may be used repeatedly in many different situations—in different regions of the body, and at different times. Often, loss-of-function mutations disrupt early development so severely that the embryo or larva dies, depriving us of the opportunity to see how the mutation would affect later processes.

One way around this problem is to study conditional mutations. If we have, for example, a temperature-sensitive mutation in the gene of interest, we can maintain the animal during early development at a low temperature, where the gene product functions normally, and then disable the gene product whenever we please by raising the temperature to discover the late functions.

Other methods involve actually modifying the DNA in subsets of cells at late stages of development—a sort of genetic surgery on individual cells that allows mutant groups of cells of a specified genotype to be generated at a chosen time in development. This remarkable feat can be achieved by *induced somatic recombination*, and the resulting organism is called a **genetic mosaic**. By means of genetic mosaics, we can not only bypass the problem of lethality when a gene function is perturbed in the organism as a whole; we can also explore the function of the gene in cell–cell interactions, by juxtaposing mutant and nonmutant cells. We can test, for example, whether cells use the gene product to send a signal to neighbors, or to receive a signal from them, or neither. And by inducing the genetic change at different times, we can find out precisely when the gene acts to produce a particular effect.

A current technique for induced somatic recombination uses transgenic flies that have been bred to contain two types of yeast-derived genetic elements: the FLP site-specific recombinase gene, and the FLP Recombinase Target (FRT) sequence. Typically, the animal is homozygous for an insertion of the FRT sequence close to the centromere on a chosen chromosome arm, while a construct consisting of the *Flp* gene under a heat-shock promoter is inserted elsewhere in the genome. If such a transgenic embryo or larva is given a heat shock (that is, exposed to a high temperature for a few minutes), expression of *Flp* is induced, and this enzyme catalyzes crossing-over and recombination between the maternal and paternal chromosomes at the FRT site. If the heat shock is adjusted to be sufficiently mild, this event will occur in only one or a few cells, scattered at random. As explained in **Figure 22–49**, if the animal is also heterozygous for a gene of interest in the crossed-over chromosomal region, the process can result in a pair of daughter cells that are homozygous, the one receiving two copies of the maternal allele of the gene, the other receiving two copies of the paternal allele. Each of these daughter cells will normally grow and divide to give clonal patches of homozygous progeny.

The occurrence of the crossover can be detected if the animal is chosen to be also heterozygous for a genetic marker that lies on the same chromosome arm as the gene of interest and so undergoes crossing over in company with it. In this way clearly marked homozygous mutant clones of cells can be created to order. Either FLP and FRT, or the analogous Cre and Lox pair of recombination elements, can also be used in other configurations to switch expression of a gene on or off (see Figure 5–79). With these techniques, one can discover what happens, for example, when cells are caused to produce a particular signal molecule at an abnormal site, or are deprived of a particular receptor.

Instead of using a heat-shock promoter to drive expression of the FLP recombinase, one can use a copy of the regulatory sequence of a gene in the fly's

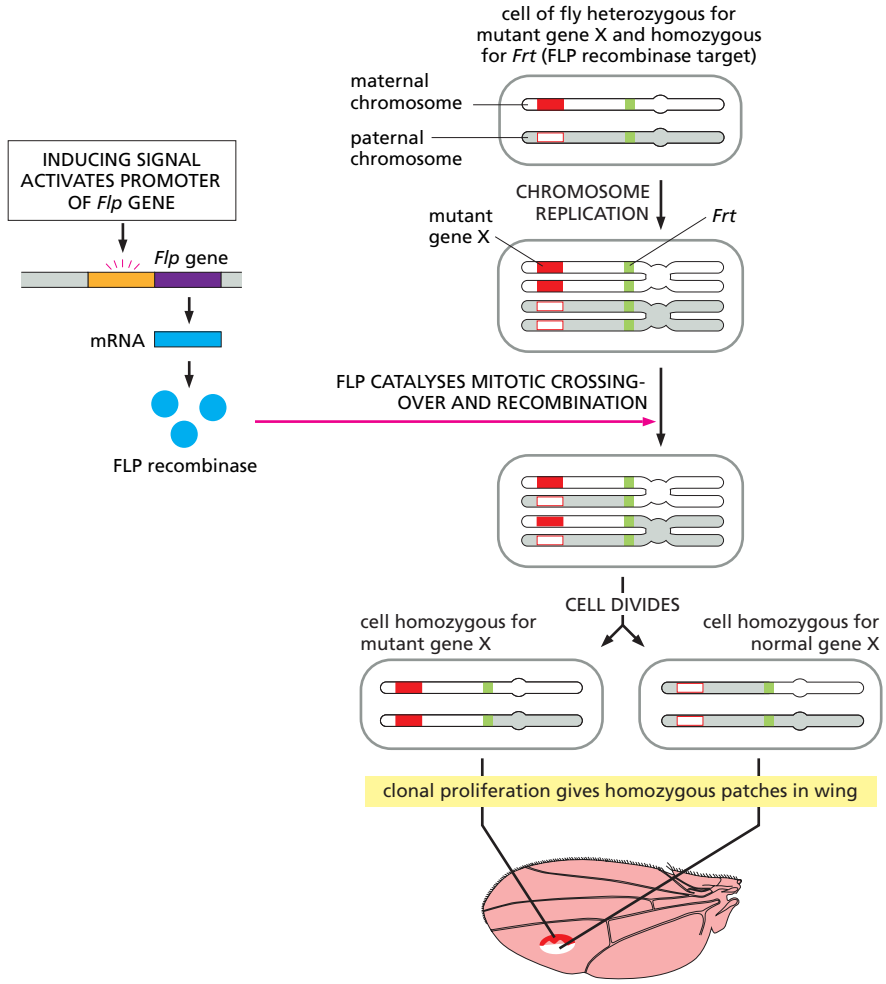


Figure 22–49 Creation of mutant cells by induced somatic recombination. The diagrams follow the fate of a single pair of homologous chromosomes, one from the father *(shaded)*, the other from the mother *(unshaded)*. These chromosomes have an *Frt* element *(green)* inserted close to their centromere, and contain a locus for a gene of interest—gene X—farther out along the same chromosome arm. The paternal chromosome (in this example) carries the wild-type allele of gene X *(open red box)* while the maternal chromosome carries a recessive mutant allele *(filled red box)*. Recombination by exchange of DNA between the maternal and paternal chromosomes, catalyzed by the FLP recombinase, can give rise to a pair of daughter cells, one containing two wild-type copies of gene X, the other containing two mutant copies. To help identify the cells where recombination has occurred, the maternal and paternal chromosomes can be chosen to carry different genetic markers (not shown here), capable of generating a visible product, and positioned on the chromosome so that recombination involving the marker locus—resulting in a visible alteration in the appearance of the cells—can be taken as a sure sign that gene X has also undergone recombination.

normal genome that is expressed at some interesting time and place. The recombination event will then be triggered, and mutant cells created, at just the sites where that gene is normally expressed. A variant of this technique uses transcriptional regulation machinery borrowed from yeast, rather than genetic recombination machinery, to switch expression of a chosen fly gene reversibly on or off according to the normal pattern of expression of some other chosen fly gene (**Figure 22–50**).

By switching gene functions off or on at specific times and places in these ways, developmental biologists can set about deciphering the system of genetically specified signals and responses that control the patterning of any organ of the body.

Body Parts of the Adult Fly Develop From Imaginal Discs

The external structures of the adult fly are formed largely from rudiments called **imaginal discs**—groups of cells that are set aside, apparently undifferentiated, in each segment of the larva. The discs are pouches of epithelium, shaped like crumpled and flattened balloons, and continuous with the epidermis (the surface layer) of the larva. There are 19 of them, arranged as 9 pairs on either side of the larva plus 1 disc in the midline (**Figure 22–51**). They grow and develop their internal pattern as the larva grows, until finally, at metamorphosis, they evert (turn inside out), extend, and differentiate overtly to form the epidermal layer of the adult. The eyes and antennae develop from one pair of discs, the wings and part of the thorax from another, the first pair of legs from another, and so on.

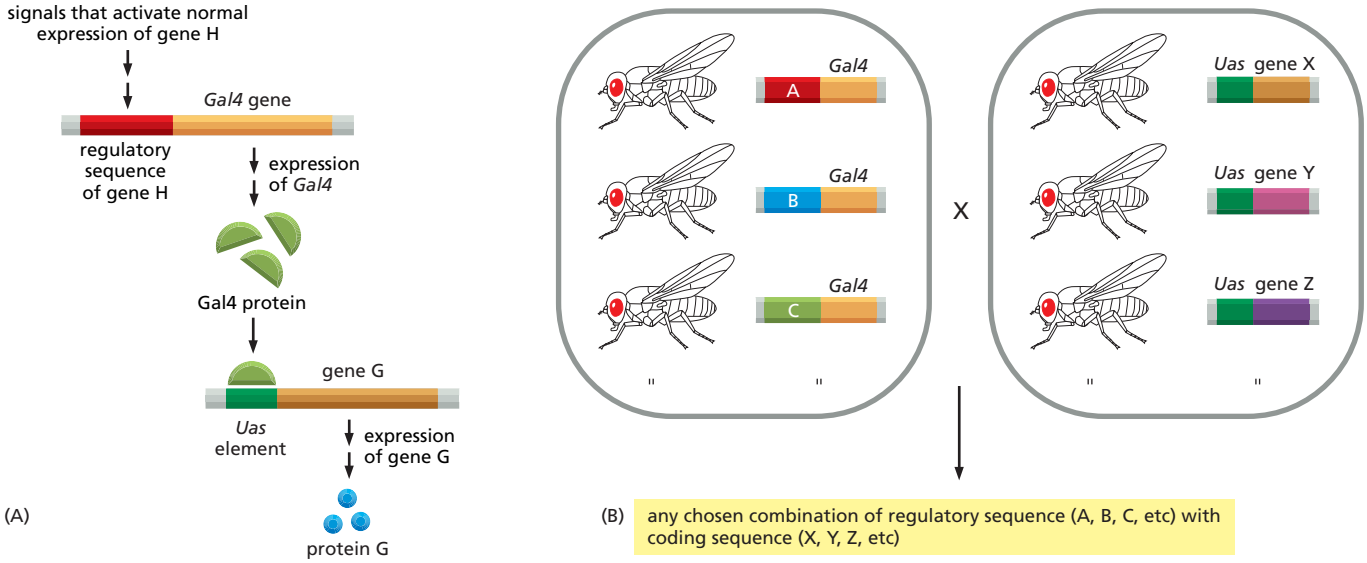


Figure 22–50 The *Gal4/Uas* technique for controlled gene misexpression in *Drosophila*. The method allows one to drive expression of a chosen gene G at the places and times where some other *Drosophila* gene H is normally expressed. (A) A transgenic animal is created, with two separate constructs inserted in its genome. One insert consists of a yeast-specific regulatory sequence, called the *Uas* (upstream activating sequence) element, coupled to a copy of the coding sequence of gene G. The other insert contains the coding sequence of the yeast *Gal4* gene, whose product is a yeast-specific gene regulatory protein that binds to the *Uas* element; this *Gal4* insert is placed next to, and controlled by, the regulatory region of gene H. Wherever gene H is normally expressed, Gal4 protein is also made and drives transcription of gene G. (B) Although one can achieve the same result by linking a copy of the H regulatory sequence directly to the G coding sequence, the *Gal4/Uas* approach allows a strategy that is more efficient in the long run. Two separate "libraries" of transgenic flies are constructed, one containing *Gal4* inserts driven by a variety of regulatory sequences of different genes A, B, C, etc., the other containing *Uas* inserts driving a variety of different coding sequences X, Y, Z, etc. By mating a fly from one library with a fly from the other, any desired coding sequence can be functionally coupled to any desired regulatory sequence. To generate the library of flies with *Gal4* insertions at useful sites, flies are first produced with *Gal4* insertions at random locations in their genome. These are then mated with flies containing a *Uas* element linked to a reporter gene with an easily detectable product. Expression of the reporter reveals whether *Gal4* has been inserted at a site that brings its expression under the control of an interesting enhancer; flies showing interesting reporter patterns are kept and studied. This is called the enhancer trap technique, because it provides a way to hunt out and characterize interesting regulatory sequences in the genome.

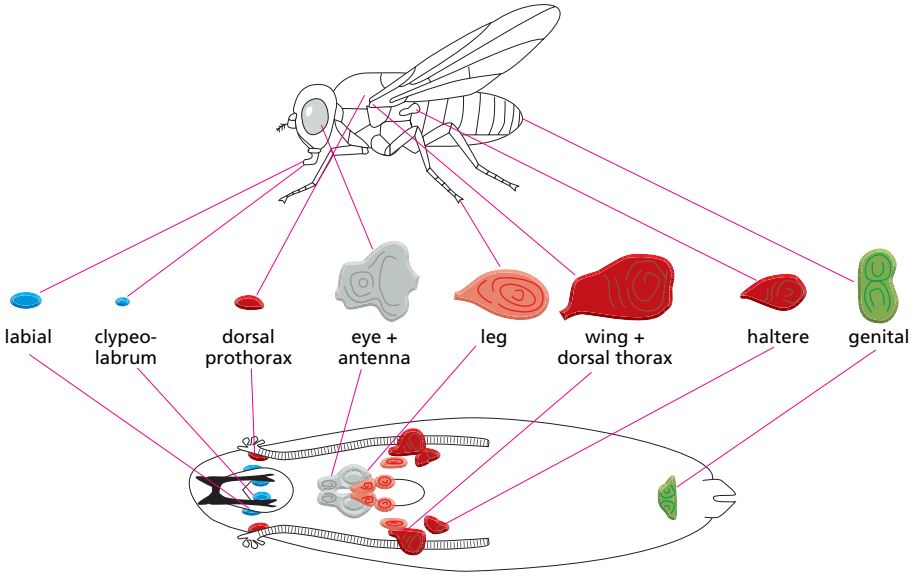


Figure 22–51 The imaginal discs in the *Drosophila* larva and the adult structures they give rise to. (After J.W. Fristrom et al., in Problems in Biology: RNA in Development [E.W. Hanley, ed.], p. 382. Salt Lake City: University of Utah Press, 1969.)

Homeotic Selector Genes Are Essential for the Memory of Positional Information in Imaginal Disc Cells

The cells of one imaginal disc look like those of another, but grafting experiments show that they are in fact already regionally determined and nonequivalent. If one imaginal disc is transplanted into the position of another in the larva and the larva is then left to go through metamorphosis, the grafted disc is found to differentiate autonomously into the structure appropriate to its origin: a wing disc will give wing structures, a haltere disc, haltere structures, regardless of its new site. This shows that the imaginal disc cells are governed by a memory of their original position. By a more complex serial grafting procedure that lets the imaginal disc cells proliferate for an extended period before differentiating, it can be shown that this cell memory is stably heritable (with rare lapses) through an indefinitely large number of cell generations.

The homeotic selector genes are essential components of the memory mechanism. If, at any stage in the long period leading up to differentiation at metamorphosis, both copies of a homeotic selector gene are eliminated by induced somatic recombination from a clone of imaginal disc cells that would normally express that gene, those cells will differentiate into incorrect structures, as though they belonged to a different segment of the body. These and other observations indicate that each cell's memory of positional information depends on the continued activity of the homeotic selector genes. This memory, furthermore, is expressed in a cell-autonomous fashion—each cell appears to maintain its state individually, depending on its own history and genome.

Specific Regulatory Genes Define the Cells That Will Form an Appendage

We must now examine how an appendage develops its internal pattern. We shall take the insect wing as our example.

The process begins with the early patterning mechanisms we have already discussed. The anteroposterior and dorsoventral systems of signals in the early embryo in effect mark out an orthogonal grid in the blastoderm, in the form of dorsoventral, anteroposterior, and periodically spaced segmental gene expression boundaries. At certain points of intersection of these boundaries, the combination of genes expressed is such as to switch a cluster of cells into the imaginal disc pathway.

In molecular terms this corresponds to switching on expression of imaginal-disc-defining regulatory genes. In most of the discs, the gene *Distal-less* is switched on. This codes for a gene regulatory protein that is essential for the sustained growth required to create an elongated appendage such as a leg or an antenna with a proximodistal axis. In its absence, such appendages fail to form, and when it is artificially expressed at abnormal sites, misplaced appendages can be produced. *Distal-less* is expressed in a similar fashion in the developing limbs and other appendages of most species of invertebrates and vertebrates that have been examined (**Figure 22–52**). For the eye disc, another gene, *Eyeless*

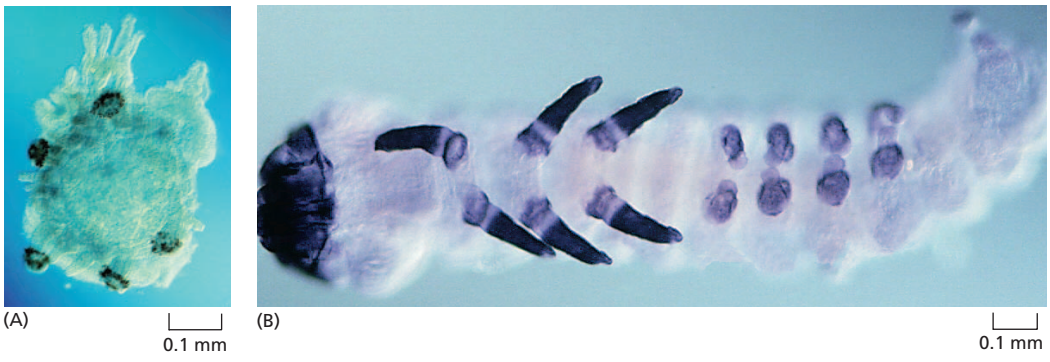


Figure 22–52 Expression of *Distal-less* in developing legs and related appendages of various species. (A) A sea-urchin larva. (B) A moth larva. (A, from G. Panganiban et al., *Proc. Natl Acad. Sci. U.S.A.* 94:5162–5166, 1997. With permission from National Academy of Sciences; B, from G. Panganiban, L. Nagy and S.B. Carroll, *Curr. Biol.* 4:671–675, 1994. With permission from Elsevier.)

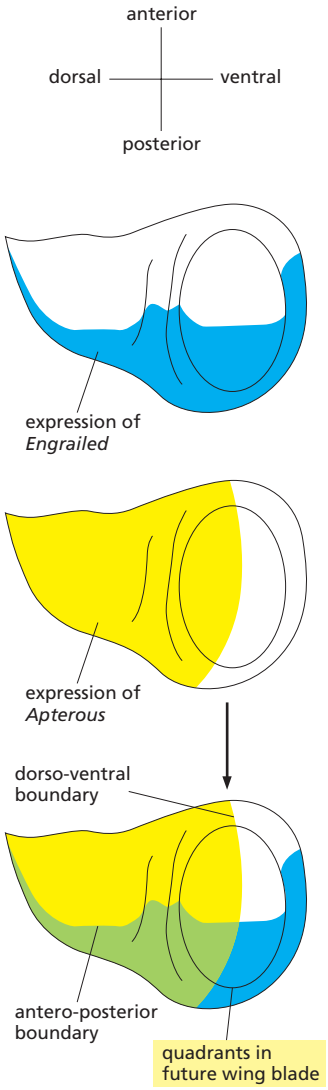


Figure 22–53 Gene expression domains in the wing imaginal disc, defining quadrants of the future wing. The wing blade itself derives from the oval-shaped domain toward the right, and it is divided into four quadrants by the expression of *Apterous* and *Engrailed,* as shown.

(together with two closely related genes), performs the corresponding role; it too has homologues with homologous functions—the *Pax6* genes that drive eye development in other species, as discussed in Chapter 7.

The Insect Wing Disc Is Divided into Compartments

From the outset, the cluster of cells forming the imaginal disc has the rudiments of an internal pattern, inherited from the earlier patterning process. For example, the cells in the posterior half of the wing-disc rudiment (and of most of the other imaginal-disc rudiments) express the segment-polarity gene *Engrailed*, while those in the anterior half do not. The initial asymmetries lay the foundations for a subsequent more detailed patterning, just as in the egg and early embryo.

The sectors of the wing disc defined by these early differences of gene expression correspond to specific parts of the future wing. The posterior, *Engrailed*-expressing region will form the posterior half of the wing, while the region that does not express *Engrailed* will form the anterior half. Meanwhile, the dorsal part of the wing disc expresses a gene called *Apterous*, while the ventral half does not. At metamorphosis, the disc folds along the line separating these domains to give a wing whose dorsal sheet of cells is derived from the *Apterous*-expressing region and whose ventral sheet is derived from the region that does not express *Apterous*. The wing margin, where these two epithelial sheets are joined, corresponds to the boundary of the *Apterous* expression domain in the disc (**Figure 22–53**).

The cells of the disc, having switched on expression of the genes that mark them as anterior or posterior, dorsal or ventral, retain this specification as the disc grows and develops. Because the cells are sensitive to these differences and selective in their choice of neighbors, sharply defined boundaries are formed between the four resultant sets of cells, with no mixing at the interfaces. The four corresponding quadrants of the disc are called **compartments**, because there is no exchange of cells between them (**Figure 22–54**).

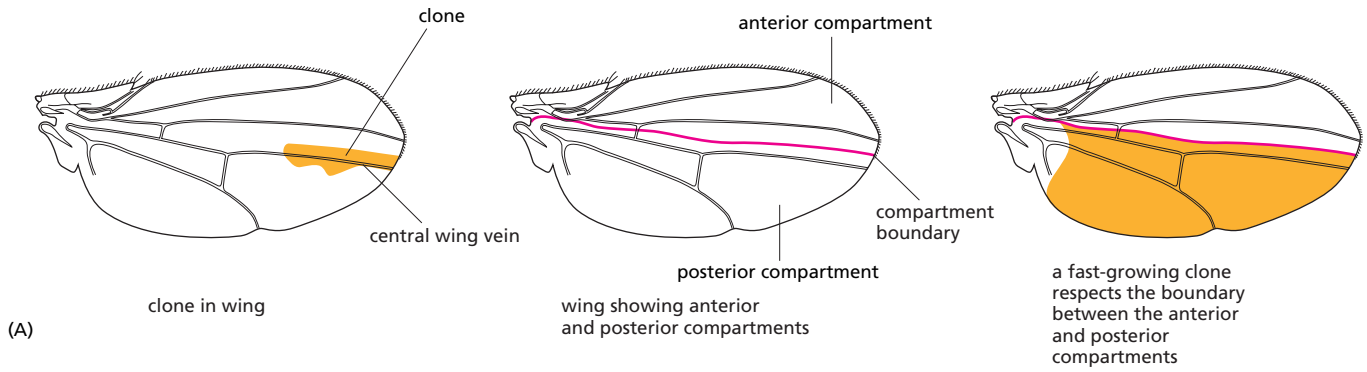


Figure 22–54 Compartments in the adult wing. (A) The shapes of marked clones in the *Drosophila* wing reveal the existence of a compartment boundary. The border of each marked clone is straight where it abuts the boundary. Even when a marked clone has been genetically altered so that it grows more rapidly than the rest of the wing and is therefore very large, it respects the boundary in the same way (drawing on right). Note that the compartment boundary does not coincide with the central wing vein. (B) The pattern of expression of the *Engrailed* gene in the wing, revealed by the same technique as for the adult fly shown in Figure 22–41. The compartment boundary coincides with the boundary of *Engrailed* gene expression. (A, after F.H.C. Crick and P.A. Lawrence, *Science* 189:340–347, 1975. With permission from AAAS; B, courtesy of Chihiro Hama and Tom Kornberg.)

Four Familiar Signaling Pathways Combine to Pattern the Wing Disc: Wingless, Hedgehog, Dpp, and Notch

Along each of the compartment boundaries—the anteroposterior boundary defined by *Engrailed* and the dorsoventral boundary defined by *Apterous*—cells in different states confront one another and interact to create narrow bands of specialized cells. These boundary cells produce new signals to organize the subsequent growth and more detailed patterning of the appendage.

Cells in the posterior wing compartment express the Hedgehog signal protein, but cannot respond to it. Cells in the anterior compartment can respond to Hedgehog. Because Hedgehog acts only over a short distance, the signal reception pathway is activated only in the narrow band of cells just anterior to the compartment boundary, where anterior and posterior cells are juxtaposed. These boundary cells respond by switching on expression of another signal molecule, Dpp—the same protein that we encountered previously, in the dorsoventral patterning of the early embryo (**Figure 22–55**). Dpp acts in its new context in much the same way as before: it spreads its effects outward from the boundary cells (by diffusion, via cytonemes, or through transfer from cell to cell by exocytosis or endocytosis), setting up a morphogen gradient to control the subsequent detailed pattern of growth and gene expression.

Analogous events occur at the dorsoventral compartment boundary (see Figure 22–55). Here, at the future wing margin, short-range communication mediated by the Notch pathway creates a band of boundary cells that produce another morphogen, the Wingless protein—the same signaling factor, belonging to the Wnt family, that acted earlier in the anteroposterior patterning of each embryonic segment. The Dpp and Wingless gradients, together with other signals and with the asymmetries of gene expression that we have discussed, combine to drive expression of other genes at precisely defined locations within each compartment.

The Size of Each Compartment Is Regulated by Interactions Among Its Cells

One of the most mysterious and ill-understood aspects of animal development is the control of growth: why does each part of the body grow to a precisely defined size? This problem is exemplified in remarkable way in the imaginal discs of *Drosophila*. By induced somatic recombination, one can, for example,

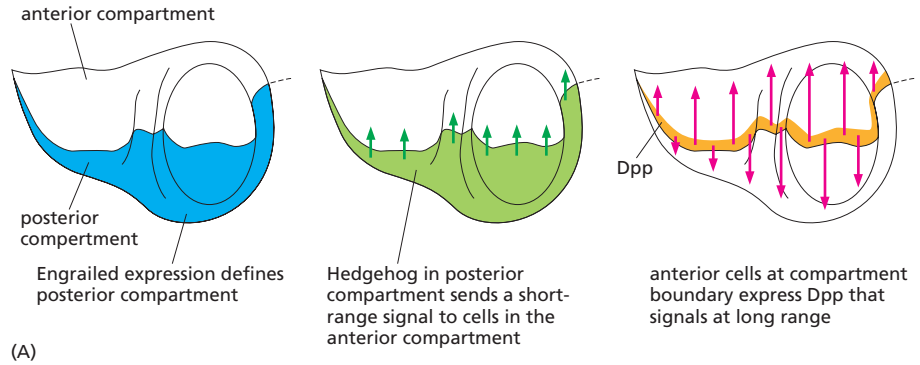


Figure 22–55 Morphogenetic signals created at compartment boundaries in the wing imaginal disc. (A) Creation of the Dpp signaling region at the anteroposterior compartment boundary through a Hedgehog-mediated interaction between the anterior and posterior cells. In an analogous way, a Notch-mediated interaction between dorsal and ventral cells creates a Wingless (Wnt) signaling region along the dorsoventral boundary. (B) The observed expression patterns of Dpp and Wingless. Although it seems clear that Dpp and Wingless act as morphogens, it is not yet certain how they spread out from their source. Cells in the imaginal disc have been seen to send out long cytonemes that may allow them to sense signals at a distance. Thus, the receiving cell may send its sensors to the source of the signal, instead of the signal moving to the receiving cell. (B, photographs courtesy of Sean Carroll and Scott Weatherbee, from S.J. Day and P.A. Lawrence, *Development* 127:2977–2987, 2000. With permission from The Company of Biologists.)

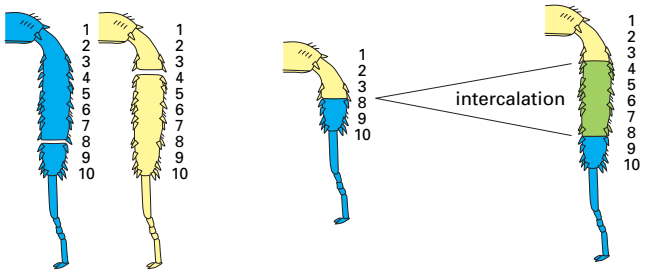


Figure 22–56 Intercalary regeneration. When mismatched portions of the growing cockroach leg are grafted together, new tissue *(green)* is intercalated (by cell proliferation) to fill in the gap in the pattern of leg structures, restoring a leg segment of normal size and pattern.

create a clonal patch of cells that proliferate more rapidly than the rest of the cells in the developing organ. The clone may grow to occupy almost the whole of the compartment in which it lies, and yet it does not overstep the boundary of the compartment. Astonishingly, its rapid growth has almost no effect on the compartment's final size, its shape, or even the details of its internal pattern (see Figure 22–54). Somehow, the cells within the compartment interact with one another to determine when their growth should stop, and each compartment behaves as a regulatory unit in this respect.

A first question is whether the size of the compartment is regulated so as to contain a set number of cells. Mutations in components of the cell-cycle control machinery can be used to speed up or slow down the rate of cell division without altering the rate of cell or tissue growth. This results in abnormally large numbers of abnormally small cells, or the converse, but the size—that is, the area—of the compartment is practically unchanged. Thus, the regulatory mechanism seems to depend on signals that indicate the physical distance between one part of the compartment and another, and on cellular responses that somehow read these signals so as to halt growth only when the spacing between the parts has reached its proper value.

This type of growth regulation is strikingly displayed in the **intercalary regeneration** that occurs when separate parts of a *Drosophila* imaginal disc or of a growing cockroach leg are surgically grafted together. After the graft, the cells in the neighborhood of the junction proliferate and fill in the parts of the pattern that should normally lie between them, continuing their growth until the normal spacing between landmarks is restored (**Figure 22–56**). The mechanisms that bring this about are a mystery, but it seems likely that they are similar to the mechanisms that regulate growth during normal development.

What mechanism could ensure that each little piece of the pattern within a compartment grows to its appropriate size, despite local disturbances in growth rate or starting conditions? The morphogen gradients (of Dpp and Wingless, for example) create a pattern by imposing different characters on cells in different positions. Could it be that the cells in each region can somehow sense how close the spacing of the pattern is—how steep the gradient of change in cell character—and continue their growth until the tissue is spread out to the right degree?

This idea has been tested by creating clones of cells in the wing disc in which downstream components of the Dpp signaling pathway are misexpressed so as to drive the level of pathway activation either higher or lower than in the neighboring cells. From the point of view of the cells, conditions at the boundary of the mutant clone are then equivalent to those produced by a very steep gradient of Dpp. The result is that cells in this neighborhood are stimulated to divide at an increased rate. Conversely, if the level of Dpp signaling is made uniform in the middle region of the developing wing disc, where it would normally be steeply graded, cell division there is inhibited. It seems that the steepness of the gradient does indeed control the rate of proliferation. But if that is so, how do cells sense the steepness of the gradient?

The answer is unknown, but there are strong hints that the mechanism depends on signals generated at cell–cell junctions, where cells with different levels of pathway activation make contact. As discussed in Chapter 19, mutations in junctional components such as the scaffold protein Discs-large (Dlg) or the cadherin superfamily member Fat can cause a dramatic failure of growth control, allowing the wing disc to grow far beyond its normal proper size. In the case of Fat, a set of other molecules, including protein kinases called Hippo and

Warts, have been identified as components of a signaling pathway that leads from Fat at the cell membrane to the control of gene expression in the nucleus. The products of the target genes include the cell-cycle regulator cyclin E and an inhibitor of apoptosis, as well as a microRNA, *Bantam,* that seems to be an essential part of the growth control mechanism. Despite these tantalizing facts, the mechanisms controlling organ size are still mysterious. If we can discover how they work in *Drosophila,* we may get some insight into the problem of the control of organ size in vertebrates, where our current perplexity on this fundamental question is even more profound. For in other aspects of organ development, as we now discuss, flies and vertebrates are unexpectedly similar at a molecular level, suggesting that their mechanisms of growth control may be similar also.

Similar Mechanisms Pattern the Limbs of Vertebrates

The limbs of vertebrates seem very different from those of insects. The insect wing, for example, consists mainly of two elaborately patterned sheets of epithelium, with very little tissue in between. In contrast, a limb of a vertebrate consists of an elaborately patterned system of muscles, bones and other connective tissues inside a thin and much more simply structured covering of epidermis. Moreover, the evolutionary evidence suggests that the last common ancestor of insects and vertebrates may have had neither legs, nor arms, nor wings, nor fins and that we have evolved these various appendages independently. And yet, when we examine the molecular mechanisms that control vertebrate limb development, we find a surprising number of similarities with the limbs of insects. We have already mentioned some of these resemblances, but there are many others: almost all the molecules we have already mentioned in the fly wing have their counterparts in the vertebrate limb, although these are expressed in different spatial relationships.

The parallels have been most thoroughly studied in the chick embryo. As we saw earlier, each leg or wing of a chick originates from a tongue-shaped limb bud, consisting of a mass of embryonic connective tissue cells, called mesenchyme cells, encased in a jacket of epithelium. In this structure, one finds expression of homologs of almost all the genes that we have mentioned in our account of *Drosophila* wing patterning, including *Distal-less*, *Wingless*, *Notch*, *Engrailed*, *Dpp*, and *Hedgehog*, mostly performing functions that seem more or less similar to their functions in the *Drosophila* wing disc (**Figure 22–57**).

The *Hox* genes likewise make an appearance in the limbs of both insects and vertebrates. In the insect appendage, the anterior and posterior compartments are distinguished by expression of different genes of the Hox complex—a result of the serial expression pattern of these genes along the anteroposterior axis of the body as a whole. In the vertebrate limb, genes of two of the vertebrate Hox complexes

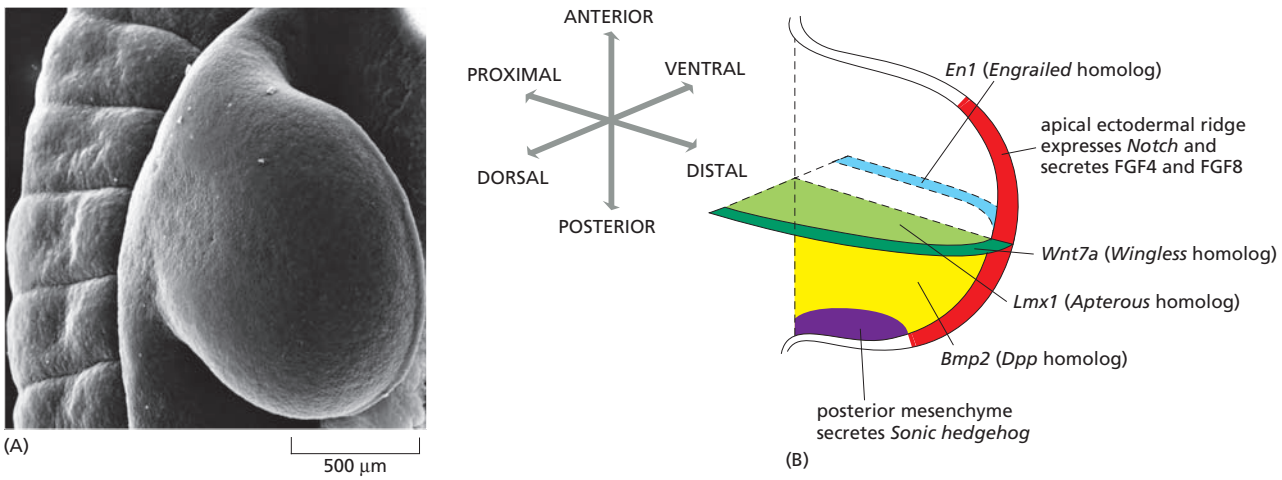


Figure 22–57 Molecules that control patterning in a vertebrate limb bud. (A) A wing bud of a chick embryo at 4 days of incubation. The scanning electron micrograph shows a dorsal view, with somites (the segments of the trunk of the embryo) visible to the left. At the distal margin of the limb bud a thickened ridge can just be seen—the apical ectodermal ridge. (B) Expression patterns of key signaling proteins and gene regulatory factors in the chick limb bud. The patterns are depicted schematically in two imaginary planes of section through the limb bud, one (horizontal) to show the dorsoventral system and the other (vertical) to show the anteroposterior and proximodistal systems. *Sonic hedgehog, Bmp2,* and *Lmx1* are expressed in the mesodermal core of the limb bud; the other molecules in the diagram are expressed in its epithelial covering. Almost all the molecules shown have homologs that are involved in patterning the *Drosophila* wing disc. (A, courtesy of Paul Martin.)

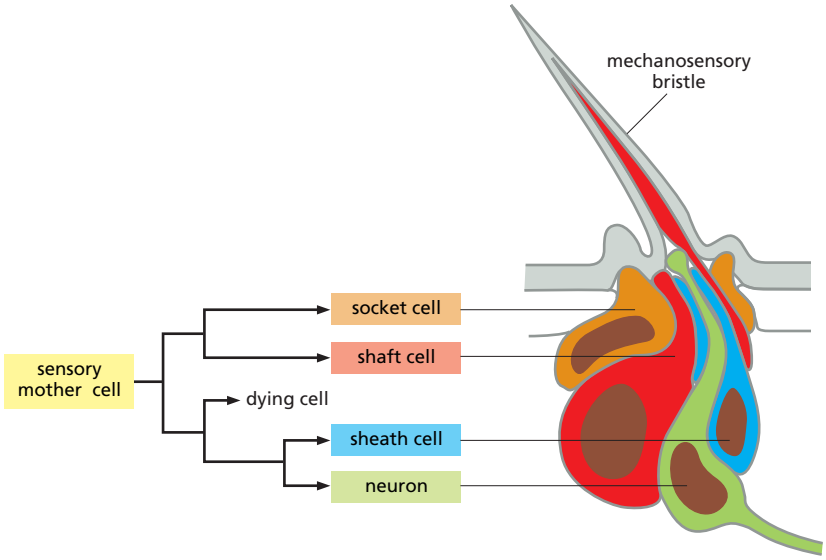


Figure 22–58 The basic structure of a mechanosensory bristle. The lineage of the four cells of the bristle—all descendants of a single sensory mother cell—is shown on the left.

(*HoxA* and *HoxD*) are expressed in a regular pattern, obedient to the usual rules of serial expression of genes in these complexes. They help, in conjunction with other factors such as the Tbx proteins mentioned earlier (see Figure 22–9), to regulate differences of cell behavior along the proximodistal limb axis.

According to one view, these molecular resemblances between developing limbs in different phyla reflect descent from a common ancestor that, while lacking limbs, had appendages of some sort built on similar principles—antennae, perhaps, or protruding mouthparts for snatching food. Modern limblike appendages, from the wings and legs of the fly to the arms and legs of a human, would then have evolved through activation of the genes for appendage formation at new sites in the body, as a result of changes in gene regulation.

Localized Expression of Specific Classes of Gene Regulatory Proteins Foreshadows Cell Differentiation

We now pick up again the thread of development in the *Drosophila* imaginal disc and follow it through to the final step at which cells become terminally differentiated. Narrowing our focus further, we take as our example the differentiation of just one type of small structure that arises in the imaginal disc epithelium: the **sensory bristle**.

The bristles that cover the body surface of an insect are miniature sense organs. Some respond to chemical stimuli, others to mechanical stimuli, but they are all constructed in a similar way. The structure is seen at its simplest in the mechanosensory bristles. Each of these consists of four cells: a shaft cell, a socket cell, a neural sheath cell, and a neuron (**Figure 22–58**). Movement of the shaft of the bristle excites the neuron, which sends a signal to the central nervous system.

The cells of the bristle of the adult fly derive from the imaginal disc epithelium, and all four of them are granddaughters or great-granddaughters (see Figure 22–58) of a single *sensory mother cell* that becomes distinct from the neighboring prospective epidermal cells during the last larval instar (**Figure 22–59**). (A fifth descendant dies, or in some tissues becomes a glial cell.) To account for the pattern of bristle differentiation, we have to explain first how the genesis of sensory mother cells is controlled and then how the five descendants of each such cell become different from one another.

Two genes, called *Achaete* and *Scute*, are crucial in initiating the formation of bristles in the imaginal disc epithelium. These genes have similar and overlapping functions and code for closely related gene regulatory proteins of the basic helix–loop–helix class (discussed in Chapter 7). As a result of disc-patterning mechanisms of the type we have already discussed, *Achaete* and *Scute* are

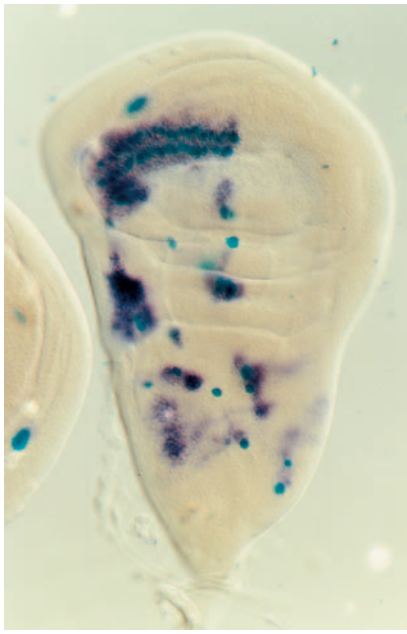


Figure 22–59 Sensory mother cells in the wing imaginal disc. The sensory mother cells (*blue* here) are easily revealed in this special strain of *Drosophila,* which contains an artificial *LacZ* reporter gene that, by chance, has inserted itself in the genome next to a control region that causes it to be expressed selectively in sensory mother cells. The *purple* stain shows the expression pattern of the *Scute* gene; this foreshadows the production of sensory mother cells and fades as the sensory mother cells successively develop. (From P. Cubas et al., *Genes Dev.* 5:996–1008, 1991. With permission from Cold Spring Harbor Laboratory Press.)

expressed in the imaginal disc in the regions within which bristles will form. Mutations that eliminate the expression of these genes at some of their usual sites block development of bristles at just those sites, and mutations that cause expression in additional, abnormal sites cause bristles to develop there. But expression of *Achaete* and *Scute* is transient, and only a minority of the cells initially expressing the genes go on to become sensory mother cells; the others become ordinary epidermis. The state that is specified by expression of *Achaete* and *Scute* is called *proneural*, and *Achaete* and *Scute* are called **proneural genes**. The proneural cells are primed to take the neurosensory pathway of differentiation, but, as we shall see, which of them will actually do so depends on competitive interactions among them.

Lateral Inhibition Singles Out Sensory Mother Cells Within Proneural Clusters

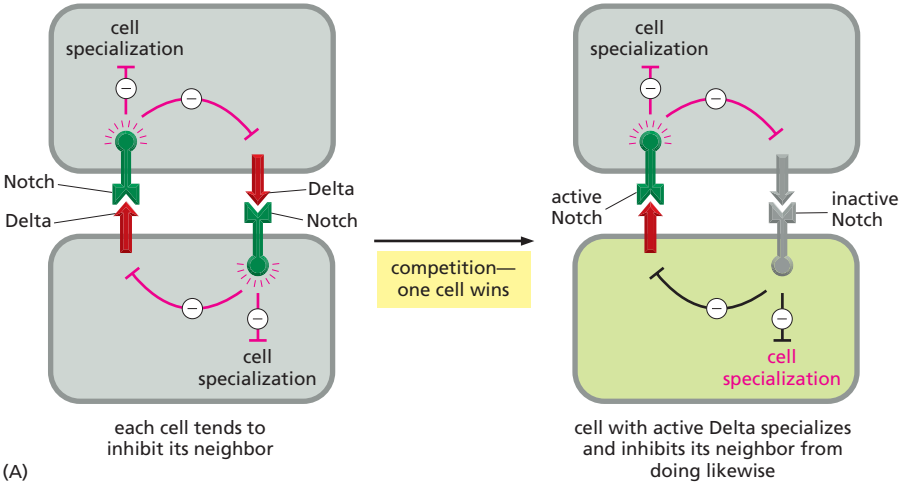
Cells expressing the proneural genes occur in groups in the imaginal disc epithelium—a small, isolated cluster of fewer than 30 cells for a big isolated bristle, a broad, continuous patch of hundreds or thousands of cells for a field of small bristles. In the former case just one member of the cluster becomes a sensory mother cell; in the latter case many cells scattered throughout the proneural region do so. In either case, each sensory mother cell becomes surrounded by cells that switch off expression of the proneural genes and become condemned to differentiate as epidermis instead. Experiments with genetic mosaics show that this is because a cell that becomes committed to the sensory-mother-cell pathway of differentiation sends a signal to its neighbors not to do the same thing: it exerts a *lateral inhibition*. If a cell that would normally become a sensory mother is genetically disabled from doing so, a neighboring proneural cell, freed from lateral inhibition, will become a sensory mother cell instead.

The lateral inhibition is mediated by the Notch signaling pathway. The cells in the cluster initially all express both the transmembrane receptor Notch and its transmembrane ligand Delta. Wherever Delta activates Notch, an inhibitory signal is sent into the Notch-expressing cell; consequently, all the cells in the cluster initially inhibit one another. However, receipt of the signal in a given cell is thought to diminish not only that cell's tendency to specialize as a sensory mother cell but also its ability to fight back by delivering the inhibitory Delta signal in return. This creates a competitive situation, from which a single cell in each small region—the future sensory mother cell—emerges as winner, sending a strong inhibitory signal to its immediate neighbors but receiving no such signal in return (**Figure 22–60**). The consequences of a failure of this regulatory mechanism are shown in **Figure 22–61**.

Lateral Inhibition Drives the Progeny of the Sensory Mother Cell Toward Different Final Fates

The same lateral inhibition mechanism dependent on Notch operates repeatedly in the formation of bristles—not only to force the neighbors of sensory mother cells to follow a different pathway and become epidermal, and but also later to make the daughters, the granddaughters, and finally the great-granddaughters of the sensory mother cell express different genes so as to form the different components of the bristle. At each stage, lateral inhibition mediates a competitive interaction that forces adjacent cells to behave in contrasting ways. Using a temperature-sensitive Notch mutation, it is possible to switch off Notch signaling after the sensory mother cell has been singled out but before it has divided. The progeny then differentiate alike, giving a cluster of neurons in place of the four different cell types of a bristle.

Like many other competitions, those mediated by lateral inhibition are often rigged: one cell starts with an advantage that guarantees it will be the winner. In the development of the different cell types of the sensory bristle, a strong initial



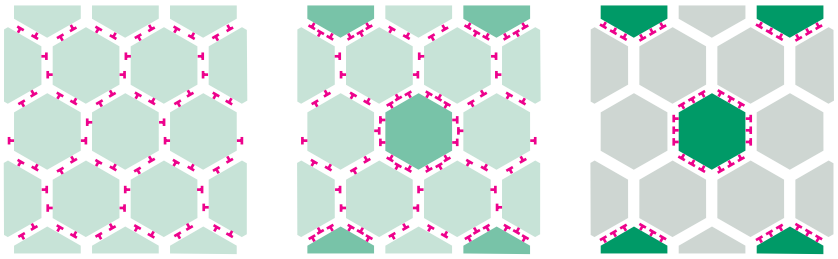


Figure 22–60 Lateral inhibition. (A) The basic mechanism of Notch-mediated competitive lateral inhibition, illustrated for just two interacting cells. In this diagram, the absence of color on proteins or effector lines indicates inactivity. (B) The outcome of the same process operating in a larger patch of cells. At first, all cells in the patch are equivalent, expressing both the transmembrane receptor Notch and its transmembrane ligand Delta. Each cell has a tendency to specialize (as a sensory mother cell), and each sends an inhibitory signal to its neighbors to discourage them from also specializing in that way. This creates a competitive situation. As soon as an individual cell gains any advantage in the competition, that advantage becomes magnified. The winning cell, as it becomes more strongly committed to differentiating as a sensory mother, also inhibits its neighbors more strongly. Conversely, as these neighbors lose their capacity to differentiate as sensory mothers they also lose their capacity to inhibit other cells from doing so. Lateral inhibition thus makes adjacent cells follow different fates. Although the interaction is thought to be normally dependent on cell–cell contacts, the future sensory mother cell may be able to deliver an inhibitory signal to cells that are more than one cell diameter away—for example, by sending out long protrusions to touch them.

bias is provided by an asymmetry in each of the cell divisions of the sensory mother cell and its progeny. A protein called Numb (together with certain other proteins) becomes localized at one end of the dividing cell, so that one daughter inherits the Numb protein and the other does not (**Figure 22–62**). Numb blocks the activity of Notch. Thus, the Numb-containing cell is deaf to inhibitory signals from its neighbors while its sister remains sensitive. Since both cells initially express the Notch ligand Delta, the cell that has inherited Numb proceeds to become neural, while driving its sister toward a nonneural fate.

Planar Polarity of Asymmetric Divisions is Controlled by Signaling via the Receptor Frizzled

For the Numb mechanism to operate, there must be machinery in the dividing cell to segregate the determinant to one side of the cell before division. In addition, as the cell enters mitosis the mitotic spindle must be aligned with this asymmetry so that the determinant is allocated to just one daughter cell, and not shared out to both daughters at the time of cell division. In the above case, the sensory mother cell, at its first division, regularly divides to give an anterior cell that inherits Numb and a posterior cell that does not. As discussed in Chapter 19, this type of polarity in the plane of the epithelium is called *planar polarity* (in

Figure 22–61 The result of switching off lateral inhibition during the singling-out of sensory mother cells. The photograph shows part of the thorax of a fly containing a mutant patch in which the neurogenic gene *Delta* has been partially inactivated. The reduction of lateral inhibition has caused almost all the cells in the mutant patch (in the center of the picture) to develop as sensory mother cells, producing a great excess of sensory bristles there. Mutant patches of cells carrying more extreme mutations in the Notch pathway, causing a total loss of lateral inhibition, form no visible bristles because all of the progeny of the sensory mother cells develop as neurons or glial cells instead of diversifying to form both neurons and the external parts of the bristle structure. (Courtesy of P. Heitzler and P. Simpson, *Cell* 64:1083–1093, 1991. With permission from Elsevier.)

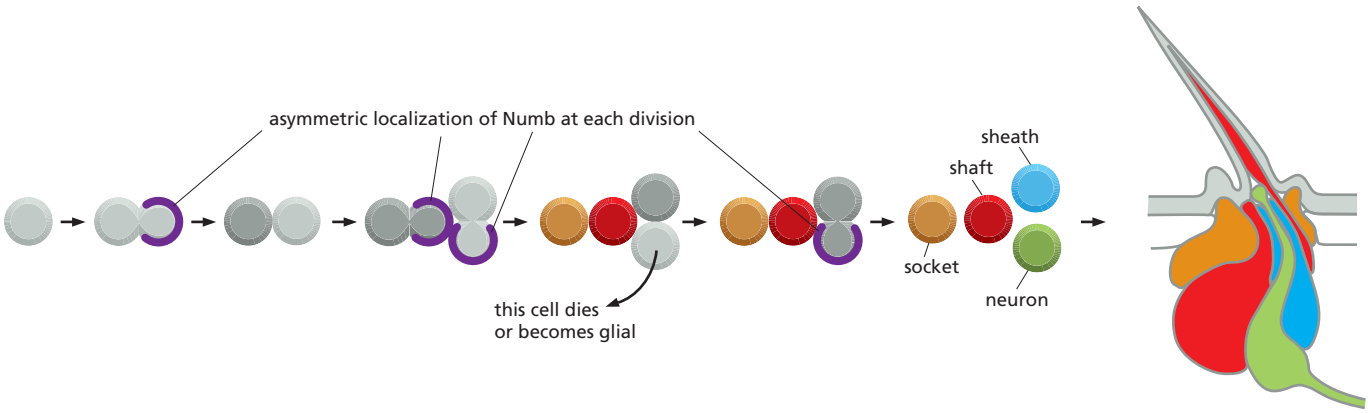


Figure 22–62 Numb biases lateral inhibition during bristle development. At each division of the progeny of the sensory mother cell, Numb protein is asymmetrically localized, producing daughter cells that differ. Note that some of the divisions are oriented with the mitotic spindle in the plane of the epithelium, others at right angles to it; the localization of Numb is controlled in different ways at these different types of division but plays a critical role at each of them in deciding cell fate. (Based on data from M. Gho, Y. Bellaiche and F. Schweisguth, *Development* 126:3573–3584, 1999. With permission from The Company of Biologists.)

contradistinction to apico-basal polarity, where the cellular asymmetry is perpendicular to the plane of the epithelium). It is manifested in the uniformly backward-pointing orientation of the bristles, giving the fly its wind-swept appearance (**Figure 22–63**).

The planar polarity in the initial division of the sensory mother cell is controlled by a signaling pathway similar to the one that we encountered controlling asymmetric divisions in the nematode (see Figure 22–21), depending on the receptor Frizzled. Frizzled proteins have been discussed in Chapter 15 as receptors for Wnt proteins, but in the control of planar polarity—in flies and probably in vertebrates too—this pathway functions in a special way: the intracellular relay mechanism exerts its main effects on the actin cytoskeleton, rather than on gene expression. The intracellular protein Dishevelled, downstream from Frizzled, is common to the gene-regulatory and the actin-regulatory branches of the signaling pathway. Separate domains of the Dishevelled molecule can be shown to be responsible for the two functions (**Figure 22–64**). Frizzled and Dishevelled both take their names from the unkempt look of flies where bristle polarity is disordered (see Figure 19–32).

Asymmetric Stem-Cell Divisions Generate Additional Neurons in the Central Nervous System

The mechanisms we have described for controlling the genesis of neurons of sensory bristles operate also, with variations, in the genesis of virtually all other neurons—not only in insects, but also in other phyla. Thus in the embryonic central nervous system, both in flies and in vertebrates, neurons are generated from regions of expression of proneural genes akin to *Achaete* and *Scute*. The nascent neurons express Delta and inhibit their immediate neighbors, which express Notch, from becoming committed to neural differentiation at the same time. When Notch signaling is blocked, inhibition fails, and in the proneural regions neurons are generated in huge excess at the expense of non-neuronal cells (**Figure 22–65**).

In the central nervous system, however, an additional mechanism comes into play to help generate the very large numbers of neurons and glial cells that are needed: a special class of cells become committed as neural precursors, but instead of differentiating directly as neurons or glial cells, these undergo a long series of asymmetric divisions through which a succession of additional neurons and glial cells are added to the population. The mechanism is best understood in *Drosophila*, although there are many hints that something similar occurs also in vertebrate neurogenesis.

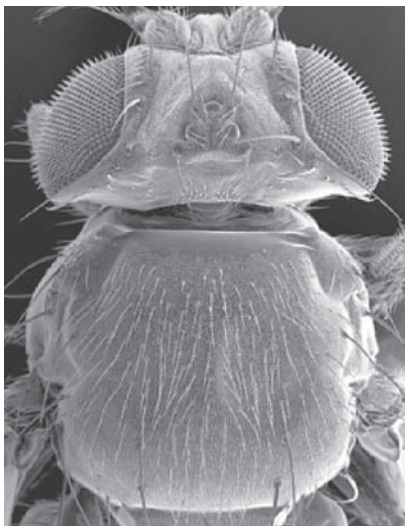
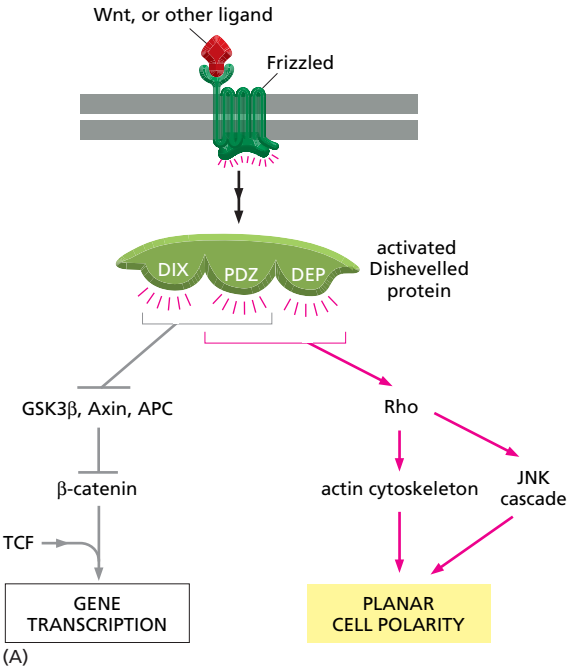


Figure 22–63 Planar cell polarity manifest in bristle polarity on a fly's back: the bristles all point backwards. (Scanning electron micrograph courtesy of S. Oldham and E. Hafen, from E. Spana and N. Perrimon, *Trends Genet.* 15:301–302, 1999. With permission from Elsevier.)



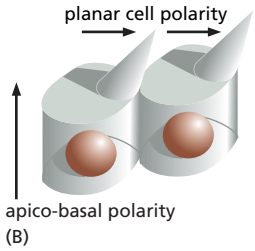


Figure 22–64 The control of planar cell polarity. (A) The two branches of the Wnt/Frizzled signaling pathway. The main branch, discussed in Chapter 15, controls gene expression via β-catenin; the planar-polarity branch controls the actin cytoskeleton via Rho GTPases. Different domains of the Dishevelled protein are responsible for the two effects. It is not yet clear which member of the Wnt signal protein family, if any, is responsible for activating the planar polarity function of Frizzled in *Drosophila*. (B) Cartoon of cells displaying planar polarity. In at least some systems, planar cell polarity is associated with asymmetric localization of the receptor Frizzled itself to one side of each cell. (See also Chapter 19, Figure 19–32.)

In the embryonic central nervous system of *Drosophila*, the nerve-cell precursors, or **neuroblasts**, are initially singled out from the neurogenic ectoderm by a typical lateral-inhibition mechanism that depends on Notch. Each neuroblast then divides repeatedly in an asymmetric fashion (**Figure 22–66**A). At each division, one daughter remains as a neuroblast, while the other, which is much smaller, becomes specialized as a *ganglion mother cell*, or *GMC*. The ganglion mother cell will divide only once, giving a pair of neurons, or a neuron plus a glial cell, or a pair of glial cells. The neuroblast becomes smaller at each division, as it parcels out its substance into one ganglion mother cell after another. Eventually, typically after about 12 cycles, the process halts, presumably because the neuroblast becomes too small to pass the cell-size checkpoint in the cell division cycle. Later, in the larva, neuroblast divisions resume, and now they are accompanied by cell growth, permitting the process to continue indefinitely, generating the much larger numbers of neurons and glial cells required in the adult fly.

The larval neuroblasts, therefore, are **stem cells**: while not terminally differentiated themselves, they behave as a self-renewing and potentially inexhaustible source of terminally differentiated cells. In Chapter 23, where we discuss stem cells in detail, we shall see that stem cells do not necessarily have to divide asymmetrically; but asymmetric division is one possible strategy, and the neuroblasts of the fly provide a beautiful example.

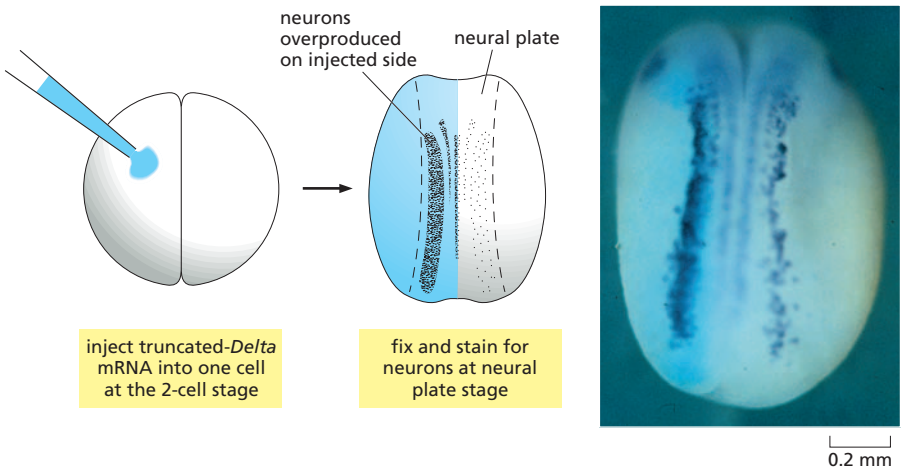
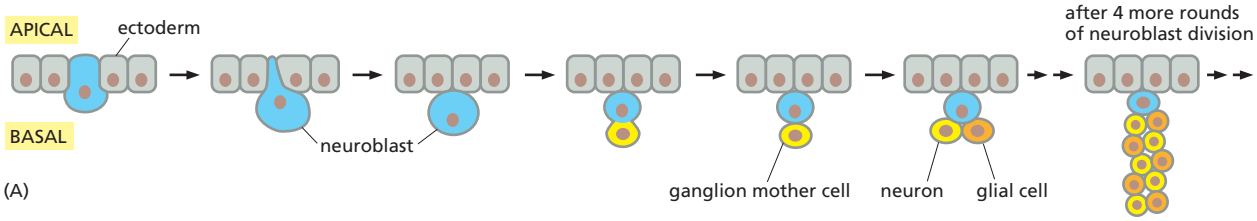


Figure 22–65 Effects of blocking Notch signaling in a *Xenopus* embryo. In the experiment shown, mRNA coding for a truncated form of the Notch ligand *Delta* is injected, together with *LacZ* mRNA as a marker, into one cell of an embryo at the two-cell stage. The truncated Delta protein produced from the mRNA blocks Notch signaling in the cells descended from the cell that received the injection. These cells lie on the left side of the embryo and are identifiable because they contain LacZ protein (*blue* stain) as well as the truncated Delta protein. The right side of the embryo is unaffected and serves as a control. The embryo is fixed and stained at a stage when the central nervous system has not yet rolled up to form a neural tube, but is still a more or less flat plate of cells—the neural plate—exposed on the surface of the embryo. The first neurons (stained *purple* in the photograph) have already begun to differentiate in elongated bands (proneural regions) on each side of the midline. On the control (right) side, they are a scattered subset of the proneural cell population. On the Notch-blocked (left) side, virtually all the cells in the proneural regions have differentiated as neurons, creating a densely stained band of neurons without intervening cells. Injections of mRNA coding for normal, functional Delta have an opposite effect, reducing the number of cells that differentiate as neurons. (Photograph from A. Chitnis et al., *Nature* 375:761–766, 1995. With permission from Macmillan Publishers Ltd.)



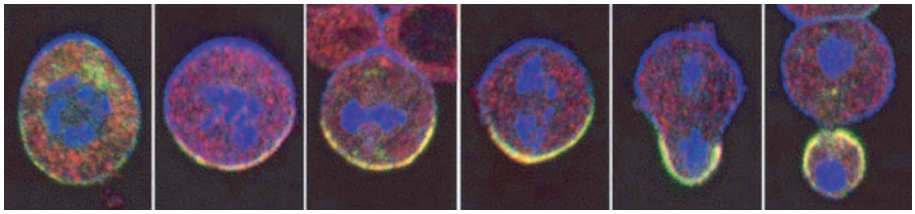


Figure 22–66 Neuroblasts and asymmetric cell division in the central nervous system of a fly embryo. (A) The neuroblast originates as a specialized ectodermal cell. It is singled out by lateral inhibition and emerges from the basal (internal) face of the ectoderm. It then goes through repeated division cycles, dividing asymmetrically to generate a series of ganglion mother cells. Each ganglion mother cell divides just once to give a pair of differentiated daughters (typically a neuron plus a glial cell). (B) The asymmetric distribution of cell fate determinants in an isolated neuroblast as it goes through mitosis. The mitotic chromosomes are stained *blue*. The Par3/Par6/aPKC complex, shown by *blue* immunostaining for aPKC, is concentrated in the apical cortex, and causes Miranda (*green*), Brat (*red,* giving *yellow* where Brat and Miranda overlap) and Prospero (not stained) to become localized in the basal cortex. As the cell divides, these latter three molecules become segregated into the ganglion mother cell, forcing it to differentiate and leaving the neuroblast free to regenerate its asymmetry and divide again in the same way. (B, from C.Y. Lee et al., *Dev. Cell* 10:441–449, 2006. With permission from Elsevier.)

Asymmetric Neuroblast Divisions Segregate an Inhibitor of Cell Division into Just One of the Daughter Cells

The divisions of the neuroblast are asymmetric in three respects: (1) physically, in that one daughter is smaller than the other; (2) biochemically, in factors controlling differentiation; and (3) biochemically, in factors controlling proliferation. These asymmetries must all be coordinated with one another and with the orientation of the axis of the mitotic spindle, if the cleavage plane is to cut the cell into the correct parts. How is this achieved?

The neuroblast has an apico-basal asymmetry that reflects its origin from the ectoderm, which, like other epithelia, has a well defined apico-basal polarity. As we saw in Chapter 19, apico-basal polarity is governed by a complex of three proteins—Par3 (also called Bazooka in *Drosophila),* Par6, and aPKC (atypical protein kinase C)—that become localized in the cortex toward the apical end of the cell. This localized Par3/Par6/aPKC complex is thought to be the primary source of asymmetry in the neuroblast. By recruiting other components, some of which exert feedback effects to maintain the localization of the complex, it coordinates the whole process of unequal division.

The Par3/Par6/aPKC complex defines the orientation of the mitotic spindle and the unequal partitioning of the cell at cytokinesis through interaction with adapter proteins called Inscuteable and Partner of Inscuteable (Pins). These in turn recruit the α subunit of a trimeric G protein (discussed in Chapter 15), which functions in this context as an intracellular messenger to guide organization of the cytoskeleton.

At the same time, the Par3/Par6/aPKC complex locally phosphorylates a regulator of intracellular architecture called Lgl (Lethal giant larvae) and thereby directs another adaptor protein called Miranda to become concentrated in the cortex at the opposite (basal) end of the cell (Figure 22–66B). Miranda binds proteins that control differentiation and proliferation, localizing them to the same site. When the neuroblast divides, Miranda and its cargo are segregated into the ganglion mother cell. One of the molecules thus carried into the ganglion mother cell is a gene regulatory protein called Prospero, which directs differentiation. Another is a posttranscriptional repressor called Brat (Brain Tumor). Brat acts as an inhibitor of cell proliferation, apparently by preventing production of the growth-promoting protein Myc, famous for its role in cancer (discussed in Chapter 20). In mutants where Brat is defective, or where it fails to become localized correctly, the smaller daughter cell of the asymmetric neuroblast division frequently fails to differentiate as a ganglion mother cell, and instead grows and divides as a neuroblast. The result is a brain tumor—a mass of neuroblasts that grows exponentially and without limit, until the fly is dead.

Whether vertebrates tissues have stem cells that behave like the fly's neuroblasts is a question of great current interest, especially in relation to cancer.

Notch Signaling Regulates the Fine-Grained Pattern of Differentiated Cell Types in Many Different Tissues

Each daughter of a normal ganglion mother cell can become either a neuron or a glial cell. This final choice, like the choice of cell fate for the progeny of a sensory mother cell in the peripheral nervous system, is controlled by Notch signaling and lateral inhibition. In fact, lateral inhibition mediated by Notch is crucial for cell diversification and fine-grained patterning in an enormous variety of different tissues. In the fly, it controls the production not only of neurons but also of many other differentiated cell types—for example, in muscle, in the lining of the gut, in the excretory system, in the tracheae, and in the eye and other sense organs. In vertebrates, homologs of Notch and its ligands are expressed in the corresponding tissues and have similar functions: mutations in the Notch pathway upset the balance not only of neurons and non-neuronal cells in the central nervous system, but also of the different specialized cell types in the lining of the gut, of endocrine and exocrine cells in the pancreas, and of sensory and supporting cells in sense organs such as the ear, to give only a few examples.

In all these tissues, a balanced mixture of different cell types is required. Notch signaling provides the means to generate the mixture, by enabling individual cells expressing one set of genes to direct their immediate neighbors to express another set.

Some Key Regulatory Genes Define a Cell Type; Others Can Activate the Program for Creation of an Entire Organ

As we mentioned at the beginning of this chapter, there are some genes whose products act as triggers for the development of a specific organ, initiating and coordinating the whole complex program of gene expression required for this. Thus, for example, when the *Eyeless* gene is artificially expressed in a patch of cells in the leg imaginal disc, a patch of well-organized eye tissue, with all its various cell types correctly arranged, will develop on the leg (see Figure 22–2). In a somewhat similar way, much later, when a cell makes its final choice of a particular mode of differentiation in the aftermath of the interactions mediated by Notch, it has to follow a complex program involving expression of a whole collection of genes, and this differentiation program is initiated and coordinated by a much smaller set of high-level regulators. Such regulators are sometimes called "master regulatory proteins" (though even they can exert their specific effect only in combination with the right partners, in a cell that is adequately primed).

An example is the MyoD/myogenin family of gene regulatory proteins. These proteins drive cells to differentiate as muscle, expressing muscle-specific actins and myosins and all the other cytoskeletal, metabolic and membrane proteins that a muscle cell needs (see Figure 7–75). The gene regulatory proteins that define particular cell types often belong (as do MyoD and its relatives) to the basic helix–loop–helix family, encoded by genes homologous to, and in some cases apparently identical to, the proneural genes that we have already mentioned. Their expression is often governed by the Notch pathway via complicated feedback loops.

Terminal cell differentiation has brought us to the end of our sketch of how genes control the making of a fly. Our account has necessarily been simplified. Many more genes than we have mentioned are involved in each of the developmental processes that we have described. Feedback loops, alternative mechanisms operating in parallel, genetic redundancy, and other phenomena complicate the full picture. Despite all this, the overriding message of developmental genetics is one of an unexpected simplicity. A limited number of genes and mechanisms, used repeatedly in different circumstances and combinations, are responsible for controlling the main features of the development of all multicellular animals.

We next turn to an essential aspect of animal development that we have so far neglected: cell movements.

Summary

The external parts of an adult fly develop from epithelial structures called imaginal discs. Each imaginal disc is divided at the outset into a small number of domains expressing different gene regulatory proteins as a result of early embryonic patterning processes. These domains are called compartments, because their cells do not mix. At the compartment boundaries, cells expressing different genes confront one another and interact, inducing localized production of morphogens that govern the further growth and internal patterning of each compartment. Thus, in the wing disc, dorsal and ventral cells interact by the Notch signaling mechanism to create a source of Wingless (Wnt) protein along the dorsoventral compartment boundary, while anterior and posterior cells interact through short-range Hedgehog signaling to create a source of Dpp protein (a TGFβ family member) along the anteroposterior compartment boundary. All these signaling molecules have homologs that play similar parts in limb patterning in vertebrates.

Each compartment of an imaginal disc, and each substructure within it, grows to a precisely predictable size, even in the face of seemingly drastic disturbances, such as mutations that alter the cell division rate. Although the morphogen gradients in the disc are clearly involved, the critical regulatory mechanisms that control organ size are not understood.

Within each compartment, the morphogen gradients control the sites of expression of further sets of genes, defining patches of cells that interact with one another yet again to create the finest details of the ultimate pattern of cell differentiation. Thus, proneural gene expression defines the sites where sensory bristles will form, and Notch-mediated interactions among the cells of the proneural cluster, together with asymmetric cell divisions, force the individual cells of the bristle to follow different paths of terminal differentiation. In the central nervous system, neuroblasts are singled out from the ectoderm by lateral inhibition in a similar way, but then go through a long series of asymmetrical divisions as stem cells to generate neurons and glia. Faults in the asymmetric distribution of the molecules that control differentiation and proliferation can convert the neuroblast stem cells into tumor cells.

Many of the same mechanisms are thought to operate in vertebrate tissues also.

CELL MOVEMENTS AND THE SHAPING OF THE VERTEBRATE BODY

Most cells of the animal body are motile, and in the developing embryo their movements are often extensive, dramatic, and surprising. <GAGC> Controlled changes of gene expression create ordered arrays of cells in different states; cell movements rearrange these cellular building blocks and put them in their proper places. The genes that the cells express determine how they move; in this sense, the control of gene expression is the primary phenomenon. But the cell movements are also crucial, and no less in need of explanation if we want to understand how the architecture of the body is created. In this section, we examine this topic in the context of vertebrate development. We take as our main example the frog *Xenopus laevis* (**Figure 22–67**), where cell movements have been well studied, though we shall also draw on evidence from chick, zebrafish, and mouse.

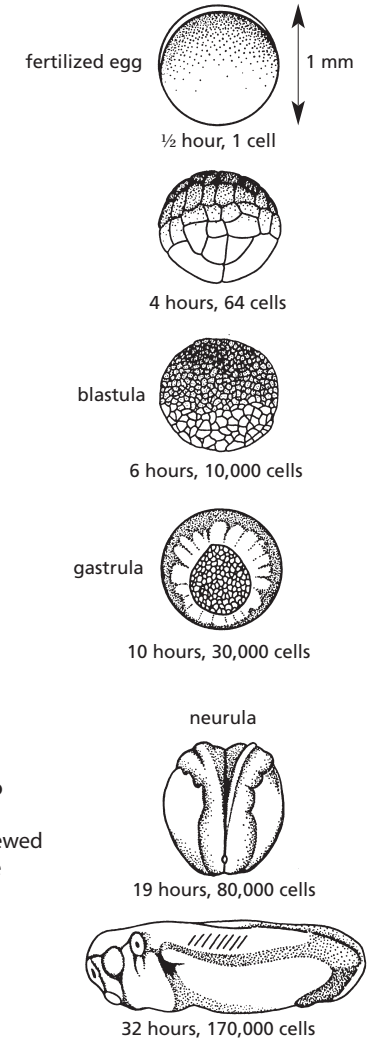
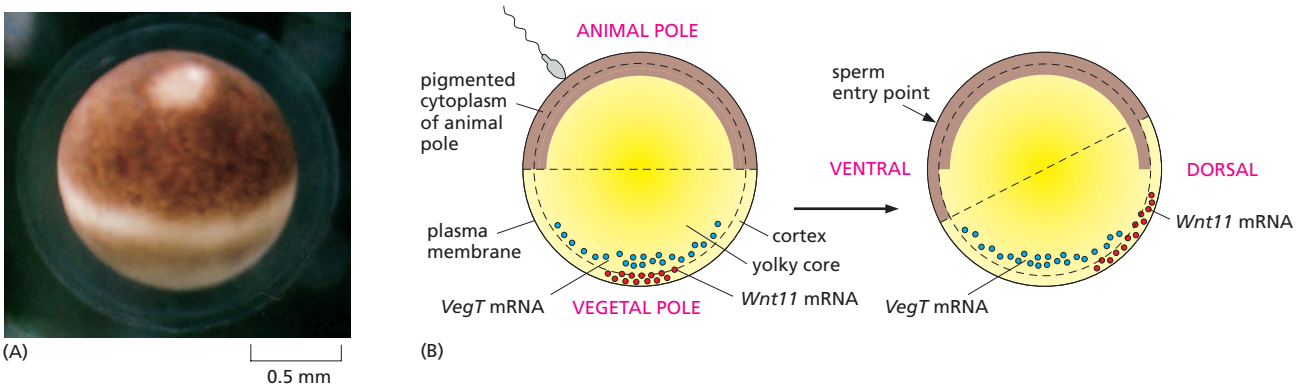


Figure 22–67 Synopsis of the development of *Xenopus laevis* from newly fertilized egg to feeding tadpole. The adult frog is shown in the photograph at the top. The developmental stages are viewed from the side, except for the 10-hour and 19-hour embryos, which are viewed from below and from above, respectively. All stages except the adult are shown at the same scale. (Photograph courtesy of Jonathan Slack; drawings after P.D. Nieuwkoop and J. Faber, Normal Table of *Xenopus laevis* [Daudin]. Amsterdam: North-Holland, 1956.)



The Polarity of the Amphibian Embryo Depends on the Polarity of the Egg

The *Xenopus* egg is a large cell, just over a millimeter in diameter (**Figure 22–68**A). The light-colored lower end of the egg is called the vegetal pole; the dark-colored upper end is called the animal pole. The animal and vegetal hemispheres contain different selections of mRNA molecules and other cell components, which become allocated to separate cells as the egg cell divides after fertilization. Near the vegetal pole, for example, there is an accumulation of mRNAs coding for the gene regulatory protein VegT (a DNA-binding protein of the T-box family) and for signal proteins of the TGFβ superfamily, as well as some ready-made protein components of the Wnt signaling pathway (Figure 22–68B). As a result, the cells that inherit vegetal cytoplasm will produce signals to organize the behavior of adjacent cells. They are committed to form the gut—the innermost tissue of the body; the cells that inherit animal cytoplasm will form the outer tissues. Thus, crudely speaking, the animal–vegetal axis of the egg corresponds to the external-to-internal (or skin-to-gut) dimension of the future organism.

Fertilization initiates a series of cell divisions and movements that will eventually tuck the vegetal cells and cells from the equatorial (middle) region of the animal–vegetal axis into the interior. In the course of these complex movements, the three principal axes of the body become established: *anteroposterior,* from head to tail; *dorsoventral,* from back to belly; and *mediolateral,* from the midline outward to the left or to the right. The orientation of these axes is determined by the asymmetries of the early embryo. The unfertilized egg, has only one axis of asymmetry—the animal–vegetal—but fertilization triggers an intracellular movement that gives the egg an additional asymmetry defining a second axis at right angles to this. Following entry of the sperm, the outer, actin-rich cortex of the egg cytoplasm rotates relative to the central core of the egg, so that the animal pole of the cortex is slightly shifted to one side. Treatments that block the rotation allow cleavage to occur normally but produce an embryo with a central gut and no dorsal structures or dorsoventral asymmetry. Thus, the cortical rotation is required to define the dorso–ventral axis of the future body, and the axis of asymmetry created in the egg by the rotation is called the dorso–ventral axis of the egg. Note, however, that the subsequent cell movements mean that the relationship between the egg axes and the future body axes is more complicated than this terminology would suggest. The direction of the cortical rotation is biased according to the point of sperm entry, perhaps through the centrosome that the sperm brings into the egg, and the movement is associated with a reorganization of microtubules in the egg cytoplasm. This leads to a microtubule-based transport of several components, including mRNA coding for Wnt11, a member of the Wnt family of signal molecules, toward the future dorsal side (see Figure 22–68B). This mRNA is soon translated, producing Wnt11 protein in the dorsal vegetal region. The Wnt11 secreted from cells that form in that region is crucial in triggering the cascade of subsequent events that will organise the dorsoventral axis of the body.

Figure 22–68 The *Xenopus* egg and its asymmetries. (A) Side view of an egg photographed just before fertilization. (B) The asymmetric distribution of molecules inside the egg, and how this changes following fertilization so as to define a dorsoventral as well as an animal–vegetal asymmetry. Fertilization, through a reorganization of the microtubule cytoskeleton, triggers a rotation of the egg cortex (a layer a few μm deep) through about 30° relative to the core of the egg in a direction determined by the site of sperm entry. Some components are carried still further to the future dorsal side by active transport along microtubules. The resulting dorsal concentration of *Wnt11* mRNA leads to dorsal production of the Wnt11 signal protein and defines the dorsoventral polarity of the future embryo. (A, courtesy of Tony Mills.)

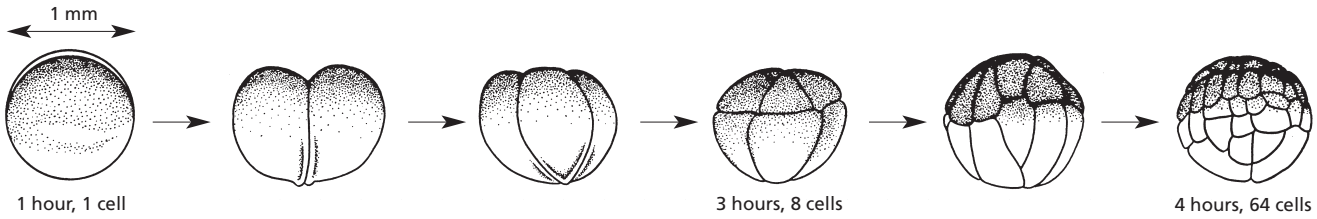


Figure 22–69 The stages of cleavage in *Xenopus*. The cleavage divisions rapidly subdivide the egg into many smaller cells. All the cells divide synchronously for the first 12 cleavages, but the divisions are asymmetric, so that the lower, vegetal cells, encumbered with yolk, are fewer and larger.

Cleavage Produces Many Cells from One

The cortical rotation is completed in about an hour after fertilization and is followed by cleavage, in which the single large egg cell rapidly subdivides by repeated mitosis into many smaller cells, or *blastomeres*, without any change in total mass (**Figure 22–69**). <ATTT> In this way, the determinants distributed asymmetrically in the egg become partitioned into separate cells, with different fates (**Figure 22–70**).

These first cell divisions in *Xenopus* have a cycle time of about 30 minutes, with a direct alternation of S and M phases, as discussed in Chapter 17. The very high rate of DNA replication and mitosis seems to preclude almost all gene transcription (although protein synthesis occurs), and the cleaving embryo is almost entirely dependent on reserves of RNA, protein, membrane, and other materials that accumulated in the egg while it developed as an oocyte in the mother. After about 12 cycles of cleavage (7 hours), the cell division rate slows down, the cell cycles begin to follow the standard pattern with G_1 and G_2 phases intervening between the S and M phases, and widespread transcription of the embryo's genome begins. This event is called the *mid-blastula transition*, and it occurs with roughly similar timing in most animal species (mammals being an exception). Studies in zebrafish show that the newly synthesized transcripts include micro-RNAs that recognize many of the transcripts deposited in the egg by the mother and direct their rapid degradation. The midblastula transition thus marks the point at which the embryo's own genome largely takes over control of development.

Gastrulation Transforms a Hollow Ball of Cells into a Three-Layered Structure with a Primitive Gut

During the period of cleavage, the frog embryo becomes transformed from a solid sphere of cells into something more like a hollow ball, with an internal fluid-filled cavity surrounded by cells that cohere to form an epithelial sheet. The embryo is now termed a **blastula** (**Figure 22–71**).

Soon after this, the coordinated movements of gastrulation begin. <TCCC> This dramatic process transforms the simple hollow ball of cells into a multilayered structure with a central gut tube and bilateral symmetry: by a more elaborate version of the process outlined earlier for the sea urchin (see Figure 22–3), many of the cells on the outside of the embryo are moved inside it. Subsequent development depends on the interactions of the inner, outer, and middle layers of cells thus formed: the *endoderm* on the inside, consisting of the cells that have moved into the interior to form the primitive gut; the *ectoderm* on the outside, consisting of cells that have remained external; and the *mesoderm* between

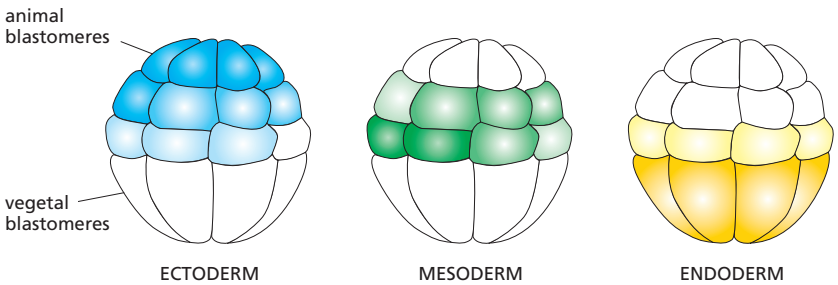


Figure 22–70 The origins of the three germ layers can be traced back to distinct blastomeres of the embryo in its early cleavage stages. The endoderm derives from the most vegetal blastomeres, the ectoderm from the most animal, and the mesoderm from a middle set that contribute also to endoderm and ectoderm. The coloring in each picture is the more intense, the higher the proportion of cell progeny that will contribute to the given germ layer. (After L. Dale, *Curr. Biol.* 9:R812–R815, 1999. With permission from Elsevier.)

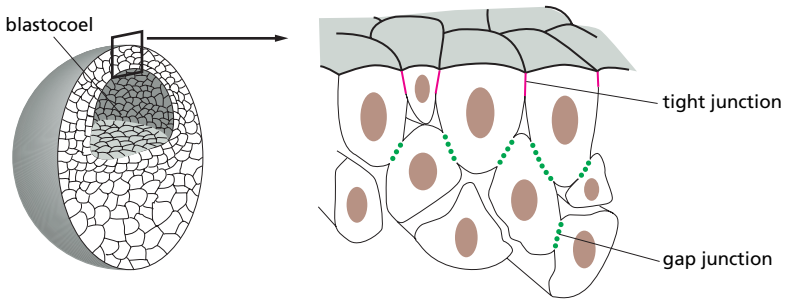


Figure 22–71 The blastula. In the outermost regions of the embryo, tight junctions between the blastomeres begin to create an epithelial sheet that isolates the interior of the embryo from the external medium. Na^+ is pumped across this sheet into the spaces in the interior of the embryo, and water follows into these spaces because of the resulting osmotic pressure gradient. As a result, the intercellular crevices inside the embryo enlarge to form a single cavity, the blastocoel. In *Xenopus* the wall of the blastocoel is several cells thick, and only the outermost cells are tightly bound together as an epithelium.

them, consisting of cells that detach from the epithelium to form a more loosely organized embryonic connective tissue (**Figure 22–72**). From these three *germ layers,* the tissues of the adult vertebrate body will be generated, preserving the basic body plan established through gastrulation.

The Movements of Gastrulation Are Precisely Predictable

The pattern of gastrulation movements that creates the germ layers and establishes the body axes is described for *Xenopus* in **Figure 22–73**. The details are complex, but the principles are simple.

Cells of the future endoderm are folded into the interior, or *involuted*, in succession. The process begins with a downward movement of cells from the animal hemisphere to cover and enclose the yolky vegetal hemisphere, which represents the food supply of the embryo. Cells that are in the vanguard of this movement, at the vegetal margin of the advancing cell sheet, are the first to involute, turning inward and then moving up toward the animal pole to form the most anterior part of the gut. As they near the animal pole, these leading endoderm cells will signal to the overlying ectoderm to define the anterior extremity of the head. The mouth will eventually develop as a hole formed at an anterior site where endoderm and ectoderm come into direct contact. Meanwhile, future mesoderm cells, destined to detach from the epithelial sheet to form the sandwich filling between endoderm and ectoderm, tuck into the interior along with the endoderm cells, and also move up toward the animal pole. The cells that are first to involute go to form parts of the head, and those that are last form parts of the tail. In this way, the anteroposterior axis of the final embryo is laid down sequentially.

The anteroposterior movements go hand in hand with movements that organize the dorsoventral axis of the body. Gastrulation begins on the side of the blastula that has been marked out as dorsal by the cortical rotation. Here, involution of cells into the interior starts with a short indentation that rapidly extends to form the *blastopore*—a line of invagination that curves around to encircle the vegetal pole. The site where the invagination starts defines the *dorsal lip of the blastopore.* As we shall see, this tissue plays a leading part in subsequent events and gives rise to the central dorsal structures of the main body axis.

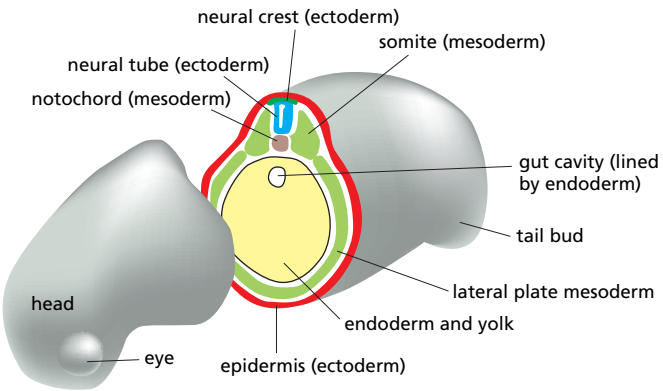


Figure 22–72 A cross section through the trunk of an amphibian embryo after the end of gastrulation, showing the arrangement of endodermal, mesodermal and ectodermal tissues. The endoderm will form the epithelial lining of the gut, from the mouth to the anus. It gives rise not only to the pharynx, esophagus, stomach, and intestines, but also to many associated glands. The salivary glands, the liver, the pancreas, the trachea, and the lungs, for example, all develop from extensions of the wall of the originally simple digestive tract and grow to become systems of branching tubes that open into the gut or pharynx. The endoderm forms only the epithelial components of these structures—the lining of the gut and the secretory cells of the pancreas, for example. The supporting muscular and fibrous elements arise from the mesoderm. The mesoderm gives rise to the connective tissues—at first to the loose, space-filling, three-dimensional mesh of cells in the embryo known as mesenchyme, and ultimately to cartilage, bone, and fibrous tissue, including the dermis (the inner layer of the skin). The mesoderm also forms the muscles, the entire vascular system—including the heart, the blood vessels, and the blood cells—and the tubules, ducts, and supporting tissues of the kidneys and gonads. The ectoderm will form the epidermis (the outer, epithelial layer of the skin) and epidermal appendages such as hair, sweat glands, and mammary glands. It will also give rise to the whole of the nervous system, central and peripheral, including not only neurons and glia but also the sensory cells of the nose, the ear, the eye, and other sense organs. (After T. Mohun et al., *Cell* 22:9–15, 1980. With permission from Elsevier.)

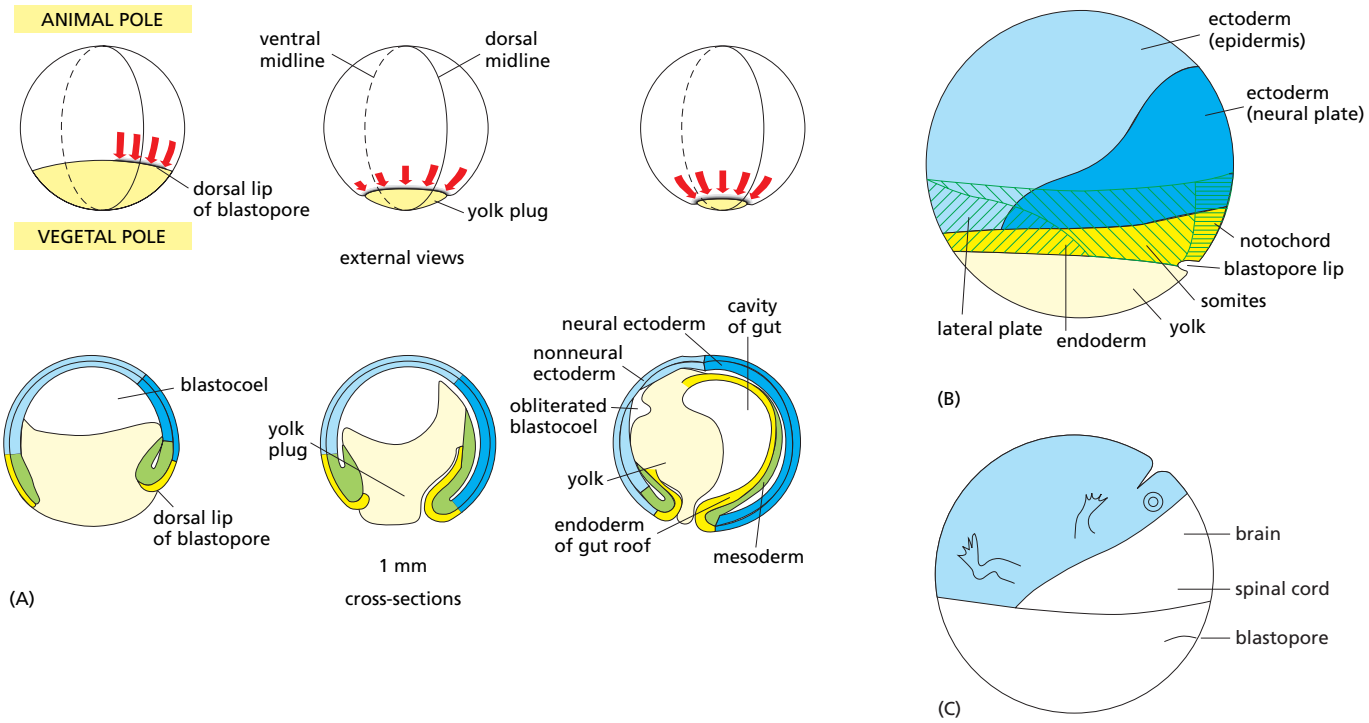
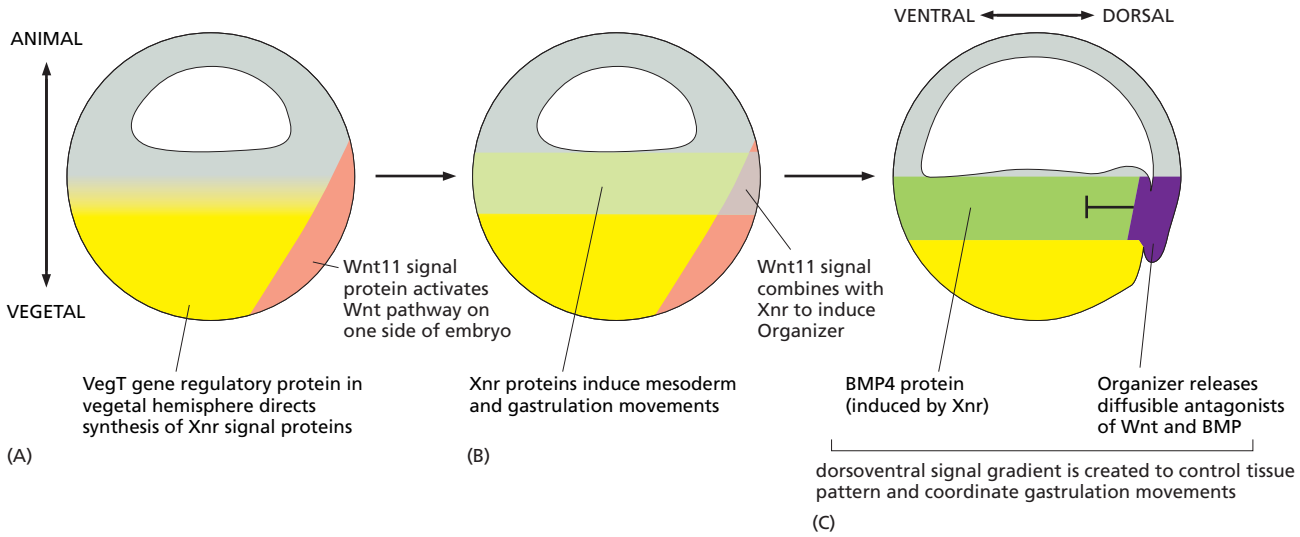


Figure 22–73 Gastrulation in *Xenopus*. <TCCC> (A) The external views *(above)* show the embryo as a semitransparent object, seen from the side; with the directions of cell movement indicated by *red arrows,* cross sections *(below)* are cut in the median plane (the plane of the dorsal and ventral midlines). Gastrulation begins when a short indentation, the beginning of the blastopore, becomes visible in the exterior of the blastula. This indentation gradually extends, curving around to form a complete circle surrounding a plug of very yolky cells (destined to be enclosed in the gut and digested). Sheets of cells meanwhile turn in around the lip of the blastopore and move deep into the interior of the embryo. At the same time the external epithelium in the region of the animal pole actively spreads to take the place of the cell sheets that have turned inward. Eventually, the epithelium of the animal hemisphere spreads in this way to cover the whole external surface of the embryo, and, as gastrulation reaches completion, the blastopore circle shrinks almost to a point. (B) A fate map for the early *Xenopus* embryo (viewed from the side) as it begins gastrulation, showing the origins of the cells that will come to form the three germ layers as a result of the movements of gastrulation. The various parts of the mesoderm (lateral plate, somites, and notochord) derive from deep-lying cells that segregate from the epithelium in the cross-hatched region. The other cells, including the more superficial cells in the cross-hatched region, will give rise to ectoderm *(blue, above)* or endoderm *(yellow, below)*. Roughly speaking, the first cells to turn into the interior, or involute, will move forward inside the embryo to form the most anterior endodermal and mesodermal structures, while the last to involute will form the most posterior structures. (C) Cartoon (not to be taken too literally) showing roughly how the different regions of the ectoderm map into the body surface of the adult animal. (After R.E. Keller, *J. Exp. Zool.* 216:81–101, 1981, with permission from John Wiley & Sons, Inc. and *Dev. Biol.* 42:222–241, 1975, with permission from Academic Press.)

Chemical Signals Trigger the Mechanical Processes

The *VegT*, *Wnt11*, and other mRNA molecules localized in the vegetal cytoplasm of the egg produce localized distributions of their protein products. These act in and on the cells in the lower and middle part of the embryo to give them specialized characters and set them moving, both by direct effects and by stimulating the production of other secreted signal molecules, in particular proteins of the TGFβ superfamily. If these latter signals are blocked, no mesodermal cell types are generated and gastrulation is disrupted. The local activation of the Wnt signaling pathway on the dorsal side of the embryo (as a result of the earlier cortical rotation; see Figure 22–68) modifies the action of the other signals so as to induce development of the special cells that form the dorsal lip of the blastopore (**Figure 22–74**).

The dorsal lip of the blastopore plays a central role in gastrulation not just in a geometrical sense, but as a powerful new source of control. If the dorsal lip of the blastopore is excised from an embryo at the beginning of gastrulation and grafted into another embryo but in a different position, the host embryo initiates gastrulation both at the site of its own dorsal lip and at the site of the graft. The movements of gastrulation at the second site entail the formation of a second



whole set of body structures, and a double embryo (Siamese twins) results (see Figure 22–6B).

Evidently, the dorsal lip of the blastopore is the source of a signal (or signals) coordinating both the movements of gastrulation and the pattern of specialization of the tissues in its neighborhood. Because of this crucial role in organizing the formation of the main body axis, the dorsal lip of the blastopore is known as the **Organizer** (or Spemann's Organizer, after its co-discoverer). It is the oldest and most famous example of an *embryonic signaling center*.

Active Changes of Cell Packing Provide a Driving Force for Gastrulation

The Organizer controls the dorsoventral pattern of cell differentiation in its neighborhood by secreting at least six different signal proteins. These act as diffusible antagonists of the two main types of signals we have already mentioned, coming from the more vegetal cells—that is, of Wnt signals and of TGFβ-like signals (specifically BMP proteins). These inhibitors released from the Organizer may help to limit the size of the Organizer by preventing neighboring cells from also adopting an Organizer character. At the same time, they create a gradient of signaling activity—a morphogen gradient, whose local value reflects the distance from the Organizer (Figure 22–74C). As the cells move during gastrulation, they experience different doses of BMP (and other) signals, delivered with different timing, evoking different cell behaviors and entailing different ultimate fates. But how is the pattern of cell movements organized in mechanical terms, and what are the forces that bring it about?

Gastrulation begins with changes in the shape of the cells at the site of the blastopore. In the amphibian these are called bottle cells: they have broad bodies and narrow necks that anchor them to the surface of the epithelium (**Figure 22–75**), and they may help to force the epithelium to curve and so to tuck inward, producing the initial indentation seen from outside. Once this first tuck has formed, cells can continue to pass into the interior as a sheet to form the gut and mesoderm. The movement seems to be driven mainly by an active repacking of the cells, especially those in the involuting regions around the Organizer (see Figure 22–75). Here **convergent extension** occurs. Small square fragments of tissue from these regions, isolated in culture, will spontaneously narrow and elongate through a rearrangement of the cells, just as they would in the embryo in the process of converging toward the dorsal midline, turning inward around the blastopore lip, and then elongating to form the main axis of the body.

Figure 22–74 A current view of the main inductive signals organizing the events of gastrulation. (A) The distribution of axis-determining molecules in the blastula results from inheritance of different parts of the cytoplasm of the fertilized frog egg. The VegT gene regulatory protein in the vegetal blastomeres is translated from *VegT* mRNA that was localized at the vegetal pole before fertilization. The Wnt11 protein on the future dorsal side is translated from mRNA localized there as a result of the cortical rotation that follows fertilization. (B) VegT drives expression of Xnr (*Xenopus* nodal-related) proteins and other members of the TGFβ superfamily, which induce formation of a band of mesoderm in the middle part of the embryo, while Wnt11 modifies the outcome on the dorsal side, collaborating with Xnr to induce formation of the Organizer. (C) A morphogen gradient that organizes the dorsoventral axis is set up by a combination of signals, including BMP4 (another TGFβ superfamily member) secreted by the mesoderm, and antagonists of the Wnt and BMP pathways, secreted by the Organizer cells at the dorsal lip of the blastopore.

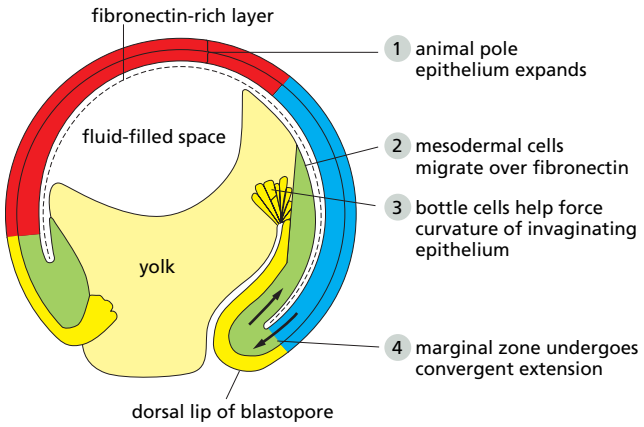


Figure 22–75 Cell movements in gastrulation. A section through a gastrulating *Xenopus* embryo, cut in the same plane as in Figure 22–73, indicating the four main types of movement that gastrulation involves. The animal pole epithelium expands by cell rearrangement, becoming thinner as it spreads. Migration of mesodermal cells over a fibronectin-rich matrix lining the roof of the blastocoel may help to pull the invaginated tissues forward. But the main driving force for gastrulation in *Xenopus* is convergent extension in the marginal zone. (After R.E. Keller, *J. Exp. Zool.* 216:81–101, 1981. With permission from Wiley-Liss.)

To bring about this remarkable transformation, the individual cells have to crawl over one another in a coordinated way (**Figure 22–76**). The alignment of their movements appears to depend on the same machinery we encountered in the worm and the fly controlling planar cell polarity: the Frizzled/Dishevelled polarity-signaling pathway. When this pathway is blocked—for example, by a dominant-negative form of Dishevelled—convergent extension fails to occur.

Changing Patterns of Cell Adhesion Molecules Force Cells Into New Arrangements

Patterns of gene expression govern embryonic cell movements in many different ways. They regulate cell motility, cell shape, and the production of signals for guidance. Very importantly, they also determine the sets of adhesion molecules that the cells display on their surfaces. Through changes in its surface molecules, a cell can break old attachments and make new ones. Cells in one region may develop surface properties that make them cohere with one another and become segregated from a neighboring group of cells whose surface chemistry is different.

Experiments done half a century ago on early amphibian embryos showed that the effects of selective cell–cell adhesion can be so powerful that they can bring about an approximate reconstruction of the normal structure of an early

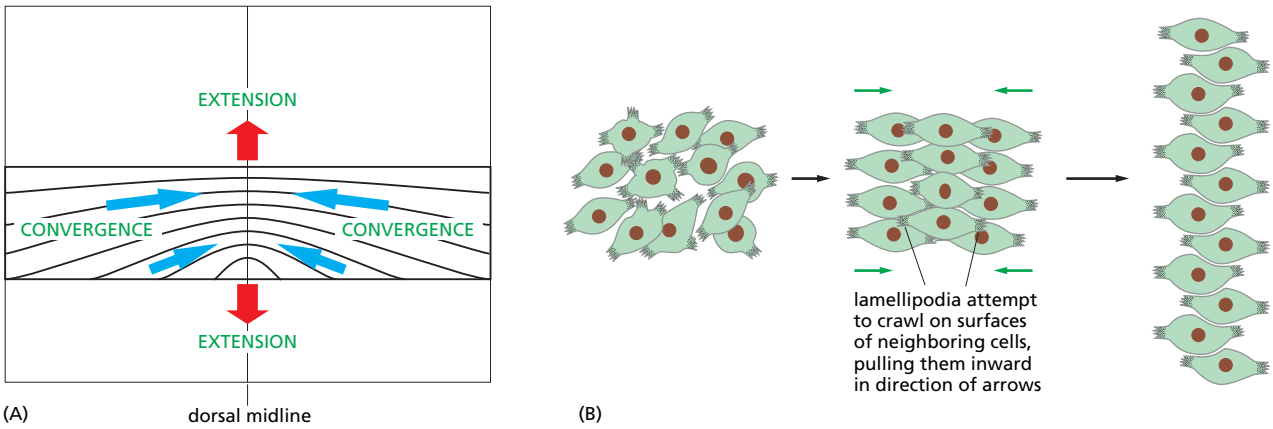


Figure 22–76 Convergent extension and its cellular basis. (A) The pattern of convergent extension in the marginal zone of a gastrula as viewed from the dorsal aspect. *Blue arrows* represent convergence toward the dorsal midline, *red arrows* represent extension of the anteroposterior axis. The simplified diagram does not attempt to show the accompanying movement of involution, whereby the cells are tucking into the interior of the embryo. (B) Schematic diagram of the cell behavior that underlies convergent extension. The cells form lamellipodia, with which they attempt to crawl over one another. Alignment of the lamellipodial movements along a common axis leads to convergent extension. The process depends on the Frizzled/Dishevelled polarity-signaling pathway and is presumably cooperative because cells that are already aligned exert forces that tend to align their neighbors in the same way. (B, after J. Shih and R. Keller, *Development* 116:901–914, 1992. With permission from The Company of Biologists.)

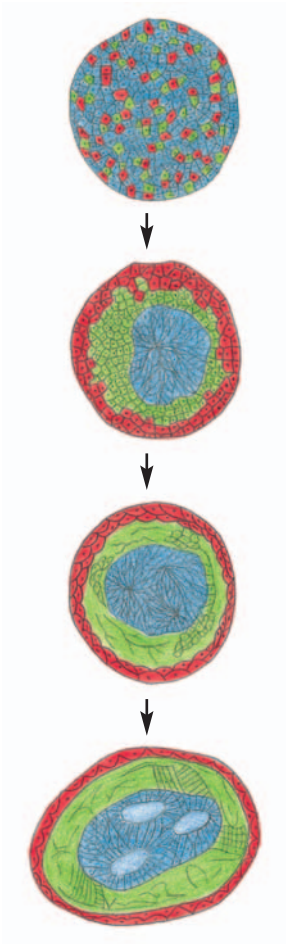


Figure 22–77 Sorting out. Cells from different parts of an early amphibian embryo will sort out according to their origins. In the classical experiment shown here, mesoderm cells *(green),* neural plate cells *(blue),* and epidermal cells *(red)* have been disaggregated and then reaggregated in a random mixture. They sort out into an arrangement reminiscent of a normal embryo, with a "neural tube" internally, epidermis externally, and mesoderm in between. (Modified from P.L. Townes and J. Holtfreter, *J. Exp. Zool.* 128:53–120, 1955. With permission from Wiley-Liss.)

postgastrulation embryo even after the cells have been artificially dissociated. When these cells are reaggregated into a random mixture, the cells sort out spontaneously according to their original characters (**Figure 22–77**). As discussed in Chapter 19, a central role in such phenomena is played by the *cadherins*—a large and varied family of evolutionarily related Ca^{2+}-dependent cell–cell adhesion proteins. These and other cell–cell adhesion molecules are differentially expressed in the various tissues of the early embryo, and antibodies against them interfere with the normal selective adhesion between cells of a similar type.

Changes in the patterns of expression of the various cadherins correlate closely with the changing patterns of association among cells during gastrulation, neurulation, and somite formation (see Figure 19–25). These rearrangements are likely to be regulated and driven in part by the cadherin pattern. In particular, cadherins appear to have a major role in controlling the formation and dissolution of epithelial sheets and clusters of cells. They not only glue one cell to another but also provide anchorage for intracellular actin filaments at the sites of cell–cell adhesion. In this way, the pattern of stresses and movements in the developing tissue is regulated according to the pattern of adhesions.

The Notochord Elongates, While the Neural Plate Rolls Up to Form the Neural Tube

Gastrulation is only the first—though perhaps the most dramatic—of a dizzying variety of cell movements that shape the parts of the body. We have space to discuss only a few of these.

In the embryo just after gastrulation, the layer of mesoderm is divided into separate slabs on the left and right sides of the body. Defining the central body axis, and effecting this separation, is the very early specialization of the mesoderm known as the **notochord**. This slender rod of cells, with ectoderm above it, endoderm below it, and mesoderm on either side (see Figure 22–72), derives from the cells of the Organizer itself. The notochordal cells are characterized by expression of a gene regulatory protein called Brachyury (Greek for "short-tail", from the mutant phenotype); this belongs to the same T-box family as the VegT protein in the vegetal blastomeres.

As the notochordal cells pass around the dorsal lip of the blastopore and move into the interior of the embryo, they form a column of tissue that elongates dramatically by convergent extension. The cells of the notochord also become swollen with vacuoles, so that the rod elongates still further and stretches out the embryo. The notochord is the defining peculiarity of the chordates—the phylum to which the vertebrates belong. It is one of the major vertebrate features that do not have any apparent counterpart in *Drosophila*. In the most primitive chordates, which have no vertebrae, the notochord persists as a primitive substitute for a vertebral column. In vertebrates it serves as a core around which other mesodermal cells will eventually gather to form the vertebrae.

In the overlying sheet of ectoderm, meanwhile, other movements are occurring to form the rudiments of the nervous system. In a process known as *neurulation*, a broad central region of ectoderm, called the *neural plate*, thickens, rolls up into a tube, and pinches off from the rest of the cell sheet. The tube thus created from the ectoderm is called the **neural tube**; it will form the brain and the spinal cord (**Figure 22–78**).

The mechanics of neurulation depend on changes of cell packing and cell shape that make the epithelium roll up into a tube (**Figure 22–79**). Signals initially

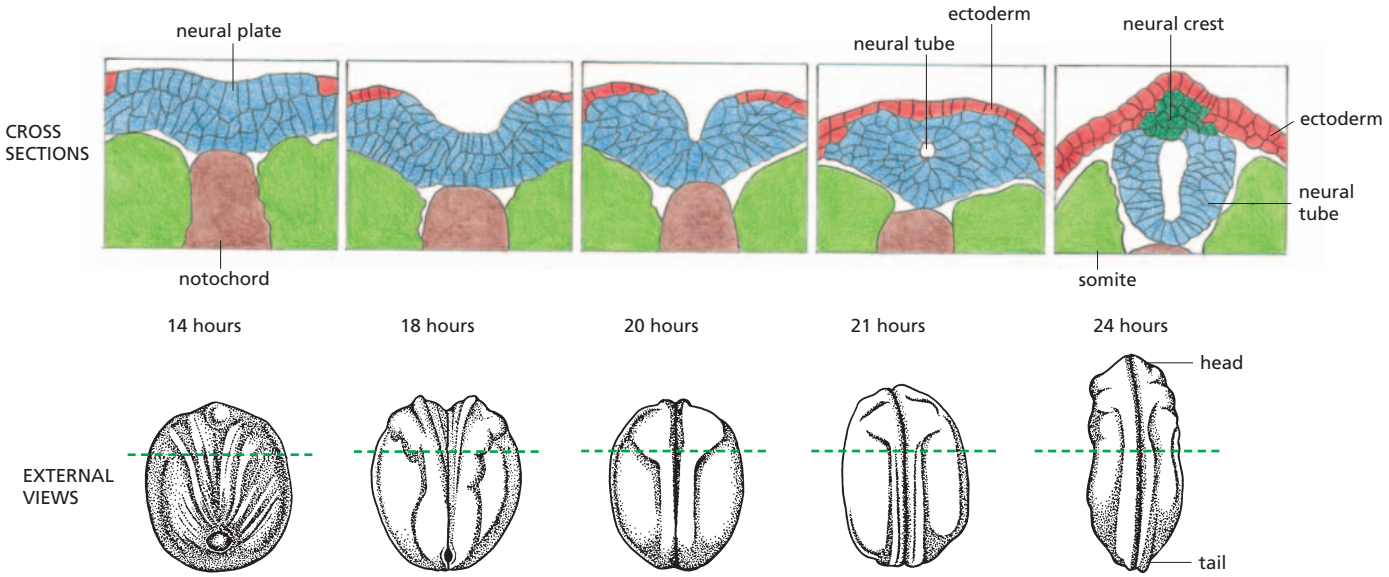


Figure 22–78 Neural tube formation in *Xenopus*. The external views are from the dorsal aspect. The cross sections are cut in a plane indicated by the broken lines. (After T.E. Schroeder, *J. Embryol. Exp. Morphol.* 23:427–462, 1970. With permission from The Company of Biologists.)

from the Organizer and later from the underlying notochord and mesoderm define the extent of the neural plate, induce the movements that make it roll up, and help to organize the internal pattern of the neural tube. The notochord in particular secretes Sonic hedgehog protein—a homolog of the *Drosophila* signal protein Hedgehog—and this acts as a morphogen to control gene expression in the neighboring tissues (**Figure 22–80**).

A Gene-Expression Oscillator Controls Segmentation of the Mesoderm Into Somites

Genetically regulated changes in cell adhesion underlie one of the most striking and characteristic processes in vertebrate development—the formation of the segments of the body axis.

On either side of the newly formed neural tube lies a slab of mesoderm (see Figure 22–72). To form the repetitive series of vertebrae, ribs, and segmental muscles, this slab breaks up into separate blocks, or **somites**—cohesive groups of cells, separated by clefts. **Figure 22–81**A shows the process as it occurs in the chick embryo. The somites form one after another, starting in the head and ending in the tail. Depending on the species, the final number of somites ranges from less than 50 (in a frog or a bird) to more than 300 (in a snake). The posterior, most immature part of the mesodermal slab, called the *presomitic mesoderm*, supplies the necessary tissue: as it retreats tailward, extending the embryo, it deposits a trail of somites. The special character of the presomitic mesoderm is maintained by FGF signaling: *Fgf8* mRNA is synthesized at the tail end of the embryo and slowly degraded as cells move away from this region. Translation of the message results in a gradient of secreted FGF8 protein, with its high point at the tail end.

Formation of the cleft between one somite and the next is foreshadowed by an alternating spatial pattern of gene expression in the presomitic mesoderm: cells about to form the posterior part of a new somite switch on expression of one set of genes, while those destined to form the anterior part of the next somite switch on expression of another set. Selective cohesion resulting from differential gene expression seems to be the underlying cause of the physical segmentation observed.

The problem then is to understand how the repetitive alternating pattern of gene expression is set up. Studies done originally in the chick embryo have provided the beginnings of an answer. In the posterior part of the presomitic mesoderm, expression of certain genes is found to oscillate in time. The first such somite oscillator gene to be discovered was *Hes1*, a homolog of the *Drosophila* pair-rule gene *Hairy* and of the *E(spl)* genes that mediate responses to Notch

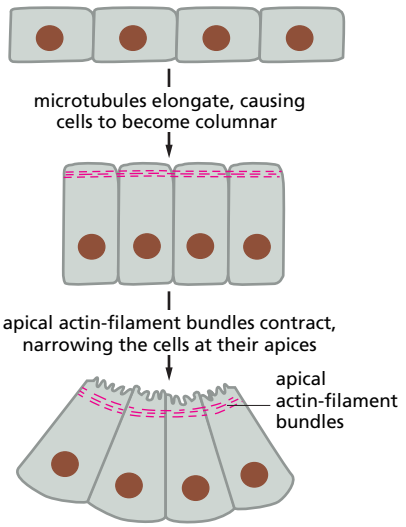


Figure 22–79 The bending of an epithelium through cell shape changes mediated by microtubules and actin filaments. The diagram is based on observations of neurulation in newts and salamanders, where the epithelium is only one cell layer thick. As the apical ends of the cells become narrower, their upper-surface membrane becomes puckered.

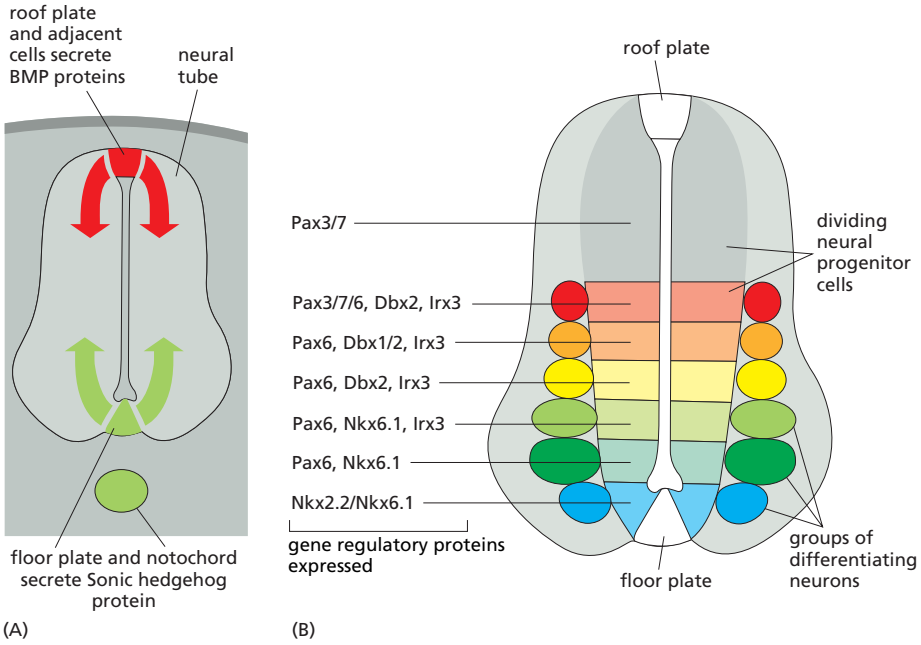


Figure 22–80 A schematic cross section of the spinal cord of a chick embryo, showing how cells at different levels along the dorsoventral axis express different gene regulatory proteins. (A) Signals that direct the dorsoventral pattern: Sonic hedgehog protein from the notochord and the floor plate (the ventral midline of the neural tube) and BMP proteins from the roof plate (the dorsal midline) act as morphogens to control gene expression. (B) The resulting patterns of gene expression in the ventral part of the developing spinal cord. Different groups of proliferating neural progenitor cells (in the ventricular zone, close to the lumen of the neural tube) and of differentiating neurons (in the mantle zone, further out) express different combinations of gene regulatory proteins. Those indicated in this diagram are almost all members of the homeodomain superfamily; various other genes of the same superfamily (including Islet/Lim proteins) are expressed in the differentiating neurons. Neurons expressing different gene regulatory proteins will form connections with different partners and may make different combinations of neurotransmitters and receptors.

signaling. The length of one complete oscillation cycle of this **segmentation clock** (90 minutes in the chick) equals the time taken to lay down one further somite. As cells emerge from the presomitic mesoderm to form somites—in other words, as they lose exposure to the FGF8 signal—their oscillation slows down and finally comes to a halt. Some become arrested in one state, some in another, according to the phase of their oscillation cycle at their time of exit from the presomitic mesoderm. *Hes1* and several of the other oscillating genes code for gene regulatory proteins; thus, the cells that drop below the critical level of FGF8 when they are at the peak of their oscillation cycle switch on one set of regulatory genes, while those passing the threshold at the trough of the cycle switch on another (Figure 22–81B). In this way, it is thought, the temporal oscillation of gene expression in the presomitic mesoderm leaves its trace in a spatially periodic pattern of gene expression in the maturing mesoderm, and this in turn dictates how the tissue will break up into physically separate blocks.

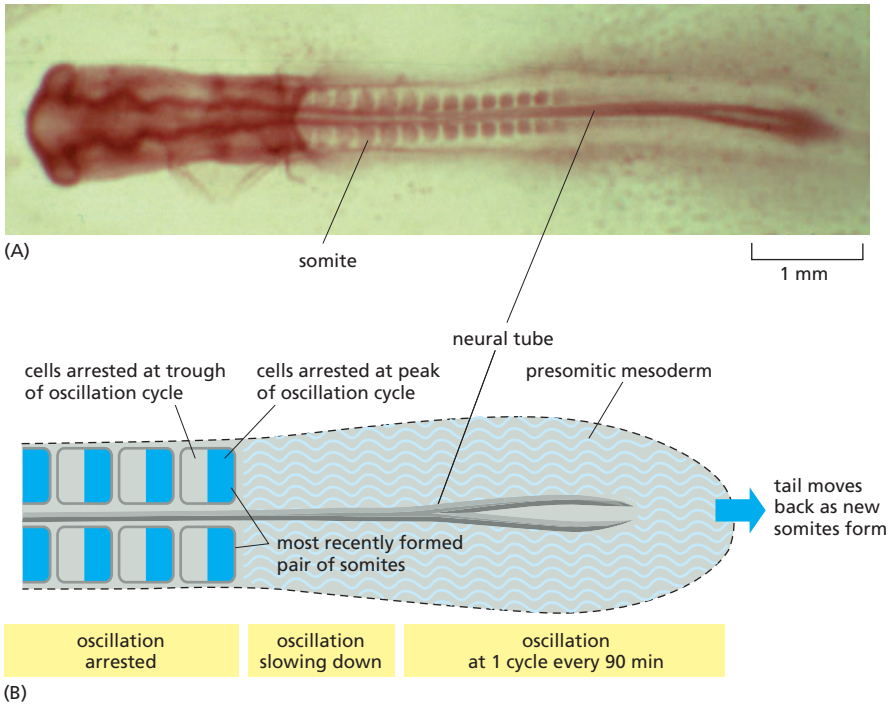
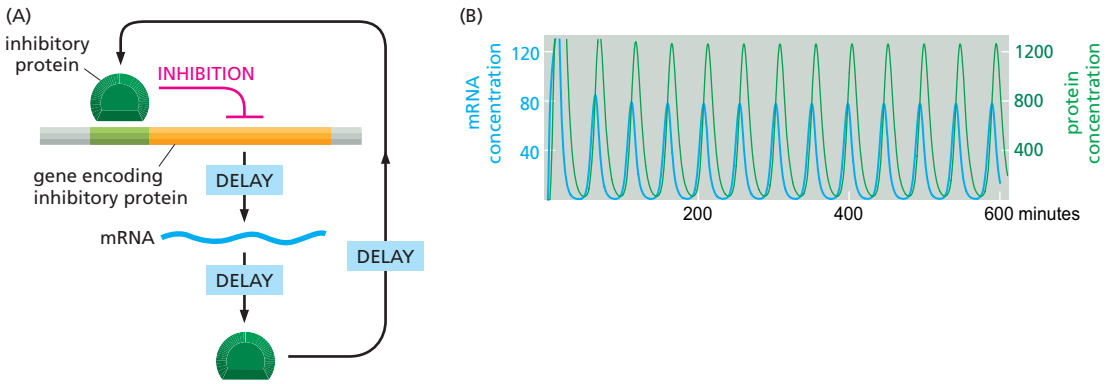


Figure 22–81 Somite formation in the chick embryo. (A) A chick embryo at 40 hours of incubation. (B) How the temporal oscillation of gene expression in the presomitic mesoderm becomes converted into a spatial alternating pattern of gene expression in the formed somites. In the posterior part of the presomitic mesoderm, each cell oscillates with a cycle time of 90 minutes. As cells mature and emerge from the presomitic region, their oscillation is gradually slowed down and finally brought to a halt, leaving them in a state that depends on the phase of the cycle they happen to be in at the critical moment. In this way, a temporal oscillation of gene expression traces out an alternating spatial pattern. (A, from Y.J. Jiang, L. Smithers and J. Lewis, *Curr. Biol.* 8:R868–R871, 1998. With permission from Elsevier.)



Delayed Negative Feedback May Generate the Oscillations of the Segmentation Clock

What, then, is the mechanism that generates the temporal oscillation? How does the clock work? In the mouse, at least three classes of genes have been found to show oscillating expression in the presomitic mesoderm, coding respectively for components of the Notch pathway, the Wnt pathway, and the Fgf pathway; but most of the mutations that are known to break the clock and disrupt somite segmentation lie in components of the Notch pathway. These include genes (such as *Hes1* and more importantly its relative *Hes7*) that are regulated by Notch and code for inhibitory gene regulatory proteins. Some of these proteins act directly on the regulatory DNA of their own gene so as to inhibit their own expression. According to one theory, this simple negative feedback loop could be the basic generator of the oscillations **Figure 22–82**: when the gene is transcribed, the amount of its protein product builds up until transcription is inhibited and synthesis of the protein ceases; the protein then decays, permitting transcription to begin again; and so on. There is a time-lag from the beginning of a new bout of transcription to the first appearance in the nucleus of the resulting regulatory protein molecules, because it takes time for the RNA polymerase to traverse the gene, for the resulting RNA transcript then to mature, leave the nucleus, and direct synthesis of a protein molecule, and for the protein then to enter the nucleus to control transcription. This delay in the feedback loop is proposed to be the main determinant of the period of oscillation of the clock and thus of the size of each somite.

Most of the cells of each newly formed somite will rapidly differentiate to form a block of muscle, corresponding to one muscle segment of the main body axis. The embryo can (and does) now begin to wriggle. Separate subsets of the somite cells will go to form the vertebrae and other connective tissues such as dermis. A further subset detach from the somite and migrate away into the lateral unsegmented mesoderm, crawling through the spaces between other cells: these emigrants will give rise to almost all the other skeletal muscle cells in the body, including those of the limbs.

Figure 22–82 Delayed negative feedback giving rise to oscillating gene expression. (A) A single gene, coding for a gene regulatory protein that inhibits its own expression, can behave as an oscillator. For oscillation to occur, there must be a delay (or several delays) in the feedback circuit, and the lifetimes of the mRNA and protein must be short compared with the total delay. The delay determines the period of oscillation. According to one theory, a feedback circuit like this, based on a gene called *Her7* in the zebrafish, or *Hes7* in the mouse (a relative of *Hes1*), is the pacemaker of the segmentation clock governing somite formation. (B) The predicted oscillation of *Her7* mRNA and protein, computed using rough estimates of the feedback circuit parameters appropriate to this gene in the zebrafish. Concentrations are measured as numbers of molecules per cell. The predicted period is close to the observed period, which is 30 minutes per somite in the zebrafish.

Embryonic Tissues Are Invaded in a Strictly Controlled Fashion by Migratory Cells

The muscle-cell precursors, or *myoblasts*, that emigrate from the somites are determined but not overtly differentiated. In the tissues that they colonize they will mingle with other classes of cells from which they appear practically indistinguishable; but they will maintain expression of myoblast-specific gene regulatory proteins (such as Pax3 and members of the MyoD family), and when the time comes for differentiation, they, and they alone, will turn into muscle cells (**Figure 22–83**).

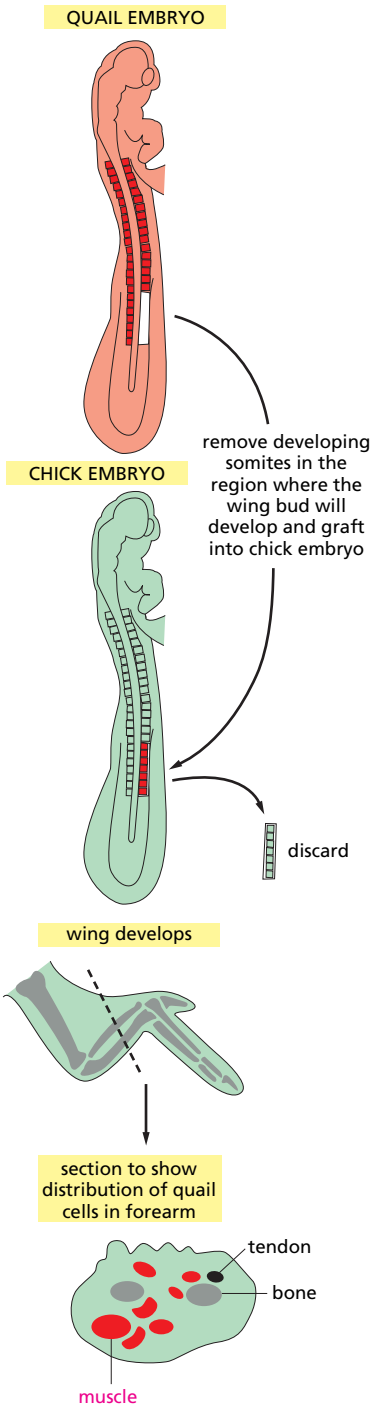


Figure 22–83 The migratory origin of limb muscle cells. The migrations can be traced by grafting cells from a quail embryo into a chick embryo; the two species are very similar in their development, but the quail cells are recognizable by the distinctive appearance of their nucleoli. If quail somite cells are substituted for the somite cells of a chick embryo at 2 days of incubation and the wing of the chick is sectioned a week later, it is found that the muscle cells in the chick wing derive from the transplanted quail somites.

The eventual pattern of muscles—in the limbs, for example—is determined by the routes that the migrant cells follow and the selection of sites that they colonize. The embryonic connective tissues form the framework through which the myoblasts travel and provide signals that guide their distribution. No matter which somite they come from, myoblasts that migrate into a forelimb bud will form the pattern of muscles appropriate to a forelimb, and those that migrate into a hindlimb bud will form the pattern appropriate to a hindlimb.

Other classes of migrant cells, meanwhile, select different routes for their travels. Along the line where the neural tube pinches off from the future epidermis, a number of ectodermal cells break loose from the epithelium and also migrate as individuals out through the mesoderm (**Figure 22–84**). These are the cells of the **neural crest**; they will give rise to almost all of the neurons and glial cells of the peripheral nervous system, as well as the pigment cells of the skin and many connective tissues in the head, including bones of the skull and jaws. Other important migrants are the precursors of the blood cells, of the germ cells, and of many groups of neurons within the central nervous system, as well as the *endothelial cells* that form blood vessels. Each of these classes of travelers will colonize a different set of sites. As a result of such invasions, most tissues in the vertebrate body are mixtures of cells of different characters derived from widely separate parts of the embryo.

As a migrant cell travels through the embryonic tissues, it repeatedly extends projections that probe its immediate surroundings, testing for subtle cues to which it is particularly sensitive by virtue of its specific assortment of cell-surface receptor proteins. Inside the cell these receptor proteins are connected to the cytoskeleton, which moves the cell along. Some extracellular matrix materials,

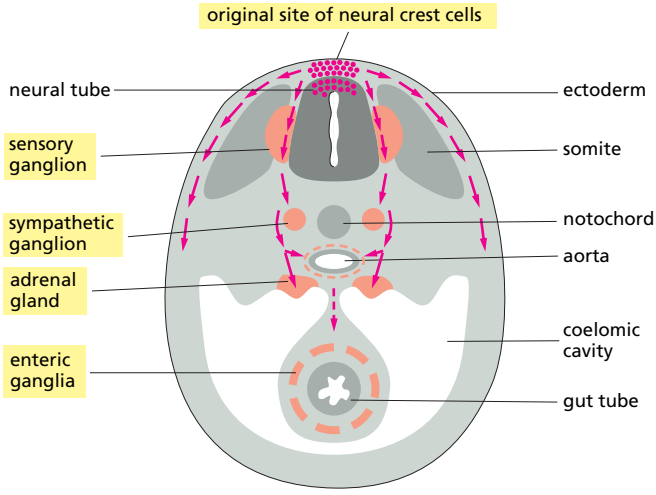


Figure 22–84 The main pathways of neural crest cell migration. A chick embryo is shown in a schematic cross section through the middle part of the trunk. Deep-lying neural crest derivatives are indicated by *yellow* text boxes. The cells that take the pathway just beneath the ectoderm will form pigment cells of the skin; those that take the deep pathway via the somites will form the neurons and glial cells of sensory and sympathetic ganglia, and parts of the adrenal gland. The neurons and glial cells of the enteric ganglia, in the wall of the gut, are formed from neural crest cells that migrate along the length of the body, originating from either the neck region or the sacral region. In *Drosophila,* neurons in the wall of the gut originate in a similar way, by migration from the head end of the embryo. (See also Figure 19–23.)

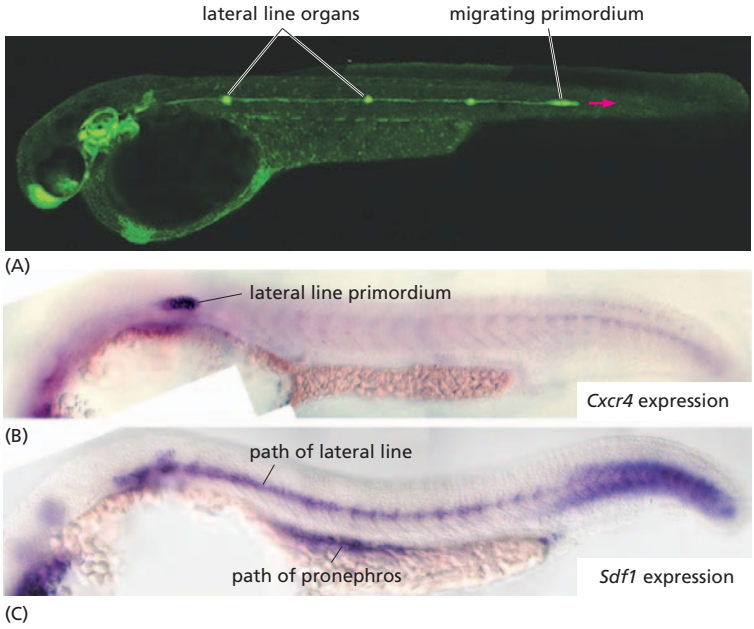


Figure 22–85 Migration of the lateral line primordium in a zebrafish larva, guided by SDF1 and CXCR4. The lateral line is a row of mechanosensory organs, closely similar to the sensory patches in the inner ear, which detect the movement of water over the surface of a fish or amphibian. (A) They originate as clusters of cells deposited by a primordium that migrates along the the flank of the larva, from a site in the head all the way down to the tail, as shown in this 2-day larva in which the lateral line cells are labeled by expression of Green Fluorescent Protein. (B) Cells in the primordium express the chemotaxis receptor CXCR4, shown here by *in situ* hybridization in a 1-day larva. (C) The track that they will follow is marked by expression of the ligand SDF1, shown by *in situ* hybridization in another 1-day specimen. If the ligand is lacking along the normal route (as a result of a mutation), the primordium departs from its proper route to follow an alternative more ventral track marked by another stripe of SDF1, defining the normal path of another migratory structure, the pronephros. (A, courtesy of David Gilmour; B and C, from N.B. David et al., *Proc. Natl Acad. Sci. U.S.A.* 99:16297–16302, 2002. With permission from National Academy of Sciences.)

such as the protein fibronectin, provide adhesive sites that help the cell to advance; others, such as chondroitin sulfate proteoglycan, inhibit locomotion and repel immigration. The nonmigrant cells along the pathway may likewise have inviting or repellent surfaces, or may even extend filopodia that touch the migrant cell and affect its behavior.

Among this mass of different guiding influences, a few stand out as particularly important. In particular, cells of many different types are guided by chemotaxis that depends on a receptor called CXCR4. This cell-surface protein belongs to the family of G-protein-coupled receptors, and it is activated by an extracellular ligand called SDF1. Cells expressing CXCR4 can snuffle their way along tracks marked out for them by production of SDF (**Figure 22–85**). Chemotaxis towards sources of SDF1 plays a major part in guiding the migrations of lymphocytes and of various other white blood cells; of neurons in the developing brain; of muscle progenitor cells entering limb buds; of primordial germ cells as they travel toward the gonads; and of cancer cells when they metastasize.

The Distribution of Migrant Cells Depends on Survival Factors as Well as Guidance Cues

The final distribution of migrant cells depends not only on the routes they take, but also on whether they survive the journey and thrive in the environment they find at the journey's end. Specific sites provide survival factors needed by specific types of migrant. For example, the neural crest cells that give rise to the pigment cells of the skin and the nerve cells of the gut depend on a peptide factor called *endothelin-3* that is secreted by tissues on the migration pathways; mutant mice and humans defective in the gene for this factor or its receptor have nonpigmented (albino) patches and potentially lethal gut malformations resulting from the lack of gut innervation (a condition called megacolon, because the colon becomes hugely distended).

Germ cells, blood cell precursors, and neural-crest-derived pigment cells all appear to share at least one common requirement for survival. This involves a transmembrane receptor, called the *Kit protein*, in the membrane of the migrant cells, and a ligand, called the *Steel factor*, produced by the cells of the tissue through which the cells migrate and/or in which they come to settle. Individuals with mutations in the genes for either of these proteins are deficient in their pigmentation, their supply of blood cells, and their production of germ cells (**Figure 22–86**).

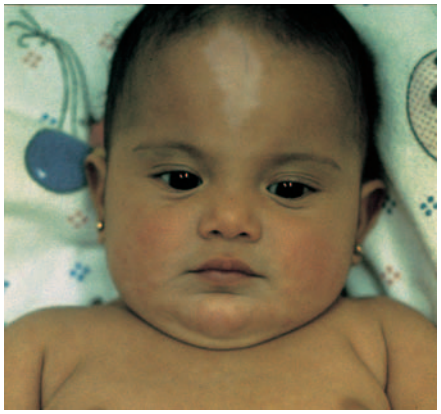


Figure 22–86 Effect of mutations in the *Kit* gene. Both the baby and the mouse are heterozygous for a loss-of-function mutation that leaves them with only half the normal quantity of Kit gene product. In both cases pigmentation is defective because pigment cells depend on the Kit product as a receptor for a survival factor. (Courtesy of R.A. Fleischman, from *Proc. Natl Acad. Sci. U.S.A.* 88:10885–10889, 1991. With permission from National Academy of Sciences.)

Left–Right Asymmetry of the Vertebrate Body Derives From Molecular Asymmetry in the Early Embryo

Vertebrates may look bilaterally symmetrical from the outside, but many of their internal organs—the heart, the stomach, the liver, and so on—are highly asymmetric. This asymmetry is quite reproducible: 99.98% of people have their heart on the left. We have seen how a vertebrate embryo develops its internal and external tissue layers and its anteroposterior and dorsoventral axes. But how does the left–right asymmetry arise?

Genetic studies in mammals show that this problem can be broken down into two distinct questions—one concerning the creation of asymmetry and the other concerning its orientation. Several mutations are known, in humans and in mice, that cause a randomization of the left–right axis: 50% of the mutant individuals have their internal organs arranged in the normal way, while the other 50% have an inverted anatomy, with the heart on the right. In these individuals, it seems, the mechanism that makes the left and right sides different has functioned correctly, but the mechanism that decides between the two possible orientations of the left–right axis is defective.

A key to the basis of these phenomena comes from the discovery of molecular asymmetries that precede the first gross anatomical asymmetries. The earliest signs are seen in patterns of gene expression in the neighborhood of the *node*—the homolog in mouse and chick of the frog Organizer. In particular, the gene *Nodal*, coding for a member of the TGFβ superfamily, is expressed asymmetrically in this region (not only in the mouse, but also in chick, frog and zebrafish) (**Figure 22–87**). Asymmetry of *Nodal* expression in the immediate neighborhood of the node is relayed outward to create a broad stripe of *Nodal*

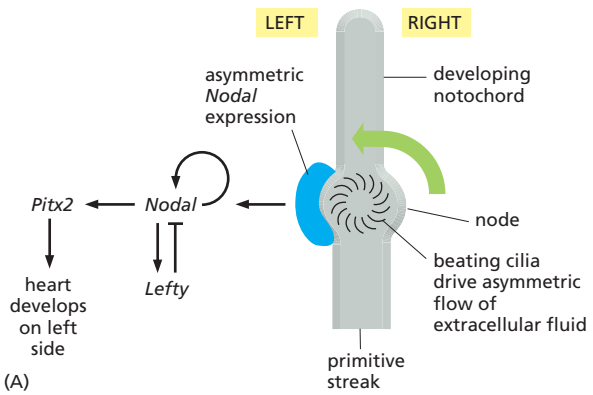


Figure 22–87 Helical beating of cilia at the node, and the origins of left-right asymmetry. (A) The beating of the cilia drives a fluid flow toward one side of the node, and this leads to asymmetric gene expression in the neighborhood of the node. According to one theory, the flow exerts this effect by carrying extracellular signal proteins to one side. Another theory notes that cilia can also function as mechanosensors, and proposes that a subset of cilia at the node respond to deflection due to the fluid flow by opening Ca^{2+} channels so as to create an increased Ca^{2+} concentration in the cells on one side. (B) The resulting asymmetric expression pattern of *Nodal*, coding for a signal protein belonging to the TGFβ superfamily, in the neighborhood of the node *(lower two blue spots)* in a mouse embryo at 8 days of gestation, as shown by *in situ* hybridization. At this stage, the asymmetry has already been relayed outward to the lateral plate mesoderm, where *Nodal* is expressed on the left side *(large elongated blue patch)* but not the right. (B, courtesy of Elizabeth Robertson.)

expression in the mesoderm along the left side—and only the left side—of the embryo's body. The mechanism that relays the asymmetry from the node and localizes *Nodal* expression is not understood and may vary from one class of vertebrates to another. In all species, however, it seems to depend on feedback loops involving *Nodal* together with a second set of genes, the *Lefty* genes. These, like *Nodal* itself, are directly regulated by the Nodal signaling pathway and their products, the Lefty proteins, are related to Nodal; but Lefty proteins diffuse more widely and act oppositely, as Nodal antagonists. Mice with a knockout mutation in the *Lefty1* gene frequently have the right side converted into a mirror image of the left, so that left–right asymmetry is lost.

Another gene that is directly regulated by the Nodal pathway, *Pitx2*, coding for a gene regulatory protein, links the outcome of the Nodal/Lefty interactions to subsequent anatomical development. Nodal drives *Pitx2* expression on the left side of the body and thereby confers asymmetry on the heart and other internal organs.

This leaves us with the puzzle of how the initial asymmetry of *Nodal* expression originates. Whatever the mechanism, the outcome of events at the node in a normal animal must be biased so that left-specific genes are regularly expressed on the left side: there has to be a link between the mechanism that creates asymmetry and the mechanism that orients it. A clue to the orienting mechanism first came to light in a Swedish infertility clinic. A small subset of infertile men were found to have sperm that were immotile because of a defect in the dynein molecules needed for beating of cilia and flagella. These men also suffered from chronic bronchitis and sinusitis because the cilia in their respiratory tract were defective. And strikingly, 50% of them had their internal organs left–right inverted, with the heart on the right. The findings originally seemed completely mysterious; but similar effects are seen in mammals with other mutations resulting in defective cilia. This suggests that ciliary beating somehow controls which way the left–right axis is oriented.

Time-lapse videomicroscopy in the living mouse embryo reveals that the cells at the node, on its internal face, have cilia that beat in a helical fashion: like a screw-thread, they have a definite handedness, and at the node they are set in a little hollow that is shaped so that their beating drives a current of fluid towards the left side (see Figure 22–87A). According to one theory, signal proteins carried in this current toward the left side provide the bias that orients the left–right axis of the mouse body. Another theory proposes that cilia in this system, as in certain other contexts, act not only as drivers of fluid flow but also as mechanical sensors, responding to deflection by generating an asymmetric current of Ca^{2+} ions across the node to influence adjacent tissue.

The handedness of the ciliary beating reflects the handedness—the left–right asymmetry—of the organic molecules of which all living things are made. It seems that this, therefore, is the ultimate director of the left–right asymmetry of our anatomy.

Summary

Animal development involves dramatic cell movements. Thus, in gastrulation, cells from the exterior of the early embryo tuck into the interior to form a gut cavity and create the three germ layers—endoderm, mesoderm, and ectoderm—from which higher animals are constructed. In vertebrates, the movements of gastrulation are organized by signals from the Organizer (the dorsal lip of the amphibian blastopore, corresponding to the node in a chick or mouse embryo). These signals specify the dorsoventral axis of the body and govern convergent extension, in which the sheet of cells moving into the interior of the body lengthens along the head-to-tail axis while narrowing at right angles to this axis. The active repacking movements of individual cells that drive convergent extension are coordinated through the Frizzled/Dishevelled planar-polarity signaling pathway—a branch of the Wnt signaling pathway that regulates the actin cytoskeleton.

Subsequent development involves many further cell movements. Part of the ectoderm thickens, rolls up, and pinches off to form the neural tube and neural crest. In the

midline, a rod of specialized cells called the notochord elongates to form the central axis of the embryo. The long slabs of mesoderm on either side of the notochord become segmented into somites. Migrant cells, such as those of the neural crest, break loose from their original neighbors and travel through the embryo to colonize new sites. Primordial germ cells and many other migrants are guided by chemotaxis dependent on the receptor CXCR4 and its ligand SDF1. Specific cell adhesion molecules, such as cadherins and integrins, help to guide the migrations and control the selective cohesion of cells in new arrangements.

Ultimately, the pattern of cell movements is directed by the pattern of gene expression, which determines cell surface properties and motility. Thus, the formation of somites depends on a periodic pattern of gene expression, which is laid down by a biochemical oscillator—the segmentation clock—in the mesoderm and dictates the way the mass of cells will break up into separate blocks. Similarly, the left–right anatomical asymmetry of the vertebrate body is foreshadowed by left–right asymmetry in the pattern of gene expression in the early embryo. This asymmetry, in mammals at least, is thought to be directed ultimately by the handedness of ciliary beating in the neighborhood of the node.

THE MOUSE

The mouse embryo—tiny and inaccessible in its mother's womb—presents a hard challenge to developmental biologists. It has, however, two immediate attractions. First, the mouse is a mammal, and mammals are the animals that we, as humans, care about most. Second, among mammals, it is one of the most convenient for genetic studies, because it is small and breeds rapidly. These two factors have spurred an enormous research effort, resulting in the development of some remarkably powerful experimental tools. In this way, the mouse has become the main model organism for experimentation in mammalian genetics and the most intensively studied surrogate for humans. It is separated from humans by only about 100 million years of evolution. Its genome is the same as ours in size, and there is very nearly a one-to-one correspondence between mouse and human genes. Our proteins are typically 80–90% identical in amino acid sequence, and large blocks of close nucleotide sequence similarity are also evident when the regulatory DNA sequences are compared.

Through ingenuity and perseverance, developmental biologists have now found ways to gain access to the early mouse embryo without killing it and to generate mice to order with mutations in any chosen gene. Almost any genetic modification that can be made in a worm, a fly, or a zebrafish can now also be made in the mouse, and in some cases made better. The costs of research in the mouse are far greater, but so are the incentives. As a result, the mouse has become a rich source of information about all aspects of the molecular genetics of development—a key model system not only for mammals, but also for other animals. It has provided, for example, much of what we know about *Hox* genes, left–right asymmetry, cell death controls, the role of Notch signaling, and a host of other topics.

We have already drawn repeatedly upon data from the mouse. We shall make use of it even more in the next chapter, where we discuss adult tissues and the developmental processes that occur in them. In this section, we examine the special features of mouse development that have been exploited to make the genetic manipulations possible. By way of example, we shall also outline how the mouse has been used to illuminate one further important developmental process—the creation of organs such as lungs and glands by interactions between embryonic connective tissue and epithelium.

Mammalian Development Begins With a Specialized Preamble

The mammalian embryo begins its development in an exceptional way. Protected within the uterus, it does not have the same need as the embryos of most

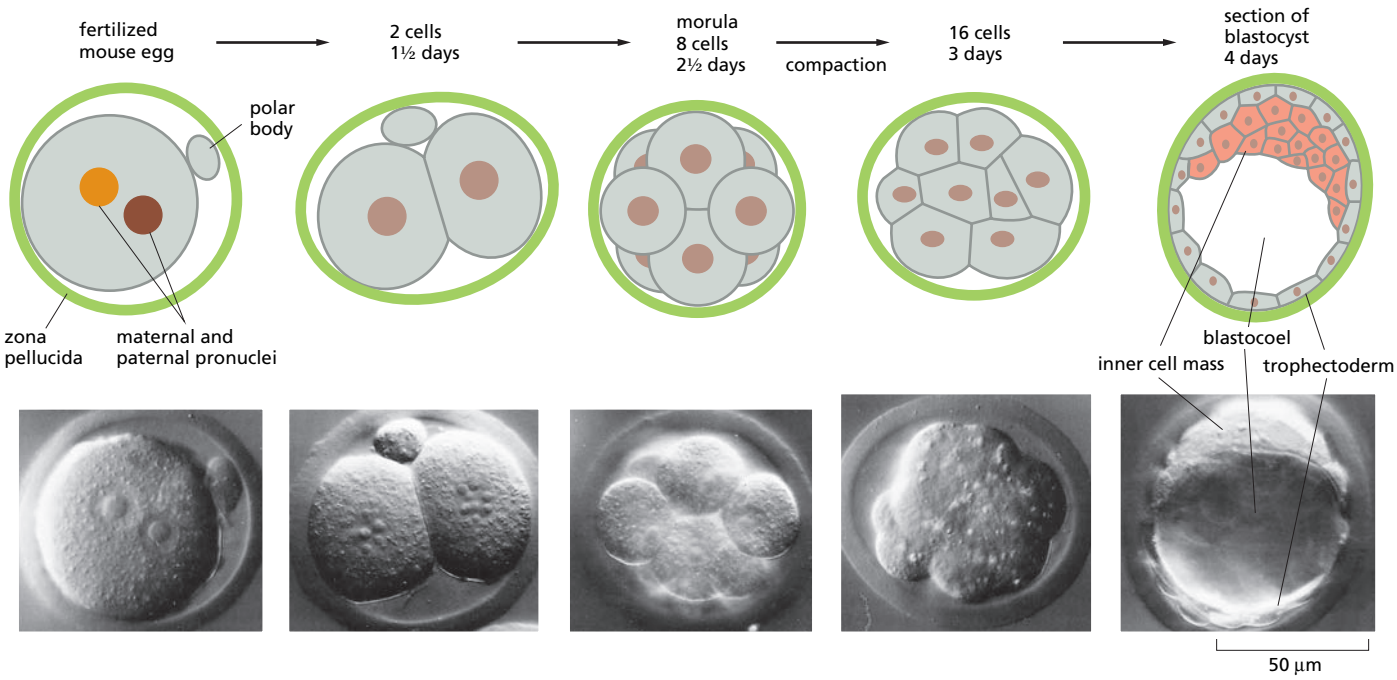
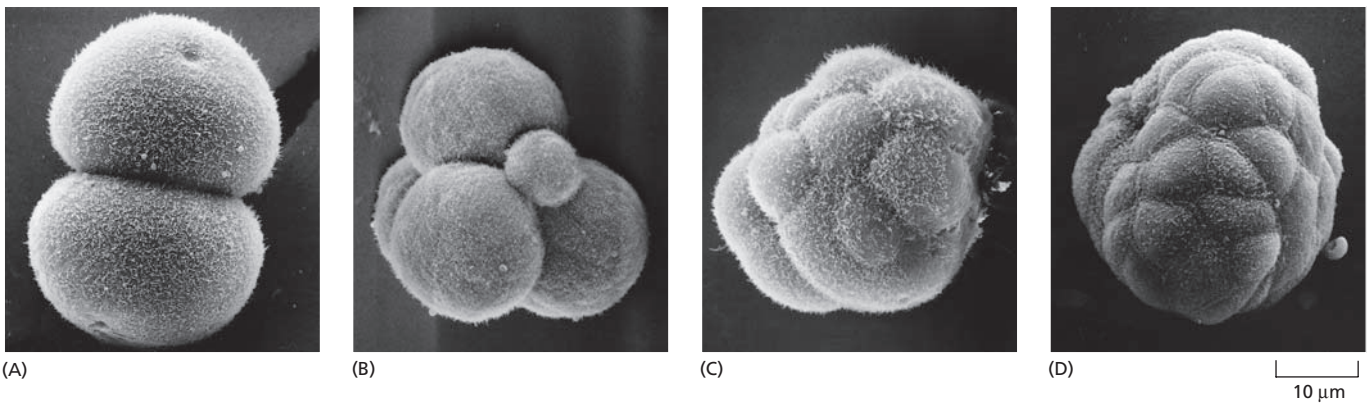


Figure 22–88 The early stages of mouse development. The zona pellucida is a jelly capsule from which the embryo escapes after a few days, allowing it to implant in the wall of the uterus. (Photographs courtesy of Patricia Calarco.)

other species to complete the early stages of development rapidly. Moreover, the development of a placenta quickly provides nutrition from the mother, so that the egg does not have to contain large stores of raw materials such as yolk. The egg of a mouse has a diameter of only about 80 μm and therefore a volume about 2000 times smaller than that of a typical amphibian egg. Its cleavage divisions occur no more quickly than the divisions of many ordinary somatic cells, and gene transcription has already begun by the 2-cell stage. Most importantly, while the later stages of mammalian development are similar to those of other vertebrates such as *Xenopus*, mammals begin by taking a large developmental detour to generate a complicated set of structures—notably the amniotic sac and the placenta—that enclose and protect the embryo proper and provide for the exchange of metabolites with the mother. These structures, like the rest of the body, derive from the fertilized egg but are called *extraembryonic* because they are discarded at birth and form no part of the adult. Similar accessory structures are formed in the development of birds and reptiles.

The early stages of mouse development are summarized in **Figure 22–88**. The fertilized egg divides to generate 16 cells by 3 days after fertilization. At first, the cells stick together only loosely, but beginning at the 8-cell stage they become more cohesive and undergo *compaction* to form a solid ball of cells called a *morula* (Latin for "little mulberry") (**Figure 22–89**). Apical tight junctions form between the cells, sealing off the interior of the morula from the external medium. Soon after this, an internal cavity develops, converting the morula into a *blastocyst*—a hollow sphere. The outer layer of cells, forming the

Figure 22–89 Scanning electron micrographs of the early mouse embryo. The zona pellucida has been removed. (A) Two-cell stage. (B) Four-cell stage (a polar body is visible in addition to the four blastomeres—see Figure 21–23). (C) Eight-to-sixteen-cell morula-compaction occurring. (D) Blastocyst. (A–C, courtesy of Patricia Calarco; D, from P. Calarco and C.J. Epstein, *Dev. Biol.* 32:208–213, 1973. With permission from Academic Press.)



wall of the sphere, is called the *trophectoderm*. It will give rise to extraembryonic tissues. An inner clump of cells, called the *inner cell mass*, is located to one side of the cavity. It will give rise to the whole of the embryo proper.

After the embryo has escaped from its jelly capsule (at about four days), the cells of the trophectoderm make close contact with the wall of the uterus, initiating the process of implantation that will lead on to formation of the placenta. Meanwhile the inner cell mass grows and begins to differentiate. Part of it gives rise to some further extraembryonic structures, such as the yolk sac, while the rest of it goes on to form the embryo proper by processes of gastrulation, neurulation, and so on, that are fundamentally similar to those seen in other vertebrates, although distortions of the geometry make some of the homologies hard to discern at first sight.

The Early Mammalian Embryo Is Highly Regulative

Localized intracellular determinants play only a small part in early mammalian development, and the blastomeres produced by the first few cell divisions are remarkably adaptable. If the early embryo is split in two, a pair of identical twins can be produced—two complete normal individuals from a single cell. Similarly, if one of the cells in a 2-cell mouse embryo is destroyed by pricking it with a needle and the resulting "half-embryo" is placed in the uterus of a foster mother to develop, in many cases a perfectly normal mouse will emerge.

Conversely, two 8-cell mouse embryos can be combined to form a single giant morula, which then develops into a mouse of normal size and structure (**Figure 22–90**). Such creatures, formed from aggregates of genetically different groups of cells, are called *chimeras*. Chimeras can also be made by injecting cells from an early embryo of one genotype into a blastocyst of another genotype. The injected cells become incorporated into the inner cell mass of the host blastocyst, and a chimeric animal develops. A single cell taken from an 8-cell embryo or from the inner cell mass of another early blastocyst can give rise in these ways to any combination of cell types in the chimera. Wherever the added cell may happen to find itself, it responds correctly to cues from its neighbors and follows the appropriate developmental pathway.

These findings have two implications. First, during the early stages, the developmental system is self-adjusting, so that a normal structure emerges even if the starting conditions are perturbed. Embryos or parts of embryos that have this property are said to be **regulative**. Second, the individual cells of the inner cell mass are initially *totipotent*, or very nearly so: though they cannot form trophoblast, they can give rise to any part of the adult body, including germ cells.

Totipotent Embryonic Stem Cells Can Be Obtained From a Mammalian Embryo

If a normal early mouse embryo is grafted into the kidney or testis of an adult, its development is disturbed beyond any possibility of proper regulation, but not halted. The result is a bizarre tumorous growth known as a *teratoma*, consisting of a disorganized mass of cells containing many varieties of differentiated tissue—skin, bone, glandular epithelium, and so on—mixed with undifferentiated stem cells that continue to divide and generate yet more of these differentiated tissues.

Investigation of the stem cells in teratomas and related types of tumors led to the discovery that their behavior reflects a remarkable property of the cells of the normal inner cell mass: given a suitable environment, they can be induced to proliferate indefinitely while retaining their totipotent character. Cultured cells with this property are called **embryonic stem cells**, or **ES cells**. They can be derived by placing a normal inner cell mass in culture and dispersing the cells as soon as they proliferate. Separating the cells from their normal neighbors and putting them in the appropriate culture medium evidently arrests the normal program of change of cell character with time and so enables the cells to carry

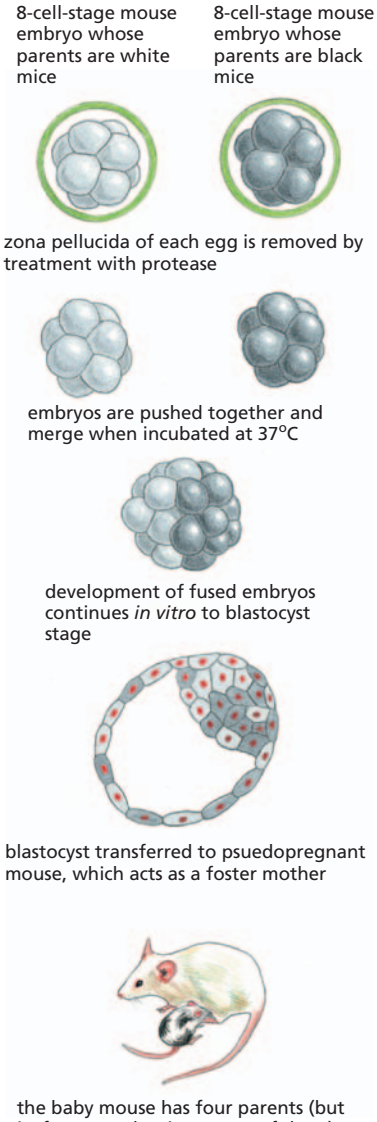


Figure 22–90 A procedure for creating a chimeric mouse. Two morulae of different genotypes are combined.

on dividing indefinitely without differentiating. Many tissues of the adult body also contain stem cells that can divide indefinitely without terminally differentiating, as we shall see in the next chapter; but these *adult stem cells,* when allowed to differentiate, normally give rise only to a narrowly restricted range of differentiated cell types.

The state in which the ES cells are arrested seems to be equivalent to that of normal inner-cell-mass cells. This can be shown by taking ES cells from the culture dish and injecting them into a normal blastocyst (**Figure 22–91**). The injected cells become incorporated in the inner cell mass of the blastocyst and can contribute to the formation of an apparently normal chimeric mouse. Descendants of the injected stem cells can be found in practically any of the tissues of this mouse, where they differentiate in a well-behaved manner appropriate to their location and can even form viable germ cells. The extraordinarily adaptable behavior of ES cells shows that cues from a cell's neighbors not only guide choices between different pathways of differentiation, but can also stop or start the developmental clock—the processes that drive a cell to progress from an embryonic to an adult state.

On a practical level, ES cells have a twofold importance. First, from a medical point of view, they offer the prospect of a versatile source of cells for repair of damaged and defective tissues in the adult body, as we shall discuss at the end of the next chapter. Second, ES cells make possible the most precisely controlled forms of genetic modification, allowing animals to be created with virtually any desired alteration introduced into their genome. As discussed in Chapter 8, the technique uses genetic recombination to substitute an artificially constructed DNA segment for the normal DNA sequence at a chosen site in the genome of an ES cell. Although only a rare cell incorporates the DNA construct correctly, selection procedures have been devised to find this cell among the thousands of cells into which the DNA construct has been transfected. Once selected, the genetically modified ES cells can be injected into a blastocyst to make a chimeric mouse. This mouse will, with luck, have some ES-derived germ cells, capable of acting as founders of a new generation of mice that consist entirely of cells carrying the carefully designed mutation. In this way, an entire mutant mouse can be resurrected from the culture dish (see Figure 8–65).

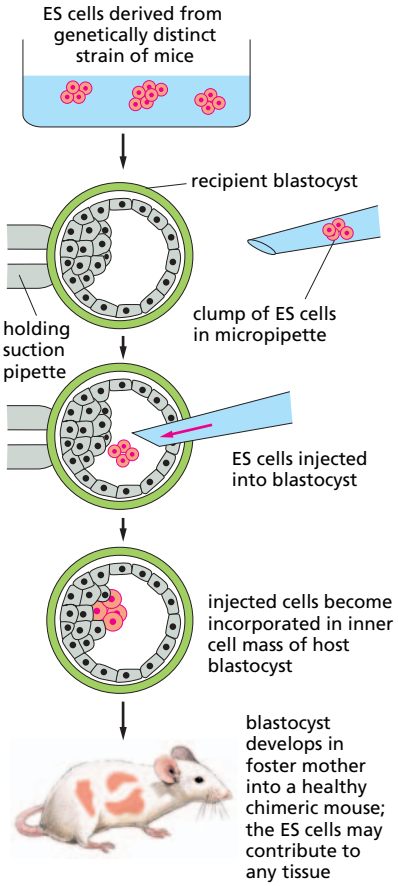


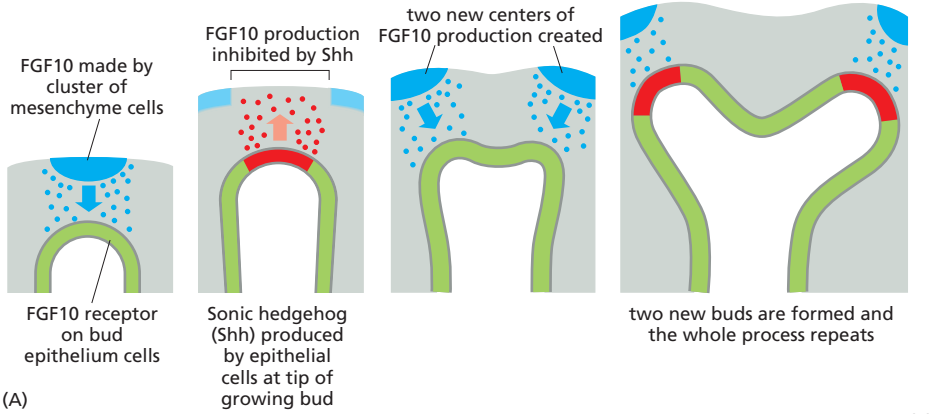
Figure 22–91 Making a chimeric mouse with ES cells. The cultured ES cells can combine with the cells of a normal blastocyst to form a healthy chimeric mouse, and can contribute to any of its tissues, including the germ line. Thus the ES cells are totipotent.

Interactions Between Epithelium and Mesenchyme Generate Branching Tubular Structures

Vertebrates are comparatively big animals, and they owe much of their bulk to connective tissues. For excretion, absorption of nutrients, and gas exchange, however, they also require large quantities of various specialized types of epithelial surfaces. Many of these take the form of tubular structures created by *branching morphogenesis,* in which an epithelium invades embryonic connective tissue (mesenchyme) to form a composite organ. The lung is a typical example. It originates from the endoderm lining the floor of the foregut. This epithelium buds and grows out into the neighboring mesenchyme to form the *bronchial tree,* a system of tubes that branch repeatedly as they extend (**Figure 22–92**). The same mesenchyme is also invaded by endothelial cells—the lining cells of blood vessels—to create the system of closely apposed airways and blood vessels required for gas exchange in the lung (discussed in Chapter 23).

The whole process depends on exchanges of signals in both directions between the growing buds of epithelium and the mesenchyme that they are invading. These signals can be analyzed by genetic manipulation in the mouse. A central part is played by signal proteins of the fibroblast growth factor (FGF) family and the receptor tyrosine kinases on which they act. This signaling pathway has various roles in development, but it seems to be especially important in the many interactions that occur between epithelium and mesenchyme.

Mammals have about 20 different *Fgf* genes, as compared with three in *Drosophila* and two in *C. elegans*. The *Fgf* that is most important in the lung is *Fgf10*. This is expressed in clusters of mesenchyme cells near the tips of the growing epithelial tubes, while its receptor is expressed in the epithelial cells



themselves. FGF10 or its receptor can be knocked out (by the standard techniques based on recombination in ES cells). In the resulting knock-out mutant mouse, the whole process of branching morphogenesis then fails—a primary bud of lung epithelium is formed but fails to grow out into the mesenchyme to create a bronchial tree. Conversely, a microscopic bead soaked in FGF10 and placed near embryonic lung epithelium in culture will induce a bud to form and grow out toward it. Evidently, the epithelium invades the mesenchyme only by invitation, in response to FGF10.

But what makes the growing epithelial tubes branch repeatedly as they invade? This seems to depend on a Sonic hedgehog signal that is sent in the opposite direction, from the epithelial cells at the tips of the buds back to the mesenchyme. In mice lacking Sonic hedgehog, the lung epithelium grows and differentiates, but forms a sac instead of a branching tree of tubules. Meanwhile, FGF10, instead of being restricted to small clusters of mesenchyme cells, with each cluster acting as a beacon to direct the outgrowth of a separate epithelial bud, is expressed in broad bands of cells immediately adjacent to the epithelium. This finding suggests that the Sonic hedgehog signal may serve to shut off FGF10 expression in the mesenchyme cells closest to the growing tip of a bud, splitting the FGF10-secreting cluster into two separate clusters, which in turn cause the bud to branch into two (see Figure 22–92A).

The branching growth of the epithelium and mesenchyme has to be coordinated with development of the associated blood vessels, and the whole process involves a large number of additional signals. Many aspects of the system are still not understood. It is known, however, that *Drosophila* uses closely related mechanisms to govern the branching morphogenesis of its tracheal system—the tubules that form the airways of an insect. Again, the process depends on the *Drosophila* FGF protein, encoded by the *Branchless* gene, and the *Drosophila* FGF receptor, encoded by the *Breathless* gene, both operating in much the same way as in the mouse. Indeed, genetic studies of tracheal development in *Drosophila* have also identified other components of the control machinery, and the *Drosophila* genes have led us to their vertebrate homologs. Genetic manipulations in the mouse have given us the means to test whether these genes have similar functions in mammals too; and to a remarkable extent they do.

Figure 22–92 Branching morphogenesis of the lung. (A) How FGF10 and Sonic hedgehog are thought to induce the growth and branching of the buds of the bronchial tree. Many other signal molecules, such as BMP4, are also expressed in this system, and the suggested branching mechanism is only one of several possibilities. (B) A cast of the adult human bronchial tree, prepared by injecting resin into the airways; resins of different colors have been injected into different branches of the tree. (B, from R. Warwick and P.L. Williams, Gray's Anatomy, 35th ed. Edinburgh: Longman, 1973.)

Summary

The mouse has a central role as model organism for study of the molecular genetics of mammalian development. Mouse development is essentially similar to that of other vertebrates, but begins with a specialized preamble to form extraembryonic structures such as the amnion and placenta. Powerful techniques have been devised for creation of gene knockouts and other targeted genetic alterations by exploiting the highly regulative properties of the cells of the inner cell mass of the mouse embryo. These cells can be put into culture and maintained as embryonic stem cells (ES cells). Under the right culture conditions, ES cells can proliferate indefinitely without differentiating, while retaining the ability to give rise to any part of the body when injected back into an early mouse embryo.

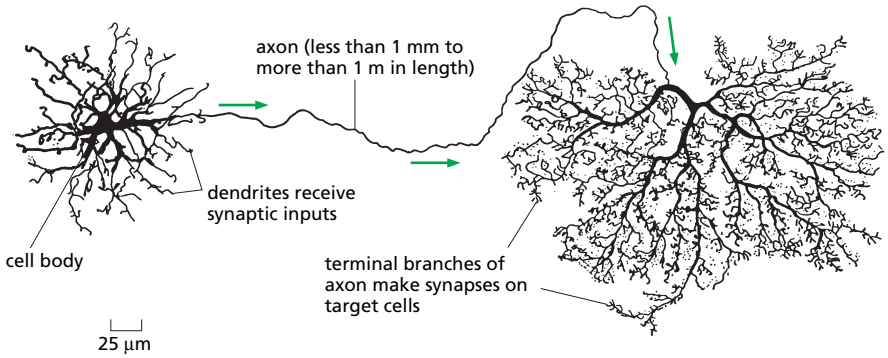


Figure 22–93 A typical neuron of a vertebrate. The arrows indicate the direction in which signals are conveyed. The neuron shown is from the retina of a monkey. The longest and largest neurons in a human extend for about 1 million μm and have an axon diameter of 15 μm. (Drawing of neuron from B.B. Boycott, in Essays on the Nervous System [R. Bellairs and E.G. Gray, eds.]. Oxford, UK: Clarendon Press, 1974.)

Many general developmental processes, including most of those discussed elsewhere in the chapter, have been illuminated by studies in the mouse. As just one example, the mouse has been used to investigate the control of branching morphogenesis. This process gives rise to structures such as lungs and glands, and is governed by exchanges of signals between mesenchyme cells and an invading epithelium. The functions of these signals can be analyzed by gene knockout experiments.

NEURAL DEVELOPMENT

Nerve cells, or **neurons**, are among the most ancient of all specialized animal cell types. Their structure is like that of no other class of cells, and the development of the nervous system poses problems that have no real parallel in other tissues. A neuron is extraordinary above all for its enormously extended shape, with a long *axon* and branching *dendrites* connecting it through synapses to other cells (**Figure 22–93**). The central challenge of neural development is to explain how the axons and dendrites grow out, find their right partners, and synapse with them selectively to create a functional network (**Figure 22–94**). The problem is formidable: the human brain contains more than 10^{11} neurons, each of which, on average, has to make connections with a thousand others, according to a regular and predictable wiring plan. The precision required is not so great as in a man-made computer, for the brain performs its computations in a different way and is more tolerant of vagaries in individual components; but the brain nevertheless outstrips all other biological structures in its organized complexity.

The components of a typical nervous system—the various classes of neurons, glial cells, sensory cells, and muscles—originate in a number of widely separate locations in the embryo and are initially unconnected. Thus, in the first phase of neural development (**Figure 22–95**), the different parts develop according to their own local programs: neurons are born and assigned specific characters according to the place and time of their birth, under the control of inductive signals and gene regulatory mechanisms similar to those we have already discussed for other tissues of the body. The next phase involves a type of morphogenesis unique to the nervous system: axons and dendrites grow out along specific routes, setting up a provisional but orderly network of connections between the separate parts of the system. In the third and final phase, which continues into adult life, the connections are adjusted and refined through interactions among the far-flung components in a way that depends on the electrical signals that pass between them.

Figure 22–94 The complex organization of nerve cell connections. This drawing depicts a section through a small part of a mammalian brain—the olfactory bulb of a dog, stained by the Golgi technique. The black objects are neurons; the thin lines are axons and dendrites, through which the various sets of neurons are interconnected according to precise rules. (From C. Golgi, *Riv. sper. freniat. Reggio-Emilia* 1:405-425, 1875; reproduced in M. Jacobson, Developmental Neurobiology, 3rd ed. New York: Plenum, 1992.)

Neurons Are Assigned Different Characters According to the Time and Place Where They Are Born

Neurons are almost always produced in association with **glial cells**, which provide a supporting framework and create an enclosed, protected environment in which the neurons can perform their functions. Both cell types, in all animals,

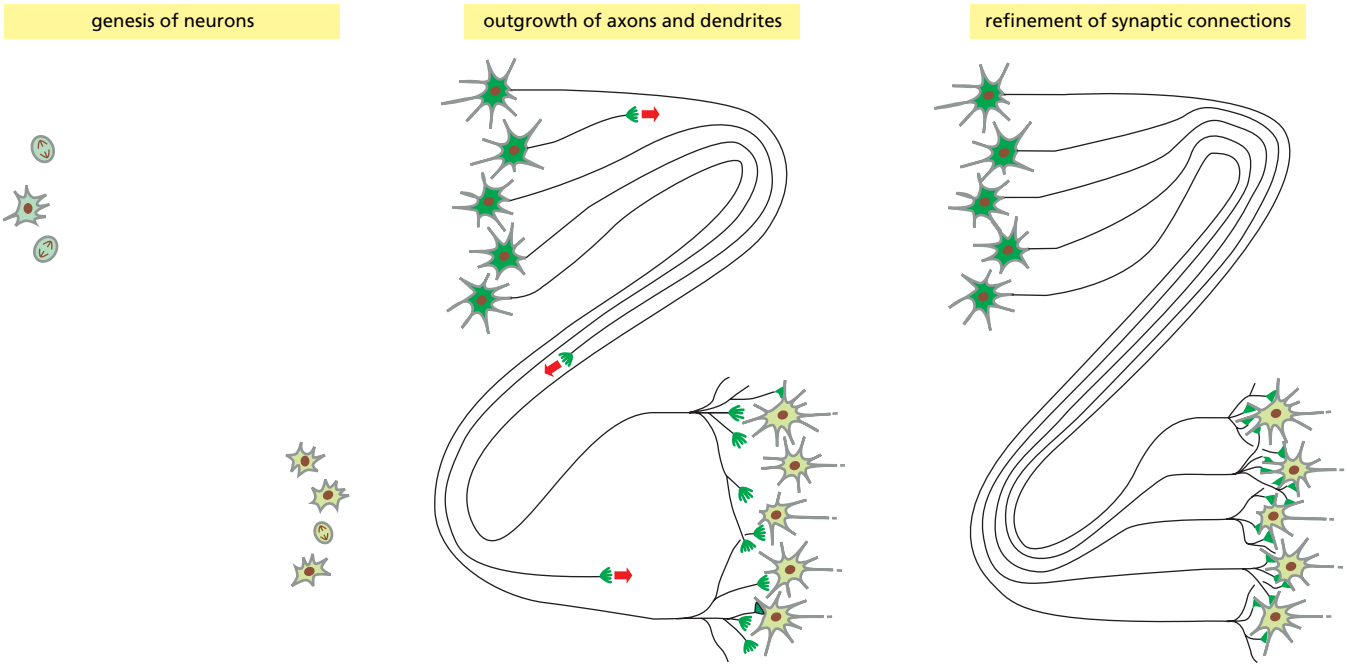


Figure 22–95 The three phases of neural development.

develop from the ectoderm, usually as sister cells or cousins derived from a common precursor. Thus, in vertebrates, the neurons and glial cells of the *central nervous system* (including the spinal cord, the brain, and the retina of the eye) derive from the part of the ectoderm that rolls up to form the neural tube, while those of the *peripheral nervous system* derive mainly from the neural crest (**Figure 22–96**).

The **neural tube**, with which we shall be mainly concerned, consists initially of a single-layered epithelium (**Figure 22–97**). The epithelial cells are the progenitors of the neurons and glia. As these cell types are generated, the epithelium becomes thickened and transformed into a more complex structure. As discussed earlier, Delta–Notch signaling controls the differentiation of the progenitor cells into neurons: the nascent neurons express Delta, and thereby inhibit their neighbors from differentiating into neurons at the same time. This ensures that the progenitors do not all differentiate simultaneously but remain as a dividing cell population from which further neurons can be generated. The progenitor and, later, glial cells also maintain the cohesiveness of the epithelium and form a scaffolding that spans its thickness. Along and between these tall cells, like animals amid the trees of the forest, the new-born neurons migrate, find their resting places, mature, and send out their axons and dendrites (**Figure 22–98**).

Signal proteins secreted from the ventral and dorsal sides of the neural tube act as opposing morphogens, causing neurons born at different dorsoventral levels to express different gene regulatory proteins (see Figure 22–80). There are differences along the head-to-tail axis as well, reflecting the anteroposterior pattern of expression of *Hox* genes and the actions of yet other morphogens. Moreover, just as in *Drosophila*, neurons continue to be generated in each region of the central nervous system over many days, weeks, or even months, and this gives rise to still greater diversity, because the cells adopt different characters

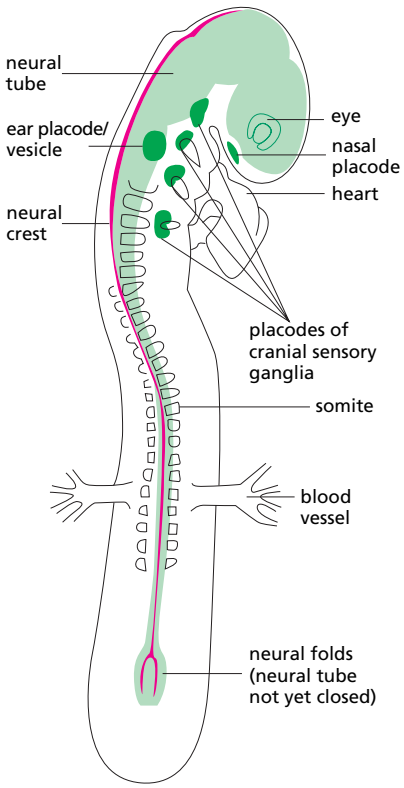


Figure 22–96 Diagram of a 2-day chick embryo, showing the origins of the nervous system. The neural tube *(light green)* has already closed, except at the tail end, and lies internally, beneath the ectoderm, of which it was originally a part (see Figure 22–78). The neural crest *(red)* lies dorsally just beneath the ectoderm, in or above the roof of the neural tube. In addition, thickenings, or placodes *(dark green),* in the ectoderm of the head give rise to some of the sensory transducer cells and neurons of that region, including those of the ear and the nose. The cells of the retina of the eye, by contrast, originate as part of the neural tube.

according to their "birthday"—the time of the terminal mitosis that marks the beginning of neuronal differentiation (**Figure 22–99**). When progenitor cells are taken from an embryonic mouse brain and maintained in culture for several days, individually isolated from their normal surroundings, they go through much the same program as in the intact tissue. That is, they divide repeatedly, producing pairs of daughters that frequently adopt different fates, such that one remains as a dividing progenitor while the other becomes committed to differentiate.

The successive divisions throw off a sequence of different neuronal and glial cell types, according to a more-or-less regular timetable. This implies that the progenitors themselves must autonomously change their intrinsic character from one cell generation to the next. The molecular mechanism of this progressive change is unknown, just as it is in other cell types where similar slow changes occur.

The Character Assigned to a Neuron at Its Birth Governs the Connections It Will Form

The differences of gene expression modulate the characters of the neurons and help to cause them to make connections with different partners. In the spinal cord, for example, ventrally located clusters of cells express genes of the *Islet/Lim* homeobox family (coding for gene regulatory proteins) and develop as motor neurons, sending out axons to connect with specific subsets of muscles—different muscles according to the particular *Islet/Lim* family members expressed. If the pattern of gene expression is artificially altered, the neurons project to different target muscles.

The different destinations reflect different pathway choices that the axons make as they grow out from the nerve cell body, as well as their selective

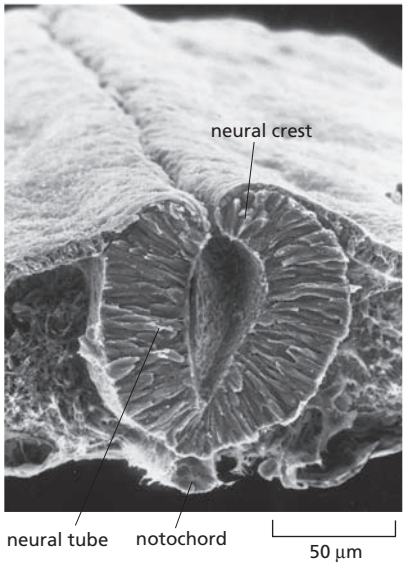


Figure 22–97 Formation of the neural tube. The scanning electron micrograph shows a cross section through the trunk of a 2-day chick embryo. The neural tube is about to close and pinch off from the ectoderm; at this stage it consists (in the chick) of an epithelium that is only one cell thick. (Courtesy of J.P. Revel and S. Brown.)

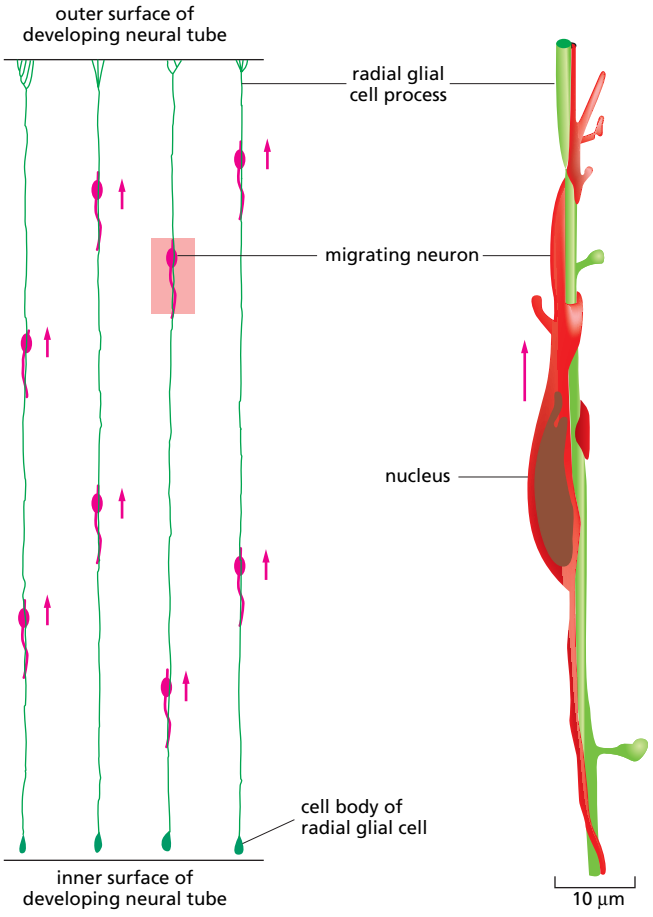


Figure 22–98 Migration of immature neurons. Before sending out axons and dendrites, newborn neurons often migrate from their birthplace and settle in some other location. The diagrams are based on reconstructions from sections of the cerebral cortex of a monkey (part of the neural tube). The neurons go through their final cell division close to the inner, luminal face of the neural tube and then migrate outward by crawling along radial glial cells. Each of these cells extends from the inner to the outer surface of the tube, a distance that may be as much as 2 cm in the cerebral cortex of the developing brain of a primate. The radial glial cells can be considered as persisting cells of the original columnar epithelium of the neural tube that become extraordinarily stretched as the wall of the tube thickens. (After P. Rakic, *J. Comp. Neurol.* 145:61–84, 1972. With permission from John Wiley & Sons, Inc.)

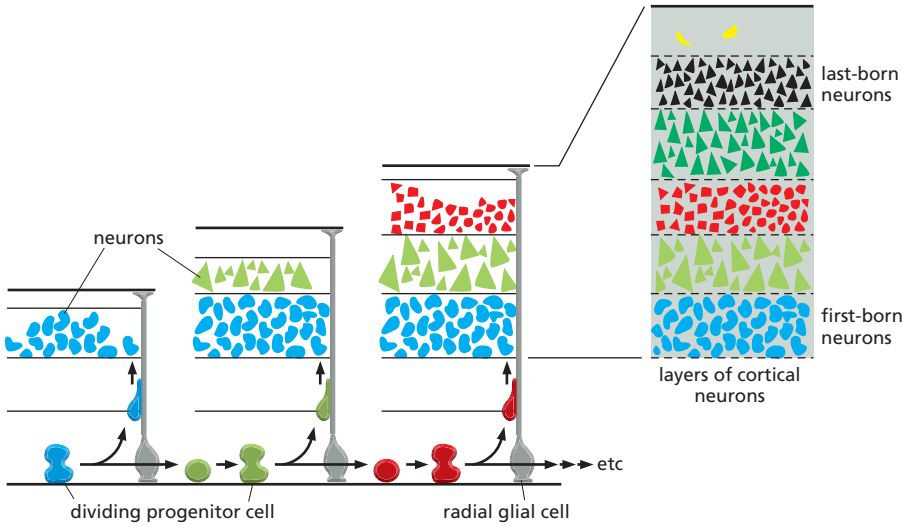


Figure 22–99 Programmed production of different types of neurons at different times from dividing progenitors in the cerebral cortex of the brain of a mammal. Close to one face of the cortical neuroepithelium, progenitor cells divide repeatedly, in stem-cell fashion, to produce neurons. The neurons migrate out toward the opposite face of the epithelium by crawling along the surfaces of radial glial cells, as shown in Figure 22–98. The first-born neurons settle closest to their birthplace, while neurons born later crawl past them to settle farther out. Successive generations of neurons thus occupy different layers in the cortex and have different intrinsic characters according to their birth dates.

recognition of different target cells at the end of the journey. In the dorsal part of the spinal cord lie neurons that receive and relay sensory information from sensory neurons in the periphery of the body. In intermediate positions, there are various other classes of interneurons, connecting specific sets of nerve cells to one another. Some send their axons dorsally, others ventrally; some up toward the head, others down toward the tail, still others across the floor of the neural tube to the other side of the body (**Figure 22–100**). In a timelapse film where the developing neurons are stained with a fluorescent dye, one can watch the movements of the growing tips of the axons as they extend: one is reminded of the lights of rush-hour traffic at night, as the cars streak along a network of highways, turning this way or that at busy junctions, each one making its own choice of route.

How are these complex movements guided? Before attempting an answer, we must examine more closely the structure of the growing neuron.

Each Axon or Dendrite Extends by Means of a Growth Cone at Its Tip

A typical neuron sends out one long axon, projecting toward a distant target to which signals are to be delivered, and several shorter dendrites, on which it mainly receives incoming signals from axon terminals of other neurons. Each

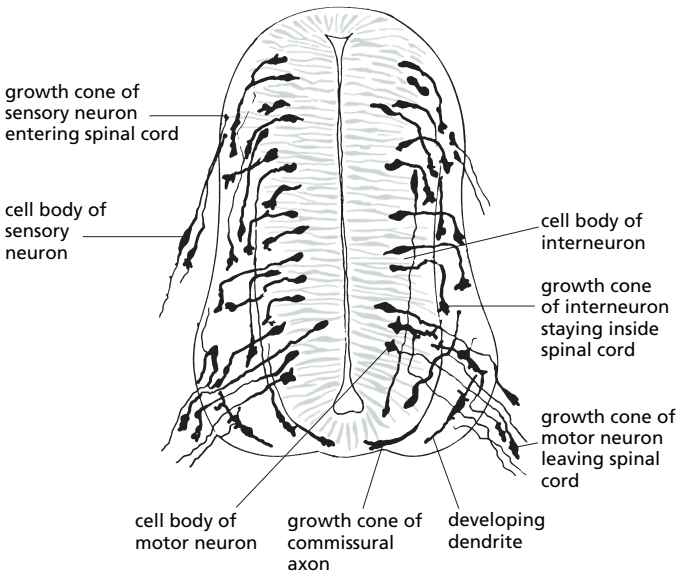


Figure 22–100 Growing axons in the developing spinal cord of a 3-day chick embryo. The drawing shows a cross section stained by the Golgi technique. Most of the neurons, apparently, have as yet only one elongated process—the future axon. An irregularly shaped expansion—a growth cone—is seen at the growing tip of each axon. The growth cones of the motor neurons emerge from the spinal cord (to make their way toward muscles), those of the sensory neurons grow into it from outside (where their cell bodies lie), and those of the interneurons remain inside the spinal cord. Many of the interneurons send their axons down toward the floor plate to cross to the other side of the spinal cord; these axons are called commissural. At this early stage, many of the embryonic spinal-cord cells (in the regions shaded *gray)* are still proliferating and have not yet begun to differentiate as neurons or glial cells. (From S. Ramón y Cajal, Histologie du Système Nerveux de l'Homme et des Vertébrés, 1909–1911. Paris: Maloine; reprinted, Madrid: C.S.I.C., 1972.)

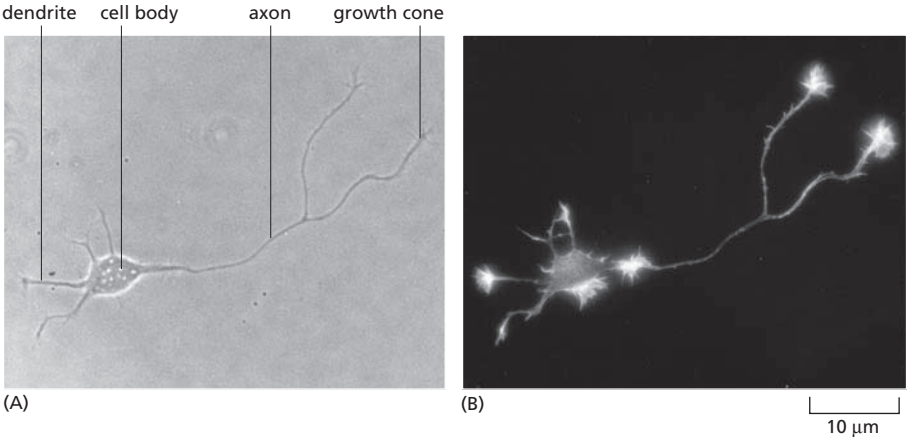


Figure 22–101 Formation of axon and dendrites in culture. A young neuron has been isolated from the brain of a mammal and put to develop in culture, where it sends out processes. One of these processes, the future axon, has begun to grow out faster than the rest (the future dendrites) and has bifurcated. (A) A phase-contrast picture; (B) the pattern of staining with fluorescent phalloidin, which binds to filamentous actin. Actin is concentrated in the growth cones at the tips of the processes that are actively extending and at some other sites of lamellipodial activity. (Courtesy of Kimberly Goslin.)

process extends by growth at its tip, where an irregular, spiky enlargement is seen. This structure, called the **growth cone**, crawls through the surrounding tissue, trailing a slender axon or dendrite behind it (see Figure 22–100). <AAGA> The growth cone comprises both the engine that produces the movement and the steering apparatus that directs the tip of each process along the proper path (see Figure 16–105).

Much of what we know about the properties of growth cones has come from studies in tissue or cell culture. One can watch as a neuron begins to put out its processes, all at first alike, until one of the growth cones puts on a sudden turn of speed, identifying its process as the axon, with its own axon-specific set of proteins (**Figure 22–101**). The contrast between axon and dendrite established at this stage involves polarized intracellular transport of different materials into the two types of process. As a result, they will grow out for different distances, follow different paths, and play different parts in synapse formation.

The growth cone at the end of a typical growing nerve cell process—either axon or dendrite—moves forward at a speed of about 1 mm per day, continually probing the regions that lie ahead and on either side by putting out filopodia and lamellipodia. When such a protrusion contacts an unfavorable surface, it withdraws; when it contacts a more favorable surface, it persists longer, steering the growth cone as a whole to move in that direction. In this way the growth cone can be guided by subtle variations in the surface properties of the substrata over which it moves. At the same time, it is sensitive to diffusible chemotactic factors in the surrounding medium, which can also encourage or hinder its advance. These behaviors depend on the cytoskeletal machinery inside the growth cone, as discussed in Chapter 16. A multitude of receptors in the growth cone membrane detect the external signals and, through the agency of intracellular regulators such as the monomeric GTPases Rho and Rac, control the assembly and disassembly of actin filaments and other components of the machinery of cell movement.

The Growth Cone Pilots the Developing Neurite Along a Precisely Defined Path *In Vivo*

In living animals, growth cones generally travel toward their targets along predictable, stereotyped routes, exploiting a multitude of different cues to find their way, but always requiring a substratum of extracellular matrix or cell surface to crawl over. Often, growth cones take routes that have been pioneered by other neurites, which they follow by contact guidance. As a result, nerve fibers in a mature animal are usually found grouped together in tight parallel bundles (called fascicles or fiber tracts). Such crawling of growth cones along axons is thought to be mediated by homophilic cell–cell adhesion molecules—membrane glycoproteins that help a cell displaying them to stick to any other cell that also displays them. As discussed in Chapter 19, two of the most important

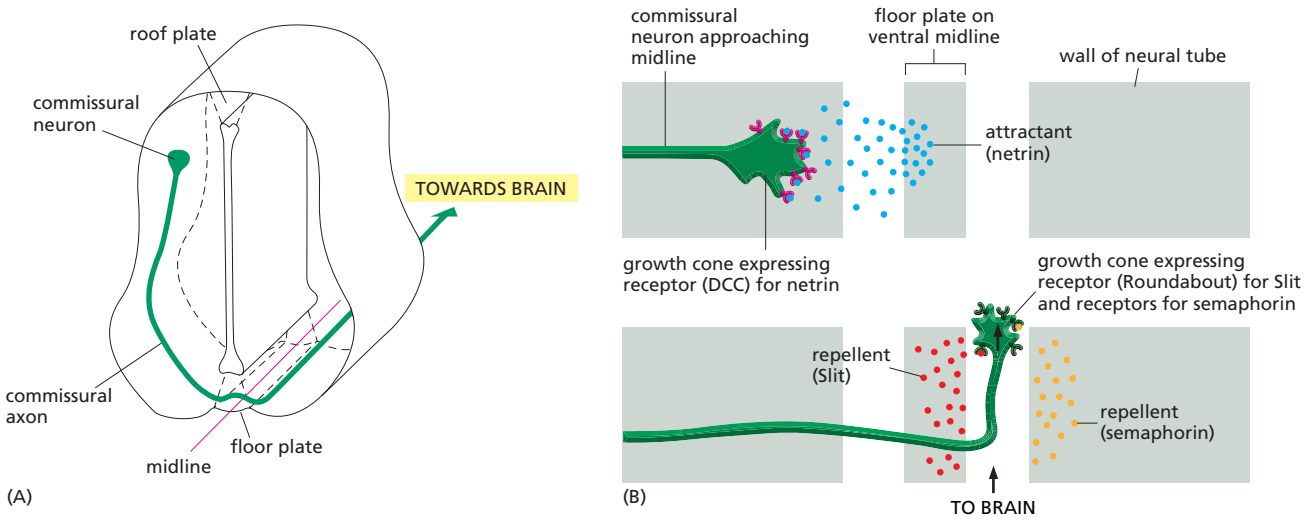


Figure 22–102 The guidance of commissural axons. (A) The pathway taken by commissural axons in the embryonic spinal cord of a vertebrate. (B) The signals that guide them. The growth cones are first attracted to the floor plate by netrin, which is secreted by the floor-plate cells and acts on the receptor DCC in the axonal membrane. As they cross the floor plate, the growth cones upregulate their expression of Roundabout, the receptor for a repellent protein, Slit, that is also secreted by the floor plate. Slit, binding to Roundabout, not only acts as a repellent to keep the cells from re-entering the floor plate, but also blocks responsiveness to the attractant netrin. At the same time, the growth cones switch on expression of receptors for another repellent protein, semaphorin, that is secreted by the cells in the side walls of the neural tube. Trapped between two repellent territories, the growth cones, having crossed the midline, travel in a tight fascicle up toward the brain.

classes of such molecules are those that belong to the immunoglobulin superfamily, such as N-CAM, and those of the Ca^{2+}-dependent cadherin family, such as N-cadherin. Members of both families are generally present on the surfaces of growth cones, of axons, and of various other cell types that growth cones crawl over, including glial cells in the central nervous system and muscle cells in the periphery of the body. The human genome contains more than 100 cadherin genes, for example, and most of them are expressed in the brain (see Figure 19–6). Different sets of cell–cell adhesion molecules, acting in varied combinations, provide a mechanism for selective neuronal guidance and recognition. Growth cones also migrate over components of the extracellular matrix. Some of the matrix molecules, such as *laminin*, favor axon outgrowth, while others, such as chondroitin sulfate proteoglycans, discourage it.

Growth cones are guided by a succession of different cues at different stages of their journey, and the stickiness of the substratum is not the only thing that matters. Another important part is played by chemotactic factors, secreted from cells that act as beacons at strategic points along the path—some attracting, others repelling. The trajectory of *commissural* axons—those that cross from one side of the body to the other—provides a beautiful example of how a combination of guidance signals can specify a complex path. Commissural axons are a general feature of bilaterally symmetrical animals, because the two sides of the body have to be neurally coordinated. Worms, flies and vertebrates use closely related mechanisms to guide their outgrowth.

In the developing spinal cord of a vertebrate, for example, a large number of neurons send their axonal growth cones ventrally toward the floor plate—a specialized band of cells forming the ventral midline of the neural tube (see Figure 22–100). The growth cones cross the floor plate and then turn abruptly through a right angle to follow a longitudinal path up toward the brain, parallel to the floor plate but never again crossing it (**Figure 22–102**A). The first stage of the journey depends on a concentration gradient of the protein *netrin*, secreted by the cells of the floor plate: the commissural growth cones sniff their way toward its source. Netrin was purified from chick embryos, by assaying extracts of neural tissue for an activity that would attract commissural growth cones in a culture dish. Its sequence revealed that it was the vertebrate homolog of a protein already known from *C. elegans*, through genetic screens for mutant worms with misguided axons—called *Unc* mutants because they move in an *unc*oordinated fashion. One of the *Unc* genes, *Unc6*, codes for the homolog of netrin. Another, *Unc40*, codes for its transmembrane receptor; and this too has a vertebrate homolog called DCC that is expressed in the commissural neurons and mediates their response to the netrin gradient.

Localized activation of DCC by netrin leads to opening of a specialized class of ion channels in the plasma membrane. These channels, called TRPC (Transient Receptor Potential C) channels, belong to a large family (the TRP family)

that is responsible for many other sensory transduction processes, from mechanosensation to the perception of heat and cold. When open, the TRPC channels allow Ca^{2+} (and other cations) to enter the cell. The localized rise in Ca^{2+} then activates the machinery for extension of filopodia and movement of the growth cone toward the netrin source.

The receptors on each growth cone determine the route it will take: non-commissural neurons in the neural tube, lacking DCC, are not attracted to the floor plate; and neurons expressing a different netrin receptor—called Unc5H in vertebrates (with a counterpart Unc5 in the worm)—are actively repelled by the floor plate and send their axons instead toward the roof plate.

Growth Cones Can Change Their Sensibilities as They Travel

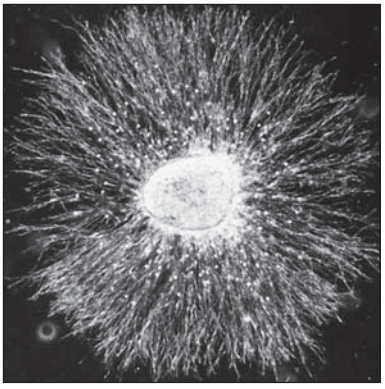
If commissural growth cones are attracted to the floor plate, why do they cross it and emerge on the other side, instead of staying in the attractive territory? And having crossed it, why do they never veer back onto it again? The likely answer lies in another set of molecules, several of which are also conserved between vertebrates and invertebrates. Studies of *Drosophila* mutants with misguided commissural axons first identified three of the key proteins: Slit, Roundabout, and Commissureless.

Slit, like netrin, is produced by midline cells of the developing fly, while its receptor, Roundabout, is expressed in the commissural neurons. Slit, acting on Roundabout, has an effect exactly opposite to that of netrin: it repels the growth cones, blocking entry to the midline territory. Commissureless, however, interferes with the delivery of Roundabout to the cell surface and thereby makes the growth cones initially blind to this "keep-out" signal. Commissural growth cones in this state advance to the midline; as they cross it, they seem, by some mechanism that we do not yet understand, to lose their blindfold of Commissureless protein and begin to be repelled. Emerging on the far side, they now have functional Roundabout on their surfaces and are thereby prohibited from re-entry.

In vertebrates, a similar mechanism operates, involving homologs of Slit and Roundabout. Commissural growth cones are at first attracted to the midline, and then somehow change their surface receptor proteins as they cross; in this way they switch their sensibilities, gaining sensitivity to repulsion by Slit—which is expressed in the floor plate—and losing sensitivity to attraction by netrin. Sensitivity to Slit in the initial approach to the midline is blocked not by any homolog of Commissureless but by a divergent member of the Roundabout receptor family called Rig1, which sits in the plasma membrane and interferes with signal reception by its cousins. The Rig1 block is switched off by some unknown mechanism once the growth cones have crossed the midline. Repulsion from the midline now prevents them from straying back across it. At the same time, the growth cones apparently become sensitive to another set of repulsive signals, in the form of proteins called semaphorins, which prevent them from traveling back up into the dorsal regions of the spinal cord. Trapped between the two sets of repulsive signals, the growth cones have no choice but to travel in a narrow track, running parallel to the floor plate but never re-entering it (Figure 22–102B).

Target Tissues Release Neurotrophic Factors That Control Nerve Cell Growth and Survival

Eventually, axonal growth cones reach the target region where they must halt and make synapses. The neurons that sent out the axons can now begin to communicate with their target cells. Although synapses generally transmit signals in one direction, from axon to either dendrite or muscle, the developmental communications are a two-way affair. Signals from the target tissue not only regulate which growth cones are to synapse where (as we discuss below), but also how many of the innervating neurons are to survive.



NGF

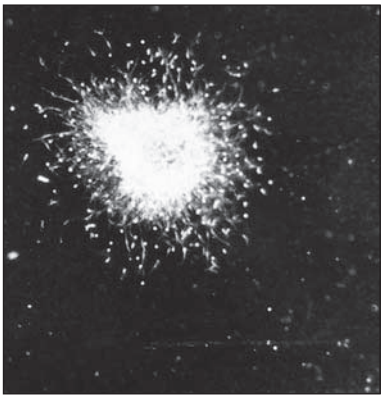


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Figure 22–103 NGF effects on neurite outgrowth. Dark-field photomicrographs of a sympathetic ganglion cultured for 48 hours with *(above)* or without *(below)* NGF. Neurites grow out from the sympathetic neurons only if NGF is present in the medium. Each culture also contains Schwann (glial) cells that have migrated out of the ganglion; these are not affected by NGF. Neuronal survival and maintenance of growth cones for neurite extension represent two distinct effects of NGF. The effect on growth cones is local, direct, rapid, and independent of communication with the cell body; when NGF is removed, the deprived growth cones halt their movements within a minute or two. The effect of NGF on cell survival is less immediate and is associated with uptake of NGF by endocytosis and its intracellular transport back to the cell body. (Courtesy of Naomi Kleitman.)

Most types of neurons in the vertebrate central and peripheral nervous system are produced in excess; up to 50% or more of them then die soon after they reach their target, even though they appear perfectly normal and healthy up to the time of their death. About half of all the motor neurons that send axons to skeletal muscle, for example, die within a few days after making contact with their target muscle cells. A similar proportion of the sensory neurons that innervate the skin die after their growth cones have arrived there.

This large-scale death of neurons is thought to reflect the outcome of a competition. Each type of target cell releases a limited amount of a specific neurotrophic factor that the neurons innervating that target require to survive. The neurons apparently compete for the factor, and those that do not get enough die by programmed cell death. If the amount of target tissue is increased—for example, by grafting an extra limb bud onto the side of the embryo—more limb-innervating neurons survive; conversely, if the limb bud is cut off, the limb-innervating neurons all die. In this way, although individuals may vary in their bodily proportions, they always retain the right number of motor neurons to innervate all their muscles and the right number of sensory neurons to innervate their whole body surface. The seemingly wasteful strategy of overproduction followed by death of surplus cells operates in almost every region of the nervous system. It provides a simple and effective means to adjust each population of innervating neurons according to the amount of tissue requiring innervation.

The first neurotrophic factor to be identified, and still the best characterized, is known simply as nerve growth factor, or NGF—the founding member of the *neurotrophin* family of signal proteins. It promotes the survival of specific classes of sensory neurons derived from the neural crest and of sympathetic neurons (a subclass of peripheral neurons that control contractions of smooth muscle and secretion from exocrine glands). NGF is produced by the tissues that these neurons innervate. When extra NGF is provided, extra sensory and sympathetic neurons survive, just as if extra target tissue were present. Conversely, in a mouse with a mutation that knocks out the gene for NGF or for its receptor (a transmembrane tyrosine kinase called TrkA), almost all sympathetic neurons and the NGF-dependent sensory neurons are lost. There are many neurotrophic factors, only a few of which belong to the neurotrophin family, and they act in different combinations to promote survival of different classes of neurons.

NGF and its relatives have an additional role: besides acting on the nerve cell as a whole to control its survival, they regulate the outgrowth of axons and dendrites (**Figure 22–103**). These can even act locally on just one part of the tree of nerve cell processes, promoting or pruning the growth of individual branches: a growth cone exposed to NGF shows an immediate increase of motility. Conversely, an axon branch that is deprived of NGF, while the rest of the neuron continues to be bathed in the factor, dies back.

The peripheral action of NGF continues to be important after the phase of neuronal death. In the skin, for example, it controls the branching of sensory nerve fibers, ensuring not only that the whole body surface becomes innervated during development but also that it recovers its innervation after damage.

Neuronal Specificity Guides the Formation of Orderly Neural Maps

In many cases, axons originating from neurons of a similar type but located in different positions come together for the journey and arrive at the target in a tight bundle. There they disperse again, to terminate at different sites in the target territory.

The projection from the eye to the brain provides an important example. <TACC> The neurons in the retina that convey visual information back to the brain are called *retinal ganglion cells*. There are more than a million of them, each one reporting on a different part of the visual field. Their axons converge on the optic nerve head at the back of the eye and travel together along the optic stalk into the brain. Their main site of termination, in most vertebrates other than mammals, is the *optic tectum*—a broad expanse of cells in the midbrain. In connecting with the tectal neurons, the retinal axons distribute themselves in a predictable pattern according to the arrangement of their cell bodies in the retina: ganglion cells that are neighbors in the retina connect with target cells that are neighbors in the tectum. The orderly projection creates a **map** of visual space on the tectum (**Figure 22–104**).

Orderly maps of this sort are found in many brain regions. In the auditory system, for example, neurons project from the ear to the brain in a tonotopic order, creating a map in which brain cells receiving information about sounds of different pitch are ordered along a line, like the keys of a piano. And in the somatosensory system, neurons conveying information about touch map onto the cerebral cortex so as to mark out a "homunculus"—a small, distorted, two-dimensional image of the body surface (**Figure 22–105**).

The retinotopic map of visual space in the optic tectum is the best characterized of all these maps. How does it arise? In principle, the growth cones could be physically channeled to different destinations as a consequence of their different starting positions, like drivers on a multilane highway where it is forbidden to change lanes. This possibility was tested in the visual system by a famous experiment in the 1940s. If the optic nerve of a frog is cut, it will regenerate. The retinal axons grow back to the optic tectum, restoring normal vision. If, in addition, the eye is rotated in its socket at the time of cutting of the nerve, so as to put originally ventral retinal cells in the position of dorsal retinal cells, vision is still restored, but with an awkward flaw: the animal behaves as though it sees the world upside down and left–right inverted. This is because the misplaced retinal

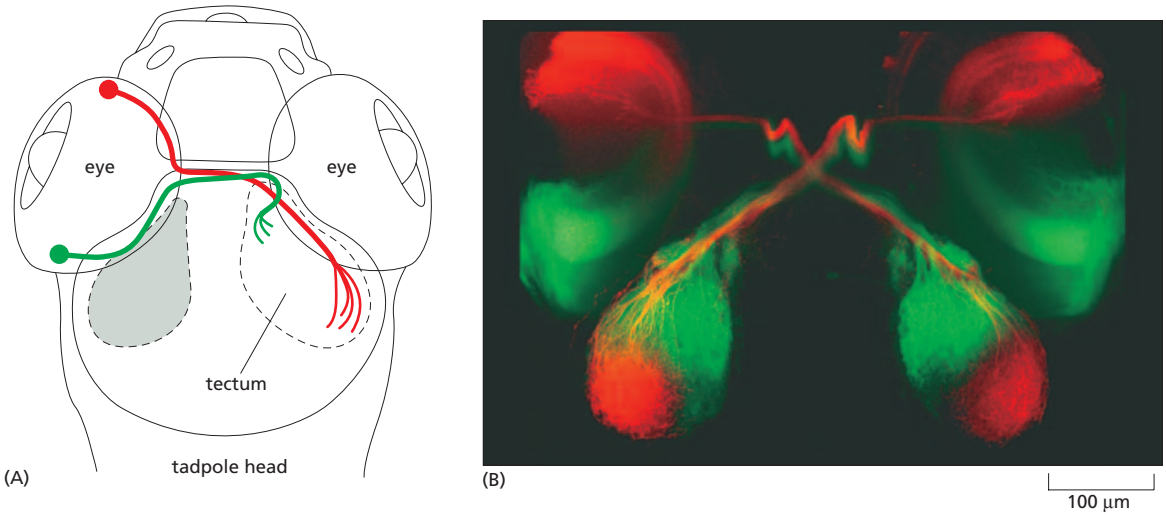


Figure 22–104 The neural map from eye to brain in a young zebrafish. (A) Diagrammatic view, looking down on the top of the head. (B) Fluorescence micrograph. Fluorescent tracer dyes have been injected into each eye—*red* into the anterior part, *green* into the posterior part. The tracer molecules have been taken up by the neurons in the retina and carried along their axons, revealing the paths they take to the optic tectum in the brain and the map that they form there. (Courtesy of Chi-Bin Chien, from D.H. Sanes, T.A. Reh and W.A. Harris, Development of the Nervous System. San Diego, CA: Academic Press, 2000.)

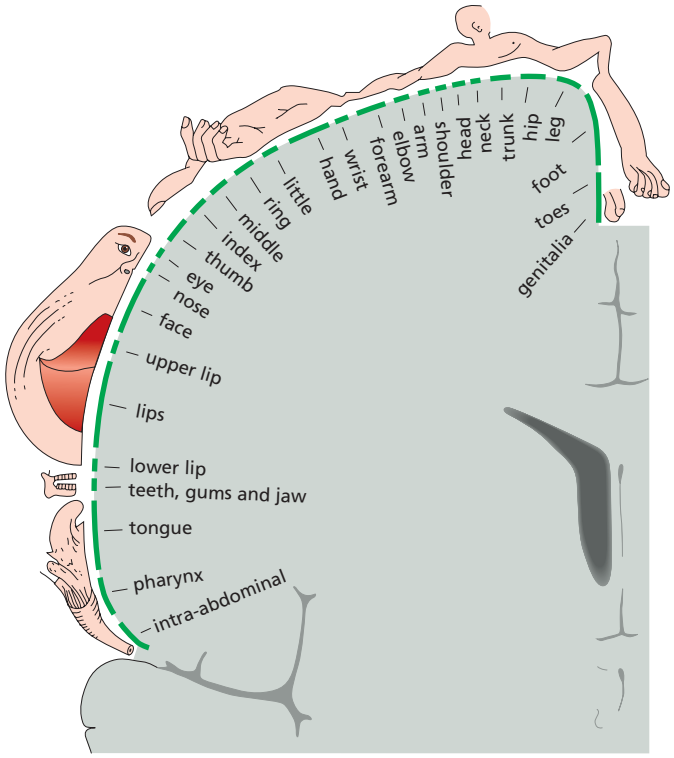


Figure 22–105 A map of the body surface in the human brain. The surface of the body is mapped onto the somatosensory region of the cerebral cortex by an orderly system of nerve cell connections, such that sensory information from neighboring body sites is delivered to neighboring sites in the brain. This means that the map in the brain is largely faithful to the topology of the body surface, even though different body regions are represented at different magnifications according to their density of innervation. The homunculus (the "little man" in the brain) has big lips, for example, because the lips are a particularly large and important source of sensory information. The map was determined by stimulating different points in the cortex of conscious patients during brain surgery and recording what they said they felt. (After W. Penfield and T. Rasmussen, The Cerebral Cortex of Man. New York: Macmillan, 1950.)

cells make the connections appropriate to their original, not their actual, positions. It seems that the cells have positional values—position-specific biochemical properties representing records of their original location. As a result, cells on opposite sides of the retina are intrinsically different, just as the motor neurons in the spinal cord that project to different muscles are intrinsically different.

Such nonequivalence among neurons is referred to as *neuronal specificity*. It is this intrinsic characteristic that guides the retinal axons to their appropriate target sites in the tectum. Those target sites themselves are distinguishable by the retinal axons because the tectal cells also carry positional labels. Thus, the neuronal map depends on a correspondence between two systems of positional markers, one in the retina and the other in the tectum.

Axons From Different Regions of the Retina Respond Differently to a Gradient of Repulsive Molecules in the Tectum

Axons from the nasal retina (the side closest to the nose) project to the posterior tectum, and axons from the temporal retina (the side farthest from the nose) project to the anterior tectum, with intermediate regions of retina projecting to intermediate regions of tectum. When nasal and temporal axons are allowed to grow out over a carpet of anterior or posterior tectal membranes in a culture dish, they also show selectivity (**Figure 22–106**). Temporal axons strongly prefer the anterior tectal membranes, as *in vivo*, whereas nasal axons either prefer posterior tectal membranes, or show no preference (depending on the species of animal). The key difference between anterior and posterior tectum appears to be a repulsive factor on the posterior tectum, to which temporal retinal axons are sensitive but nasal retinal axons are not: if a temporal retinal growth cone touches posterior tectal membrane, it collapses its filopodia and withdraws.

Assays based on these phenomena *in vitro* have identified some of the molecules responsible. The repulsive factor on posterior tectal membrane seems to be partly or entirely comprised of *ephrinA* proteins, a subset of the family of GPI-linked proteins that act as ligands for the *EphA* family of tyrosine kinase receptors. In the mouse, two different ephrins are expressed to form an anterior-to-posterior gradient on the tectal cells. Anterior cells have little or no

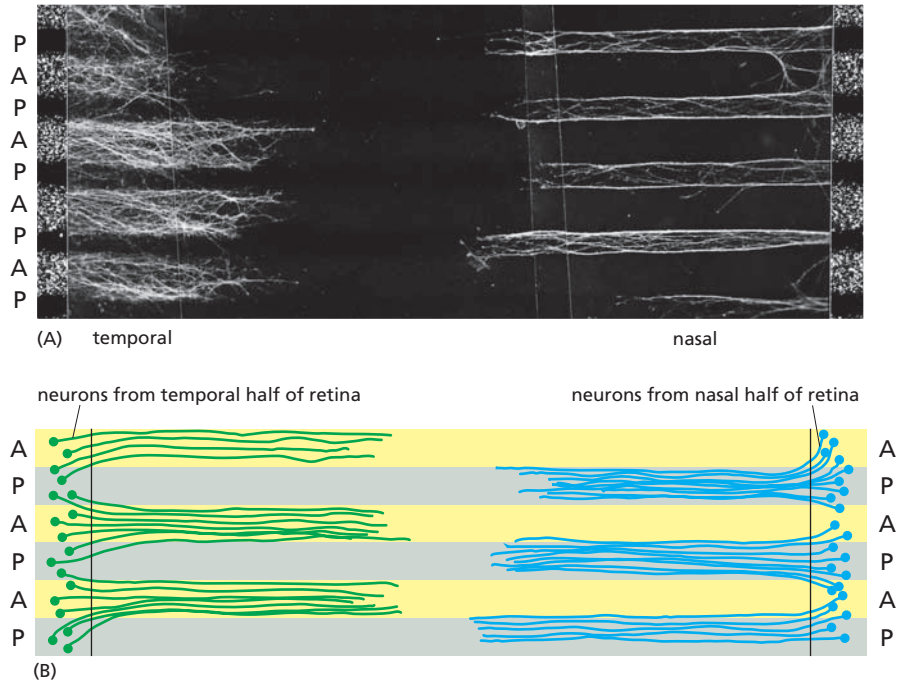


Figure 22–106 Selectivity of retinal axons growing over tectal membranes. (A) A photograph of the experimental observation. (B) A diagram of what is happening. The culture substratum has been coated with alternating stripes of membrane prepared either from posterior tectum (P) or from anterior tectum (A). In the photograph, the anterior tectal stripes are made visible by staining them with a fluorescent marker in the vertical strips at the sides of the picture. Axons of neurons from the temporal half of the retina (growing in from the left) follow the stripes of anterior tectal membrane but avoid the posterior tectal membrane, while axons of neurons from the nasal half of the retina (growing in from the right) do the converse. Thus anterior tectum differs from posterior tectum and nasal retina from temporal retina, and the differences guide selective axon outgrowth. These experiments were performed with cells from the chick embryo. (From Y. von Boxberg, S. Deiss and U. Schwarz, *Neuron* 10:345–357, 1993. With permission from Elsevier.)

ephrin, cells in the center of the tectum express ephrin A2, and cells at the posterior edge of the tectum express ephrin A2 and ephrin A5. Thus, there is a gradient of ephrin expression across the tectum. Meanwhile, the incoming axons express Eph receptors, also in a gradient: temporal axons express high Eph levels, making them sensitive to repulsion by ephrinA, whereas nasal axons express low Eph levels. In a similar way, distributed across the other main axis of the tectum, from medial to lateral, there is graded expression of ephrinB protein and also of another type of signal molecule, Wnt3, with correspondingly graded expression of EphB receptors and Wnt receptors along the dorso-ventral axis of the retina.

This system of signals and receptors is enough to produce an orderly two-dimensional map, if we make one further assumption—an assumption supported by experiments *in vivo*: that the retinal axons somehow interact with one another and compete for tectal territory. Thus, temporal axons are restricted to anterior tectum, and drive nasal axons off it; nasal axons, consequently, are restricted to posterior tectum. Between the extremes, a balance is struck, creating a smooth map of the temporo-nasal axis of the retina onto the anteroposterior axis of the tectum.

Diffuse Patterns of Synaptic Connections Are Sharpened by Activity-Dependent Remodeling

In a normal animal the retinotectal map is initially fuzzy and imprecise: the system of matching markers we have just described is enough to define the broad layout of the map, but not sufficient to specify its fine details. Studies in frogs and fish show that each retinal axon at first branches widely in the tectum and makes a profusion of synapses, distributed over a large area of tectum that overlaps with the territories innervated by other axons. These territories are subsequently trimmed back by selective elimination of synapses and retraction of axon branches. This is accompanied by the formation of new sprouts, through which each axon develops a denser distribution of synapses in the territory that it retains.

A central part in this remodeling and refinement of the map is played by two competition rules that jointly help to create spatial order: (1) axons from separate regions of retina, which tend to be excited at different times, compete to dominate the available tectal territory, but (2) axons from neighboring sites in

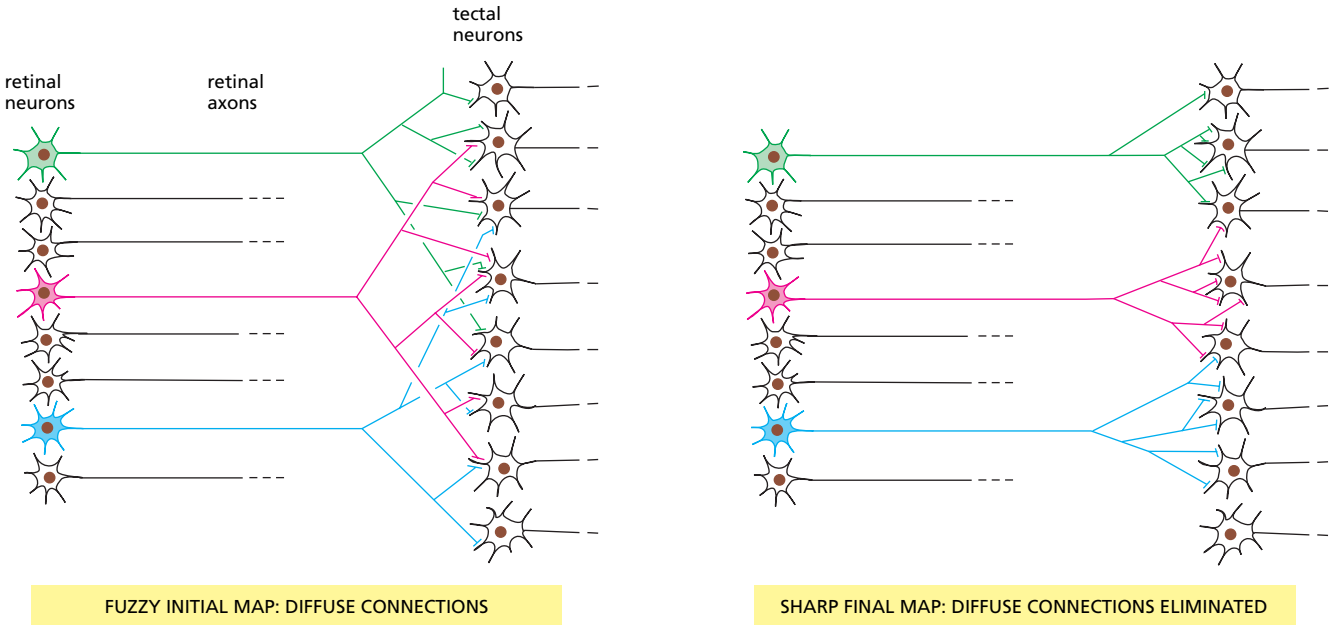


Figure 22–107 Sharpening of the retinotectal map by synapse elimination. At first the map is fuzzy because each retinal axon branches widely to innervate a broad region of tectum overlapping the regions innervated by other retinal axons. The map is then refined by synapse elimination. Where axons from separate parts of the retina synapse on the same tectal cell, competition occurs, eliminating the connections made by one of the axons. But axons from cells that are close neighbors in the retina cooperate, maintaining their synapses on shared tectal cells. Thus each retinal axon ends up innervating a small tectal territory, adjacent to and partly overlapping the territory innervated by axons from neighboring sites in the retina.

the retina, which tend to be excited at the same time, innervate neighboring territories in the tectum because they collaborate to retain and strengthen their synapses on shared tectal cells (**Figure 22–107**). The mechanism underlying both these rules depends on electrical activity and signaling at the synapses that are formed. If all action potentials are blocked by a toxin that binds to voltage-gated Na^+ channels, synapse remodeling is inhibited and the map remains fuzzy.

The phenomenon of activity-dependent synapse elimination is encountered in almost every part of the developing vertebrate nervous system. Synapses are first formed in abundance and distributed over a broad target field; then the system of connections is pruned back and remodeled by competitive processes that depend on electrical activity and synaptic signaling. The elimination of synapses in this way is distinct from the elimination of surplus neurons by cell death, and it occurs after the period of normal neuronal death is over.

Much of what we know about the cellular mechanisms of synapse formation and elimination comes from experiments on the innervation of skeletal muscle in vertebrate embryos. A two-way exchange of signals between the nerve axon terminals and the muscle cells controls the initial formation of synapses. At sites of contact, acetylcholine receptors are clustered in the muscle cell membrane and the apparatus for secretion of this neurotransmitter becomes organized in the axon terminals (discussed in Chapter 11). Each muscle cell at first receives synapses from several neurons; but in the end, through a process that typically takes a couple of weeks, it is left innervated by only one. The synapse retraction again depends on synaptic communication: if synaptic transmission is blocked by a toxin that binds to the acetylcholine receptors in the muscle cell membrane, the muscle cell retains its multiple innervation beyond the normal time of elimination.

Experiments on the musculoskeletal system, as well as in the retinotectal system, suggest that it is not only the amount of electrical activity at a synapse that is important for its maintenance, but also its temporal coordination. Whether a synapse is strengthened or weakened seems to depend critically on whether or not activity in the presynaptic cell is synchronized with activity of the other presynaptic cells synapsing on the same target (and thus also synchronized with activity of the target cell itself).

These and many other findings have suggested a simple interpretation of the competition rules for synapse elimination in the retinotectal system (**Figure 22–108**). Axons from different parts of the retina fire at different times and so compete. Each time one of them fires, the synapse(s) made by the other on a shared tectal target cell are weakened, until one of the axons is left in sole command of

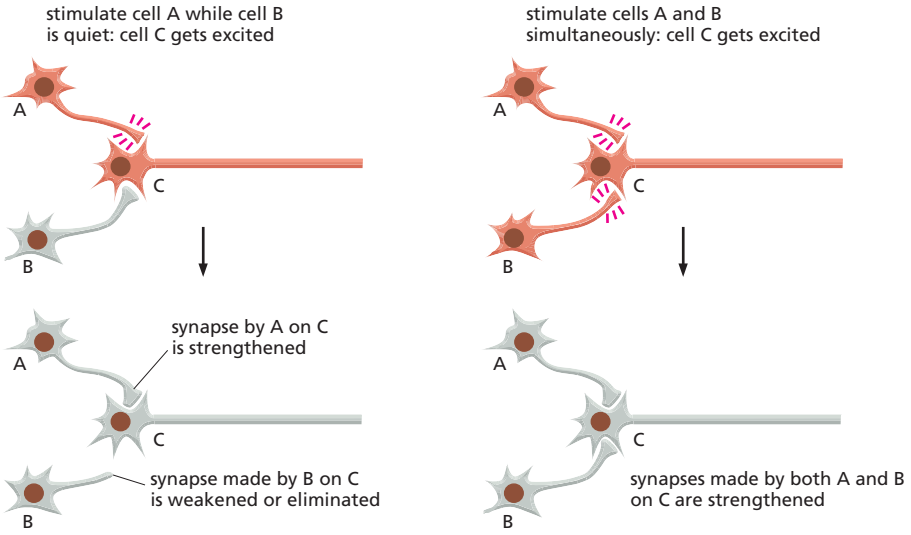


Figure 22–108 Synapse modification and its dependence on electrical activity. Experiments in several systems indicate that synapses are strengthened or weakened by electrical activity according to the rule shown in the diagram. The underlying principle appears to be that each excitation of a target cell tends to weaken any synapse where the presynaptic axon terminal has just been quiet but to strengthen any synapse where the presynaptic axon terminal has just been active. As a result, "neurons that fire together, wire together." A synapse that is repeatedly weakened and rarely strengthened is eventually eliminated altogether.

that cell. Axons from neighboring retinal cells, on the other hand, tend to fire in synchrony with one another: they therefore do not compete but instead maintain synapses on shared tectal cells, creating a precisely ordered map in which neighboring cells of the retina project to neighboring sites in the tectum.

Experience Molds the Pattern of Synaptic Connections in the Brain

The phenomenon that we have just described is summed up in the catch-phrase "neurons that fire together, wire together". The same firing rule relating synapse maintenance to neural activity helps to organize our developing brains in the light of experience.

In the brain of a mammal, axons relaying inputs from the two eyes are brought together in a specific cell layer in the visual region of the cerebral cortex. Here, they form two overlapping maps of the external visual field, one as perceived through the right eye, the other as perceived through the left. Although there is some evidence of a tendency for right- and left-eye inputs to be segregated even before synaptic communication begins, a large proportion of the axons carrying information from the two eyes at early stages synapse together on shared cortical target cells. A period of early signaling activity, however, occurring spontaneously and independently in each retina even before vision begins, leads to a clean segregation of inputs, creating stripes of cells in the cortex that are driven by inputs from the right eye alternating with stripes that are driven by inputs from the left eye (**Figure 22–109**). The firing rule suggests a simple interpretation: a pair of axons bringing information from neighboring sites in the left eye will frequently fire together, and therefore wire

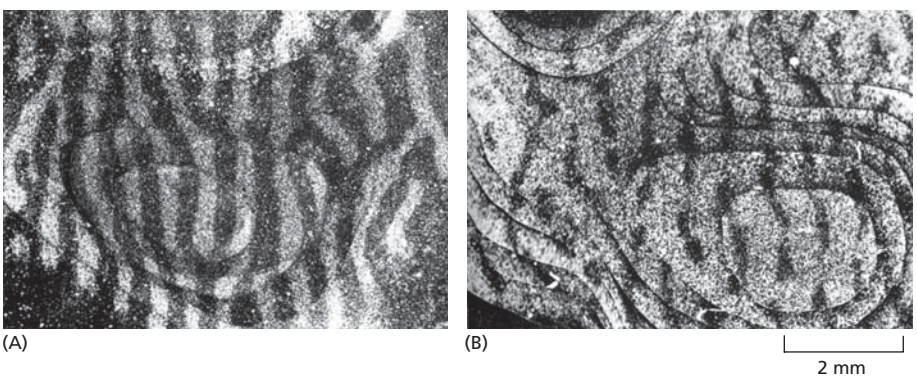


Figure 22–109 Ocular dominance columns in the visual cortex of a monkey's brain, and their sensitivity to visual experience. (A) Normally, stripes of cortical cells driven by the right eye alternate with stripes, of equal width, driven by the left eye. The stripes are revealed here by injecting a radioactive tracer molecule into one eye, allowing time for this tracer to be transported to the visual cortex, and detecting radioactivity there by autoradiography, in sections cut parallel to the cortical surface. (B) If one eye is kept covered during the critical period of development, and thus deprived of visual experience, its stripes shrink and those of the active eye expand. In this way, the deprived eye may lose the power of vision almost entirely. (From D.H. Hubel, T.N. Wiesel and S. Le Vay, *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 278:377–409, 1977. With permission from The Royal Society.)

together, as will a pair of axons from neighboring sites in the right eye; but a right-eye axon and a left-eye axon will rarely fire together, and will instead compete. Indeed, if activity from both eyes is silenced using drugs that block action potentials or synaptic transmission, the inputs fail to segregate correctly.

Maintenance of the pattern of connections is extraordinarily sensitive to experience early in life. If, during a certain *critical period* (ending at about the age of 5 years in humans), one eye is kept covered for a time so as to deprive it of visual stimulation, while the other eye is allowed normal stimulation, the deprived eye loses its synaptic connections to the cortex and becomes almost entirely, and irreversibly, blind. In accordance with what the firing rule would predict, a competition has occurred in which synapses in the visual cortex made by inactive axons are eliminated while synapses made by active axons are consolidated. In this way cortical territory is allocated to axons that carry information and is not wasted on those that are silent.

In establishing the nerve connections that enable us to see, it is not only the quantity of visual stimulation that is important, but also its temporal coordination. For example, the ability to see depth—stereo vision—depends on cells in other layers of the visual cortex that receive inputs relayed from both eyes at once, conveying information about the same part of the visual field as seen from two slightly different angles. These binocularly driven cells allow us to compare the view through the right eye with that through the left so as to derive information about the relative distances of objects from us. If, however, the two eyes are prevented during the critical period from ever seeing the same scene at the same time—for example, by covering first one eye and then the other on alternate days, or simply as a consequence of a childhood squint—almost no binocularly driven cells are retained in the cortex, and the capacity for stereo perception is irretrievably lost. Evidently, in accordance with the firing rule, the inputs from each eye to a binocularly driven neuron are maintained only if the two inputs are frequently triggered to fire in synchrony, as occurs when the two eyes look together at the same scene.

Adult Memory and Developmental Synapse Remodeling May Depend on Similar Mechanisms

We saw in Chapter 11 that synaptic changes underlying memory in at least some parts of the adult brain, notably the hippocampus, hinge on the behavior of a particular type of receptor for the neurotransmitter glutamate—the NMDA receptor. Ca^{2+} flooding into the postsynaptic cell through the channels opened by this receptor triggers lasting changes in the strengths of the synapses on that cell, affecting the presynaptic as well as the postsynaptic structures. The changes that are induced by the NMDA-dependent mechanism in the adult brain obey rules closely akin to the developmental firing rule: events in the external world that cause two neurons to be active at the same time, or in quick succession, favor the making or strengthening of synapses between them. This condition, called the *Hebb rule*, has been suggested to be the fundamental principle underlying associative learning.

Is it possible, then, that adult learning and the more drastic forms of synaptic plasticity seen during development both depend on the same basic machinery of synapse adjustment? There are many hints that it may be so. For example, inhibitors that specifically block activation of the NMDA receptor interfere with the refinement and remodeling of synaptic connections in the developing visual system. Both in the developing animal and in the adult, the alterations in the strength of the synaptic connections correspond to changes in physical structure. The scale of these physical changes is, however, very different. In the developing organism, electrical activity often regulates the extension and regression of large branches of the axonal and dendritic trees. But in the adult brain, the structural adjustments occurring in response to activity seem typically to be much more finely localized, affecting the sizes of individual dendritic spines—the tiny knob-shaped protrusions, no more than a few micrometers long, on which dendrites receive individual synapses (**Figure 22–110**). It seems that Ca^{2+}

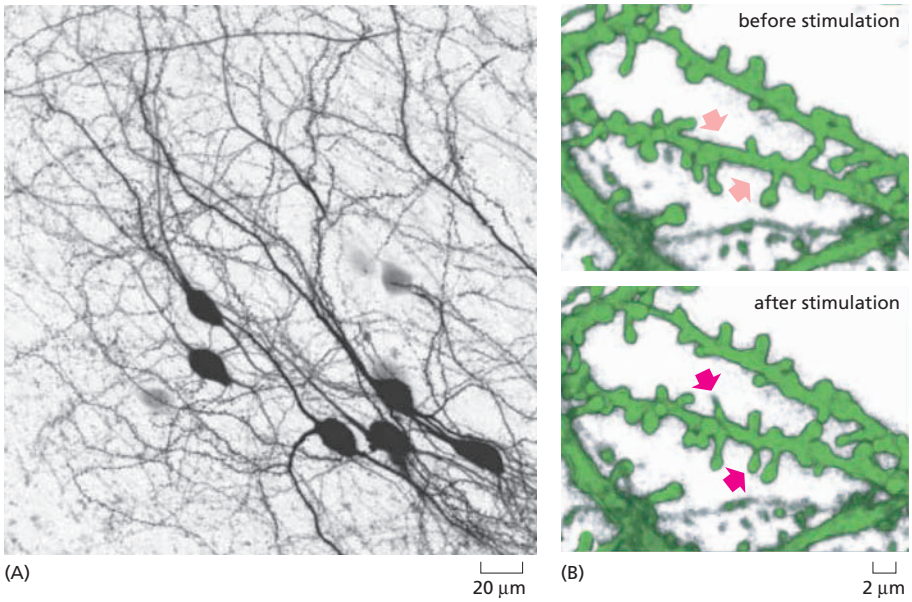


Figure 22–110 Growth of dendritic spines in response to synaptic stimulation. (A) Neurons in a slice of living tissue from the hippocampus of a young mouse. The cells are labeled by expression of Green Fluorescent Protein and observed with a two-photon laser scanning microscope, which allows individual dendrites to be seen at high resolution. The insert shows a processed image of a small part of some of the dendrites. These are covered with tiny dendritic spines, which are the sites of synapses. (B) Repeated intense bursts of synaptic stimulation, triggered by a nearby microelectrode, cause new spines to form within 30 minutes. Low-frequency stimulation has an opposite effect, causing a subset of spines to regress. (From U.V. Nägerl, N. Eberhorn, S.B. Cambridge and T. Bonhoeffer, *Neuron* 44:759–767, 2004. With permission from Elsevier.)

entering a spine through NMDA channels in response to excitation of the synapse on that particular spine can cause just that spine to remodel its actin cytoskeleton. But we still have a lot to learn about the mechanism of such changes and their relationship to learning and memory. The molecular basis of the processes of synapse remodeling through which experience molds our brains remains one of the central challenges that the nervous system presents to cell biology.

Summary

The development of the nervous system proceeds in three phases: first, nerve cells are generated through cell division; then, having ceased dividing, they send out axons and dendrites to form profuse synapses with other, remote cells so that communication can begin; last, the system of synaptic connections is refined and remodeled according to the pattern of electrical activity in the neural network.

The neurons, and the glial cells that always accompany them, are generated from ectodermal precursors, and those born at different times and places express different sets of genes, which help to determine the connections they will form. Axons and dendrites grow out from the neurons by means of growth cones, which follow specific pathways delineated by signals along the way. Structures such as the floor plate of the embryonic spinal cord secrete both chemoattractants and chemorepellents, to which growth cones from different classes of neurons respond differently. On reaching their target area, the axons terminate selectively on a subset of the accessible cells, and in many parts of the nervous system neural maps are set up—orderly projections of one array of neurons onto another. In the retinotectal system, the map is based on the matching of complementary systems of position-specific cell-surface markers—ephrins and Eph receptors—possessed by the two sets of cells.

After the growth cones have reached their targets and initial connections have formed, two major sorts of adjustment occur. First, many of the innervating neurons die as a result of a competition for survival factors such as NGF (nerve growth factor) secreted by the target tissue. This cell death adjusts the quantity of innervation according to the size of the target. Second, individual synapses are pruned away in some places and reinforced in others, so as to create a more precisely ordered pattern of connections. This latter process depends on electrical activity: synapses that are frequently active are reinforced, and different neurons contacting the same target cell tend to maintain their synapses on the shared target only if they are both frequently active at the same time. In this way the structure of the brain can be adjusted to reflect the connections between events in the external world. The underlying molecular mechanism of this synaptic plasticity may be similar to that responsible for the formation of memories in adult life.

PLANT DEVELOPMENT

Plants and animals are separated by about 1.5 billion years of evolutionary history. They have evolved their multicellular organization independently but using the same initial tool kit—the set of genes inherited from their common unicellular eucaryotic ancestor. Most of the contrasts in their developmental strategies spring from two basic peculiarities of plants. First, they get their energy from sunlight, not by ingesting other organisms. This dictates a body plan different from that of animals. Second, their cells are encased in semirigid cell walls and cemented together, preventing them from moving as animal cells do. This dictates a different set of mechanisms for shaping the body and different developmental processes to cope with a changeable environment.

Animal development is largely buffered against environmental changes, and the embryo generates the same genetically determined body structure unaffected by external conditions. The development of most plants, by contrast, is dramatically influenced by the environment. Because they cannot match themselves to their environment by moving from place to place, plants adapt instead by altering the course of their development. Their strategy is opportunistic. A given type of organ—a leaf, a flower, or a root, say—can be produced from the fertilized egg by many different paths according to environmental cues. A begonia leaf pegged to the ground may sprout a root; the root may throw up a shoot; the shoot, given sunlight, may grow leaves and flowers.

The mature plant is typically made of many copies of a small set of standardized modules, as described in **Figure 22–111**. The positions and times at which those modules are generated are strongly influenced by the environment, causing the overall structure of the plant to vary. The choices between alternative modules and their organization into a whole plant depend on external cues and long-range hormonal signals that play a much smaller part in the control of animal development.

But although the global structure of a plant—its pattern of roots or branches, its numbers of leaves or flowers—can be highly variable, its detailed organization on a small scale is not. A leaf, a flower, or indeed an early plant embryo, is as precisely specified as any organ of an animal, possessing a *determinate* structure, in contrast with the *indeterminate* pattern of branching and sprouting of the plant as a whole. The internal organization of a plant module raises essentially the same problems in the genetic control of pattern formation as does animal development, and they are solved in analogous ways. In this section we focus on the cellular mechanisms of development in flowering plants. We examine both the contrasts and the similarities with animals.

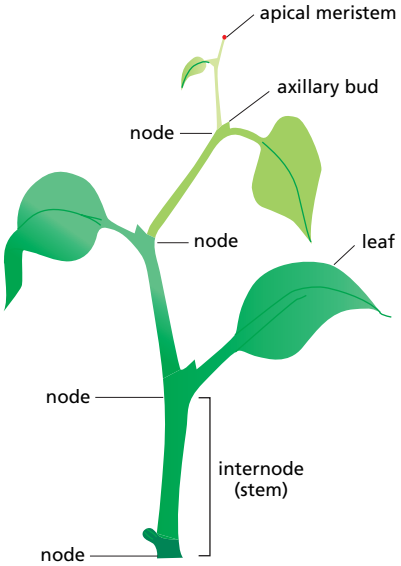


Figure 22–111 A simple example of the modular construction of plants. Each module (shown in different shades of *green)* consists of a stem, a leaf, and a bud containing a potential growth center, or meristem. The bud forms at the branch point, or node, where the leaf diverges from the stem. Modules arise sequentially from the continuing activity of the apical meristem.

Arabidopsis Serves as a Model Organism for Plant Molecular Genetics

Flowering plants, despite their amazing variety, are of relatively recent origin. The earliest known fossil examples are 130 million years old, as against 350 million years or more for vertebrate animals. Underlying the diversity of form, therefore, there is a high degree of similarity in molecular mechanisms. As we shall see, a small genetic change can transform a plant's large-scale structure; and just as plant physiology allows survival in many different environments, so also it allows survival of many differently structured forms. A mutation that gives an animal two heads is generally lethal; one that doubles the number of flowers or branches on a plant is generally not.

To identify the genes that govern plant development and to discover how they function, plant biologists have selected a small weed, the common wall cress *Arabidopsis thaliana* (**Figure 22–112**) as their primary model organism.

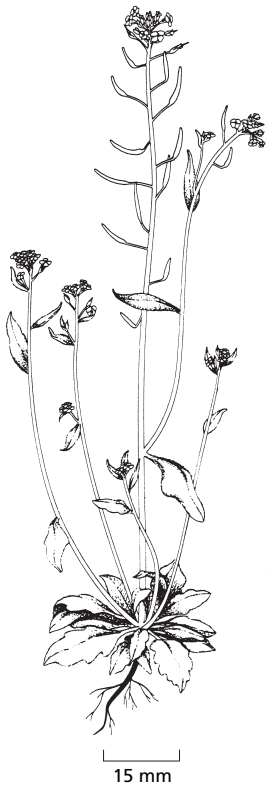


Figure 22–112 *Arabidopsis thaliana*. This small plant is a member of the mustard (or crucifer) family (see also Figure 1–46). It is a weed of no economic use but of great value for genetic studies of plant development. (From M.A. Estelle and C.R. Somerville, *Trends Genet.* 12:89–93, 1986. With permission from Elsevier.)

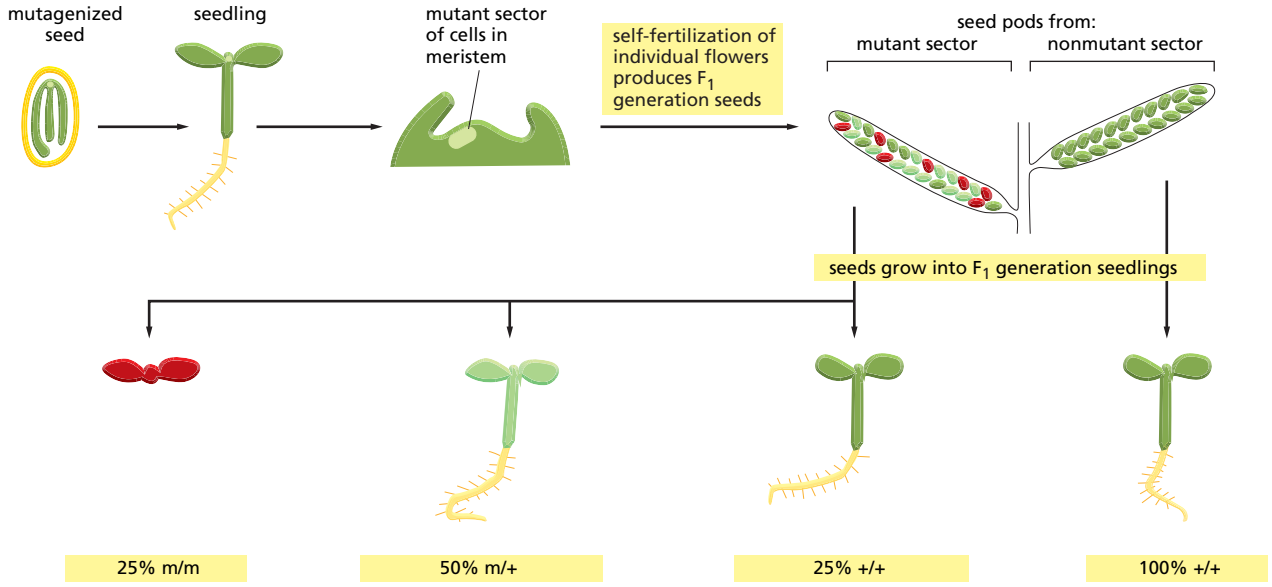


Figure 22–113 Production of mutants in *Arabidopsis*. A seed, containing a multicellular embryo, is treated with a chemical mutagen and left to grow into a plant. In general, this plant will be a mosaic of clones of cells carrying different induced mutations. An individual flower produced by this plant will usually be composed of cells belonging to the same clone, all carrying the same mutation, m, in heterozygous form (m/+). Self-fertilization of individual flowers by their own pollen results in seed pods, each of which contains a family of embryos of whose members half, on average, will be heterozygous (m/+), one-quarter will be homozygous mutant (m/m), and one-quarter will be homozygous wild-type (+/+). Often, the mutation will have a recessive lethal effect, as indicated here by the lack of a root in the m/m seedling. The mutant stock is then maintained by breeding from the heterozygote: it will produce seed pods (F_2 generation) that all contain a mixture of +/+, m/+, and m/m seeds.

Like *Drosophila* or *Caenorhabditis elegans*, it is small, quick to reproduce, and convenient for genetics. It can be grown indoors in Petri dishes or tiny plant pots in large numbers and produces hundreds of seeds per plant after 8–10 weeks. It has, in common with *C. elegans*, a significant advantage over *Drosophila* or vertebrate animals for genetics: like many flowering plants, it can reproduce as a hermaphrodite because a single flower produces both eggs and the male gametes that can fertilize them. Therefore, when a flower that is heterozygous for a recessive lethal mutation is self-fertilized, one-fourth of its seeds will display the homozygous embryonic phenotype. This makes it easy to perform genetic screens (**Figure 22–113**) and so to obtain a catalog of the genes required for specific developmental processes.

The *Arabidopsis* Genome Is Rich in Developmental Control Genes

Arabidopsis has one of the smallest plant genomes—125 million nucleotide pairs, on a par with *C. elegans* and *Drosophila*—and the complete DNA sequence is now known. It contains approximately 26,000 genes. This total includes many recently generated duplicates, however, so that the number of functionally distinct types of protein represented may be considerably less. Cell culture and genetic transformation methods have been established, as well as vast libraries of seeds carrying mutations produced by random insertions of mobile genetic elements, so that plants with mutations in any chosen gene can be obtained to order. Powerful tools are thus available to analyze gene functions. Although only a small fraction of the total gene set has been characterized experimentally as yet, functions can be tentatively assigned to many genes—about 18,000—on the basis of their sequence similarities to well-characterized genes in *Arabidopsis* and other organisms.

Even more than the genomes of multicellular animals, the *Arabidopsis* genome is rich in genes that code for gene regulatory proteins (**Table 22–2**). Some major families of animal gene regulatory proteins (such as the Myb family of DNA-binding proteins) are greatly expanded, while others (such as nuclear hormone receptors) seem to be entirely absent, and there are large families of gene regulatory proteins in the plant that have no animal homologs.

Where homologous gene regulatory proteins (such as homeodomain proteins) can be recognized in both plants and animals, they have little in common with regard to the genes they regulate or the types of developmental decisions that they control, and there is very little conservation of protein sequence outside the DNA-binding domains.

Arabidopsis is like multicellular animals in possessing many genes for cell communication and signal transduction (1900 genes out of 18,000 classified),

Table 22–2 Some Major Families of Gene Regulatory Proteins in *Arabidopsis, Drosophila, C. elegans,* and the Yeast *Saccharomyces cerevisiae*

FAMILY	NUMBER OF FAMILY MEMBERS PREDICTED FROM GENOME ANALYSIS			
	Arabidopsis	*Drosophila*	*C. elegans*	YEAST
Myb	190	6	3	10
AP2/EREBP (Apetala2/ethylene-responsive-element binding protein)	144	0	0	0
bHLH (basic helix–loop–helix)	139	46	25	8
NAC	109	0	0	0
C2H2 (Zn finger)	105	291	139	53
Homeobox	89	103	84	9
MADS box	82	2	2	4
bZIP	81	21	25	21
WRKY (Zn finger)	72	0	0	0
GARP	56	0	0	0
C2C2 (Zn finger)/GATA	104	6	9	10
Nuclear hormone receptor	0	21	25	0
C6 (Zn finger)	0	0	0	52
Estimated total (including many not listed above)	1533	635	669	209
% of genes in genome	5.9	4.5	3.5	3.5

The Table lists only those families that have at least 50 members in at least one organism. (Data from J.L. Riechmann et al., *Science* 290:2105–2110, 2000. With permission from AAAS.)

but the specific details of these gene sets are very different, as discussed in Chapter 15. The Wnt, Hedgehog, Notch, and TGFβ signaling mechanisms are all absent in *Arabidopsis*. In compensation, other signaling pathways peculiar to plants are highly developed. Cell-surface receptors of the tyrosine kinase class seem to be entirely absent, although many of the signaling components downstream of these receptors in animals are present. Conversely, receptors of the serine/threonine kinase class are very plentiful, but they do not act through the same system of intracellular messengers as the receptor serine/threonine kinases in animals. Substantial sets of genes are devoted to developmental processes of special importance in plants: more than 1000 for synthesis and remodeling of the plant cell wall, for example, and more than 100 for detecting and responding to light.

We must now examine how the genes of the plant are used to control plant development.

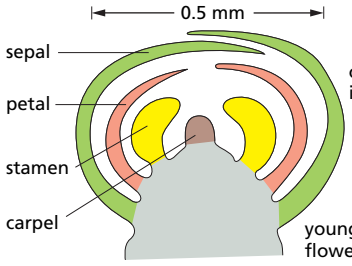
Embryonic Development Starts by Establishing a Root–Shoot Axis and Then Halts Inside the Seed

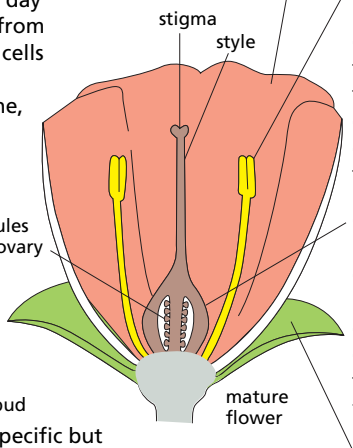
The basic strategy of sexual reproduction in flowering plants is briefly summarized in **Panel 22–1**. The fertilized egg, or zygote, of a higher plant begins by dividing asymmetrically to establish the polarity of the future embryo. One product of this division is a small cell with dense cytoplasm, which will become the embryo proper. The other is a large vacuolated cell that divides further and forms a structure called the *suspensor*, which in some ways is comparable to the umbilical cord in mammals. The suspensor attaches the embryo to the adjacent nutritive tissue and provides a pathway for the transport of nutrients.

During the next step in development the diploid embryo cell proliferates to form a ball of cells that quickly acquires a polarized structure. This comprises two key groups of proliferating cells—one at the suspensor end of the embryo that will collaborate with the uppermost suspensor cell to generate a root, and one at the opposite end that will generate a shoot (**Figure 22–114**). The main root–shoot axis established in this way is analogous to the head-to-tail axis of an animal. At the same time it begins to be possible to distinguish the future *epidermal cells*, forming the outermost layer of the embryo, the future *ground*

THE FLOWER

Flowers, which contain the reproductive cells of higher plants, arise from vegetative shoot apical meristems, where they terminate further vegetative growth. Environmental factors, often the rhythms of day length and temperature, trigger the switch from vegetative to floral development. The germ cells thus arise late in plant development from somatic cells rather than from a germ-cell line, as in animals.

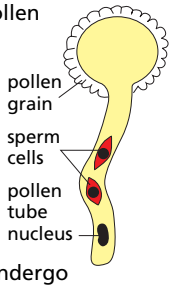




Flower structure is both varied and species-specific but generally comprises four concentrically arranged sets of structures that may each be regarded as modified leaves.

Petal: distinctive leaflike structures, usually brightly colored, facilitate pollination via, for example, attracted insects.

Stamen: an organ containing cells that undergo meiosis and form haploid pollen grains, each of which contains two male sperm cells. Pollen transferred to a stigma germinates, and the pollen tube delivers the two nonmotile sperm to the ovary.

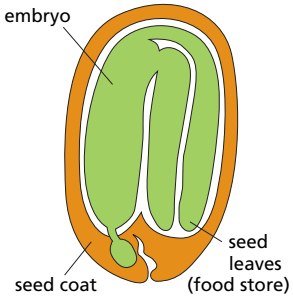


Carpel: an organ containing one or more ovaries, each of which contains ovules. Each ovule houses cells that undergo meiosis and form an embryo sac containing the female egg cell. At fertilization, one sperm cell fuses with the egg cell and will form the future diploid embryo, while the other fuses with two cells in the embryo sac to form the triploid endosperm tissue.

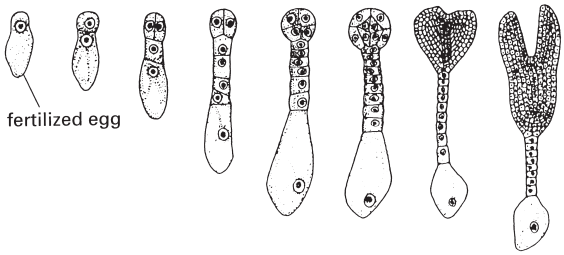
Sepals: leaflike structures that form a protective covering during early flower development.

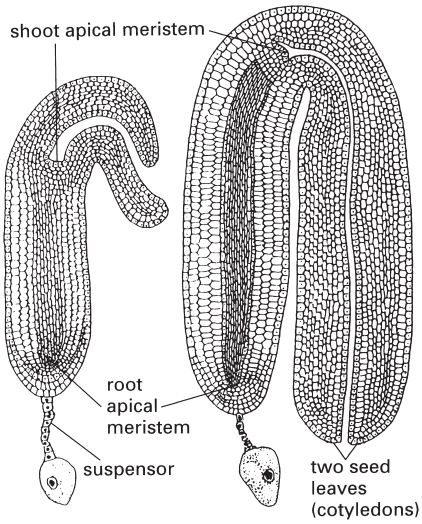
THE SEED

A seed contains a dormant embryo, a food store, and a seed coat. By the end of its development a seed's water content can drop from 90% to 5%. The seed is usually protected in a *fruit* whose tissues are of maternal origin.



THE EMBRYO





The fertilized egg within the ovule will grow to form an embryo using nutrients transported from the endosperm by the suspensor. A complex series of cell divisions, illustrated here for the common weed called shepherd's purse, produces an embryo with a root apical meristem, a shoot apical meristem, and either one (monocots) or two (dicots) seed leaves, called cotyledons.

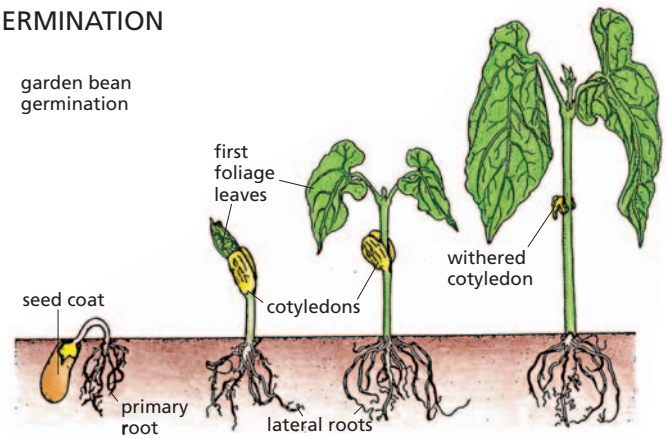
Development is arrested at this stage, and the ovule, containing the embryo, now becomes a seed, adapted for dispersal and survival.

For the embryo to resume its growth the seed must germinate, a process dependent upon both internal factors (dormancy) and environmental factors including water, temperature, and oxygen. The food reserves for the early phase of germination may either be the endosperm (maize) or the cotyledons (pea and bean).

The primary root usually emerges first from the seed to ensure an early water supply for the seedling. The cotyledon(s) may appear above the ground, as in the garden bean shown here, or they may remain in the soil, as in peas. In both cases the cotyledons eventually wither away.

The apical meristem can now show its capacity for continuous growth, producing a typical pattern of nodes, internodes, and buds (see Figure 22–106).

GERMINATION



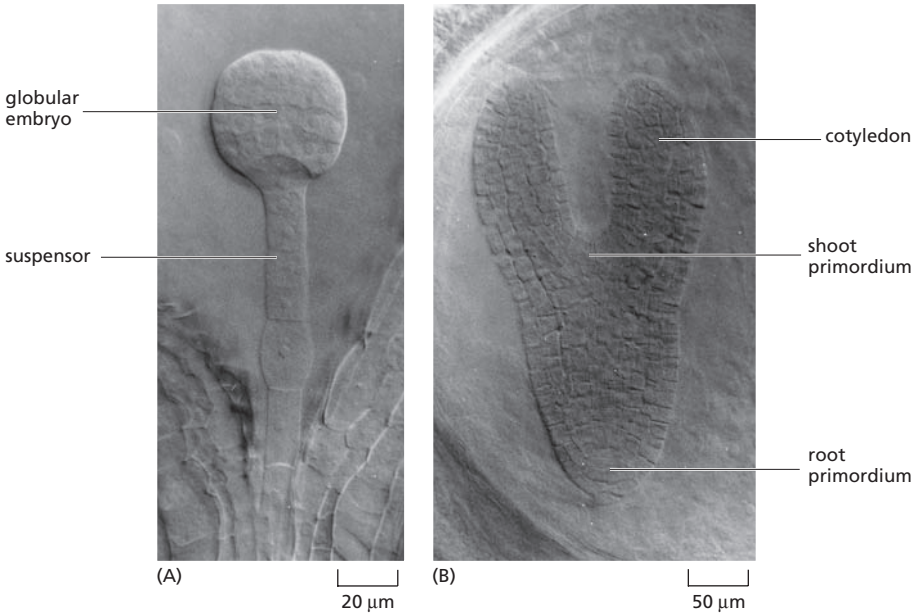


Figure 22–114 Two stages of embryogenesis in *Arabidopsis thaliana*. (From G. Jürgens et al., *Development [Suppl.]* 1:27–38, 1991. With permission from The Company of Biologists.)

tissue cells, occupying most of the interior, and the future *vascular tissue cells*, forming the central core (**Panel 22–2**). These three sets of cells can be compared to the three germ layers of an animal embryo. Slightly later in development, the rudiment of the shoot begins to produce the embryonic seed leaves, or *cotyledons*—one in monocots and two in dicots. Soon after this stage, development usually halts and the embryo becomes packaged in a **seed** (a case formed by tissues of the mother plant), specialized for dispersal and for survival in harsh conditions. The embryo in a seed is stabilized by dehydration, and it can remain dormant for a very long time—even hundreds of years. When rehydrated, the seeds germinate and embryonic development resumes.

Genetic screens can be used in *Arabidopsis*, just as in *Drosophila* or *C. elegans*, to identify the genes that govern the organization of the embryo and to group these into categories according to their homozygous mutant phenotypes. Some are required for formation of the seedling root, some for the seedling stem, and some for the seedling apex with its cotyledons. Another class is required for formation of the three major tissue types—epidermis, ground tissue, and vascular tissue—and yet another class for the organized changes of cell shape that give the embryo and seedling their elongated form (**Figure 22–115**).

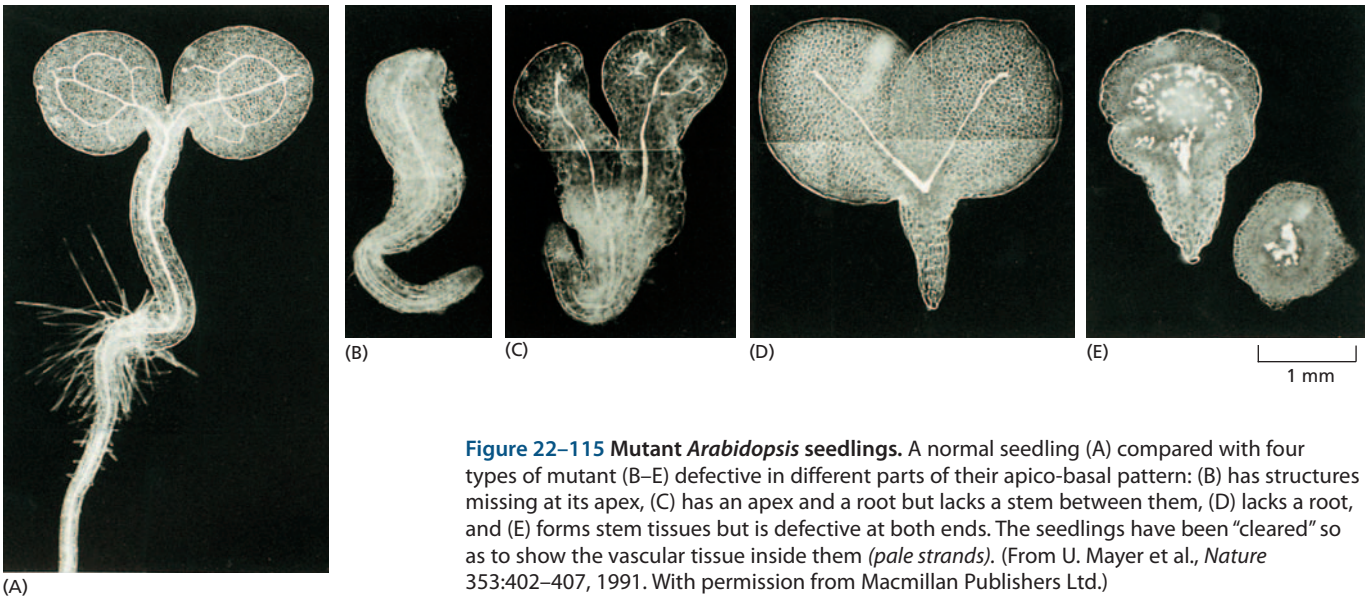


Figure 22–115 Mutant *Arabidopsis* seedlings. A normal seedling (A) compared with four types of mutant (B–E) defective in different parts of their apico-basal pattern: (B) has structures missing at its apex, (C) has an apex and a root but lacks a stem between them, (D) lacks a root, and (E) forms stem tissues but is defective at both ends. The seedlings have been "cleared" so as to show the vascular tissue inside them *(pale strands)*. (From U. Mayer et al., *Nature* 353:402–407, 1991. With permission from Macmillan Publishers Ltd.)

The Parts of a Plant Are Generated Sequentially by Meristems

Roughly speaking, the embryo of an insect or a vertebrate animal is a rudimentary miniature scale model of the later organism, and the details of body structure are filled in progressively as it enlarges. The plant embryo grows into an adult in a quite different way: the parts of the adult plant are created sequentially by groups of cells that proliferate to lay down additional structures at the plant's periphery. These all-important groups of cells are called **apical meristems** (see Figure 22–111). Each meristem consists of a self-renewing population of stem cells. As these divide, they leave behind a trail of progeny that become displaced from the meristem region, enlarge, and finally differentiate. Although the shoot and root apical meristems generate all the basic varieties of cells that are needed to build leaves, roots, and stems, many cells outside the apical meristems also keep a capacity for further proliferation and retain meristem potential. In this way trees and other perennial plants, for example, are able to increase the girth of their stems and roots as the years go by and can sprout new shoots from dormant regions if the plant is damaged.

The rudiments of the apical meristems of root and shoot are already determined in the embryo. As soon as the seed coat ruptures during germination, a dramatic enlargement of nonmeristematic cells occurs, driving the emergence first of a root, to establish an immediate foothold in the soil, and then of a shoot (**Figure 22–116**). This is accompanied by rapid and continual cell divisions in the apical meristems: in the apical meristem of a maize root, for example, cells divide every 12 hours, producing 5×10^5 cells per day. The rapidly growing root and shoot probe the environment—the root increasing the plant's capacity for taking up water and minerals from the soil, the shoot increasing its capacity for photosynthesis (see Panel 22–1).

Figure 22–116 A seedling of *Arabidopsis*. The *brown* objects to the right of the young seedling are the two halves of the discarded seed coat. (Courtesy of Catherine Duckett.)

Development of the Seedling Depends on Environmental Signals

From germination onward, the course of plant development is powerfully influenced by signals from the environment. The shoot has to push its way rapidly up through the soil, and must open its cotyledons and begin photosynthesis only after it has reached the light. The timing of this transition from rapid subterranean sprouting to illuminated growth cannot be genetically programmed, because the depth at which the seed is buried is unpredictable. The developmental switch is controlled instead by light, which, among other effects, acts on the seedling by inhibiting production of a class of plant growth regulators called *brassinosteroids*, discussed in Chapter 15. Mutations in genes required for production or reception of the brassinosteroid signal cause the stem of the seedling to go green, slow its elongation, and open its cotyledons prematurely, while it is still in the dark.

Long-Range Hormonal Signals Coordinate Developmental Events in Separate Parts of the Plant

Separate parts of a plant experience different environments and react to them individually by changes in their mode of development. The plant, however, must continue to function as a whole. This demands that developmental choices and events in one part of the plant affect developmental choices elsewhere. There must be long-range signals to bring about such coordination.

As gardeners know, for example, by pinching off the tip of a branch one can stimulate side growth: removal of the apical meristem relieves the quiescent axillary meristems of an inhibition and allows them to form new shoots. In this case the long-range signal from the apical meristem, or at least a key component has been identified. It is an auxin, a member of one of several classes of **plant growth regulators** (sometimes called *plant hormones*), all of which have powerful influences on plant development. Other known classes include the *gibberellins*, the *cytokinins*, *abscisic acid*, the gas *ethylene*, and the *brassinosteroids*. As shown in **Figure 22–117**, all are small molecules that readily penetrate cell walls. They are

THE THREE TISSUE SYSTEMS

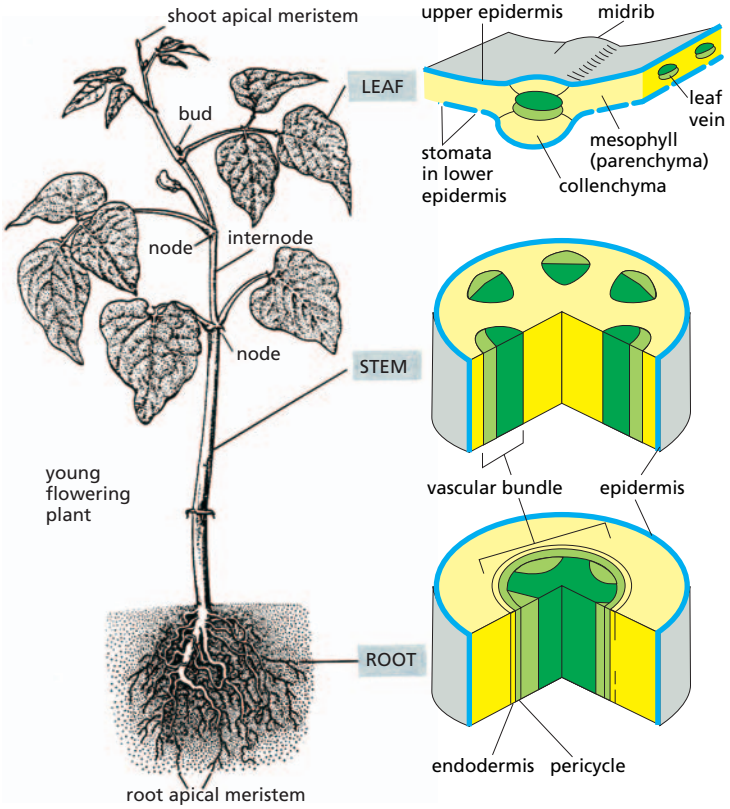
Cell division, growth, and differentiation give rise to tissue systems with specialized functions.

DERMAL TISSUE (▭): This is the plant's protective outer covering in contact with the environment. It facilitates water and ion uptake in roots and regulates gas exchange in leaves and stems.

VASCULAR TISSUE: Together the phloem (▭) and the xylem (▭) form a continuous vascular system throughout the plant. This tissue conducts water and solutes between organs and also provides mechanical support.

GROUND TISSUE (▭): This packing and supportive tissue accounts for much of the bulk of the young plant. It also functions in food manufacture and storage.

THE PLANT



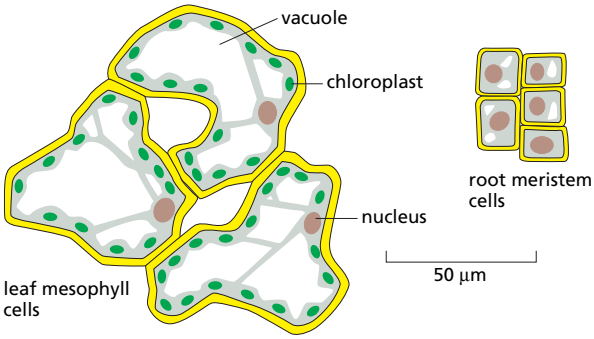
The young flowering plant shown on the *right* is constructed from three main types of organs: leaves, stems, and roots. Each plant organ in turn is made from three tissue systems: ground (▭), dermal (▭), and vascular (▭).

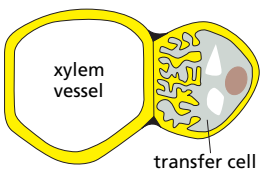
All three tissue systems derive ultimately from the cell proliferative activity of the shoot or root apical meristems, and each contains a relatively small number of specialized cell types. These three common tissue systems, and the cells that comprise them, are described in this panel.

GROUND TISSUE

The ground tissue system contains three main cell types called parenchyma, collenchyma, and sclerenchyma.

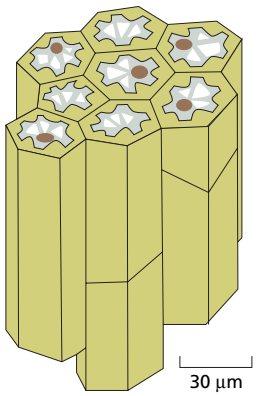
Parenchyma cells are found in all tissue systems. They are living cells, generally capable of further division, and have a thin primary cell wall. These cells have a variety of functions. The apical and lateral meristematic cells of shoots and roots provide the new cells required for growth. Food production and storage occur in the photosynthetic cells of the leaf and stem (called mesophyll cells); storage parenchyma cells form the bulk of most fruits and vegetables. Because of their proliferative capacity, parenchyma cells also serve as stem cells for wound healing and regeneration.

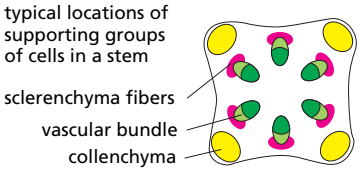




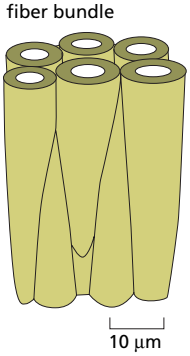
A transfer cell, a specialized form of the parenchyma cell, is readily identified by elaborate ingrowths of the primary cell wall. The increase in the area of the plasma membrane beneath these walls facilitates the rapid transport of solutes to and from cells of the vascular system.

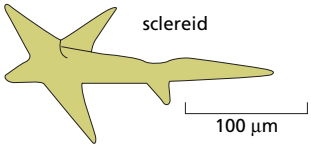
Collenchyma are living cells similar to parenchyma cells except that they have much thicker cell walls and are usually elongated and packed into long ropelike fibers. They are capable of stretching and provide mechanical support in the ground tissue system of the elongating regions of the plant. Collenchyma cells are especially common in subepidermal regions of stems.





Sclerenchyma, like collenchyma, have strengthening and supporting functions. However, they are usually dead cells with thick, lignified secondary cell walls that prevent them from stretching as the plant grows. Two common types are fibers, which often form long bundles, and sclereids, which are shorter branched cells found in seed coats and fruit.

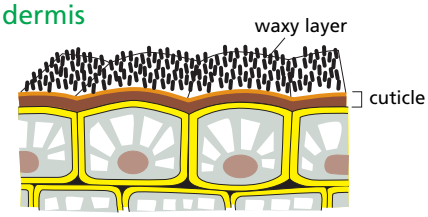




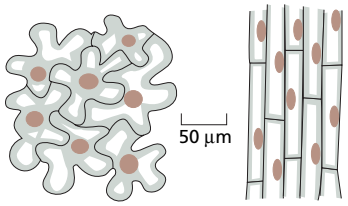
DERMAL TISSUE

The epidermis is the primary outer protective covering of the plant body. Cells of the epidermis are also modified to form stomata and hairs of various kinds.

Epidermis



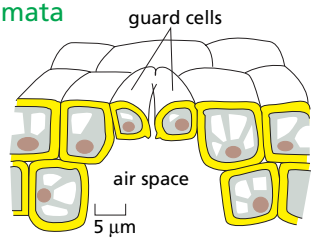
The epidermis (usually one layer of cells deep) covers the entire stem, leaf, and root of the young plant. The cells are living, have thick primary cell walls, and are covered on their outer surface by a special cuticle with an outer waxy layer. The cells are tightly interlocked in different patterns.



upper epidermis of a leaf

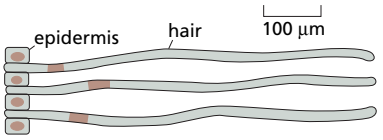
epidermis of a stem

Stomata

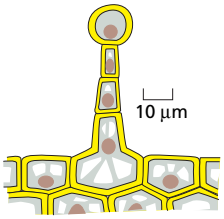


Stomata are openings in the epidermis, mainly on the lower surface of the leaf, that regulate gas exchange in the plant. They are formed by two specialized epidermal cells called *guard cells,* which regulate the diameter of the pore. Stomata are distributed in a distinct species-specific pattern within each epidermis.

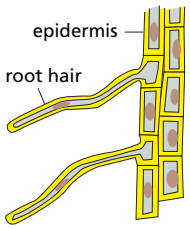
Hairs (or trichomes) are appendages derived from epidermal cells. They exist in a variety of forms and are commonly found in all plant parts. Hairs function in protection, absorption, and secretion; for example,



young, single-celled hairs in the epidermis of the cotton seed. When these grow, the walls will be secondarily thickened with cellulose to form cotton fibers.



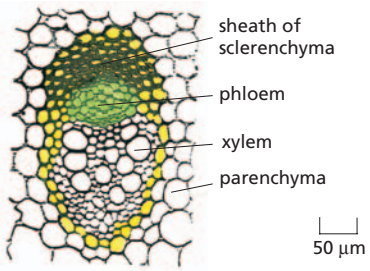
a multicellular secretory hair from a geranium leaf



Single-celled root hairs have an important function in water and ion uptake.

Vascular bundles

Roots usually have a single vascular bundle, but stems have several bundles. These are arranged with strict radial symmetry in dicots, but they are more irregularly dispersed in monocots.

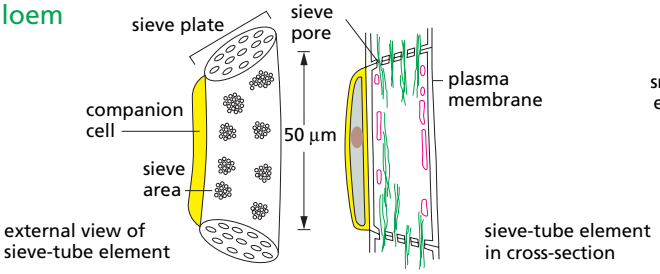


a typical vascular bundle from the young stem of a buttercup

VASCULAR TISSUE

The phloem and the xylem together form a continuous vascular system throughout the plant. In young plants they are usually associated with a variety of other cell types in *vascular bundles.* Both phloem and xylem are complex tissues. Their conducting elements are associated with parenchyma cells that maintain the elements and exchange materials with them. In addition, groups of collenchyma and sclerenchyma cells provide mechanical support.

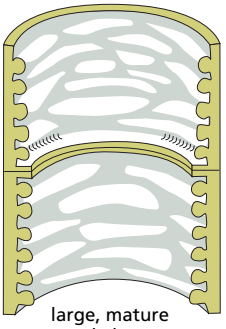
Phloem



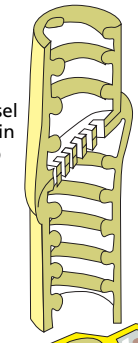
Phloem is involved in the transport of organic solutes in the plant. The main conducting cells (elements) are aligned to form tubes called *sieve tubes*. The sieve-tube elements at maturity are living cells, interconnected by perforations in their end walls formed from enlarged and modified plasmodesmata (sieve plates). These cells retain their plasma membrane, but they have lost their nuclei and much of their cytoplasm; they therefore rely on associated *companion cells* for their maintenance. These companion cells have the additional function of actively transporting soluble food molecules into and out of sieve-tube elements through porous sieve areas in the wall.

Xylem

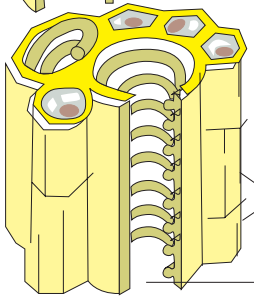
Xylem carries water and dissolved ions in the plant. The main conducting cells are the vessel elements shown here, which are dead cells at maturity that lack a plasma membrane. The cell wall has been secondarily thickened and heavily lignified. As shown below, its end wall is largely removed, enabling very long, continuous tubes to be formed.



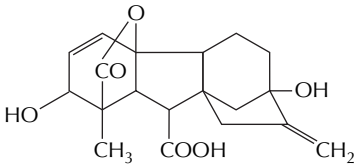
large, mature vessel element



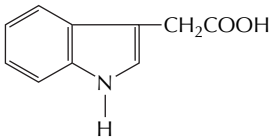
small vessel element in root tip



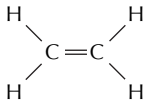
The vessel elements are closely associated with xylem parenchyma cells, which actively transport selected solutes into and out of the elements across the parenchyma cell plasma membrane.



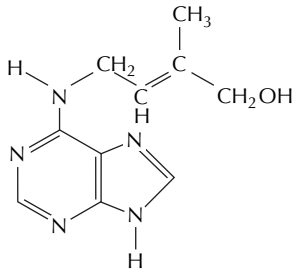
gibberellic acid (GA3) [a gibberellin]



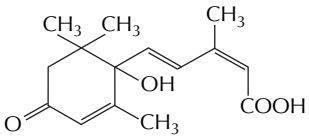
indole-3-acetic acid (IAA) [an auxin]



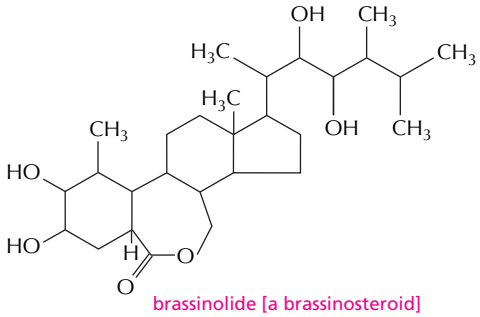
ethylene



zeatin [a cytokinin]



abscisic acid (ABA)



brassinolide [a brassinosteroid]

Figure 22–117 Plant growth regulators. The formula of one naturally occurring representative molecule from each of six groups of plant growth regulatory molecules is shown.

all synthesized by most plant cells and can either act locally or be transported to influence target cells at a distance. Auxin, for example, is transported from cell to cell at a rate of about 1 cm per hour from the tip of a shoot toward its base. Each growth regulator has multiple effects, and these are modulated by the other growth regulators, as well as by environmental cues and nutritional status. Thus, auxin alone can promote root formation, but in conjunction with gibberellin it can promote stem elongation, with cytokinin, it can suppress lateral shoot outgrowth, and with ethylene it can stimulate lateral root growth. Remarkably, as we shall see below, auxin also controls the detailed patterns of cell specialization on a microscopic scale in the apical meristem. The receptors that recognize some of these growth regulators are discussed in Chapter 15.

The Shaping of Each New Structure Depends on Oriented Cell Division and Expansion

Plant cells, imprisoned within their cell walls, cannot crawl about and cannot be shuffled as the plant grows; but they can divide, and they can swell, stretch, and bend. The morphogenesis of a developing plant therefore depends on orderly cell divisions followed by strictly oriented cell expansions. Most cells produced in the root-tip meristem, for example, go through three distinct phases of development—division, growth (elongation), and differentiation. These three steps, which overlap in both space and time, give rise to the characteristic architecture of a root tip. Although the process of cell differentiation often begins while a cell is still enlarging, it is comparatively easy to distinguish in a root tip a zone of cell division, a zone of oriented cell elongation (which accounts for the growth in length of the root), and a zone of cell differentiation (**Figure 22–118**).

In the phase of controlled expansion that generally follows cell division, the daughter cells may often increase in volume by a factor of 50 or more. This expansion is driven by an osmotically based turgor pressure that presses outward on the plant cell wall, and its direction is determined by the orientation of the cellulose fibrils in the cell wall, which constrain expansion along one axis (see Figure 19–73). The orientation of the cellulose in turn is apparently controlled by the orientation of arrays of microtubules just inside the plasma membrane, which are thought to guide cellulose deposition (discussed in Chapter 19). These orientations can be rapidly changed by plant growth regulators, such as ethylene and gibberellic acid (**Figure 22–119**), but the molecular mechanisms underlying these dramatic cytoskeletal rearrangements are still unknown.

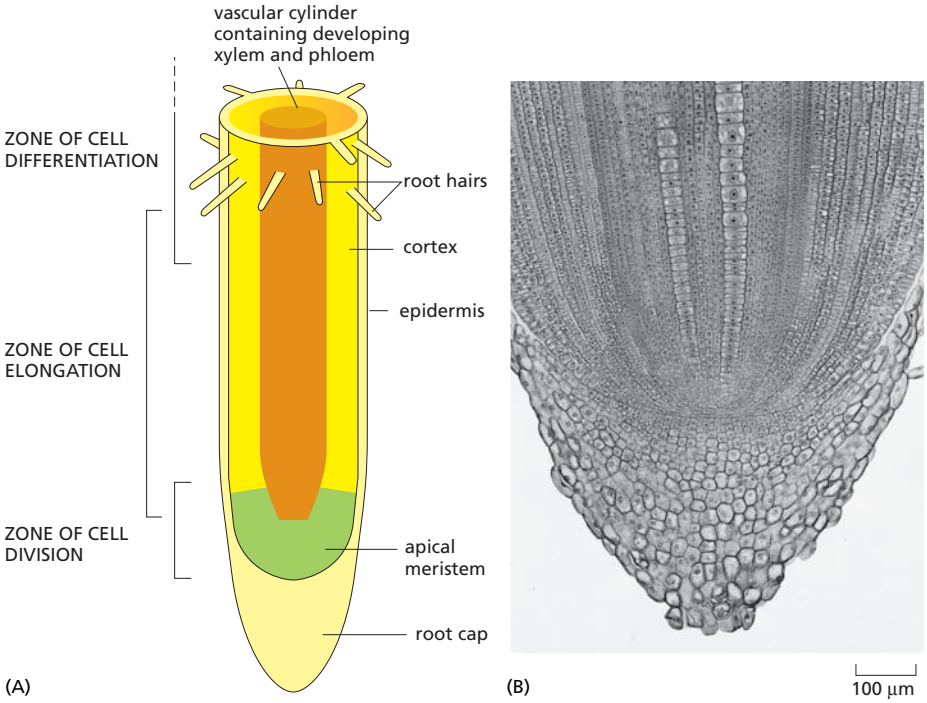


Figure 22–118 A growing root tip. (A) The organization of the final 2 mm of a growing root tip. The approximate zones in which cells can be found dividing, elongating, and differentiating are indicated. (B) The apical meristem and root cap of a corn root tip, showing the orderly files of cells produced. (B, from R.F. Evert, Biology of Plants, 4th ed. New York: Worth, 1986.)

Each Plant Module Grows From a Microscopic Set of Primordia in a Meristem

The apical meristems are self-perpetuating: in a perennial plant, they carry on with their functions indefinitely, as long as the plant survives, and they are responsible for its continuous growth and development. But apical meristems also give rise to a second type of outgrowth, whose development is strictly limited and culminates in the formation of a structure such as a leaf or a flower, with a determinate size and shape and a short lifespan. Thus, as a vegetative (nonflowering) shoot elongates, its apical meristem lays down behind itself an orderly sequence of *nodes*, where leaves have grown out, and *internodes* (segments of stem). In this way the continuous activity of the meristem produces an ever increasing number of similar modules, each consisting of a stem, a leaf, and a bud (see Figure 22–111). The modules are connected to one another by supportive and transport tissue, and successive modules are precisely located relative to each other, giving rise to a repetitively patterned structure. This iterative mode of development is characteristic of plants and is seen in many other structures besides the stem–leaf system (**Figure 22–120**).

Although the final module may be large, its organization, like that of an animal embryo, is mapped out at first on a microscopic scale. At the apex of the shoot, within a space of a millimeter or less, one finds a small, low central dome surrounded by a set of distinctive swellings in various stages of enlargement (**Figure 22–121**). The central dome is the apical meristem itself; each of the surrounding swellings is the primordium of a leaf. This small region, therefore, contains the already distinct rudiments of several entire modules. Through a well-

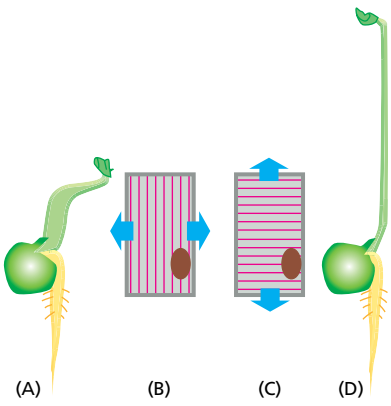
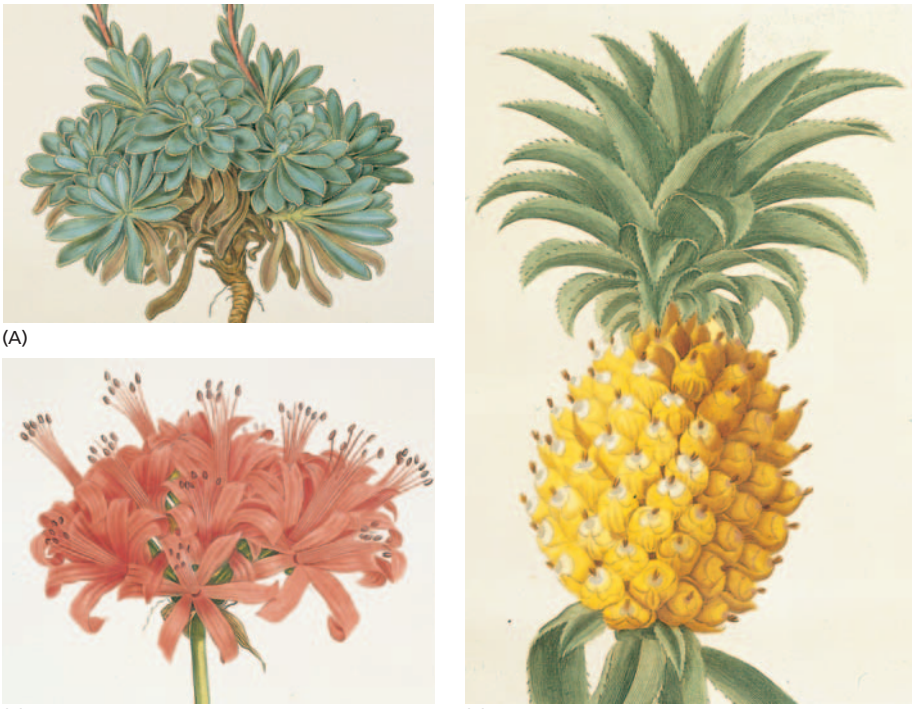


Figure 22–119 The different effects of the plant growth regulators ethylene and gibberellic acid. These regulators exert rapid and opposing effects on the orientation of the cortical microtubule array in cells of young pea shoots. A typical cell in an ethylene-treated plant (B) shows a net longitudinal orientation of microtubules, while a typical cell in a gibberellic-acid-treated plant (C) shows a net transverse orientation. New cellulose microfibrils are deposited parallel to the microtubules. Since this influences the direction of cell expansion, gibberellic acid and ethylene encourage growth in opposing directions: ethylene-treated seedlings will develop short, fat shoots (A), while gibberellic-acid-treated seedlings will develop long, thin shoots (D).



(A) (B) (C)

Figure 22–120 Repetitive patterning in plants. Accurate placing of successive modules from a single apical meristem produces these elaborate but regular patterns in leaves (A), flowers (B), and fruits (C). (A, from John Sibthorp, Flora Graeca. London: R. Taylor, 1806–1840; B, from Pierre Joseph Redouté, Les Liliacées. Paris: chez l'Auteur, 1807; C, from Christopher Jacob Trew, Uitgezochte planten. Amsterdam: Jan Christiaan Sepp, 1771—all courtesy of the John Innes Foundation.)

defined program of cell proliferation and cell enlargement, each leaf primordium and its adjacent cells will grow to form a leaf, a node, and an internode. Meanwhile, the apical meristem itself will give rise to new leaf primordia, so as to generate more and more modules in a potentially unending succession. The serial organization of the modules of the plant is thus controlled by events at the shoot apex.

Polarized Auxin Transport Controls the Pattern of Primordia in the Meristem

What are the signals that operate in the tiny apical region to determine the arrangement of primordia, and how are these signals generated in the appropriate pattern? A clue comes from mutation of a gene called *Pin1*, whose loss prevents formation of leaf primordia but allows the main stem to continue growing, producing a long thin bare structure shaped like a pin, with the apical meristem

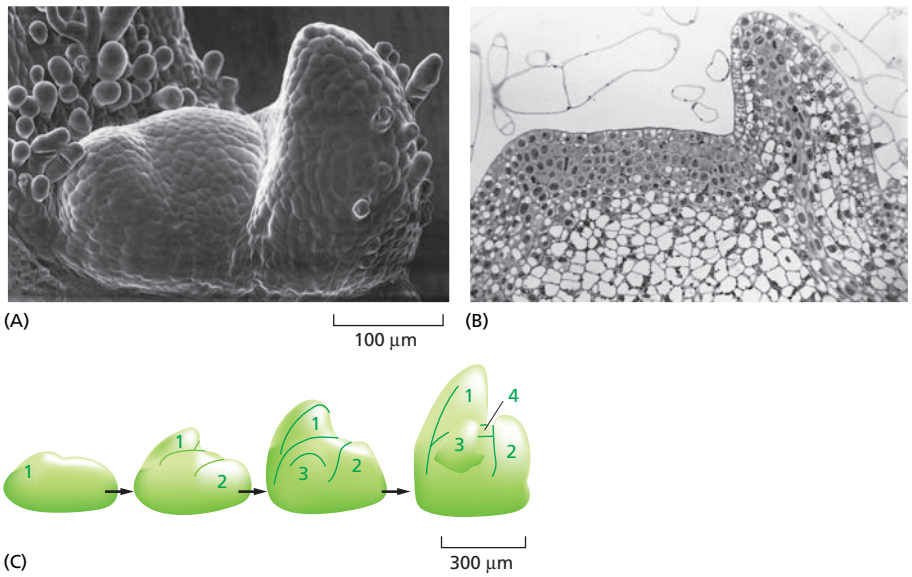
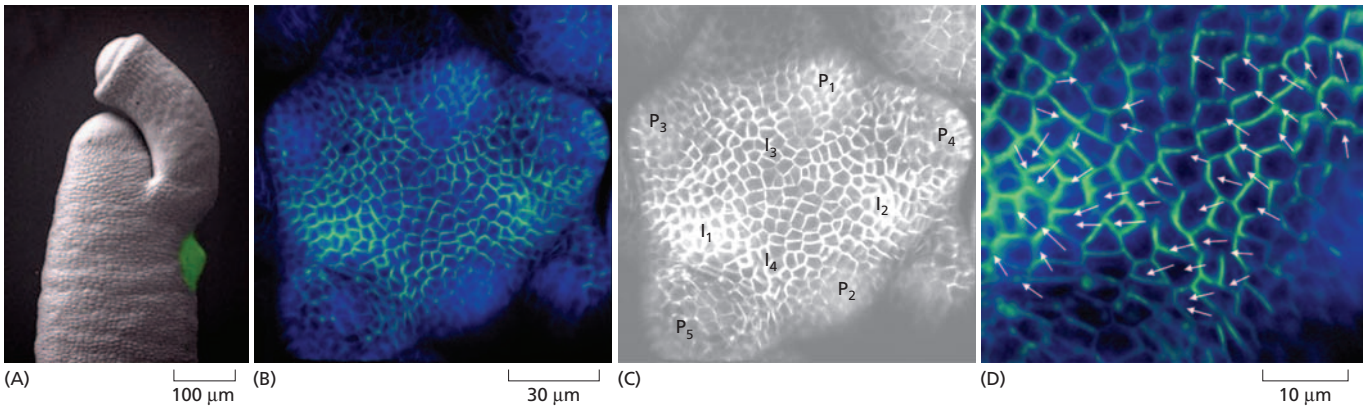


Figure 22–121 A shoot apex from a young tobacco plant. (A) A scanning electron micrograph shows the shoot apex with two sequentially emerging leaf primordia, seen here as lateral swellings on either side of the domed apical meristem. (B) A thin section of a similar apex shows that the youngest leaf primordium arises from a small group of cells (about 100) in the outer four or five layers of cells. (C) A very schematic drawing showing that the sequential appearance of leaf primordia takes place over a small distance and very early in shoot development. Growth of the apex will eventually form internodes that will separate the leaves in order along the stem (see Figure 22–111). (A and B, from R.S. Poethig and I.M. Sussex, *Planta* 165:158–169, 1985. With permission from Springer-Verlag.)



at its head. The Pin1 protein is an auxin transporter, driving efflux across the plasma membrane into the extracellular space. This suggests that the leaf primordia are missing in the mutant because the auxin distribution is wrong. Indeed, a microdroplet of auxin applied to one side of a *Pin1* or similar type of mutant apical meristem, on the side of the head of the "pin", will induce a leaf or flower primordium to form at the site of the auxin application (**Figure 22–122**A).

One can observe the distribution of Pin1 transporter protein in the living tissue by creating a transgenic (but otherwise normal) plant that expresses a form of Pin1 tagged with Green Fluorescent Protein (Figure 22–122B–D). In the outermost layer of meristem cells, the amount of Pin1 varies from region to region in a pattern that correlates with the pattern of developing primordia because the *Pin1* gene is upregulated by auxin. Moreover, the Pin1 protein is asymmetrically distributed in the membranes of the individual cells, so that they pump out more auxin on one side than the other, creating local maxima that specify where primordia will begin to form. The pumps appear to be concentrated on the side facing the neighbors whose own auxin concentration is the highest, suggesting that there is a positive feedback in the accumulation of auxin. Computer models show that positive feedback of this type can amplify asymmetry and generate a pattern of peaks and troughs of auxin concentration of the sort observed. Localized transport of auxin in a perpendicular direction, between the outer sheet of meristematic cells and the developing strands of vascular tissue below, contributes to the asymmetry. As the cells proliferate and the tissue grows, the distributions of Pin1 protein and of auxin adjust, producing new peaks and new lateral primordia in regular succession.

Variations on this basic repetitive theme can give rise to more complex architectures, including structures such as tendrils, leaves, branches, and flowers. Thus, by switching on different sets of genes at the shoot apex, the plant can produce different types of primordia, in different spatial patterns.

Figure 22–122 Control of patterning in a meristem by auxin and Pin1. (A) A microdroplet containing auxin *(green spot)* has been applied on one side of a mutant meristem, phenotypically similar to a *Pin1* mutant because it lacks a protein required for control of auxin transport. The auxin has induced formation of a lateral flower primordium. (B) Distribution of the Pin1 auxin transporter at a meristem. (B) An apical meristem of *Arabidopsis* is viewed from above by fluorescence microscopy, revealing the distribution of GFP-tagged Pin1 protein in the surface layer of cells. (C) The same image labeled to show the locations of the established primordia (P_1 being the most recently formed, P_4 being the most mature) and the predicted incipient primordia (I_1 being the next to form, I_4 being the one that lies farthest in the future). (D) Magnified part of (B), showing the asymmetric distribution of Pin1 in the membranes of the individual cells, driving auxin toward the site of an incipient primordium. *Arrows* indicate the direction of transport. As primordia become established, the amount of Pin1 in their surface layer declines, in part because further changes in the distribution of the transport proteins cause auxin to be pumped downward into the developing vascular tissue below. Complex patterns of auxin transport control the detailed structure of many other developing plant tissues also. (A, from D. Reinhardt et al., *Nature* 426:255–260, 2003. With permission from Macmillan Publishers Ltd; B–D, from M.G. Heisler et al., *Curr. Biol.* 15:1899–1911, 2005. With permission from Elsevier.)

Cell Signaling Maintains the Meristem

Central to all these phenomena is the question of how the apical meristem maintains itself. The meristem cells must continue to proliferate for weeks, years, or even centuries as a plant grows, replacing themselves while continually generating progeny cells that differentiate. Through all this, the size of the cluster of cells that constitute the meristem remains practically constant (about 100 cells in *Arabidopsis*, for example). New meristems may arise as the plant branches, but they too preserve the same size.

Genetic screens have identified genes required for meristem maintenance. For example, mutations that disrupt the *Wuschel* gene, which codes for a homeodomain protein, convert the apical meristem into non-meristematic tissue, so that the seedling fails to sprout. Conversely, mutations in the *Clavata* group of genes, coding for components of a cell–cell signaling pathway (see Figure 15–83), make the meristem abnormally big. These genes are expressed in different layers of cells in the meristem region (**Figure 22–123**A). The two most

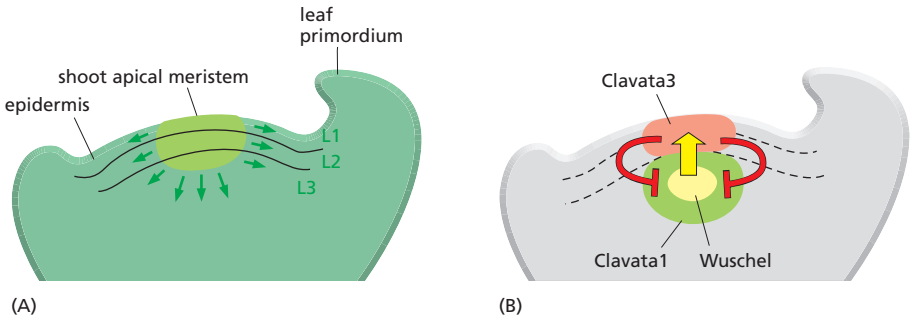


Figure 22–123 The feedback loops that are thought to maintain the shoot apical meristem. (A) The arrangement of cell layers constituting a shoot apical meristem. (B) The pattern of cell–cell communication that maintains the meristem. Artificial overexpression of *Wuschel* in the L3 region causes an increase in the number of cells in the L1 and L2 layers that behave as meristem cells and express *Clavata3*; artificial overexpression of *Clavata3* in the L1 and L2 layers causes a reduction of *Wuschel* expression in the L3 region below and a decrease in the number of meristem cells. *Clavata3* codes for a small signal protein, while *Clavata1* codes for its receptor, a transmembrane protein kinase. *Wuschel,* which is expressed in the central part of the region that expresses the receptor Clavata1, codes for a gene regulatory protein of the homeodomain class. The size of the meristem is thought to be controlled by a self-regulating balance between a short-range stimulatory signal produced by cells expressing Wuschel *(yellow arrow),* and a longer-range inhibitory signal delivered by Clavata3 *(red bars).*

superficial cell layers, called the L1 and L2 layers, together with the uppermost part of the L3 layer, contain the cells of the meristem proper, that is, the stem cells, capable of dividing indefinitely to give rise to future parts of the plant. The meristematic cells of the L1 and L2 layers express Clavata3, a small secreted signal protein. Just beneath, in the L3 layer, lies a cluster of cells expressing Clavata1 (the receptor for Clavata3). In the center of this Clavata1 patch are cells that express the Wuschel gene regulatory protein.

The pattern of cell divisions implies that the cells expressing Wuschel are not themselves part of the meristem proper; new Wuschel-expressing cells are apparently continually recruited from the meristematic (stem-cell) part of the L3 population, just above the Wuschel domain. Nevertheless, the Wuschel-expressing cells are at the heart of the mechanism that maintains the meristem. A signal that they produce maintains meristematic behavior in the cells above, stimulates expression of the *Clavata* genes, and, presumably, causes new cells recruited into the Wuschel domain to switch on Wuschel. Negative feedback from the upper meristematic cells, delivered by the Clavata signaling pathway, acts back on the regions below to limit the size of the Wuschel domain, thereby preventing the meristem from becoming too big (Figure 22–123B).

This account of the plant meristem, though uncertain in some details and certainly oversimplified, provides one of the clearest examples of an important general developmental strategy: it shows how a feedback loop involving a short-range activating signal (such as that produced by the Wuschel-expressing cells) and a long-range inhibitory signal (such as Clavata3) can stably maintain a signaling center of a well-defined size even when there is continual proliferation and turnover of the cells that form that center. As we pointed out at the beginning of this chapter, analogous systems of signals are thought to operate in animal development to maintain localized signaling centers—such as the Organizer of the amphibian gastrula, or the zone of polarizing activity in a limb bud. And just as this strategy serves in the mature plant to maintain its meristems, it may also serve in adult animal tissues such as the gut lining (discussed in Chapter 23) to maintain the all-important clusters of adult animal stem cells.

Regulatory Mutations Can Transform Plant Topology by Altering Cell Behavior in the Meristem

If a plant stem is to branch, new shoot apical meristems must be created, and this too depends on events in the neighborhood of the shoot apex. At each developing node, in the acute angle (the axil) between the leaf primordium and the stem, a bud is formed (**Figure 22–124**). This contains a nest of cells, derived from the apical meristem, that keep a meristematic character. They have the capacity to become the apical meristem of a new branch or the primordium of a structure such as a flower; but they also have the alternative option of remaining quiescent as *axillary buds*. The plant's pattern of branching is regulated through this choice of fate, and mutations that affect it can transform the structure of the plant. Maize provides a beautiful example.

Maize represents one of mankind's most remarkable feats of genetic engineering. Native Americans created it by selective breeding, over a period of several centuries or perhaps millennia between 5000 and 10,000 years ago. They started from a wild grass known as teosinte, with highly branched leafy stems

and tiny ears bearing hard, inedible kernels. Detailed genetic analysis has identified a handful of genetic loci—about five—as the sites of the mutations that account for most of the difference between this unpromising ancestor and modern corn. One of these loci, with a particularly dramatic effect, corresponds to a gene called *Teosinte branched-1 (Tb1)*. In maize with loss-of-function mutations in *Tb1*, the usual simple unbranched stem, with a few large leaves at intervals along it, is transformed into a dense, branching, leafy mass reminiscent of teosinte (**Figure 22–125**A). The pattern of branching in the mutant implies that axillary buds, originating in normal positions, have escaped from an inhibition that prevents them, in normal maize, from growing into branches.

In normal maize, the single stem is crowned with a tassel—a male flower—while a few of the axillary buds along the stem develop into female flowers and, upon fertilization, form the ears of corn that we eat. In the mutant maize with a defective *Tb1* gene, these fruitful axillary buds are transformed into branches bearing tassels. The wild teosinte plant is like the *Tb1*–defective maize in its leafy, highly branched appearance, but unlike this mutant it makes ears on many of its side branches, as though *Tb1* were active. DNA analysis reveals the explanation. Both teosinte and normal maize possess a functional *Tb1* gene, with an almost identical coding sequence, but in maize the regulatory region has undergone a mutation that boosts the level of gene expression. Thus, in normal maize the gene is expressed at a high level in every axillary bud, inhibiting branch formation, while in teosinte the expression in many axillary buds is low, so that branches are permitted to form (Figure 22–125B).

This example shows how simple mutations, by switching the behavior of meristem cells, can transform plant structure—a principle of enormous

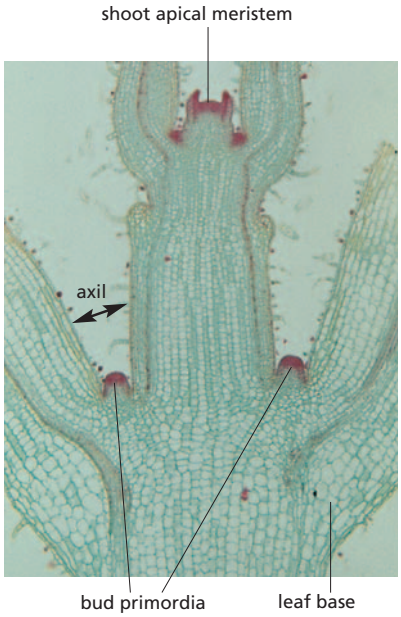


Figure 22–124 Axillary buds in the neighborhood of a shoot apex. The photograph shows a longitudinal section of *Coleus blumei,* a common houseplant. (From P.H. Raven, R.F. Evert and S.E. Eichhorn, Biology of Plants, 6th ed. New York: Freeman/Worth, 1999, used with permission.)

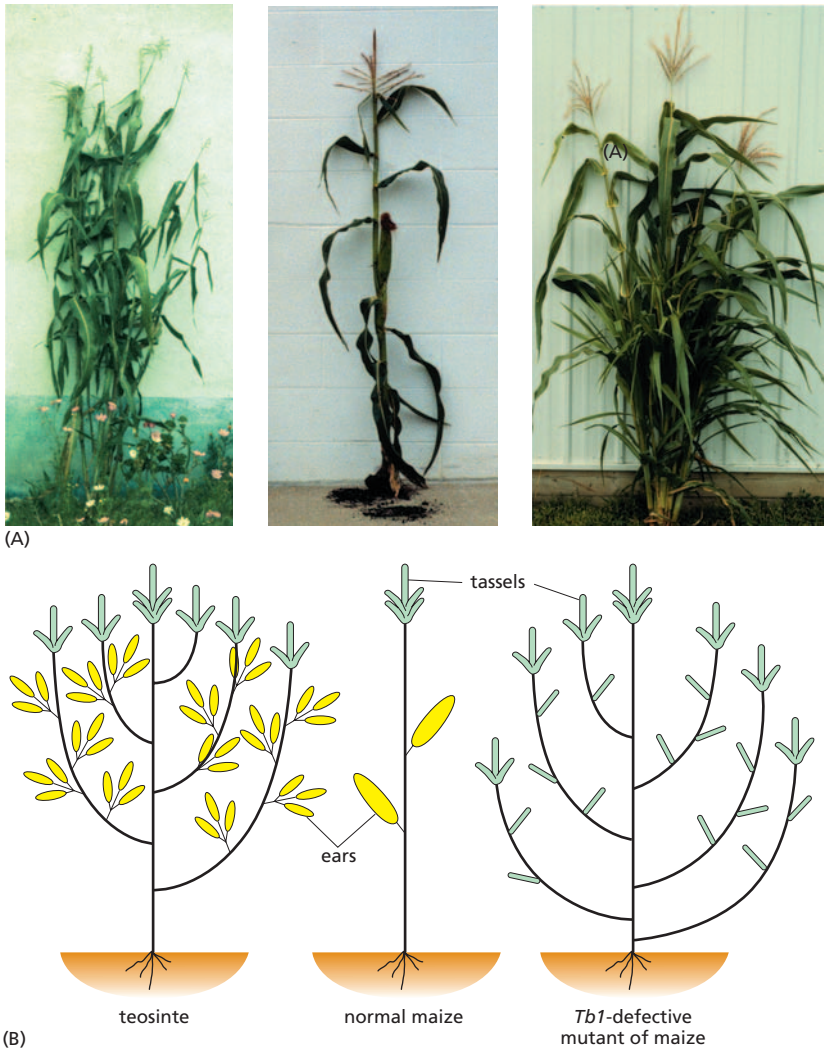


Figure 22–125 Transformation of plant architecture by mutation: a comparison of teosinte, normal maize, and *Tb1*-defective maize. (A) Photographs of the three types of plants. (B) The architecture of teosinte, normal maize and the *Tb1*-defective maize compared schematically. The *Tb1* gene product is needed for development of ears. It is absent in the *Tb1* mutant; it is present in both teosinte and normal maize, but these two plants differ because the gene is differently regulated. (A. left image, from J. Doebley and R.L. Wang, *Cold Spring Harbor Symp. Quant Biol.* 62:361–367, 1997. With permission from Cold Spring Harbor Laboratory Press; A, middle and right images. from J. Doebley, A. Stec and L. Hubbard, *Nature* 386:485-488, 1997. With permission from Macmillan Publishers Ltd.)

importance in the breeding of plants for food. More generally, the case of *Tb1* illustrates how new body plans, whether of plant or animal, can evolve through changes in regulatory DNA without change in the characters of the proteins made.

The Switch to Flowering Depends on Past and Present Environmental Cues

Meristems face other developmental choices besides that between quiescence and growth, as we have already seen in our discussion of maize, and these also are frequently regulated by the environment. The most important is the decision to form a flower (**Figure 22–126**).

The switch from meristematic growth to flower formation is triggered by a combination of cues. The plant does not merely take account of the current temperature, light intensity, and nutritional conditions; it bases its decision to flower on past conditions as well. One important cue, for many plants, is day length. To sense this, the plant uses its circadian clock—an endogenous 24-hour rhythm of gene expression—to generate a signal for flowering only when there is light for the appropriate part of the day. The clock itself is influenced by light, and the plant in effect uses the clock to compare past to present lighting conditions. Important parts of the genetic circuitry underlying these phenomena have been identified, from the phytochromes and cryptochromes that act as light receptors (discussed in Chapter 15) to the *Constans* gene, whose expression in the leaves of the plant represents a signal for flowering. The signal is thought to be relayed from the leaves to the meristem via the vasculature by the product of another gene, *Flowering locus T* (*Ft*), that is regulated by Constans.

But this signal itself will reach the meristem and trigger flowering only if the plant is in a receptive condition, typically depending on its history over a much longer period. Many plants will flower only if they have previously spent a long time in the cold: they must pass through winter before they will behave as though it is spring—a process called *vernalization*. The prolonged cold brings about changes in chromatin structure, dependent on another large collection of genes, including homologs of members of the *Polycomb* group that we mentioned earlier for their role in perpetuating patterns of gene expression in *Drosophila*. These epigenetic changes (discussed in Chapters 4 and 7) result in the gradual silencing of the *Flowering locus C* (*Flc*) gene. The effect is long-lasting, persisting through many rounds of cell division even as the weather grows warmer. *Flc* codes for an inhibitor of flowering, antagonizing the expression and action of *Ft*. Thus vernalization, by blocking production of the inhibitor, enables the meristem to receive the Ft signal and respond to it by switching on the expression of a set of *floral meristem-identity* genes in the apical meristem.

Mutations affecting the regulation of *Flc* expression alter the time of flowering and thus the ability of a plant to flourish in a given climate. The whole

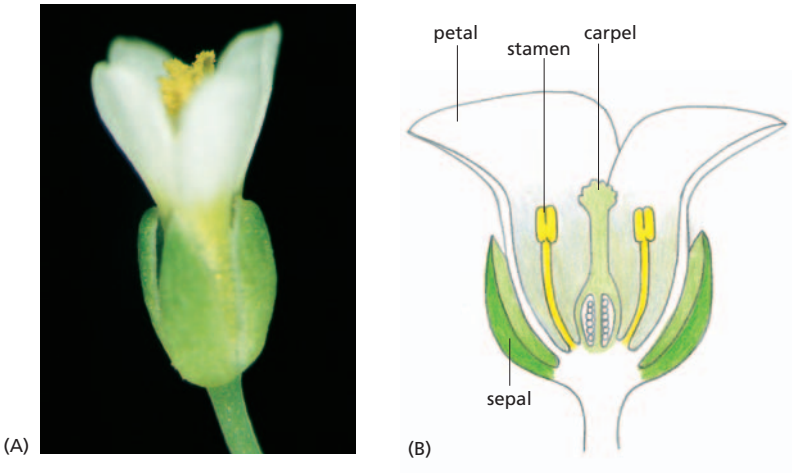


Figure 22–126 The structure of an *Arabidopsis* flower. (A) Photograph. (B) Schematic cross-sectional view. The basic plan, as shown in (B), is common to most flowering dicotyledonous plants. (A, courtesy of Leslie Sieburth.)

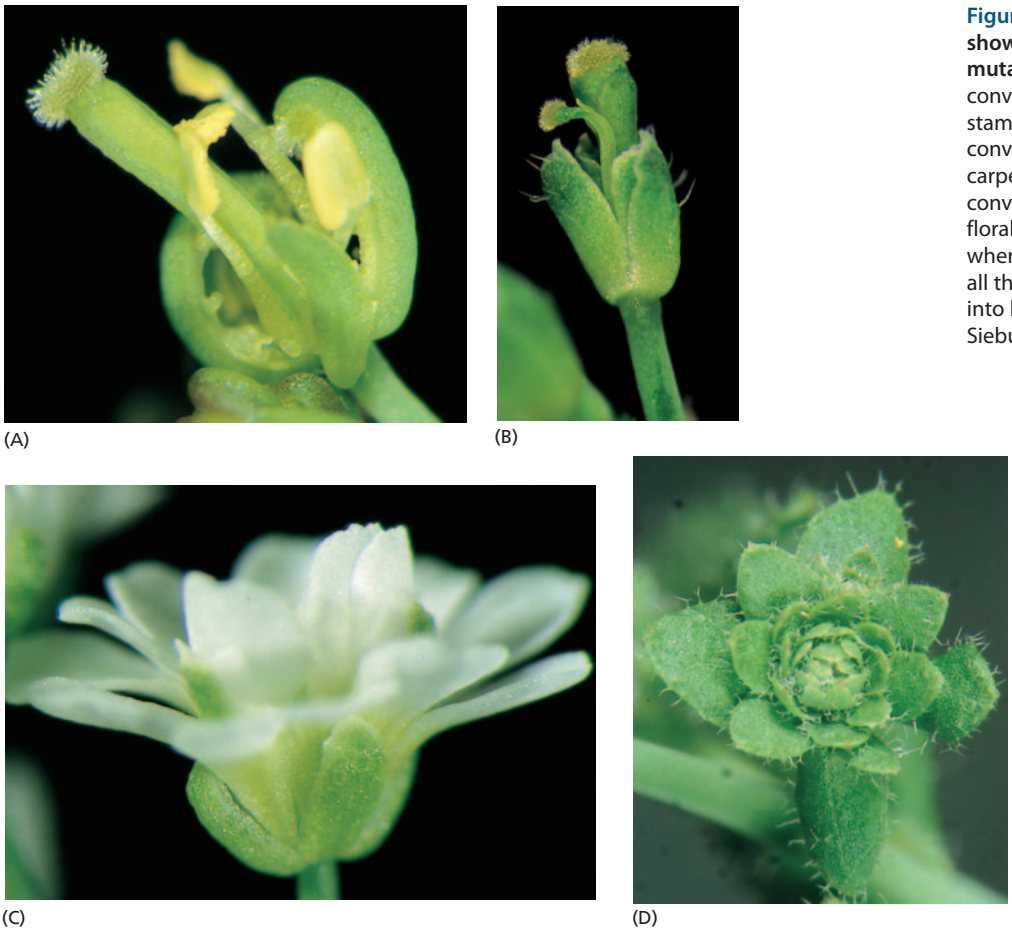


Figure 22–127 *Arabidopsis* flowers showing a selection of homeotic mutations. (A) In *Apetala2,* sepals are converted into carpels and petals into stamens; (B) In *Apetala3,* petals are converted into sepals and stamens into carpels; (C) In *Agamous,* stamens are converted into petals and carpels into floral meristem. (D) In a triple mutant where these three functions are defective, all the organs of the flower are converted into leaves. (A–C, courtesy of Leslie Sieburth; D, courtesy of Mark Running.)

control system governing the switch to flowering thus is of vital importance for agriculture, especially in an era of rapid climate change.

Homeotic Selector Genes Specify the Parts of a Flower

By switching on the floral meristem-identity genes, the apical meristem abandons its chances of continuing vegetative growth and gambles its future on the production of gametes. Its cells embark on a strictly finite program of growth and differentiation: by a modification of the ordinary mechanisms for generating leaves, a series of whorls of specialized appendages are formed in a precise order—typically sepals first, then petals, then stamens carrying anthers containing pollen, and lastly carpels containing eggs (see Panel 22–1). By the end of this process the meristem has disappeared, but among its progeny it has created germ cells.

The series of modified leaves forming a flower can be compared to the series of body segments forming a fly. In plants, as in flies, one can find homeotic mutations that convert one part of the pattern to the character of another. The mutant phenotypes can be grouped into at least four classes, in which different but overlapping sets of organs are altered (**Figure 22–127**). The first or 'A' class, exemplified by the *Apetala2* mutant of *Arabidopsis*, has its two outermost whorls transformed: the sepals are converted into carpels and the petals into stamens. The second or 'B' class, exemplified by *Apetala3,* has its two middle whorls transformed: the petals are converted into sepals and the stamens into carpels. The third or 'C' class, exemplified by *Agamous,* has its two innermost whorls transformed, with a more drastic consequence: the stamens are converted into petals, the carpels are missing, and in their place the central cells of the flower behave as a floral meristem, which begins the developmental performance all over again, generating another abnormal set of sepals and petals nested inside

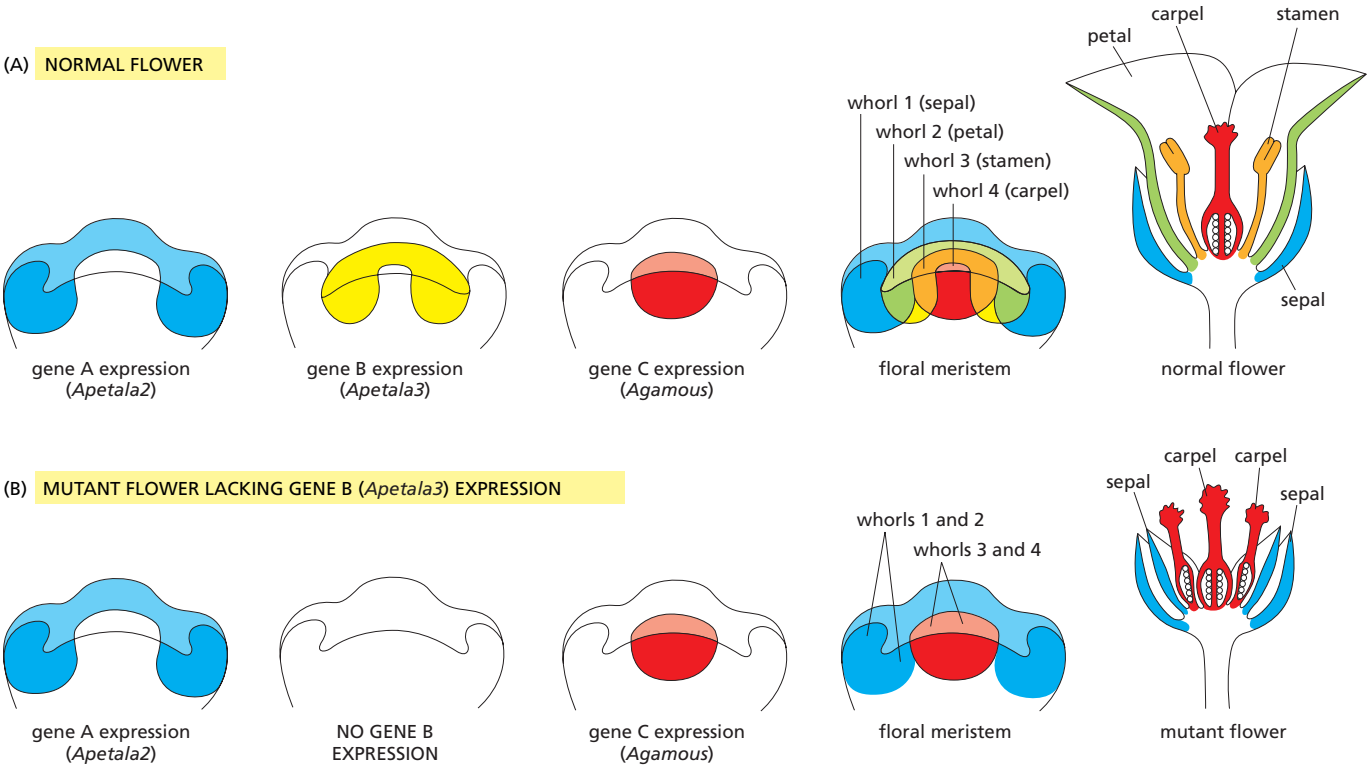


Figure 22–128 Homeotic selector gene expression in an *Arabidopsis* flower. (A) Diagram of the normal expression patterns of the three genes whose mutant phenotypes are illustrated in Figure 22–127A–C. All three genes code for gene regulatory proteins. The colored shading on the flower indicates which organ develops from each whorl of the meristem, but does not imply that the homeotic selector genes are still expressed at this stage. (B) The patterns in a mutant where the *Apetala3* gene is defective. Because the character of the organs in each whorl is defined by the set of homeotic selector genes that they express, the stamens and petals are converted into sepals and carpels. The consequence of a deficiency of a gene of class A, such as *Apetala2*, is slightly more complex: the absence of this class A gene product allows the class C gene to be expressed in the outer two whorls as well as the inner two, causing these outer whorls to develop as carpels and stamens, respectively. Deficiency of a class C gene prevents the central region from undergoing terminal differentiation as a carpel and causes it instead to continue growth as a meristem, generating more and more sepals and petals.

the first and, potentially, another nested inside that, and so on, indefinitely. A fourth class, the *Sepallata* mutants, has its three inner whorls all transformed into sepals.

These phenotypes identify four classes of homeotic selector genes, which, like the homeotic selector genes of *Drosophila*, all code for gene regulatory proteins. These are expressed in different domains and define the differences of cell state that give the different parts of a normal flower their different characters, as shown in **Figure 22–128**. The gene products collaborate to form protein complexes that drive expression of the appropriate downstream genes. In a triple mutant where the A, B, and C genetic functions are all absent, one obtains in place of a flower an indefinite succession of tightly nested leaves (see Figure 22–127D). Conversely, in a transgenic plant where genes of the A, B, and *Sepallata* classes are all expressed together outside their normal domains, leaves are transformed into petals. Leaves therefore represent a "ground state" in which none of these homeotic selector genes are expressed, while the other types of organ result from expressing the genes in different combinations.

Similar studies have been carried out in other plant species, and a similar set of phenotypes and genes have been identified: plants, no less than animals, have conserved their homeotic selector gene systems. Gene duplication has played a large part in the evolution of these genes: several of them, required in different organs of the flower, have clearly homologous sequences. These are not of the homeobox class but are members of another family of gene regulatory proteins (the so-called MADS family), also found in yeast and in vertebrates.

Clearly, plants and animals have independently found very similar solutions to many of the fundamental problems of multicellular development.

Summary

The development of a flowering plant, like that of an animal, begins with division of a fertilized egg to form an embryo with a polarized organization: the apical part of the embryo will form the shoot, the basal part, the root, and the middle part, the stem. At first, cell division occurs throughout the body of the embryo. As the embryo grows, however, addition of new cells becomes restricted to small regions known as meristems. Apical meristems, at shoot tips and root tips, will persist throughout the life of the plant, enabling it to grow by sequentially adding new body parts at its periphery. Typically, the shoot generates a repetitive series of modules, each consisting of a segment of stem, a leaf, and an axillary bud. Polarized transport of auxin controls the positioning of the primordia of these structures as they arise in the neighborhood of the meristem. An axillary bud is a potential new meristem, capable of giving rise to a side branch; the environment—and long-range hormonal signals within the plant can control the development of the plant by regulating bud activation. Mutations that alter the rules for activating axillary buds can have a drastic effect on the shape and structure of the plant; a single such mutation—one of about five key genetic alterations—accounts for a large part of the dramatic difference between modern maize and its wild ancestor, teosinte.

The small weed Arabidopsis thaliana *is widely used as a model organism for genetic studies and is the first plant to have had its genome completely sequenced. As in animals, genes governing plant development can be identified through genetic screens and their functions tested by genetic manipulations. Such studies have begun to reveal the molecular mechanisms by which the internal organization of each plant module is sketched out on a microscopic scale through cell–cell interactions in the neighborhood of the apical meristem. The meristem itself appears to be maintained by a local feedback loop, in which cells expressing the gene regulatory protein Wuschel provide a positive stimulus, and a negative feedback dependent on the Clavata cell–cell signaling pathway keeps the meristem from becoming too big.*

Environmental cues—especially light that is appropriately timed—can cause the expression of genes that switch the apical meristem from a leaf-forming to a flower-forming mode. The parts of a flower—its sepals, petals, stamens, and carpels—are formed by a modification of the mechanism for development of leaves, and the differences between these parts are controlled by homeotic selector genes that are closely analogous (although not homologous) to those of animals.

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