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

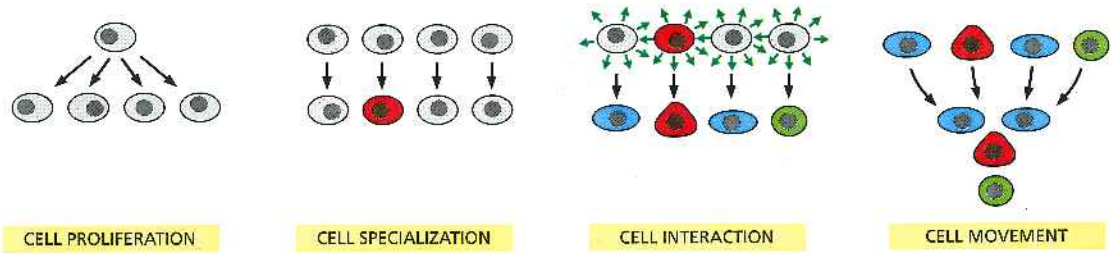


Figure 22–1 The four essential processes by which a multicellular organism is made: cell proliferation, cell specialization, cell interaction, and cell movement.

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.

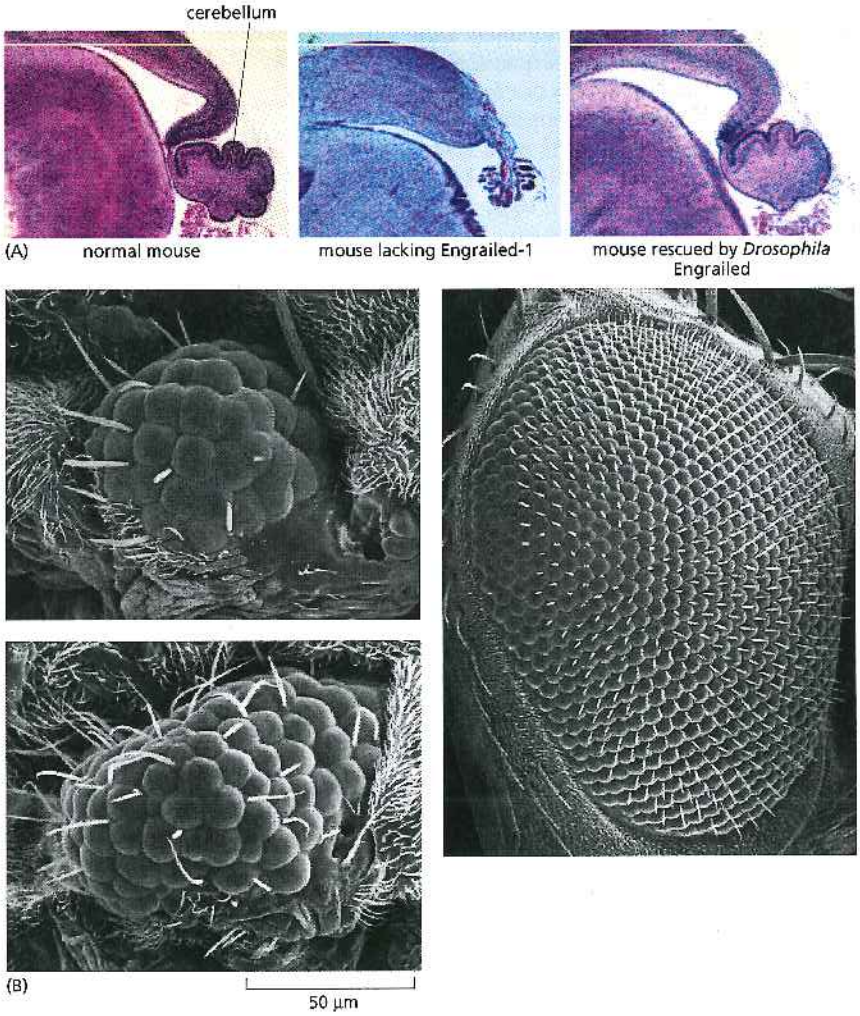


Figure 22–2 Homologous proteins functioning interchangeably in the development of mice and flies. (A) A fly protein used in a mouse. The DNA sequence from *Drosophila* coding for the Engrailed protein (a gene regulatory protein) can be substituted for the corresponding sequence coding for the Engrailed-1 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

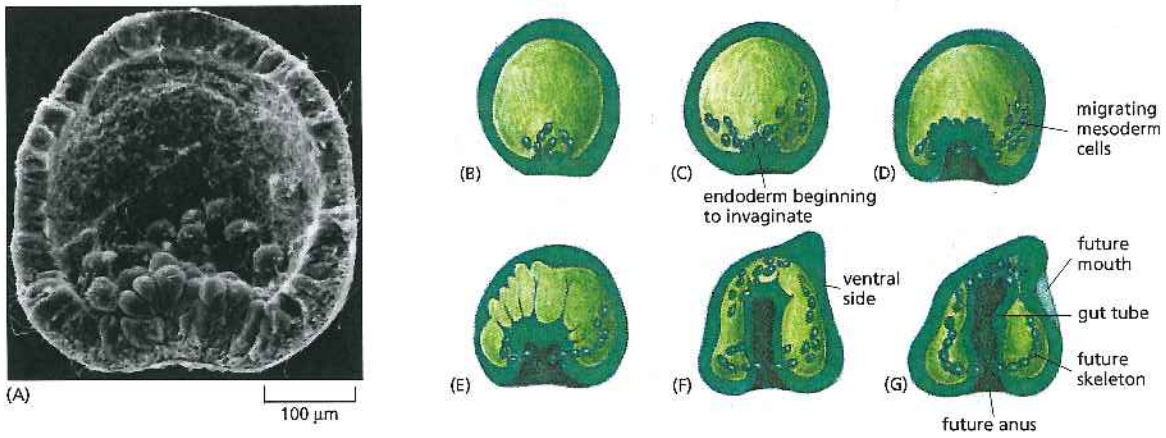
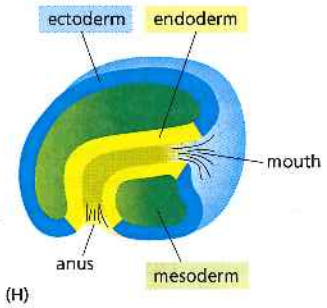


Figure 22–3 Sea urchin gastrulation. A fertilized egg divides to produce a blastula—a hollow sphere of epithelial cells surrounding a cavity. Then, in the process of gastrulation, some of the cells tuck into the interior to form the gut and other internal tissues. (A) Scanning electron micrograph showing 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

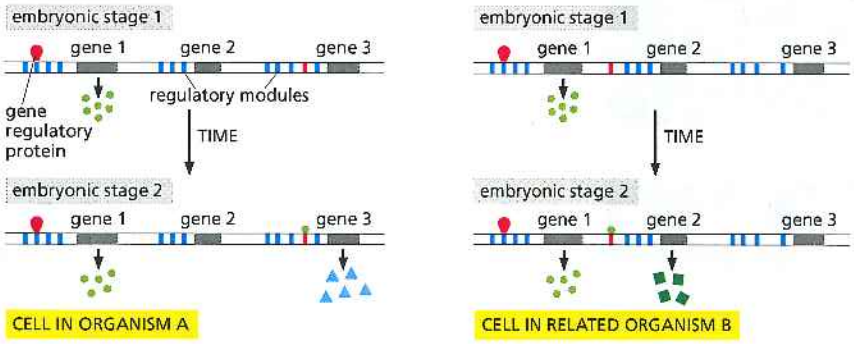


Figure 22–4 How regulatory DNA defines the succession of gene expression patterns in development. The genomes of organisms A and B code for the same set of proteins but have different regulatory DNA. The two cells in the cartoon start in the same state, expressing the same proteins at stage 1, but step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

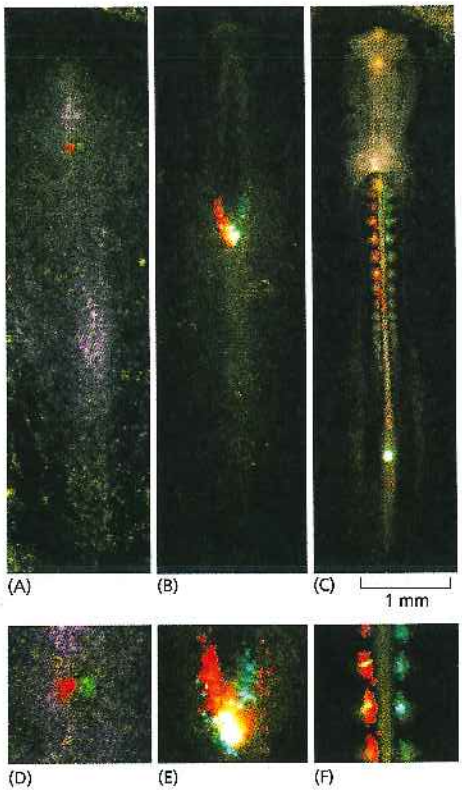


Figure 22–5 Cell lineage tracing in the early chick embryo. The pictures in the top row are at low magnification and show the whole embryo; the pictures below are details, showing the distribution of labeled cells. The tracing experiment reveals complex and dramatic cell rearrangements. (A,D) Two tiny 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.)

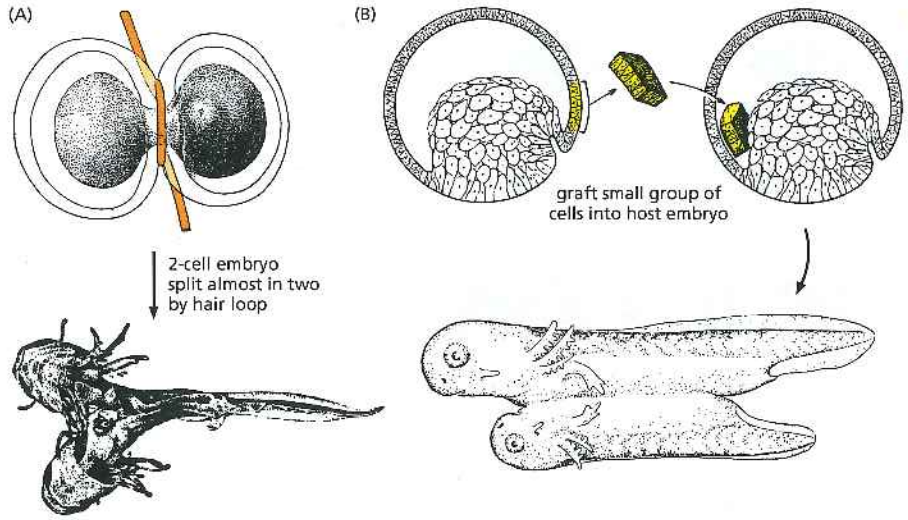


Figure 22–6 Some striking results obtained by experimental embryology. <ATTG> In (A), an early amphibian embryo is split almost into two parts with a hair loop. In (B), an amphibian embryo at a somewhat later stage receives a graft of a small cluster of cells from another embryo at that stage. The two 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

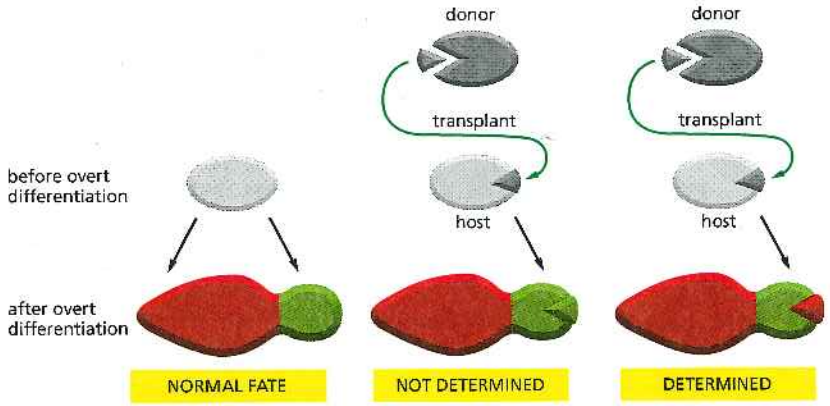


Figure 22–7 The standard test for cell determination.

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

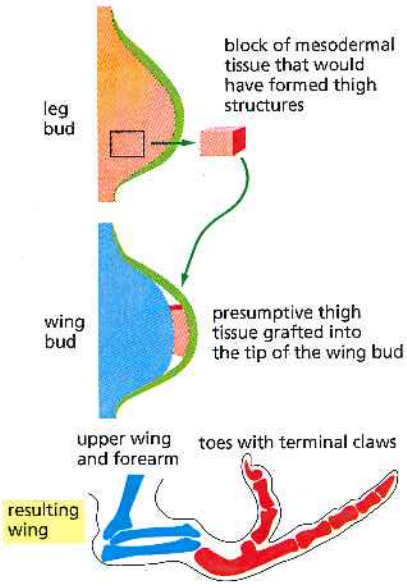


Figure 22–8 Prospective thigh tissue grafted into the tip of a chick wing bud forms toes. (After J.W. Saunders et al., *Dev. Biol.* 1:281–301, 1959. With permission from Academic Press.)

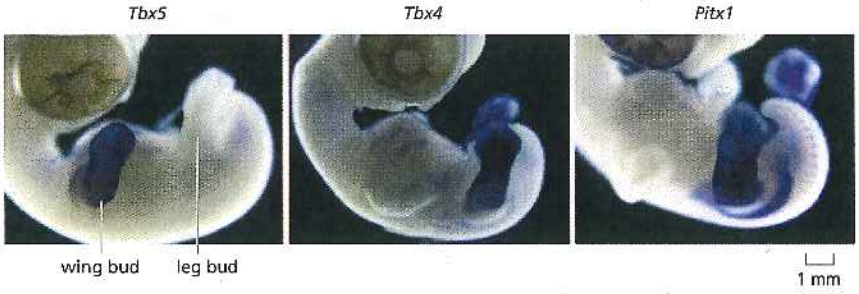


Figure 22–9 Chick embryos at 6 days of incubation, showing the limb buds stained by *in situ* hybridization with probes to detect expression of the *Tbx4*, *Tbx5*, and *Pitx1* genes, all coding for related gene regulatory proteins. The cells expressing *Tbx5* will form a wing; those expressing *Tbx4* 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.

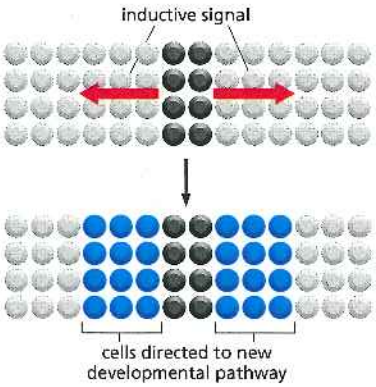


Figure 22–10 Inductive signaling.

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

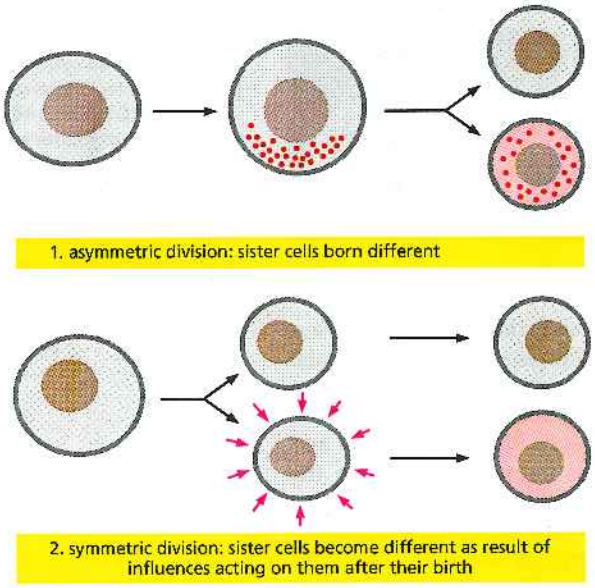


Figure 22–11 Two ways of making sister cells different.

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.

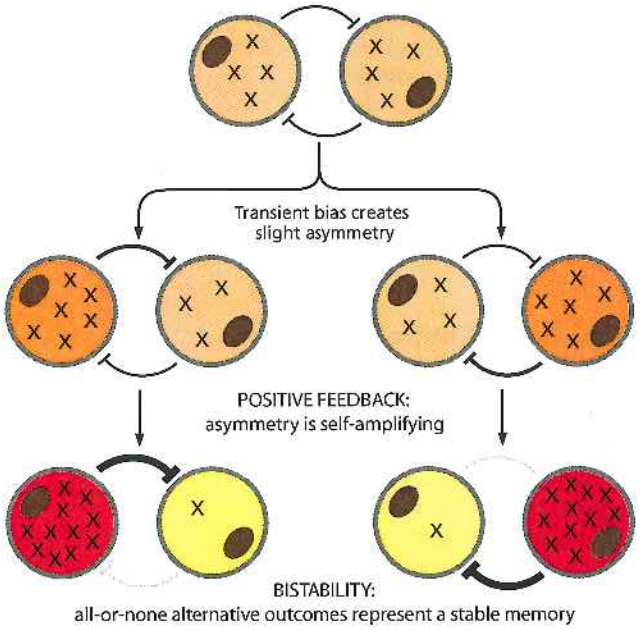


Figure 22–12 Genesis of asymmetry through positive feedback. In this example, two cells interact, each producing a substance X that acts on the other cell to inhibit its production of X, an effect known as lateral inhibition. An increase of X in one of the cells leads to a positive feedback that tends 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 FAMILY	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.

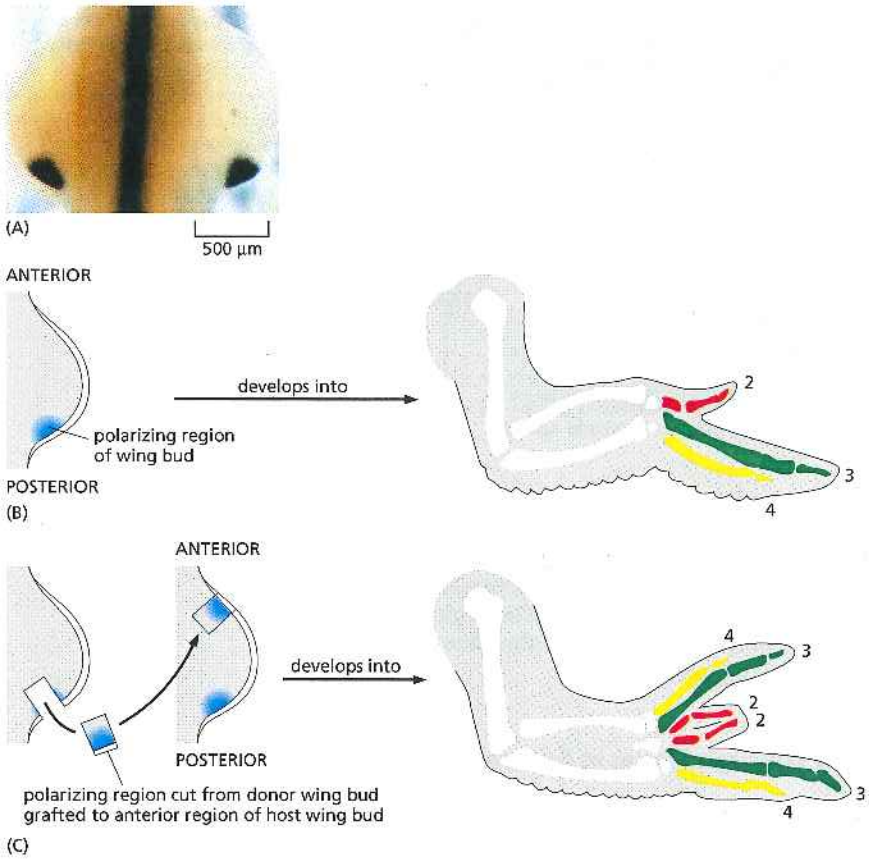


Figure 22–13 Sonic hedgehog as a morphogen in chick limb development. (A) Expression of the Sonic hedgehog gene in a 4-day chick embryo, shown by *in situ* hybridization (dorsal view of the trunk at the level of the wing buds). The gene is expressed in the midline of the body and at the posterior border (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

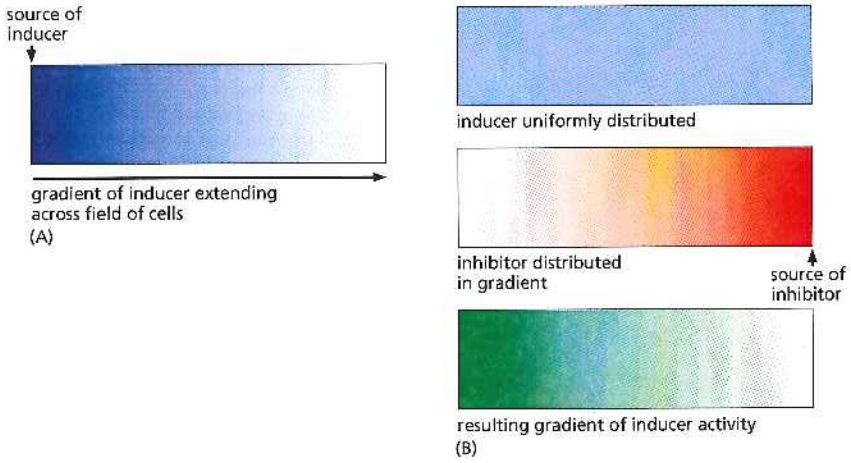


Figure 22–14 Two ways to create a morphogen gradient. (A) By localized production of an inducer—a morphogen—that diffuses away from its source. (B) By localized production of an inhibitor that diffuses away from its source and blocks the action of a uniformly distributed inducer.

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

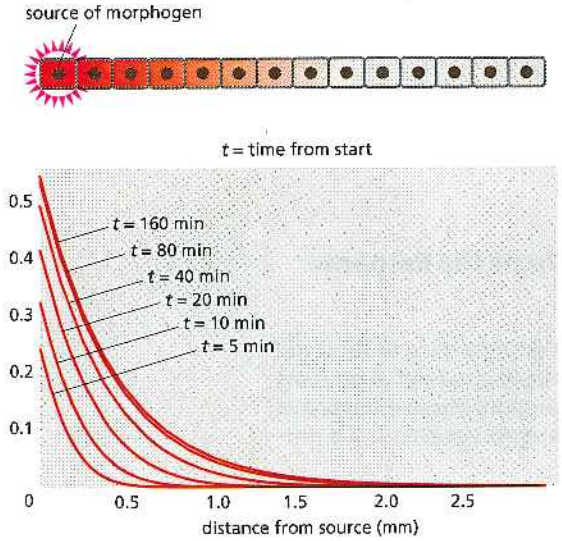


Figure 22–15 Setting up a signal gradient by diffusion. The graphs show successive stages in the build-up of the concentration of a signal molecule that is produced at a steady rate at the origin, with production starting at time 0. The molecule undergoes degradation as it diffuses away from the source, 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

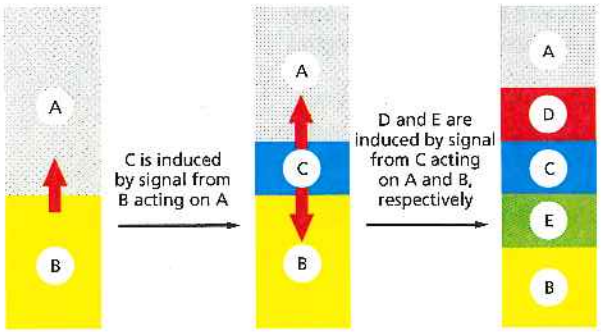


Figure 22–16 Patterning by sequential induction. A series of inductive interactions can generate many types of cells, starting from only a few.

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.

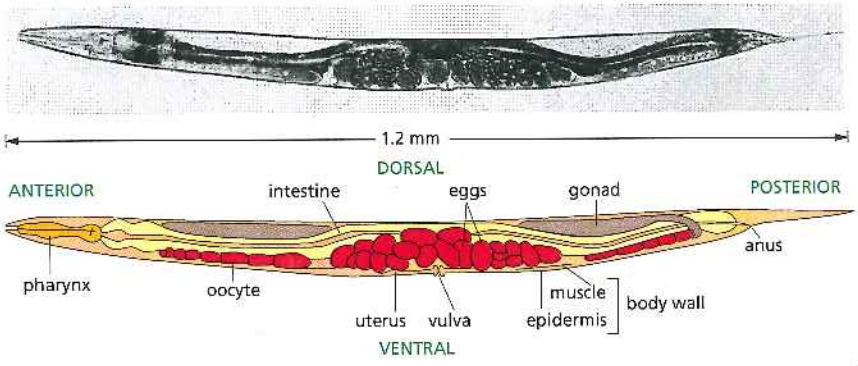


Figure 22–17 ***Caenorhabditis elegans.*** A side view of an adult hermaphrodite is shown. (From J.E. Sulston and H.R. Horvitz, *Dev. Biol.* 56:110–156, 1977. With permission from Academic Press.)