# Specialized Tissues, Stem Cells, and Tissue Renewal

# 23

Cells evolved originally as free-living individuals, but the cells that matter most to us, as human beings, are specialized members of a multicellular community. They have lost features needed for independent survival and acquired peculiarities that serve the needs of the body as a whole. Although they share the same genome, they are spectacularly diverse: there are more than 200 different named cell types in the human body (see our web site for a list). These collaborate with one another to form many different tissues, arranged into organs performing widely varied functions. To understand them, it is not enough to analyze them in a culture dish: we need also to know how they live, work, and die in their natural habitat, the intact body.

In Chapters 7 and 21, we saw how the various cell types become different in the embryo and how cell memory and signals from their neighbors enable them to remain different thereafter. In Chapter 19, we discussed the building technology of multicellular tissues—the devices that bind cells together and the extracellular materials that give them support. In this chapter, we consider the functions and lifestyles of the specialized cells in the adult body of a vertebrate. We describe how cells work together to perform their tasks, how new specialized cells are born, how they live and die, and how the architecture of tissues is preserved despite the constant replacement of old cells by new. We examine in particular the role played in many tissues by *stem cells*—cells that are specialized to provide an indefinite supply of fresh differentiated cells where these are lost, discarded, or needed in greater numbers.

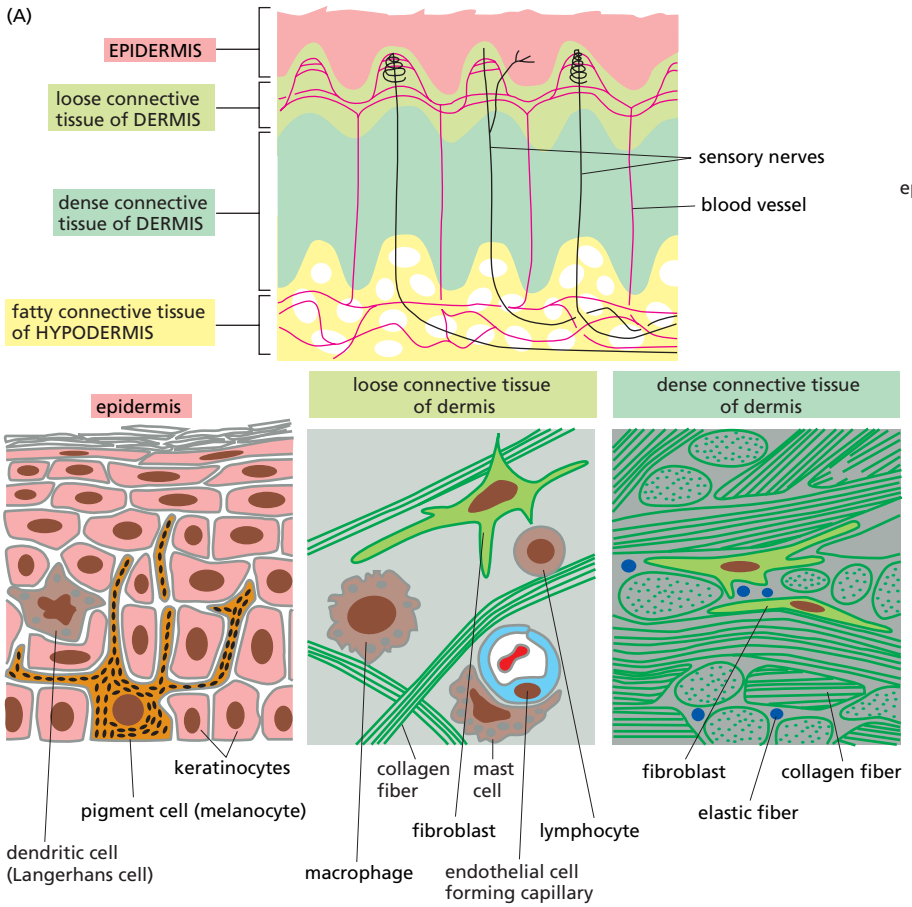
We discuss these topics through a series of examples—some chosen because they illustrate important general principles, others because they highlight favorite objects of study, still others because they pose intriguing problems that cell biology has yet to solve. Finally, we shall confront the practical question that underlies the current storm of interest in stem cells: How can we use our understanding of the processes of cell differentiation and tissue renewal to improve upon nature, and make good those injuries and failings of the human body that have hitherto seemed to be beyond repair?

## In This Chapter



## EPIDERMIS AND ITS RENEWAL BY STEM CELLS

We begin with a very familiar tissue: the skin. Like almost all tissues, skin is a complex of several different cell types. To perform its basic function as a barrier, the outer covering of the skin depends on a variety of supporting cells and structures, many of which are required in most other tissues also. It needs mechanical support, largely provided by a framework of extracellular matrix, mainly secreted by *fibroblasts*. It needs a blood supply to bring nutrients and oxygen and to remove waste products and carbon dioxide, and this requires a network of blood vessels, lined with *endothelial cells*. These vessels also provide access routes for cells of the immune system to defend against infection: *macrophages* and *dendritic* cells, to phagocytose invading pathogens and help activate *lymphocytes,* and lymphocytes themselves, to mediate more sophisticated adaptive immune system responses (discussed in Chapter 24). *Nerve fibers* are needed too, to convey sensory information from the tissue to the central nervous system, and to



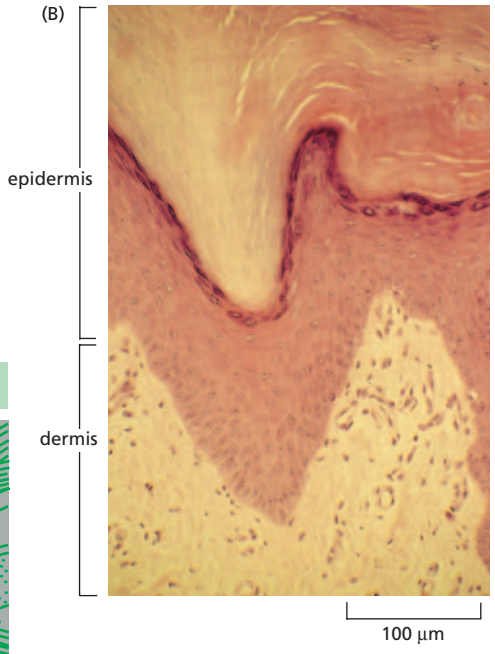




**Figure 23–1 Mammalian skin.** (A) These diagrams show the cellular architecture of thick skin. (B) Micrograph of a cross section through the sole of a human foot, stained with hematoxylin and eosin. The skin can be viewed as a large organ composed of two main tissues: the epidermis, and the underlying connective tissue, which consists of the dermis and the hypodermis. Each tissue is composed of several different cell types. The dermis and hypodermis are richly supplied with blood vessels and nerves. Some nerve fibers extend into the epidermis.

deliver signals in the opposite direction for glandular secretion and smooth muscle contraction.

**Figure 23–1** illustrates the architecture of the skin and shows how it satisfies all these requirements. An epithelium, the **epidermis**, forms the outer covering, creating a waterproof barrier that is self-repairing and continually renewed. Beneath this lies a relatively thick layer of connective tissue, which includes the tough collagen-rich **dermis** (from which leather is made) and the underlying fatty *subcutaneous layer* or *hypodermis*. In the skin, as elsewhere, the connective tissue, with vessels and nerves running through it, provides most of the general supportive functions listed above. The epidermis, however, is the fundamental, quintessential component of the skin—the tissue that is peculiar to this organ, even though not the major part of its bulk. Appendages such as hairs, fingernails, sebaceous glands, and sweat glands develop as specializations of the epidermis (**Figure 23–2**). Complex mechanisms regulate the distribution of these structures and their distinctive patterns of growth and renewal. The regions of less specialized, more or less flat epithelium covering the body surface between the hair follicles and other appendages are called *interfollicular epidermis*. This has a simple organization, and it provides a good introduction to the way in which tissues of the adult body are continually renewed.

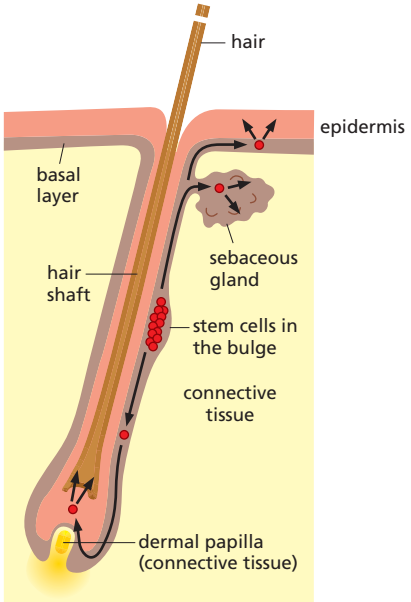



**Figure 23–2 A hair follicle and its associated sebaceous gland.** These structures form as specializations of the epidermis. The hair grows upward from the papilla at its base. The sebaceous gland contains cells loaded with lipid, which is secreted to keep the hair properly oiled. The whole structure undergoes cycles of growth, regression (when the hair falls out), and reconstruction. Like the rest of the epidermis, it depends on stem cells for its growth and reconstruction in each cycle. An important group of stem cells *(red),* able to give rise to both hair follicle and interfollicular epidermis, lie in a region called the bulge, just below the sebaceous gland.

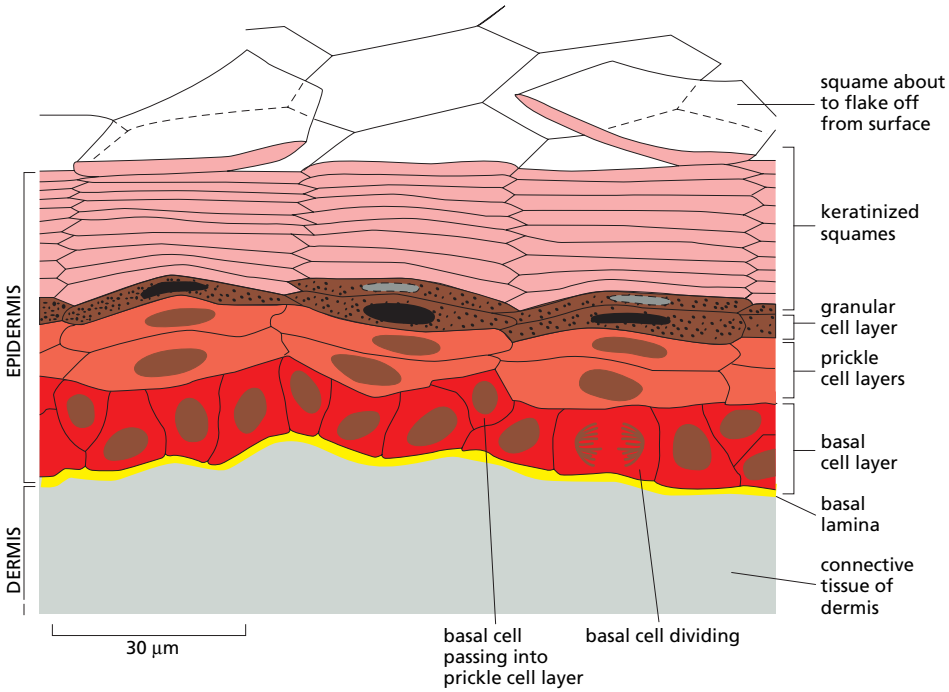



**Figure 23–3 The multilayered structure of the epidermis, as seen in a mouse.** The outlines of the keratinized squames are revealed by swelling them in a solution containing sodium hydroxide. The highly ordered hexagonal arrangement of interlocking columns of cells shown here occurs only in some sites where the epidermis is thin. In human skin, the stacks of squames are usually many times higher and less regular, and where the skin is very thick mitotic cells are seen not only in the basal layer but also in the first few cell layers above it. In addition to the cells destined for keratinization, the deep layers of the epidermis include small numbers of different types of cells, as indicated in Figure 23–1, including dendritic cells, called Langerhans cells, derived from bone marrow; melanocytes (pigment cells) derived from the neural crest; and Merkel cells, which are associated with nerve endings in the epidermis.

## Epidermal Cells Form a Multilayered Waterproof Barrier

The interfollicular epidermis is a multilayered *(stratified)* epithelium composed largely of *keratinocytes* (so named because their characteristic differentiated activity is the synthesis of keratin intermediate filament proteins, which give the epidermis its toughness) (**Figure 23–3**). These cells change their appearance from one layer to the next. Those in the innermost layer, attached to an underlying basal lamina, are termed *basal cells*, and it is usually only these that divide. Above the basal cells are several layers of larger *prickle cells* (**Figure 23–4**), whose numerous desmosomes—each a site of anchorage for thick tufts of keratin filaments—are just visible in the light microscope as tiny prickles around the cell surface (hence the name). Beyond the prickle cells lies the thin, darkly staining granular cell layer (see Figure 23–3). It is at this level that the cells are sealed together to form a waterproof barrier. Mice that fail to form this barrier because of a genetic defect die from rapid fluid loss soon after birth, even though their skin appears normal in other respects.

The granular layer, with its barrier to the movement of water and solutes, marks the boundary between the inner, metabolically active strata and the outermost layer of the epidermis, consisting of dead cells whose intracellular organelles have disappeared. These outermost cells are reduced to flattened scales, or *squames*, filled with densely packed keratin. The plasma membranes of both the squames and the outer granular cells are reinforced on their cytoplasmic surface by a thin (12 nm), tough, cross-linked layer of proteins, including a cytoplasmic protein called involucrin. The squames themselves are normally so compressed and thin that their boundaries are hard to make out in the light microscope, but soaking in sodium hydroxide solution (or a warm bath tub) makes them swell slightly, and their outlines can then be seen (see Figure 23–3).

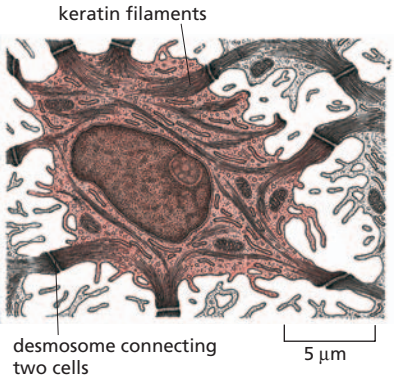



**Figure 23–4 A prickle cell.** This drawing, from an electron micrograph of a section of the epidermis, shows the bundles of keratin filaments that traverse the cytoplasm and are inserted at the desmosome junctions that bind the prickle cell *(red)* to its neighbors. Nutrients and water diffuse freely through the intercellular spaces in the metabolically active layers of the epidermis occupied by the prickle cells. Farther out, at the level of the granular cells, there is a waterproof barrier that is thought to be created by a sealant material that the granular cells secrete. (From R.V. Krstić, Ultrastructure of the Mammalian Cell: an Atlas. Berlin: Springer-Verlag, 1979.)

## Differentiating Epidermal Cells Express a Sequence of Different Genes as They Mature

Let us now set this static picture in motion to see how the epidermis is continually renewed. While some basal cells are dividing, adding to the population in the basal layer, others (their sisters or cousins) are slipping out of the basal cell layer into the prickle cell layer, taking the first step on their outward journey. When they reach the granular layer, the cells start to lose their nucleus and cytoplasmic organelles, through a degradative mechanism that involves partial activation of the machinery of apoptosis; in this way, the cells are transformed into the keratinized squames of the keratinized layer. These finally flake off from the surface of the skin (and become a main constituent of household dust). The time from birth of a cell in the basal layer of the human skin to its loss by shedding from the surface is about a month, depending on body region.

As the new keratinocyte in the basal layer is transformed into the squame in the outermost layers (see Figure 23–4), it steps through a succession of different states of gene expression, synthesizing a succession of different members of the keratin protein family. Meanwhile other characteristic proteins, such as involucrin, also begin to be synthesized as part of a coordinated program of **terminal cell differentiation**—the process in which a precursor cell acquires its final specialized characteristics and usually permanently stops dividing. The whole program is initiated in the basal layer. It is here that the fates of the cells are decided.

## Stem Cells in the Basal Layer Provide for Renewal of the Epidermis

Humans renew the outer layers of their epidermis a thousand times over in the course of a lifetime. In the basal layer, there have to be cells that can remain undifferentiated and carry on dividing for this whole period, continually throwing off descendants that commit to differentiation, leave the basal layer, and are eventually discarded. The process can be maintained only if the basal cell population is self-renewing. It must therefore contain some cells that generate a mixture of progeny, including daughters that remain undifferentiated like their parent, as well as daughters that differentiate. Cells with this property are called **stem cells**. They have so important a role in such a variety of tissues that it is useful to have a formal definition.

The defining properties of a stem cell are as follows:

1. It is not itself terminally differentiated (that is, it is not at the end of a pathway of differentiation).
2. It can divide without limit (or at least for the lifetime of the animal).
3. When it divides, each daughter has a choice: it can either remain a stem cell, or it can embark on a course that commits it to terminal differentiation (**Figure 23–5**).

Stem cells are required wherever there is a recurring need to replace differentiated cells that cannot themselves divide. The stem cell itself has to be able to divide—that is part of the definition—but it should be noted that it does not necessarily have to divide rapidly; in fact, stem cells usually divide at a relatively slow rate.

The need for stem cells arises in many different tissues. Thus, stem cells are of many types, specialized for the genesis of different classes of terminally differentiated cells—epidermal stem cells for epidermis, intestinal stem cells for intestinal epithelium, hemopoietic stem cells for blood, and so on. Each stem-cell system nevertheless raises similar fundamental questions. What are the distinguishing features of the stem cell in molecular terms? What factors determine

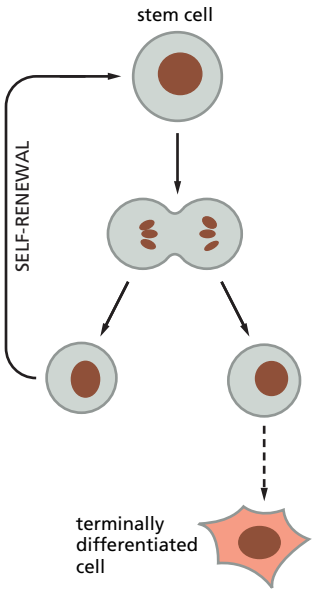



**Figure 23–5 The definition of a stem cell.** Each daughter produced when a stem cell divides can either remain a stem cell or go on to become terminally differentiated. In many cases, the daughter that opts for terminal differentiation undergoes additional cell divisions before terminal differentiation is completed.

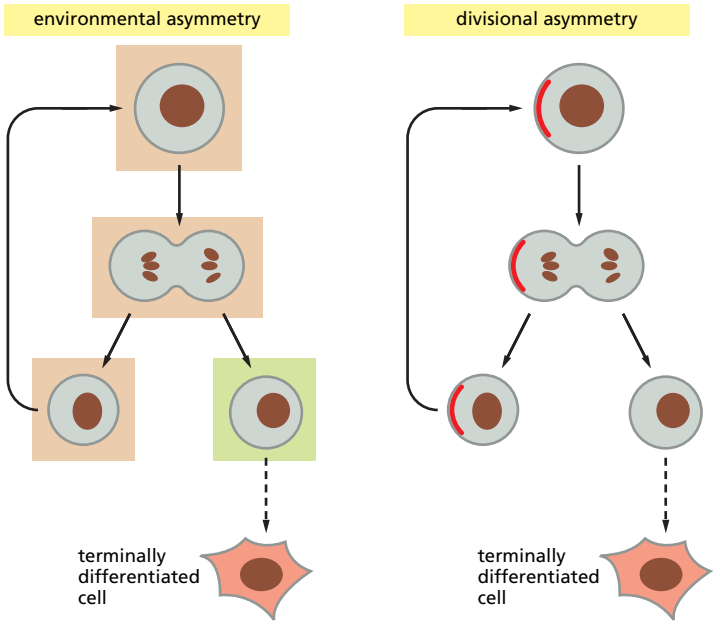



**Figure 23–6 Two ways for a stem cell to produce daughters with different fates.** In the strategy based on environmental asymmetry, the daughters of the stem cell are initially similar and are directed into different pathways according to the environmental influences that act on them after they are born. The environment is shown as *colored shading* around the cell. With this strategy, the number of stem cells can be increased or reduced to fit the niche available for them. In the strategy based on divisional asymmetry, the stem cell has an internal asymmetry and divides in such a way that its two daughters are already endowed with different determinants at the time of their birth. In some cases, the choice between the alternative fates may be made at random for each daughter, but with a defined probability, like a coin-toss, reflecting the intrinsic randomness or "noise" in all genetic control systems (discussed in Chapter 7).

whether it divides or stays quiescent? What decides whether a given daughter cell commits to differentiation or remains a stem cell? And where the stem cell can give rise to more than one kind of differentiated cell—as is very often the case—what determines which differentiation pathway is followed?

## The Two Daughters of a Stem Cell Do Not Always Have to Become Different

At steady state, to maintain a stable stem-cell population, precisely 50% of the daughters of stem cells in each cell generation must remain as stem cells. In principle, this could be achieved in two ways—through *environmental asymmetry* or through *divisional asymmetry* (**Figure 23–6**). In the first strategy, the division of a stem cell could generate two initially similar daughters whose fates would be governed by their subsequent environment or by some random process with an appropriate environmentally controlled probability; 50% of the *population* of daughters would remain as stem cells, but the two daughters of an individual stem cell in the population might often have the same fate. At the opposite extreme, the stem cell division could be always strictly asymmetric, producing one daughter that inherits the stem-cell character and another that inherits factors that force it to embark on differentiation. The neuroblasts of the *Drosophila* central nervous system, discussed in Chapter 22, are an example of cells that show this type of divisional asymmetry. This strategy in its strict form has a drawback, however: it means that the existing stem cells can never increase their numbers, and any loss of stem cells is irreparable, unless by recruitment of some other type of cell to become a stem cell. The strategy of control by environmental asymmetry is more flexible.

In fact, if a patch of epidermis is destroyed, the surrounding epidermal cells repair the damage by migrating in and proliferating to cover the denuded area. In this process, a new self-renewing patch of epidermis is established, implying that additional stem cells have been generated to make up for the loss. These must have been produced by symmetric divisions in which one stem cell gives rise to two. In this way, the stem cell population adjusts its numbers to fit the available niche.

Observations such as these suggest that the maintenance of stem cell character in the epidermis might be controlled by contact with the basal lamina, with a loss of contact triggering the start of terminal differentiation, and maintenance of contact serving to preserve stem cell potential. This idea contains a grain of truth, but it is not the whole truth. As we now explain, not all the cells in the basal layer have the capacity to serve as stem cells.

## The Basal Layer Contains Both Stem Cells and Transit Amplifying Cells

Basal keratinocytes can be dissociated from intact epidermis and can proliferate in a culture dish, giving rise to new basal cells and to terminally differentiated cells. Even within a population of cultured basal keratinocytes that all seem undifferentiated, there is great variation in the ability to proliferate. When human keratinocytes are taken singly and tested for their ability to found new colonies, some seem unable to divide at all, others go through only a few division cycles and then halt, and still others divide enough times to form large colonies. This proliferative potential directly correlates with the expression of the β1 subunit of integrin, which helps mediate adhesion to the basal lamina. Clusters of cells with high levels of this molecule are found in the basal layer of the intact human epidermis also, and they are thought to contain the stem cells (**Figure 23–7**). We still do not have definitive markers for the stem cells themselves, and we still do not understand in molecular terms what it is that fundamentally defines the stem-cell state. This is one of the key problems of stem-cell biology, and we shall say more about it in later sections of the chapter.

Paradoxically, many if not all of the epidermal cells that generate large colonies in culture seem to be cells that themselves as a rule divide rarely. One line of evidence comes from experiments in which a pulse of the thymidine analog bromodeoxyuridine (BrdU) is given to a young animal, in which the epidermis is growing rapidly, or to a mature animal following an injury that provokes rapid repair. One then waits for many days or weeks before fixing the tissue and staining with an antibody that recognizes DNA in which BrdU has been incorporated. The BrdU is taken up by any cell that is in S phase of the division cycle at the time of the initial pulse. Because the BrdU would be expected then to be diluted by half at each subsequent cell division, any cells that remain strongly labeled at the time of fixation are assumed to have undergone few or no divisions since replicating their DNA at the time of the pulse. Such *label-retaining cells* can be seen scattered among unlabeled or lightly labeled cells in the basal layer of the epidermis even after a period of several months, and large numbers are visible in hair follicles, in a region called the bulge (see Figure 23–2). Ingenious labeling procedures indicate that the label-retaining cells, in the hair follicle at least, are in fact stem cells: when a new cycle of hair growth begins after an old hair has been shed, the label-retaining cells in the bulge at last divide and contribute the

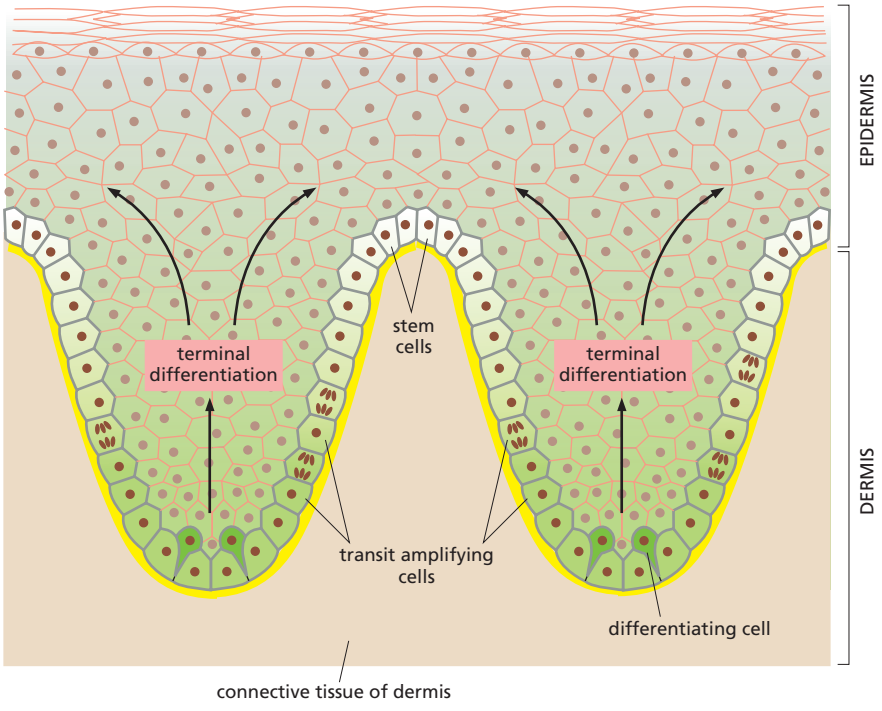



**Figure 23–7 The distribution of stem cells in human epidermis, and the pattern of epidermal cell production.** The diagram is based on specimens in which the location of the stem cells was identified by staining for β1 integrin, and that of the differentiating cells by staining for keratin-10, a marker of keratinocyte differentiation; dividing cells were identified by labeling with BrdU, a thymidine analog that is incorporated into cells in S phase of the cell division cycle. The stem cells seem to be clustered near the tips of the dermal papillae. They divide infrequently, giving rise (through a sideways movement) to transit amplifying cells, which occupy the intervening regions. The transit amplifying cells divide frequently, but for a limited number of division cycles, at the end of which they begin to differentiate and slip out of the basal layer. The precise distribution of stem cells and transit amplifying cells varies from one region of epidermis to another. (Adapted from S. Lowell et al., *Curr. Biol.* 10:491–500, 2000. With permission from Elsevier.)

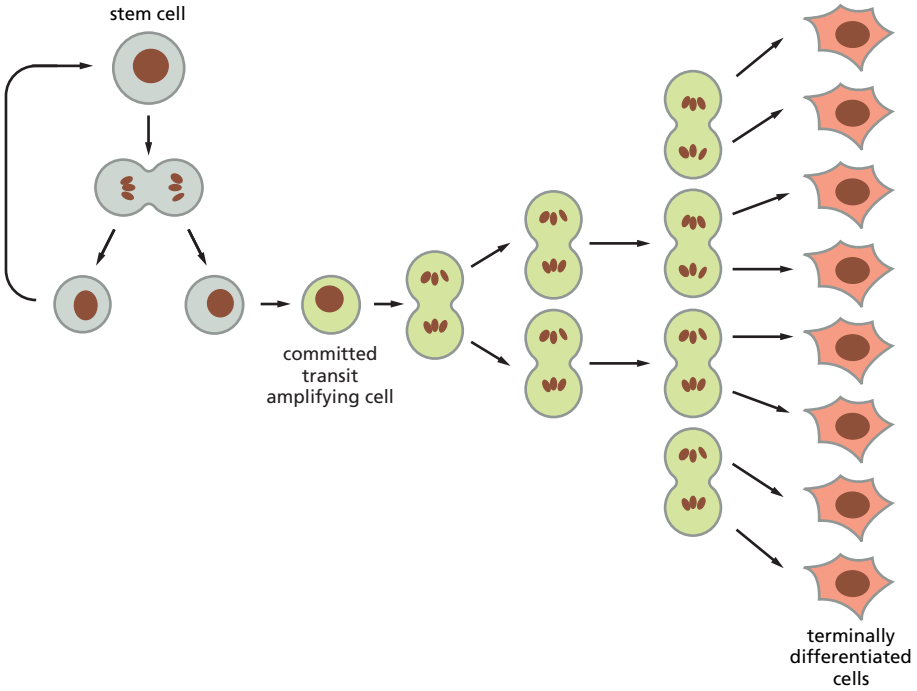



**Figure 23–8 Transit amplifying cells.** Stem cells in many tissues divide only rarely but give rise to transit amplifying cells—daughters committed to differentiation that go through a limited series of more rapid divisions before completing the process. In the example shown here, each stem cell division gives rise in this way to eight terminally differentiated progeny.

cells that go to form the regenerated hair follicle. Although it is not certain that all the stem cells of the hair follicle have this label-retaining character, some clearly do, and the same seems to be true of the stem cells in the interfollicular epidermis. Moreover, basal cells expressing β1 integrin at a high level—the cells that can give rise to large colonies in culture —are rarely seen dividing.

Mixed with these cells there are others that divide more frequently—but only for a limited number of division cycles, after which they leave the basal layer and differentiate. These latter cells are called **transit amplifying cells**—"transit", because they are in transit from a stem-cell character to a differentiated character; and "amplifying", because the division cycles they go through have the effect of amplifying the number of differentiated progeny that result from a single stem-cell division (**Figure 23–8**). In this way, a small population of stem cells that divide only rarely can generate a plentiful supply of new differentiated cells.

## Transit Amplifying Divisions Are Part of the Strategy of Growth Control

Transit amplifying cells are a common feature of stem cell systems. This means that in most such systems there are few true stem cells and they are mixed with a much larger number of progeny cells that have only a limited capacity to divide. As discussed in Chapter 20, the same seems to be true not only of normal self-renewing tissues but also for many cancers, where only a small minority of cells in the tumor cell population are capable of serving as cancer stem cells. Why should this be? There are several possible answers, but a part of the explanation probably lies in the strategy by which large multicellular animals (such as mammals) control the sizes of their cell populations.

The proportions of the parts of the body are mostly determined early, during development, by means of signals that operate over distances of a few hundred cell diameters at most: for each organ or tissue, a small rudiment or founder cell population is delimited in this way. The founder cell populations must then grow, but—in mammals at least—only up to a certain definite limit, at which point they must stop.

One way to halt growth at a certain size is by feedback signals that operate over much larger distances in the mature organism; we shall see that such signals indeed play an important part in controlling the growth of at least some tissues. Another strategy, however, is to endow each founder cell with an internal

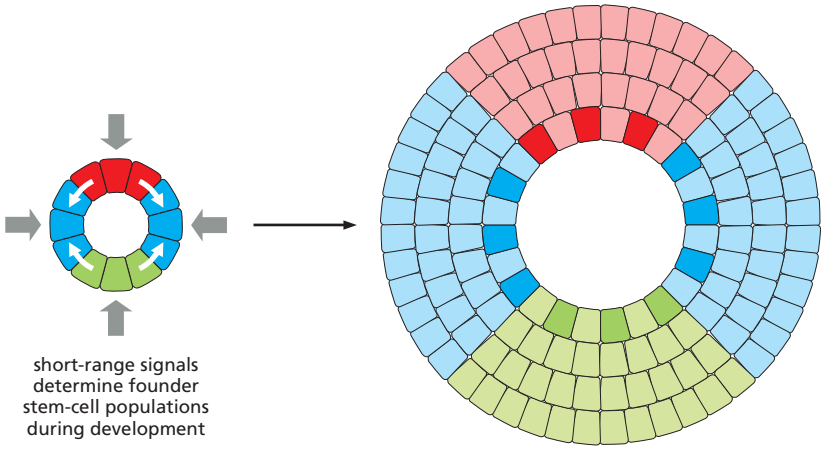



**Figure 23–9 One way to define the size of a large organ.** In the embryo, short-range signals determine small groups of cells as founders of the different cell populations. Each founder can be programmed then to divide a certain number of times, giving rise to a large set of cells in the adult. If the adult organ is to be renewed while maintaining its proper size, the founders can be programmed to divide as stem cells, giving rise at each division, on average, to one daughter that remains as a stem cell and another that is programmed to go through a set number of transit amplifying divisions.

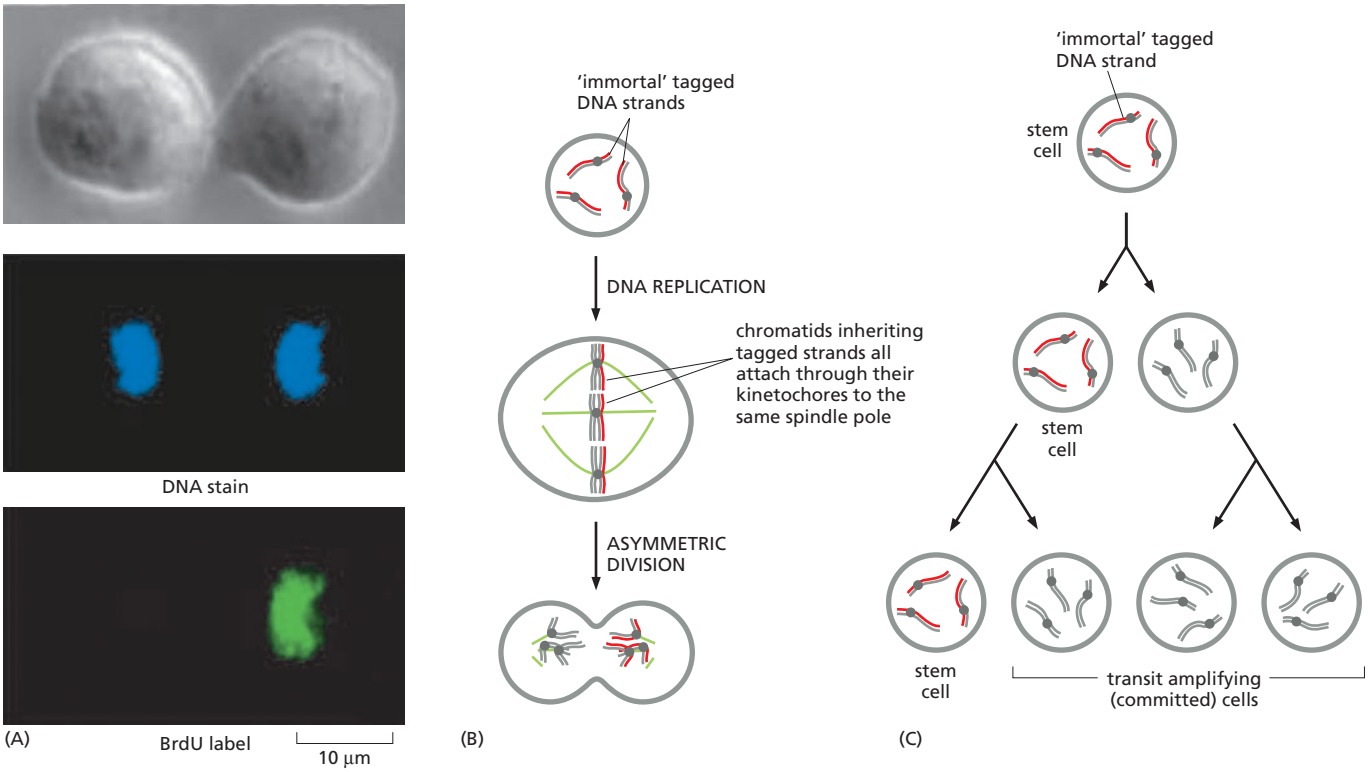
program dictating that it shall divide a limited number of times and then stop. In this way, short-range signals during development can define the size of large final structures (**Figure 23–9**). But if that is the strategy, how can the adult tissue be continually renewed? A solution is to specify the founder cells as stem cells, able to continue dividing indefinitely, but producing at each division one daughter that remains as a stem cell and one that is programmed to go through a limited number of transit amplifying divisions and then stop.

This is certainly an oversimplified and incomplete account of the control of tissue growth and renewal, but it helps to explain why cells that are programmed to undergo long sequences of cell divisions and then halt are such a common feature of animal development and why tissue renewal by stem cells so often involves transit amplifying divisions.

## Stem Cells of Some Tissues Selectively Retain Original DNA Strands

Stem cells in many tissues appear to be label-retaining cells. As we have just explained, this has generally been assumed to be because, having incorporated a tracer such as BrdU into their DNA during a period of BrdU exposure, the stem cells then divide rarely, so that the label is only slowly diluted by newly synthesized DNA. There is, however, another possible interpretation: regardless of whether they divide fast or slowly, the stem cells might segregate their DNA strands asymmetrically, in such a way that in every division, and for every chromosome, the specific DNA strand that was originally labeled is retained in the daughter cell that remains a stem cell. This original strand would presumably have to have acquired some sort of special tag, designating it as a stem-cell strand and ensuring that it segregates asymmetrically, into the daughter that remains a stem cell (see Figure 23–6), along with all the similarly tagged DNA strands of the other chromosomes; in this way, the old labeled strands would be retained in the stem cells from cell generation to cell generation. The tag might, for example, take the form of some special kinetochore protein that remains associated with the old DNA strand at the centromere of each chromosome during DNA replication and then engages with some asymmetry in the mitotic spindle so as to ensure that the stem-cell daughter receives all the daughter chromosomes carrying the tag. In each stem-cell generation, the same original tagged DNA strands would then serve as templates for production of the new sets of DNA strands to be despatched into the transit amplifying cells in the following generation (**Figure 23–10**).

This "immortal strand" hypothesis may seem a lot to swallow, given that no mechanism for such tagging and segregation of individual DNA strands has yet been identified. Yet there is increasing evidence suggesting that the immortal strand hypothesis is correct. Muscle (described later in this chapter) provides an




example. When BrdU is supplied during a period of production of muscle stem cells and the subsequent fate of the cells is followed as they divide and proliferate, it is possible to detect small clones of cells, or pairs of sister cells, within which all the BrdU label is concentrated in a single cell, even though all the cells share a common origin from a single ancestor cell that took up the label initially. Similar observations have been reported in studies of other types of stem cells, and, importantly, this behavior has not been seen in cell populations that do not contain stem cells. The immortal strand hypothesis would not only explain why stem cells retain labeled DNA indefinitely, but would also imply that asymmetric division is a fundamental stem-cell property, with the corollary that any increase in the number of stem cells must require special conditions to confer the immortality tag on additional, newly synthesized DNA strands. The immortal strand hypothesis was originally proposed in the 1970s as a mechanism for stem cells to avoid accumulating cancer-promoting mutations during DNA replication. Reduction of the risk of cancer could be one of its benefits.

## The Rate of Stem-Cell Division Can Increase Dramatically When New Cells Are Needed Urgently

Whatever the mechanism of stem-cell maintenance may be, the use of transit amplifying divisions brings several benefits. First, it means that the number of stem cells can be small and their division rate can be low, even when terminally differentiated cells have to be produced rapidly in large numbers. This reduces the cumulative burden of genetic damage, since most mutations occur in the course of DNA replication and mitosis, and mutations occurring in cells that are not stem cells are discarded in the course of tissue renewal. The likelihood of cancer is thus reduced. If the immortal strand hypothesis is correct, so that stem cells always retain the original "immortal" template DNA strands, the risk is still further reduced, since most sequence errors introduced during DNA replication will be in the newly synthesized strands, which the stem cells ultimately discard.

Second, and perhaps more important, a low stem-cell division rate in normal circumstances allows for dramatic increase when there is an urgent need - for example, in wound repair. The stem cells can then be roused to divide rapidly, and the additional division cycles can both amplify the stock of stem

**Figure 23–10 The immortal strand hypothesis.** (A) Experimental evidence. Here, stem cells of skeletal muscle (members of the muscle satellite cell population, discussed later in this chapter) have been placed in culture and allowed to divide for 4 days in the presence of BrdU to label newly synthesized DNA strands. The cells have then been allowed to divide for 1 day in the absence of BrdU. The photographs show a pair of sister cells at the end of this procedure: one has inherited BrdU, the other has not. This implies that daughter chromosomes carrying DNA strands synthesized during the cell divisions that occurred in the presence of BrdU have all been inherited by the one cell, while those carrying only either pre-existing or subsequently synthesized DNA strands have been inherited by the other. This phenomenon, in which old and new DNA strands are asymmetrically allocated to different daughter cells, is seen only in cell populations that include stem cells. (B) The pattern of DNA strand inheritance in stem cells according to the immortal strand hypothesis. One strand in each chromosome in the stem cell is somehow tagged as the immortal strand and is retained by the stem-cell daughter. (C) This original DNA strand remains available through all subsequent stem-cell generations as a template for production of chromosomes of transit amplifying cells. (A, from V. Shinin, B. Gayraud-Morel, D. Gomès and S. Tajbakhsh, *Nat. Cell Biol.* 8:677–687, 2006. With permission from Macmillan Publishers Ltd.)

cells and increase steeply the production of cells committed to terminal differentiation. Thus, for example, when a patch of hairy skin is cut away, the slowly dividing stem cells in the bulge region of surviving hair follicles near the wound are switched into rapid proliferation, and some of their progeny move out as new stem cells to form fresh interfollicular epidermis to cover the wounded patch of body surface.

## Many Interacting Signals Govern Epidermal Renewal

Cell turnover in the epidermis seems at first glance a simple matter, but the simplicity is deceptive. There are many points in the process that have to be controlled according to circumstances: the rate of stem-cell division; the probability that a stem-cell daughter will remain a stem cell; the number of cell divisions of the transit amplifying cells; the timing of exit from the basal layer, and the time that the cell then takes to complete its differentiation program and be sloughed from the surface. Regulation of these steps must enable the epidermis to respond to rough usage by becoming thick and callused, and to repair itself when wounded. In specialized regions of epidermis, such as those that form hair follicles, with their own specialized subtypes of stem cells, yet more controls are needed to organize the local pattern.

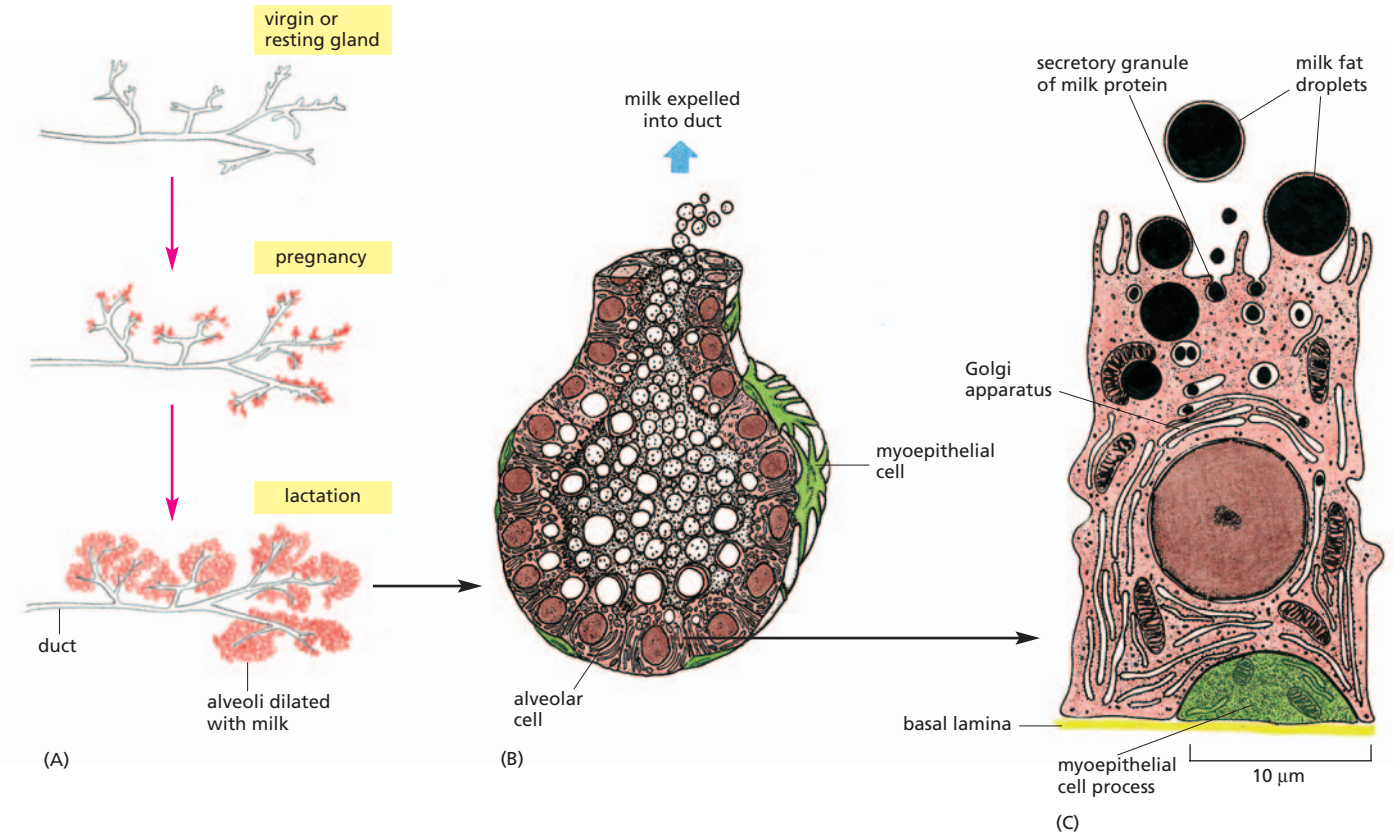
Each of the control points has its own importance, and a multitude of molecular signals is needed to regulate them all, so as to keep the body surface always properly covered. As we suggested earlier, one important influence is contact with the basal lamina, signaled via integrins in the plasma membrane of the cells. If cultured basal keratinocytes are held in suspension, instead of being allowed to settle and attach to the bottom of the culture dish, they all stop dividing and differentiate. To remain as an epidermal stem cell, it is apparently necessary (although not sufficient) to be attached to the basal lamina or other extracellular matrix. This requirement helps ensure that the size of the stem cell population does not increase without limit. If crowded out of their regular niche on the basal lamina, the cells lose their stem cell character. When this rule is broken, as in some cancers, the result can be an ever-growing tumor.

Most of the other cell communication mechanisms described in Chapter 15 are also implicated in the control of epidermal renewal, either in signaling between cells within the epidermis or in signaling between epidermis and dermis. The EGF, FGF, Wnt, Hedgehog, Notch, BMP/TGFβ, and integrin signaling pathways are all involved (and we shall see that the same is true of most other tissues). Overactivation of the Hedgehog pathway, for example, can cause epidermal cells to carry on dividing after they have left the basal layer, and mutations in components of this pathway are responsible for many epidermal cancers. At the same time, Hedgehog signaling helps to guide the choice of differentiation pathway: a deficit of Hedgehog signaling leads to loss of sebaceous glands, while an excess can cause sebaceous glands to develop in regions where they would never normally form. Similarly, loss of Wnt signaling leads to failure of hair follicle development, while excessive activation of this pathway causes extra hair follicles to form and to grow excessively so that they give rise to tumors.

Notch signaling, in contrast, seems to restrict the size of the stem cell population, inhibiting neighbors of stem cells from remaining as stem cells and causing them to become transit amplifying cells instead. And TGFβ has a key role in signaling to the dermis during the repair of skin wounds, promoting the formation of collagen-rich scar tissue. The precise individual functions of all the various signaling mechanisms in the epidermis are only beginning to be disentangled.

## The Mammary Gland Undergoes Cycles of Development and Regression

In specialized regions of the body surface, various other types of cells develop from the embryonic epidermis. In particular, secretions such as sweat, tears,




saliva, and milk are produced by cells segregated in deep-lying glands that originate as ingrowths of the epidermis. These epithelial structures have functions and patterns of renewal quite different from those of keratinizing regions.

The mammary glands are the largest and most remarkable of these secretory organs. They are the defining feature of mammals and an important concern in many ways: not only for nourishment of babies and attraction of the opposite sex, but also as the basis for a large industry—the dairy industry—and as the site of some of the commonest forms of cancer. Mammary tissue illustrates most dramatically that developmental processes continue in the adult body; and it shows how cell death by apoptosis permits cycles of growth and regression.

Milk production must be switched on when a baby is born and switched off when the baby is weaned. During pregnancy, the producer cells of the milk factory are generated; at weaning, they are destroyed. A "resting" adult mammary gland consists of branching systems of ducts embedded in fatty connective tissue; this is the future plumbing network that will deliver milk to the nipple. The ducts are lined with an epithelium that includes mammary stem cells. These stem cells can be identified by a functional test, in which the cells of the mammary tissue are dissociated, sorted according to the cell surface markers that they express, and transplanted back into appropriate host tissue (a mammary fat pad). This assay reveals that a small subset of the total epithelial cells have stem-cell potential. A single one of these cells, estimated to be about one in 5000 of the total mammary epithelial population but more concentrated within a subpopulation expressing certain markers, can proliferate indefinitely and give rise to an entire new mammary gland with all its epithelial cell types. This reconstituted gland is able to go through the full program of differentiation required for milk production. As a first step toward milk production, the steroid hormones that circulate during pregnancy (estrogen and progesterone) cause the duct cells to proliferate, increasing their numbers several hundred-fold. In a process that depends on local activation of the Wnt signaling pathway, the terminal regions of the ducts grow and branch, forming little dilated outpocketings, or

**Figure 23–11 The mammary gland.** (A) The growth of alveoli from the ducts of the mammary gland during pregnancy and lactation. Only a small part of the gland is shown. The "resting" gland contains a small amount of inactive glandular tissue embedded in a large amount of fatty connective tissue. During pregnancy an enormous proliferation of the glandular tissue takes place at the expense of the fatty connective tissue, with the secretory portions of the gland developing preferentially to create alveoli. (B) One of the milk-secreting alveoli with a basket of contractile myoepithelial cells *(green)* embracing it (see also Figure 23–47E). (C) A single type of secretory alveolar cell produces both the milk proteins and the milk fat. The proteins are secreted in the normal way by exocytosis, while the fat is released as droplets surrounded by plasma membrane detached from the cell. (B, after R. Krstić, Die Gewebe des Menschen und der Säugetiere. Berlin: Springer-Verlag, 1978; C, from D.W. Fawcett, A Textbook of Histology, 12th ed. New York: Chapman and Hall, 1994.)

alveoli, containing secretory cells (**Figure 23–11**). Milk secretion begins only when these cells are stimulated by the different combination of hormones circulating in the mother after the birth of the baby, especially prolactin from the pituitary gland. Prolactin binds to receptors on the surface of the mammary epithelial cells and thereby activates a pathway that switches on expression of milk protein genes. As in the epidermis, signals from the extracellular matrix, mediated by integrins, are also essential: the milk-producing cells can only respond to prolactin if they are also in contact with the basal lamina. A further tier of hormonal control governs the actual ejection of milk from the breast: the stimulus of suckling causes cells of the hypothalamus (in the brain) to release the hormone *oxytocin*, which travels via the bloodstream to act on *myoepithelial cells*. These musclelike cells originate from the same epithelial precursor population as the secretory cells of the breast, and they have long spidery processes that embrace the alveoli. In response to oxytocin they contract, thereby squirting milk out of the alveoli into the ducts.

Eventually, when the baby is weaned and suckling stops, the secretory cells die by apoptosis, and most of the alveoli disappear. Macrophages rapidly clear away the dead cells, matrix metalloproteinases degrade the surplus extracellular matrix, and the gland reverts to its resting state. This ending of lactation seems to be induced by the accumulation of milk, rather than by a hormonal mechanism. If one subset of mammary ducts is obstructed so that no milk can be discharged, the secretory cells that supply it commit mass suicide by apoptosis, while other regions of the gland survive and continue to function. The apoptosis is triggered by a complex array of factors that accumulate where milk secretion is blocked.

Cell division in the growing mammary gland is regulated not only by hormones but also by local signals passing between cells within the epithelium and between the epithelial cells and the connective tissue, or *stroma,* in which the epithelial cells are embedded. All the signals listed earlier as important in controlling cell turnover in the epidermis are also implicated in controlling events in the mammary gland. Again, signals delivered via integrins play a crucial part: deprived of the basal lamina adhesions that activate integrin signaling, the epithelial cells fail to respond normally to hormonal signals. Faults in these interacting control systems underlie some of the commonest forms of cancer, and we need to understand them better.

## Summary

*Skin consists of a tough connective tissue, the dermis, overlaid by a multilayered waterproof epithelium, the epidermis. The epidermis is continually renewed from stem cells, with a turnover time, in humans, on the order of a month. Stem cells, by definition, are not terminally differentiated and have the ability to divide throughout the organism's lifetime, yielding some progeny that differentiate and others that remain stem cells. The epidermal stem cells lie in the basal layer, attached to the basal lamina; under normal conditions, their division rate is low. Progeny that become committed to differentiation go through several rapid transit amplifying divisions in the basal layer, and then stop dividing and move out toward the surface of the skin. They progressively differentiate, switching from expression of one set of keratins to expression of another until, eventually, their nuclei degenerate, producing an outer layer of dead keratinized cells that are continually shed from the surface.*

*The fate of the daughters of a stem cell is controlled by interactions with the basal lamina, mediated by integrins and by signals from neighboring cells. Some types of stem cells may also be internally programmed to divide asymmetrically so as to create one stem-cell daughter and one daughter committed to eventual differentiation; this may involve selective segregation of original "immortal" template DNA strands into the stem-cell daughter. Environmental controls, however, allow two stem cells to be generated from one during repair processes and can trigger steep increases in the rate of stem-cell division. Factors such as Wnt and Hedgehog signal proteins not only regulate the rate of cell proliferation according to need, but can also drive specialization of epidermal cells to form structures such as hair follicles and sebaceous glands.*

*These and other organs connected to the epidermis, such as the mammary glands, have their own stem cells and their own distinct patterns of cell turnover. In the breast, for example, circulating hormones stimulate the cells to proliferate, differentiate, and make milk; the cessation of suckling triggers the milk-secreting cells to die by apoptosis, in response to a combination of factors that build up where milk fails to be drained away.*

# SENSORY EPITHELIA

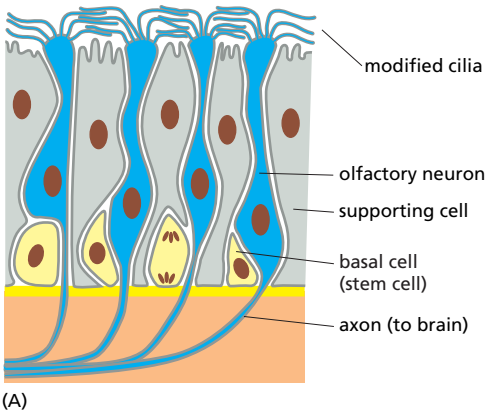
We sense the smells, sounds, and sights of the external world through another class of specializations of the epithelium that cover our body surface. The sensory tissues of the nose, the ears, and the eyes—and, indeed, if we look back to origins in the early embryo, the whole of the central nervous system—all arise from the same sheet of cells, the *ectoderm*, that gives rise to the epidermis. These structures have several features in common, and related systems of genes govern their development (discussed in Chapter 22). They all retain an epithelial organization, but it is very different from that of the ordinary epidermis or of the glands that derive from it.

The nose, the ear, and the eye are complex organs, with elaborate devices to collect signals from the external world and to deliver them, filtered and concentrated, to the sensory epithelia, where they can act on the nervous system. The sensory epithelium in each organ is the key component, although it is small relative to all the ancillary apparatus. It is the part that has been most highly conserved in evolution, not only from one vertebrate to another, but also between vertebrates and invertebrates.

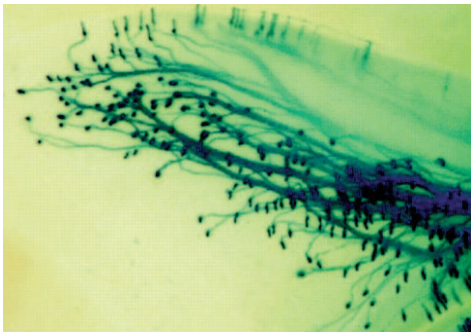
Within each sensory epithelium lie sensory cells that act as *transducers*, converting signals from the outside world into an electrical form that the nervous system can interpret. In the nose, the sensory transducers are *olfactory sensory neurons*; in the ear, *auditory hair cells*; and in the eye, *photoreceptors*. All of these cell types are either neurons or neuron-like. Each carries at its apical end a specialized structure that detects the external stimulus and converts it to a change in the membrane potential. At its basal end, each makes synapses with neurons that relay the sensory information to specific sites in the brain.

## Olfactory Sensory Neurons Are Continually Replaced

In the olfactory epithelium of the nose (**Figure 23–12**A), a subset of the epithelial cells differentiate as **olfactory sensory neurons**. These cells have modified, immotile cilia on their free surfaces (see Figure 15–46), containing odorant receptor proteins, and a single axon extending from their basal end toward the brain (Figure 23–12B). *Supporting cells* that span the thickened epithelium and have properties similar to those of glial cells in the central nervous system hold



(A)


(B)
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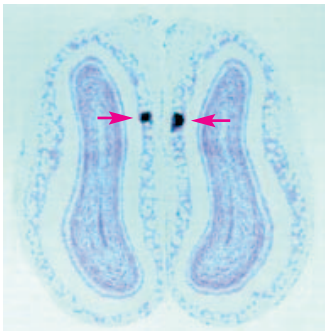

(C)
500 μm

**Figure 23–12 Olfactory epithelium and olfactory neurons.** (A) Olfactory epithelium consists of supporting cells, basal cells, and olfactory sensory neurons. The basal cells are the stem cells for production of the olfactory neurons. Six to eight modified cilia project from the apex of the olfactory neuron and contain the odorant receptors. (B) This micrograph shows olfactory neurons in the nose of a genetically modified mouse in which the *LacZ* gene has been inserted into an odorant receptor locus, so that all the cells that would normally express that particular receptor now also make the enzyme β-galactosidase. The β-galactosidase is detected through the blue product of the enzymatic reaction that it catalyzes. The cell bodies *(dark blue)* of the marked olfactory neurons, lying scattered in the olfactory epithelium, send their axons *(light blue)* toward the brain (out of the picture to the right). (C) A cross section of the *left* and *right* olfactory bulbs, stained for β-galactosidase. Axons of all the olfactory neurons expressing the same odorant receptor converge on the same glomeruli *(red arrows)* symmetrically placed within the bulbs on the *right* and *left* sides of the brain. Other glomeruli (unstained) receive their inputs from olfactory neurons expressing other odorant receptors. (B and C, from P. Mombaerts et al., *Cell* 87:675–686, 1996. With permission from Elsevier.)

the neurons in place and separate them from one another. The sensory surfaces are kept moist and protected by a layer of fluid secreted by cells sequestered in glands that communicate with the exposed surface. Even with this protection, however, each olfactory neuron survives for only a month or two, and so a third class of cells—the *basal cells*—is present in the epithelium to generate replacements for the olfactory neurons that are lost. The population of basal cells, lying in contact with the basal lamina, includes stem cells for the production of the neurons.

As discussed in Chapter 15, the genome contains a remarkably large number of odorant receptor genes—about 1000 in a mouse or a dog, and about 350 (plus many more that are degenerate and non-functional) in a human. Each olfactory neuron most probably expresses only one of these genes, enabling the cell to respond to one particular class of odorants (generally small organic molecules) sharing some structural feature that the odorant receptor protein recognizes. But regardless of the odor, every olfactory neuron responds in the same way—it sends a train of action potentials back along its axon to the brain. The discriminating sensibility of an individual olfactory neuron is therefore useful only if its axon delivers its messages to the specific relay station in the brain that is dedicated to the particular range of odors that the neuron senses. These relay stations are called *glomeruli*. They are located in structures called the olfactory bulbs (one on each side of the brain), with about 1800 glomeruli in each bulb (in the mouse). Olfactory neurons expressing the same odorant receptor are widely scattered in the olfactory epithelium, but their axons all converge on the same glomerulus (Figure 23–12C). As new olfactory neurons are generated, replacing those that die, they must in turn send their axons to the correct glomerulus. The odorant receptor proteins thus have a second function: guiding the growing tips of the new axons along specific paths to the appropriate target glomeruli in the olfactory bulbs. If it were not for the continual operation of this guidance system, a rose might smell in one month like a lemon, in the next like rotting fish.

## Auditory Hair Cells Have to Last a Lifetime

The sensory epithelium responsible for hearing is the most precisely and minutely engineered of all the tissues in the body (**Figure 23–13**). Its sensory cells, the **auditory hair cells**, are held in a rigid framework of supporting cells and overlaid by a mass of extracellular matrix (the tectorial membrane), in a structure called the *organ of Corti*. The hair cells convert mechanical stimuli into electrical signals. Each has a characteristic organ-pipe array of giant microvilli (called *stereocilia)* protruding from its surface as rigid rods, filled with cross-linked actin filaments, and arranged in ranks of graded height. The dimensions of each such array are specified with extraordinary accuracy according to the location of the hair cell in the ear and the frequency of sound that it has to

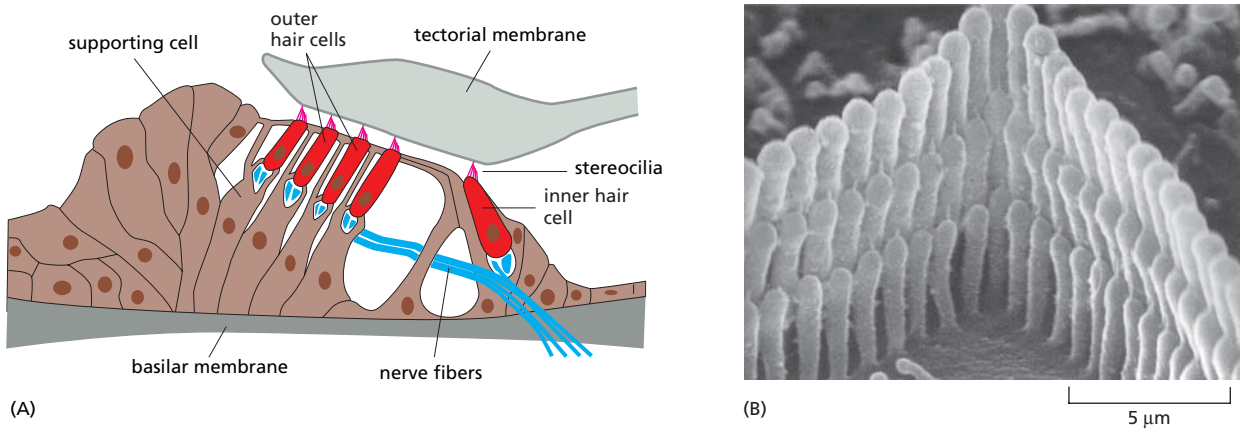



**Figure 23–13 Auditory hair cells.** (A) A diagrammatic cross section of the auditory apparatus (the organ of Corti) in the inner ear of a mammal shows the auditory hair cells held in an elaborate epithelial structure of supporting cells and overlaid by a mass of extracellular matrix (the tectorial membrane). The epithelium containing the hair cells sits on the basilar membrane—a thin, resilient sheet of tissue that forms a long, narrow partition separating two fluid-filled channels. Sound creates pressure waves in these channels and makes the basilar membrane vibrate up and down. (B) This scanning electron micrograph shows the apical surface of an outer auditory hair cell, with the characteristic organ-pipe array of giant microvilli (stereocilia). The inner hair cells, of which there are just 3500 in each human ear, are the principal auditory receptors. The outer hair cells, roughly four times more numerous in humans, are thought to form part of a feedback mechanism that regulates the mechanical stimulus delivered to the inner hair cells. (B, from J.D. Pickles, *Prog. Neurobiol.* 24:1–42, 1985. With permission from Elsevier.)

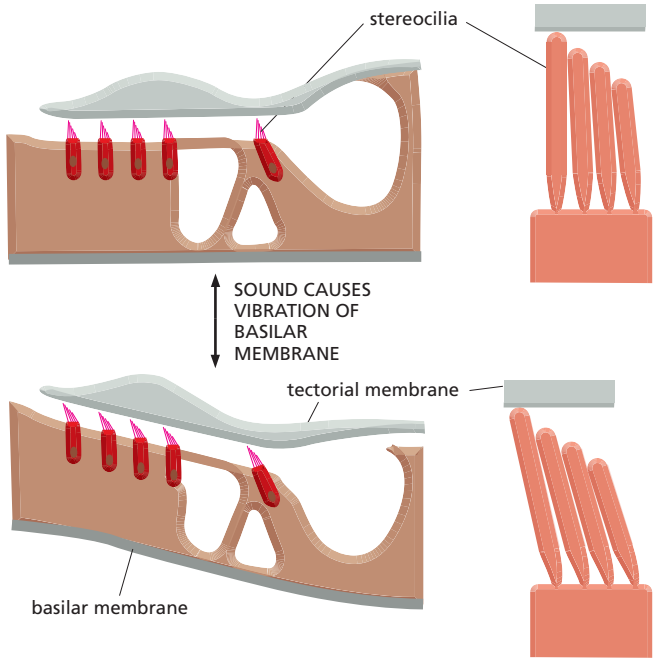



**Figure 23–14 How a relative movement of the overlying extracellular matrix (the tectorial membrane) tilts the stereocilia of auditory hair cells in the organ of Corti in the inner ear of a mammal.** The stereocilia behave as rigid rods hinged at the base and bundled together at their tips.

respond to. Sound vibrations rock the organ of Corti, causing the bundles of stereocilia to tilt (**Figure 23–14**) and mechanically gated ion channels in the membranes of the stereocilia to open or close (**Figure 23–15**). The flow of electric charge carried into the cell by the ions alters the membrane potential and thereby controls the release of neurotransmitter at the cell's basal end, where the cell synapses with a nerve ending. <TCCA> <CATA>

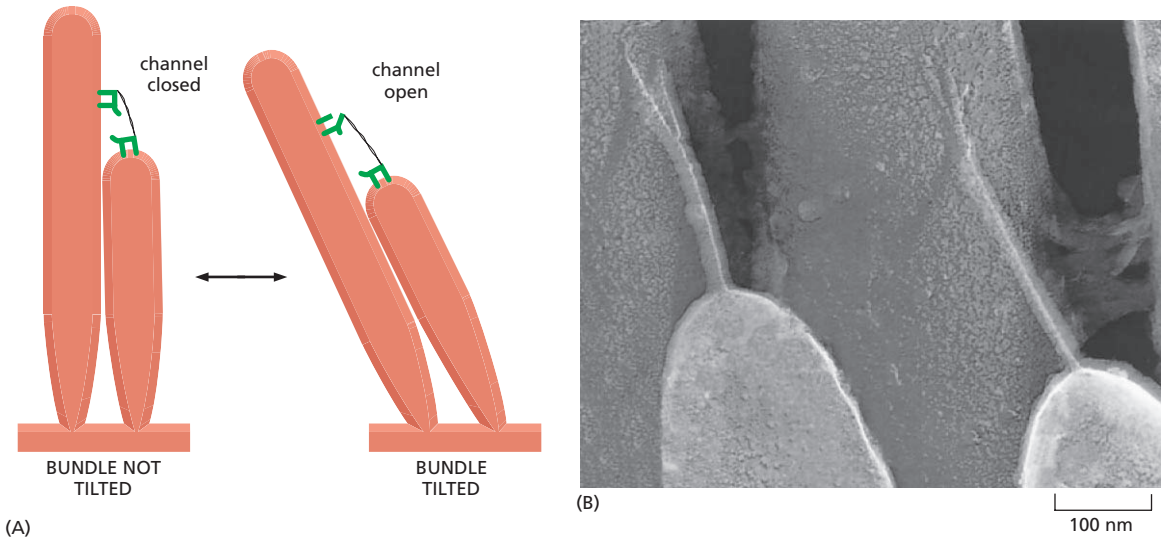



**Figure 23–15 How a sensory hair cell works.** (A) The cell functions as a transducer, generating an electrical signal in response to sound vibrations that rock the organ of Corti and so cause the stereocilia to tilt. A fine filament runs more or less vertically upward from the tip of each shorter stereocilium to attach at a higher point on its adjacent taller neighbor. Tilting the bundle puts tension on the filaments, which pull on mechanically gated ion channels in the membrane of the stereocilia. Opening of these channels allows an influx of positive charge, depolarizing the hair cell. (B) An electron micrograph of the filaments extending from the tops of two stereocilia. Each filament consists, in part at least, of members of the cadherin superfamily of cell–cell adhesion molecules. Mutant individuals lacking these specific cadherins lack the filaments and are deaf.

By extraordinarily delicate mechanical measurements, correlated with electrical recordings from a single hair cell as the bundle of stereocilia is deflected by pushing with a flexible glass probe, it is possible to detect an extra "give" of the bundle as the mechanically gated channels yield to the applied force and are pulled open. In this way it can be shown that the force required to open a single one of the hypothesized channels is about $2 \times 10^{-13}$ newtons and that its gate swings through a distance of about 4 nm as it opens. The mechanism is astonishingly sensitive: the faintest sounds that we can hear have been estimated to stretch the filaments by an average of 0.04 nm, which is just under half the diameter of a hydrogen atom. (B, from B. Kachar et al., *Proc. Natl Acad. Sci. U.S.A.* 97:13336–13341, 2000. With permission from National Academy of Sciences.)

In humans and other mammals, the auditory hair cells, unlike olfactory neurons, have to last a lifetime. If they are destroyed by disease, toxins, or excessively loud noise, they do not regenerate and the resultant hearing loss is permanent. But in other vertebrates, destruction of auditory hair cells triggers the supporting cells to divide and behave as stem cells, generating progeny that can differentiate as replacements for the hair cells that are lost. With better understanding of how this regeneration process is regulated, we may one day be able to induce the auditory epithelium to repair itself in humans also.

So far, one treatment is known that can bring about the partial regeneration of auditory hair cells in an adult mammal. The technique uses a virus (an adenovirus) engineered to contain a copy of the *Atoh1* gene, coding for a gene regulatory protein that is known to drive the differentiation of hair cells during development. Guinea pigs that have been deafened by exposure to a toxin that destroys hair cells can be treated by injection of this viral construct into a damaged ear. Many of the surviving supporting cells then become infected with the viral construct and express *Atoh1*. This converts them into functioning hair cells, and the animal partially recovers its hearing in the treated ear.

## Most Permanent Cells Renew Their Parts: the Photoreceptor Cells of the Retina

The neural retina is the most complex of the sensory epithelia. It consists of several cell layers organized in a way that seems perverse. The neurons that transmit signals from the eye to the brain (called *retinal ganglion cells*) lie closest to the external world, so that the light, focused by the lens, must pass through them to reach the photoreceptor cells. The **photoreceptors**, which are classified as *rods* or *cones*, according to their shape, lie with their photoreceptive ends, or outer segments, partly buried in the *pigment epithelium* (**Figure 23–16**). Rods and cones contain different *visual pigments*—photosensitive complexes of *opsin* protein with the light-absorbing small molecule *retinal*. Rods, whose visual pigment is called *rhodopsin*, are especially sensitive at low light levels,

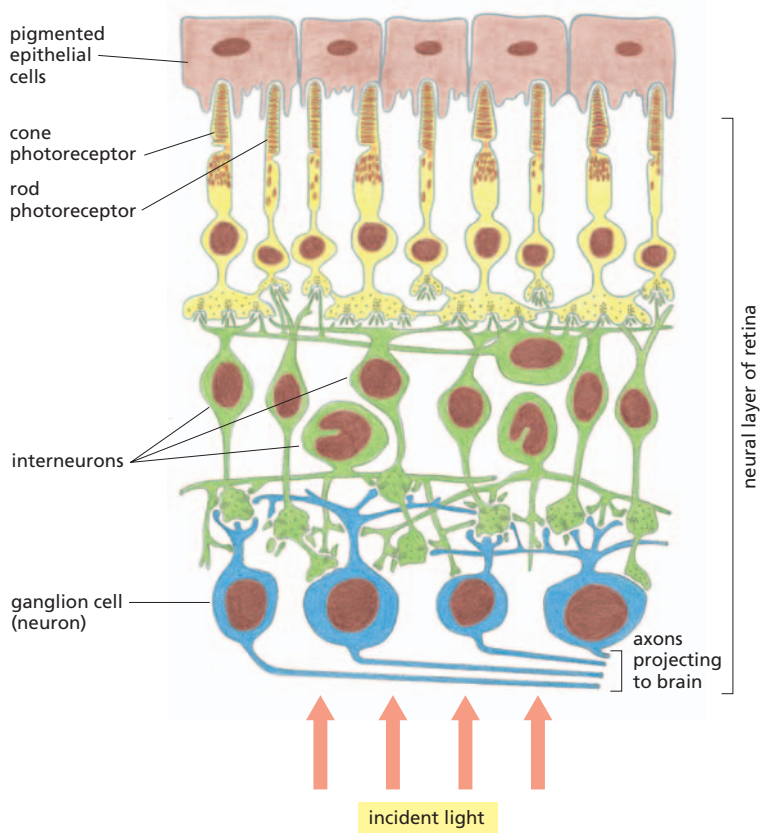



**Figure 23–16 The structure of the retina.** When light stimulates the photoreceptors, the resulting electrical signal is relayed via interneurons to the ganglion cells, which then convey the signal to the brain. A population of specialized supporting cells (not shown here) occupies the spaces between the neurons and photoreceptors in the neural retina. (Modified from J.E. Dowling and B.B. Boycott, *Proc. R. Soc. Lond. B Biol. Sci.* 166:80–111, 1966. With permission from Royal Society.)

while cones (of which there are three types in humans, each with a different opsin, giving a different spectral response) detect color and fine detail.

The outer segment of a photoreceptor appears to be a modified cilium with a characteristic ciliumlike arrangement of microtubules in the region connecting the outer segment to the rest of the cell (**Figure 23–17**). The remainder of the outer segment is almost entirely filled with a dense stack of membranes in which the photosensitive complexes are embedded; light absorbed here produces an electrical response, as discussed in Chapter 15. At their opposite ends, the photoreceptors form synapses on interneurons, which relay the signal to the retinal ganglion cells (see Figure 23–16).

Photoreceptors in humans, like human auditory hair cells, are permanent cells that do not divide and are not replaced if destroyed by disease or by a misdirected laser beam. The photosensitive molecules of visual pigment, however, are not permanent but are continually degraded and replaced. In rods (although not, curiously, in cones), this turnover is organized in an orderly production line, which can be analyzed by following the passage of a cohort of radiolabeled protein molecules through the cell after a short pulse of radioactive amino acid (**Figure 23–18**). The radiolabeled proteins can be traced from the Golgi apparatus in the inner segment of the cell to the base of the stack of membranes in the outer segment. From here they are gradually displaced toward the tip as new material is fed into the base of the stack. Finally (after about 10 days in the rat), on reaching the tip of the outer segment, the labeled proteins and the layers of membrane in which they are embedded are phagocytosed (chewed off and digested) by the cells of the pigment epithelium.

This example illustrates a general point: even though individual cells of certain types persist, very little of the adult body consists of the same molecules that were laid down in the embryo.

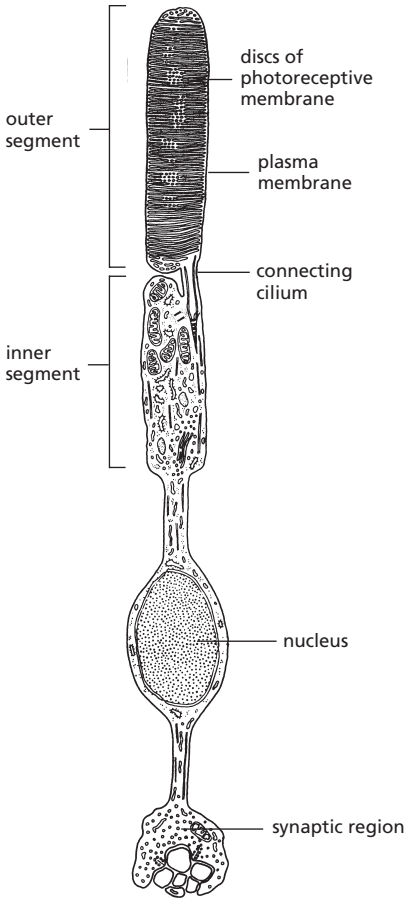



**Figure 23–17 A rod photoreceptor.**

## Summary

*Most sensory receptor cells, like epidermal cells and nerve cells, derive from the epithelium forming the outer surface of the embryo. They transduce external stimuli into electrical signals, which they relay to neurons via chemical synapses. Olfactory receptor cells in the nose are themselves full-fledged neurons, sending their axons to the brain. They have a lifetime of only a month or two, and are continually replaced by new cells derived from stem cells in the olfactory epithelium. Each olfactory neuron expresses just*

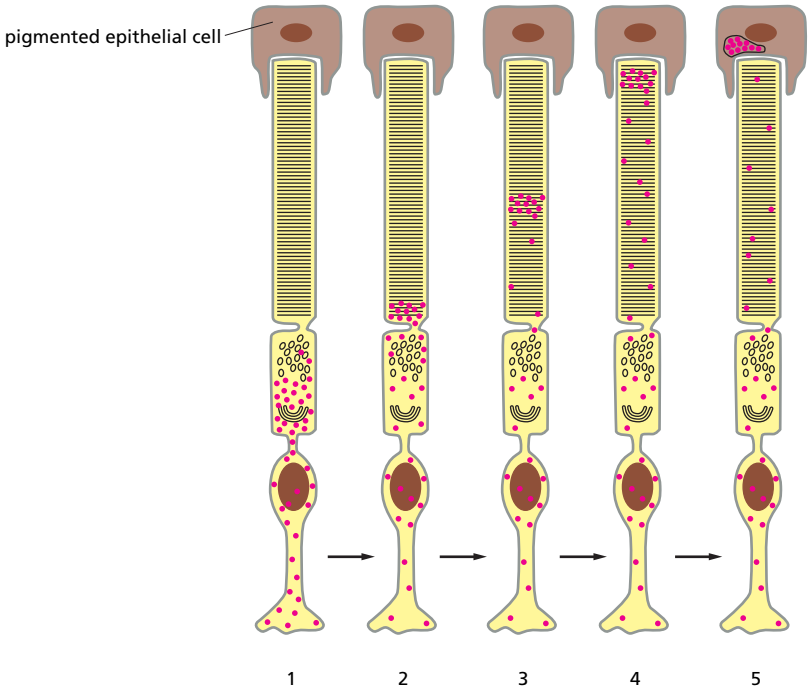



**Figure 23–18 Turnover of membrane protein in a rod cell.** Following a pulse of $^{3}$H-leucine, the passage of radiolabeled proteins through the cell is followed by autoradiography. *Red dots* indicate sites of radioactivity. The method reveals only the $^{3}$H-leucine that has been incorporated into proteins; the rest is washed out during tissue preparation. (1) The incorporated leucine is first seen concentrated in the neighborhood of the Golgi apparatus. (2) From there it passes to the base of the outer segment into a newly synthesized disc of photoreceptive membrane. (3–5) New discs are formed at a rate of three or four per hour (in a mammal), displacing the older discs toward the pigment epithelium.

*one of the hundreds of different olfactory receptor proteins for which genes exist in the genome, and the axons from all olfactory neurons expressing the same receptor protein navigate to the same glomeruli in the olfactory bulbs of the brain.*

*Auditory hair cells—the receptor cells for sound—unlike olfactory receptor cells, have to last a lifetime, in mammals at least, although artificial expression of a hair-cell differentiation gene, Atoh1, can convert surviving supporting cells into hair cells where hair cells have been destroyed. Hair cells have no axon but make synaptic contact with nerve terminals in the auditory epithelium. They take their name from the hair-like bundle of stereocilia (giant microvilli) on their outer surface. Sound vibrations tilt the bundle, pulling mechanically gated ion channels on the stereocilia into an open configuration to excite the cell electrically.*

*Photoreceptor cells in the retina of the eye absorb photons in molecules of visual pigment (opsin protein plus retinal) held in stacks of membrane in the photoreceptor outer segments, triggering an electrical excitation by a more indirect intracellular signaling pathway. Although the photoreceptor cells themselves are permanent and irreplaceable, the stacks of opsin-rich membrane that they contain undergo continual renewal.*

## THE AIRWAYS AND THE GUT

The examples we have discussed so far represent a small selection of the tissues and cell types that derive from the outer layer of the embryo—the ectoderm. They are enough, however, to illustrate how diverse these cells can be, in form, function, lifestyle, and pattern of replacement. The innermost layer of the embryo—the *endoderm*, forming the primitive gut tube—gives rise to another whole zoo of cell types lining the digestive tract and its appendages. We begin with the lungs.

### Adjacent Cell Types Collaborate in the Alveoli of the Lungs

The airways of the lungs are formed by repeated branching of a system of tubes that originated in the embryo from an outpocketing of the gut lining, as discussed in Chapter 22 (see Figure 22–92). Repeated tiers of branching terminate in several hundred million air-filled sacs—the **alveoli**. Alveoli have thin walls, closely apposed to the walls of blood capillaries so as to allow exchange of $O_2$ and $CO_2$ with the bloodstream (**Figure 23–19**).

To survive, the cells lining the alveoli must remain moist. At the same time, they must serve as a gas container that can expand and contract with each breath in and out. This creates a problem. When two wet surfaces touch, they become stuck together by surface tension in the layer of water between them—an effect that operates more powerfully the smaller the scale of the structure. There is a risk, therefore, that the alveoli may collapse and be impossible to reinflate. To solve the problem, two types of cells are present in the lining of the alveoli. *Type I alveolar cells* cover most of the wall: they are thin and flat (*squamous*) to allow gas exchange. *Type II alveolar cells* are interspersed among them. These are plump and secrete *surfactant,* a phospholipid-rich material that forms a film on the free water surfaces and reduces surface tension, making the alveoli easy to reinflate even if they collapse. The production of adequate amounts of surfactant in the fetus, starting at about 5 months of pregnancy in humans, marks the beginning of the possibility of independent life. Premature babies born before this stage are unable to inflate their lungs and breathe; those born after it can do so and, with intensive care, can survive.

### Goblet Cells, Ciliated Cells, and Macrophages Collaborate to Keep the Airways Clean

Higher up in the airways we find different combinations of cell types, serving different purposes. The air we breathe is full of dust, dirt, and air-borne

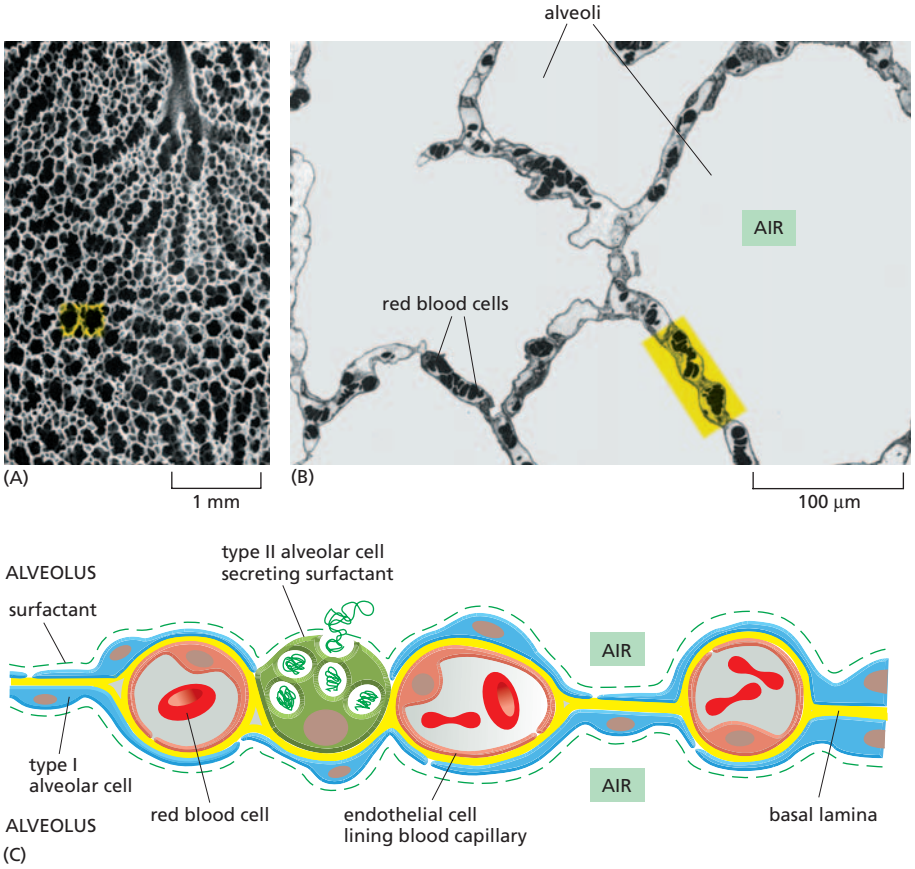



**Figure 23–19 Alveoli in the lung.** (A) Scanning electron micrograph at low magnification, showing the sponge-like texture created by the many air-filled alveoli. A bronchiole (small tubular airway) is seen at the top, communicating with the alveoli. (B) Transmission electron micrograph of a section through a region corresponding to the *yellow* box in (A) showing the alveolar walls, where gas exchange occurs. (C) Diagram of the cellular architecture of a piece of alveolar wall, corresponding to the *yellow* box in (B). (A, from P. Gehr et al., *Respir. Physiol.* 44:61–86, 1981. With permission from Elsevier; B, courtesy of Peter Gehr, from D.W. Fawcett, A Textbook of Histology, 12th ed. New York: Chapman and Hall, 1994.)

microorganisms. To keep the lungs clear and healthy, this debris must be constantly swept out. To perform this task, a relatively thick *respiratory epithelium* lines the larger airways (**Figure 23–20**). This epithelium consists of three differentiated cell types: *goblet cells* (so named because of their shape), which secrete mucus; *ciliated cells*, with cilia that beat; and a small number of *endocrine cells*, secreting serotonin and peptides that act as local mediators. These signal molecules affect nerve endings and other neighboring cells in the respiratory tract, so as to help regulate the rate of mucus secretion and ciliary beating, the contraction of surrounding smooth muscle cells that can constrict the airways, and other functions. Basal cells are also present, and serve as stem cells for renewal of the epithelium.

The mucus secreted by the goblet cells forms a viscoelastic blanket about 5 μm thick over the tops of the cilia. The cilia, all beating in the same direction, at a rate of about 12 beats per second, sweep the mucus out of the lungs, carrying with it the debris that has become stuck to it. This conveyor belt for the removal of rubbish from the lungs is called the *mucociliary escalator*. Of course, some inhaled particles may reach the alveoli themselves, where there is no escalator. Here, the unwanted matter is removed by yet another class of specialized cells, *macrophages*, which roam the lungs, engulfing foreign matter and killing and digesting bacteria. Many millions of macrophages, loaded with debris, are swept out of the lungs every hour on the mucociliary escalator.

At the upper end of the respiratory tract, the wet mucus-covered respiratory epithelium gives way abruptly to stratified squamous epithelium. This cell sheet is structured for mechanical strength and protection, and, like epidermis, it consists of many layers of flattened cells densely packed with keratin. It differs from epidermis in that it is kept moist and its cells retain their nucleus even in the outermost layers. Abrupt boundaries of epithelial cell specialization, such as that between the mucous and the stratified squamous epithelium of the respiratory tract, are also found in other parts of the body, but very little is known about how they are created and maintained.

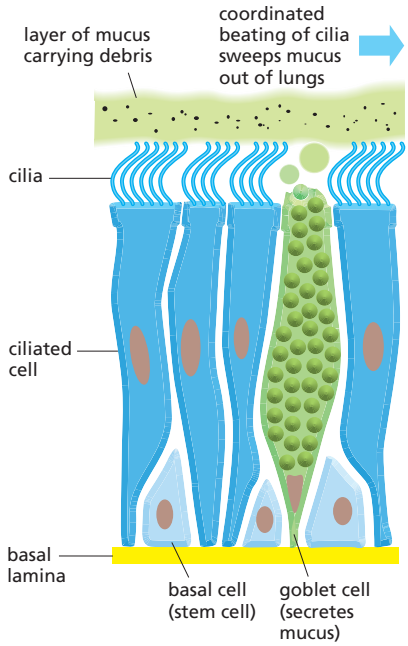


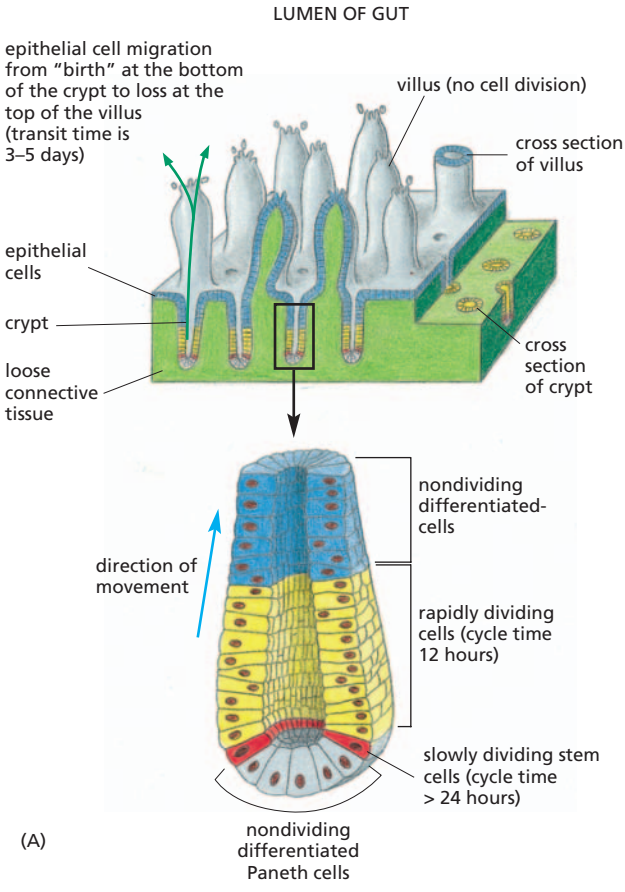

**Figure 23–20 Respiratory epithelium.** The goblet cells secrete mucus, which forms a blanket over the tops of the ciliated cells. The regular, coordinated beating of the cilia sweeps the mucus up and out of the airways, carrying any debris that is stuck to it. The mechanism that coordinates the ciliary beating is a mystery, but it seems to reflect an intrinsic polarity in the epithelium. If a segment of rabbit trachea is surgically reversed, it carries on sweeping mucus, but in the wrong direction, back down toward the lung, in opposition to adjacent unreversed portions of trachea.

## The Lining of the Small Intestine Renews Itself Faster Than Any Other Tissue

Only air-breathing vertebrates have lungs, but all vertebrates, and almost all invertebrate animals, have a gut—that is, a digestive tract lined with cells specialized for the digestion of food and absorption of the nutrient molecules released by the digestion. These two activities are hard to carry on at the same time, as the processes that digest food in the lumen of the gut are liable also to digest the lining of the gut itself, including the cells that absorb the nutrients. The gut uses several strategies to solve the problem.

The fiercest digestive processes, involving acid hydrolysis as well as enzyme action, are conducted in a separate reaction vessel, the stomach. The products are then passed on to the small intestine, where the nutrients are absorbed and enzymatic digestion continues, but at a neutral pH. The different regions of the gut lining consist of correspondingly different mixtures of cell types. The stomach epithelium includes cells that secrete acid, and other cells that secrete digestive enzymes that work at acid pH. Conversely, glands (in particular the pancreas) that discharge into the initial segment of the small intestine contain cells that secrete bicarbonate to neutralize the acidity, along with other cells that secrete digestive enzymes that work at neutral pH. The lining of the intestine, downstream from the stomach, contains both absorptive cells and cells specialized for the secretion of mucus, which covers the epithelium with a protective coat. In the stomach, too, mucus cells line most exposed surfaces. And, in case these measures are not enough, the whole lining of the stomach and intestine is continually renewed and replaced by freshly generated cells, with a turnover time of a week or less.

The renewal process has been studied best in the small intestine (**Figure 23–21**). The lining of the small intestine (and of most other regions of the gut) is a single-layered epithelium. This epithelium covers the surfaces of the *villi* that project into the lumen, and it lines the *crypts* that descend into the underlying



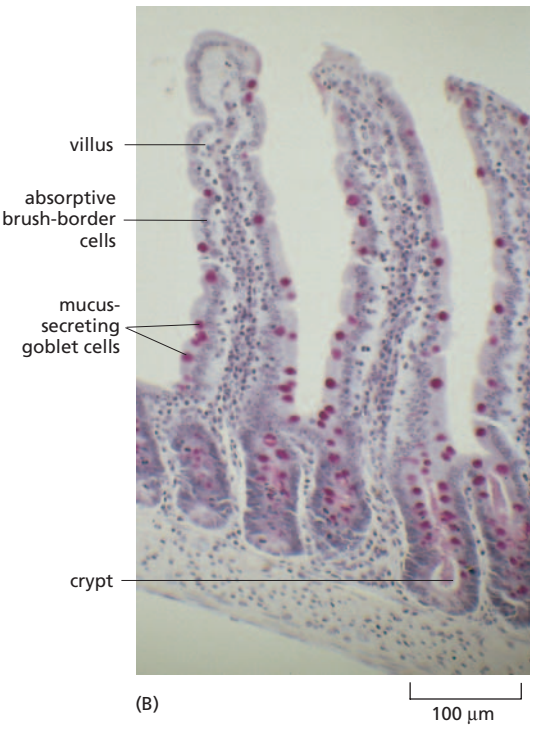




**Figure 23–21 Renewal of the gut lining.** (A) The pattern of cell turnover and the proliferation of stem cells in the epithelium that forms the lining of the small intestine. The *colored arrow* shows the general upward direction of cell movement onto the villi, but some cells, including a proportion of the goblet and enteroendocrine cells, stay behind and differentiate while still in the crypts. The nondividing differentiated cells (Paneth cells) at the very bottom of the crypts also have a finite lifetime and are continually replaced by progeny of the stem cells. (B) Photograph of a section of part of the lining of the small intestine, showing the villi and crypts. Note how mucus-secreting goblet cells (stained *red*) are interspersed among other cell types. Enteroendocrine cells are less numerous and less easy to identify without special stains. See Figure 23–22 for the structure of these cells.

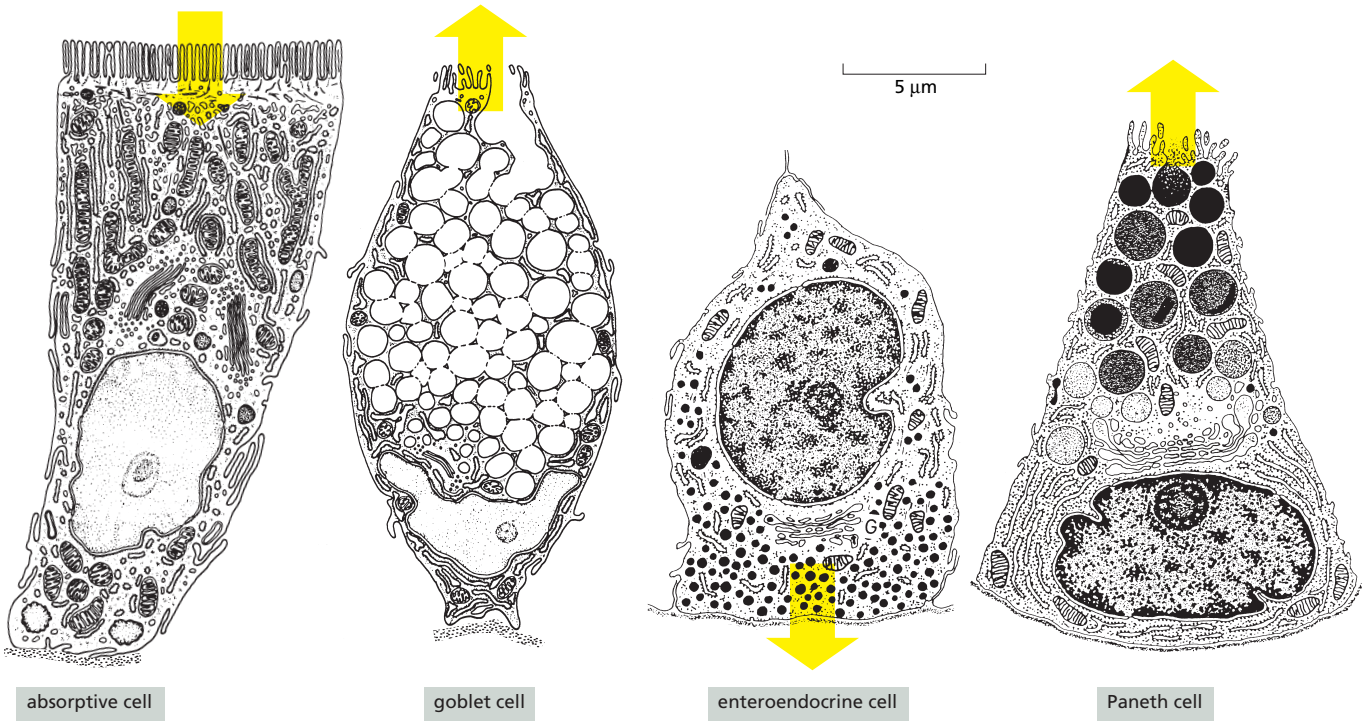



**Figure 23–22 The four main differentiated cell types found in the epithelial lining of the small intestine.** All of these are generated from undifferentiated multipotent stem cells living near the bottoms of the crypts (see Figure 23–21). The microvilli on the apical surface of the absorptive (brush-border) cell provide a 30-fold increase of surface area, not only for the import of nutrients but also for the anchorage of enzymes that perform the final stages of extracellular digestion, breaking down small peptides and disaccharides into monomers that can be transported across the cell membrane. Broad *yellow* arrows indicate direction of secretion or uptake of materials for each type of cell. (After T.L. Lentz, Cell Fine Structure. Philadelphia: Saunders, 1971; R. Krstić, Illustrated Encyclopedia of Human Histology. Berlin: Springer-Verlag, 1984.)

connective tissue. Dividing stem cells lie in a protected position in the depths of the crypts. These generate four types of differentiated progeny (**Figure 23–22**):

1. *Absorptive cells* (also called *brush-border cells* or *enterocytes*) have densely packed microvilli on their exposed surfaces to increase their active surface area for the uptake of nutrients. They both absorb nutrients and secrete (or carry on their exterior surfaces) hydrolytic enzymes that perform some of the final steps of extracellular digestion, breaking down food molecules in preparation for transport across the plasma membrane.
2. *Goblet cells* (as in respiratory epithelium) secrete mucus.
3. *Paneth cells* form part of the innate immune defense system (discussed in Chapter 24) and secrete (along with some growth factors) *cryptdins*—proteins of the defensin family that kill bacteria (see Figure 24–46).
4. *Enteroendocrine cells*, of more than 15 different subtypes, secrete serotonin and peptide hormones, such as cholecystokinin (CCK), that act on neurons and other cell types in the gut wall and regulate the growth, proliferation, and digestive activities of cells of the gut and other tissues. Cholecystokinin, for example, is released from enteroendocrine cells in response to the presence of nutrients in the gut and binds to receptors on nearby sensory nerve endings, which relay a signal to the brain to stop you feeling hungry after you have eaten enough.

The absorptive, goblet, and enteroendocrine cells travel mainly upward from the stem-cell region, by a sliding movement in the plane of the epithelial sheet, to cover the surfaces of the villi. In analogy with the epidermis, it is thought that the most rapidly proliferating precursor cells in the crypt are in a transit amplifying stage, already committed to differentiation but undergoing several divisions on their way out of the crypt, before they stop dividing and differentiate terminally. Within 2–5 days (in the mouse) after emerging from the crypts, the cells reach the tips of the villi, where they undergo the initial stages of

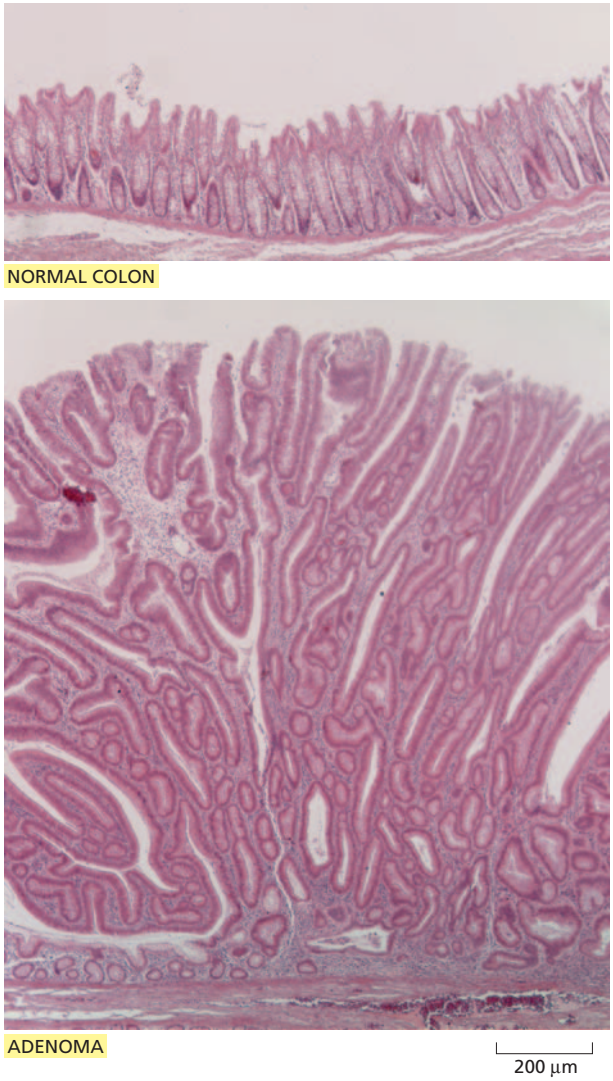



**Figure 23–23 An adenoma in the human colon, compared with normal tissue from an adjacent region of the same person's colon.** The specimen is from a patient with an inherited mutation in one of his two copies of the *Apc* gene. A mutation in the other *Apc* gene copy, occurring in a colon epithelial cell during adult life, has given rise to a clone of cells that behave as though the Wnt signaling pathway is permanently activated. As a result, the cells of this clone form an adenoma—an enormous, steadily expanding mass of giant cryptlike structures.

apoptosis and are finally discarded into the gut lumen. The Paneth cells are produced in much smaller numbers and have a different migration pattern. They live at the bottom of the crypts, where they too are continually replaced, although not so rapidly, persisting for about 20 days (in the mouse) before undergoing apoptosis and being phagocytosed by their neighbors. The stem cells, too, remain at or near the bottoms of the crypts. What keeps them there, and what restricts cell division to the crypts? How are the migrations controlled so that some cells move up while others stay down? What are the molecular signals that organize the whole stem-cell system, and how do they work?

## Wnt Signaling Maintains the Gut Stem-Cell Compartment

The beginnings of an answer to these questions came from the study of cancer of the colon and rectum (the lower end of the gut). As discussed in Chapter 20, some people have a hereditary predisposition to this disease and, in advance of the cancer, develop large numbers of small precancerous tumors (adenomas) in the lining of their large intestine (**Figure 23–23**). The appearance of these tumors suggests that they have arisen from intestinal crypt cells that have failed to halt their proliferation in the normal way, and so have given rise to excessively large cryptlike structures. The cause can be traced to mutations in the *Apc* (*Adenomatous Polyposis Coli*) gene: the tumors arise from cells that have lost both gene copies. *Apc* codes for a protein that prevents inappropriate activation of the Wnt signaling pathway, so that loss of APC is presumed to mimic the effect of continual exposure to a Wnt signal. The suggestion, therefore, is that Wnt signaling normally keeps

crypt cells in a proliferative state, and cessation of exposure to Wnt signaling normally makes them stop dividing as they leave the crypt. Indeed, mice that are homozygous for a knockout mutation in the *Tcf4* gene, coding for a gene regulatory protein that is required as an effector of Wnt signaling in the gut, make no crypts, fail to renew their gut epithelium, and die soon after birth.

Experiments with transgenic mice confirm the importance of Wnt signaling and reveal other regulators that act together with Wnt to organize the gut-cell production line and keep it running correctly. Using the Cre/lox technique with an inducible promoter for Cre (as described in Chapter 8, p. 567), it is possible, for example, to knock out the *Apc* gene in gut epithelial cells abruptly, at any chosen time in the life of the mouse. Within a few days, the gut structure is transformed: the crypt-like regions of proliferative cells are greatly enlarged, villi are reduced, and the numbers of terminally differentiated cells are drastically diminished. Conversely, one can make a transgenic mouse in which the gut epithelial cells all secrete a diffusible inhibitor of Wnt signaling. These animals, in which Wnt signaling is blocked, form scarcely any crypts and have hardly any proliferating cells in their gut epithelium. Instead, almost all the gut lining cells are fully differentiated non-dividing absorptive cells; but goblet cells, enteroendocrine cells, and Paneth cells are missing. Thus Wnt signaling not only keeps cells in a proliferative state but is also needed to make them competent to give rise to the full range of ultimate differentiated cell types.

## Notch Signaling Controls Gut Cell Diversification

What then causes the cells to diversify as they differentiate? Notch signaling has this function in many other systems, where it mediates lateral inhibition—a competitive interaction that drives neighboring cells toward different fates (see Chapters 15 and 22, Figures 15–75 and 22–60). All the essential components of the Notch pathway are expressed in the crypts; it seems that Wnt signaling switches on their expression. When Notch signaling is abruptly blocked by knocking out one of these essential components, within a few days all the cells in the crypts differentiate as goblet cells and cease dividing; conversely, when Notch signaling is artificially activated in all the cells, no goblet cells are produced and the crypt-like regions of cell proliferation are enlarged.

From the effects of all these manipulations of Wnt and Notch signaling, we arrive at a simple picture of how the two pathways combine to govern the production of differentiated cells from the intestinal stem cells (**Figure 23–24**). Wnt

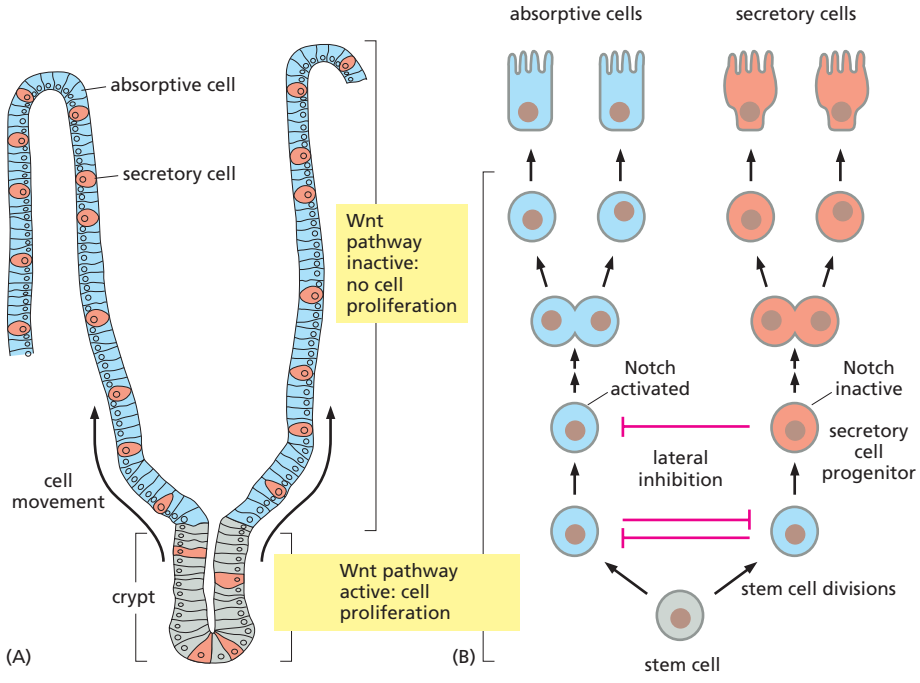



**Figure 23–24 How Wnt and Notch signaling pathways combine to control the production of differentiated cells from stem cells in the intestine.** (A) Wnt signaling maintains proliferation in the crypt, where the stem cells reside and their progeny become committed to diverse fates. (B) Wnt signaling in the crypt drives expression of the components of the Notch signaling pathway in that region; Notch signaling is thus active in the crypt and, through lateral inhibition, forces cells there to diversify. Both pathways must be activated in the same cell to keep it as a stem cell. The progeny of the stem cell continue dividing under the influence of Wnt even after they become committed to a specific differentiated fate, but the timing of these transit amplifying divisions in relation to commitment is not understood in detail.

signaling promotes cell proliferation and confers competence for the full range of modes of differentiation, while preventing differentiation from occurring immediately; in this way, it defines the crypt and maintains the stem cells. But Wnt signaling also, at the same time, activates the expression of Notch pathway components, and Notch signaling within the crypt population mediates lateral inhibition, which forces the cells to diversify, in such a way that some become singled out to deliver lateral inhibition, while others receive it. Cells of the former class express Notch ligands and activate Notch in their neighbors, but escape from Notch activation themselves; as a result, they become committed to differentiate as secretory cells. Cells of the latter class—the majority—are kept in an opposite state, with Notch activated and ligand expression inhibited; as a result, they retain competence to differentiate in any of a variety of ways and to engage in lateral-inhibition competition with their neighbors. Both classes of cells (with the exception of some secretory subtypes) continue dividing so long as they are in the crypt, under the influence of Wnt. But when cells leave the crypt and lose exposure to Wnt signaling, the competition halts, division stops, and the cells differentiate according to their individual states of Notch activation at that time—as absorptive cells if Notch is still activated, as secretory cells if it is not.

This is certainly not the whole story of events in the crypt. It does not explain, for example, how the various subclasses of secretory cells (goblet, enteroendocrine, and Paneth) become different from one another. Nor does it say anything about the distinction that many experts believe to exist between true stem cells and the more rapidly dividing transit amplifying cells within the crypt. Several different members of each of the families of Wnt and Notch pathway components are expressed in the crypt epithelium and in the connective tissue around the base of the crypts, and probably have differing effects. Moreover, other signaling pathways also have crucial functions in organizing the system.

## Ephrin–Eph Signaling Controls the Migrations of Gut Epithelial Cells

One of the most remarkable features of the gut stem-cell system is the steady, ordered, selective migration of cells from crypt to villus. Differentiating absorptive, goblet, and enteroendocrine cells stream out of the crypts and up the villi (**Figure 23–25**); stem cells remain deep in the crypts; and Paneth cells migrate right down to the crypt bottoms. This pattern of movements, which segregates the different groups of cells, depends on yet another cell–cell signaling pathway. Wnt signaling stimulates the expression of cell-surface receptors of the EphB family (discussed in Chapter 15) in the cells in the crypt; however, as cells differentiate, they switch off expression of these receptors, and switch on instead expression of the ligands, cell-surface proteins of the ephrinB family (**Figure 23–26**A). There is one exception: the Paneth cells retain expression of the EphB

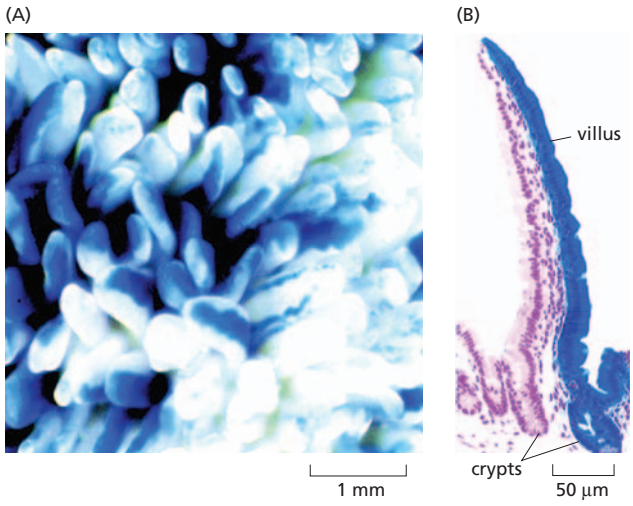



**Figure 23–25 Migration of cells from crypts onto villi.** In this mouse intestine, a random subset of epithelial cells was induced to undergo a mutation during late fetal life, causing the mutant cells to express a *LacZ* transgene, coding for an enzyme that can be detected by the blue product of the reaction that it catalyses. By 6 weeks after birth, each crypt has become populated by the progeny of a single stem cell and thus appears either totally blue or totally white, according to whether that stem cell was or was not genetically marked in this way. Several crypts contribute to a single villus, each sending a stream of differentiated cells outward along it. (A) Low-magnification surface view of part of the lining of the intestine, showing many villi, each receiving streams of cells from several crypts. (B) Detail of a single villus and adjacent crypts in cross-section. In the example shown, the streams from different crypts have remained unmixed, so that the villus appears blue on one side and white on the other; more commonly, there is some mixing, giving a less orderly result. (From M.H. Wong, J.R. Saam, T.S. Stappenbeck, C.H. Rexer and J.I. Gordon, *Proc. Natl Acad. Sci. U.S.A.* 97:12601–12606, 2000. With permission from National Academy of Sciences.)

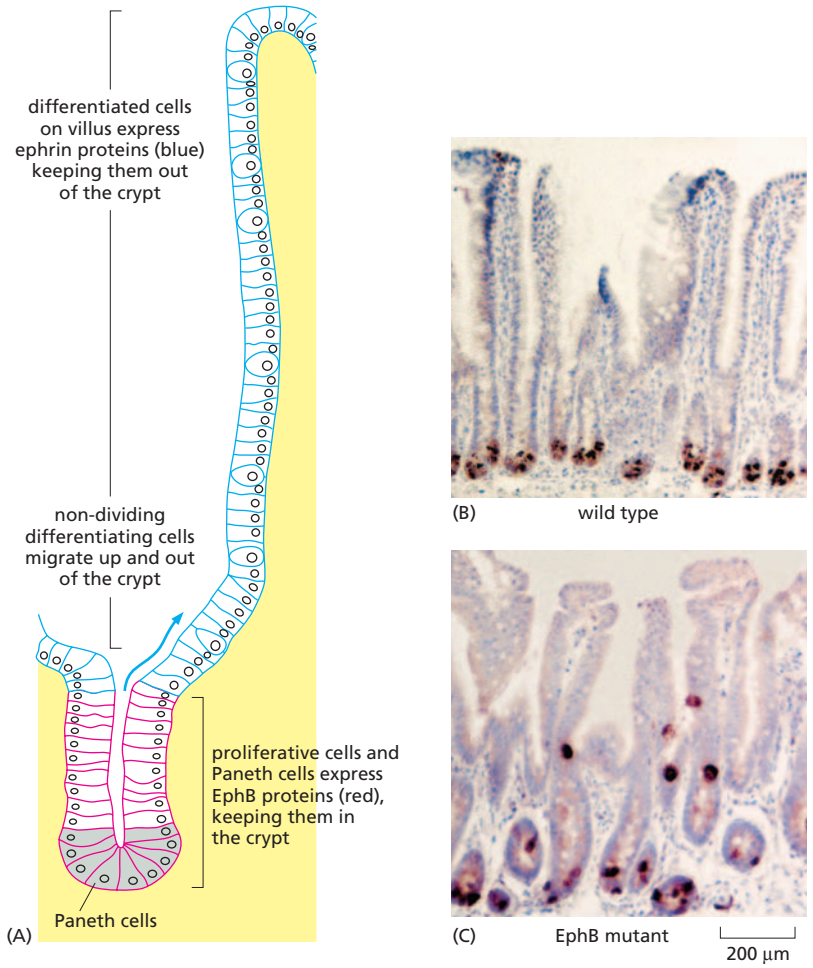
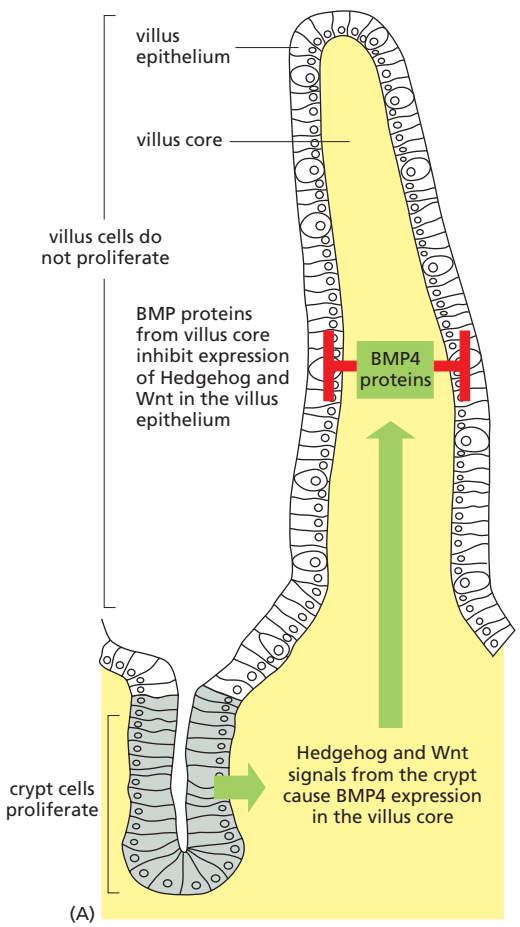



**Figure 23–26 Ephrin–Eph signaling controls cell segregation between crypts and villi.** (A) Proliferative cells (including the stem cells) and Paneth cells express EphB proteins, while the differentiated, nondividing cells that cover the villi express ephrinB proteins. The repulsive cell–cell interaction mediated by encounters between these two types of cell-surface molecules keeps the two classes of cells segregated. (B) In a normal gut, as a result, Paneth cells (*brown* stain) and dividing cells remain confined to the bottoms of the crypts. (C) In a mutant where EphB proteins are defective, cells that should stay in the crypts wander out onto the villi. (Adapted from E. Batlle et al., *Cell* 111:251–263, 2002. With permission from Elsevier.)

proteins. Thus EphB expression is characteristic of cells that stay in the crypts, while ephrinB expression is characteristic of cells moving out onto the villi. In various other tissues, cells expressing Eph proteins are repelled by contacts with cells expressing ephrins (see Chapter 22, Figure 22–106). It seems that the same is true in the gut lining, and that this mechanism serves to keep the cells in their proper places. In EphB knockout mutants, the populations become mixed, so that, for example, Paneth cells wander out onto the villi (Figure 23–26C). Loss of EphB genes in intestinal cancers correlates with the onset of invasive behavior by the tumor cells.

## Wnt, Hedgehog, PDGF, and BMP Signaling Pathways Combine to Delimit the Stem-Cell Niche

Clearly, the gut stem cells cannot exist without the special environment that the crypt provides for them. This **stem cell niche** is as essential as the stem cells themselves. How is it created and maintained? The mechanism seems to depend on a complex interplay of signals between the epithelium and the underlying connective tissue. Exchange of Wnt, Hedgehog, and PDGF signals between the two tissues, and between different regions of the crypt–villus axis, leads to a restriction of Wnt signaling to the neighborhood of the crypts. The epithelial cells in the crypts produce both Wnt proteins and the receptors that respond to them, creating a positive feedback loop that presumably helps to make Wnt pathway activation in this region self-sustaining. At the same time, signals exchanged with the connective tissue lead to expression of BMP proteins in the connective-tissue cells forming the core of the villi (**Figure 23–27**). These cells signal to the adjacent villus epithelium to inhibit the development of misplaced crypts: blocking BMP signaling disrupts the whole organization



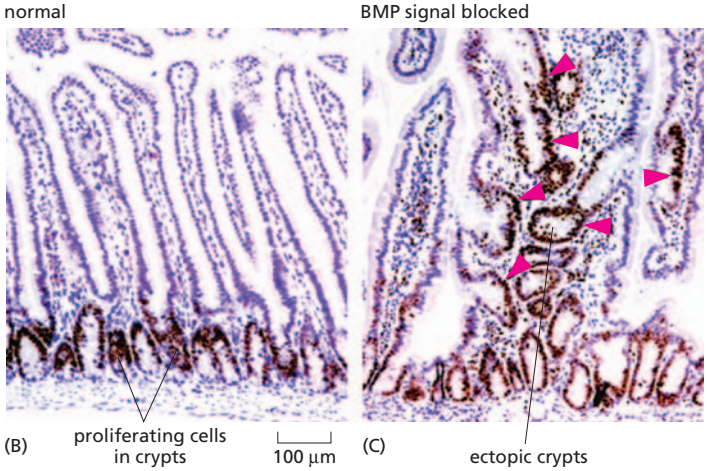




**Figure 23–27 Signals defining the intestinal stem-cell niche.** (A) Diagram of the signaling system. Signal proteins of the Hedgehog and Wnt families are expressed by the epithelial cells in the base of each crypt, which also express Wnt receptors and experience high levels of Wnt pathway activation. The connective-tissue cells underlying the epithelium express both Hedgehog receptors and Wnt receptors. The combined effect of the signals from the crypt base, perhaps in conjunction with other signals, is to provoke the connective-tissue cells that lie in the core of each villus to express BMP proteins. The BMP proteins act on the epithelium of the villus, preventing its cells from forming crypts. (B) Cross section of a region of normal intestinal epithelium. The brown stain marks proliferative cells, which are confined to the crypts. (C) Similarly stained section of intestine of a transgenic mouse expressing an inhibitor of BMP signaling. Crypts containing dividing cells have developed ectopically, along the sides of the misshapen villi. (B and C, courtesy of A. Haramis et al., *Science* 303:1684–1686, 2004. With permission from AAAS.)

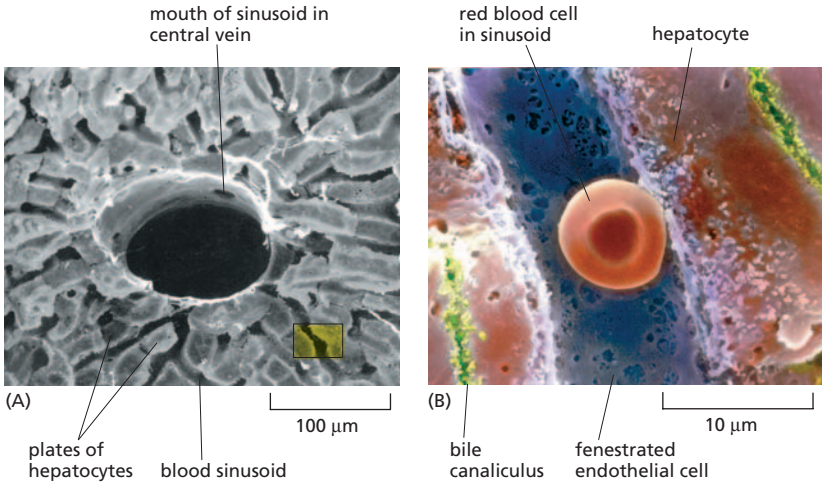
and causes misplaced crypts to form as invaginations of proliferating epithelium along the sides of the villi.

## The Liver Functions as an Interface Between the Digestive Tract and the Blood

As we have seen, the functions of the gut are divided between a variety of cell types. Some cells are specialized for the secretion of hydrochloric acid, others for the secretion of enzymes, others for the absorption of nutrients, and so on. Some of these cell types are closely intermingled in the wall of the gut, whereas others are segregated in large glands that communicate with the gut and originate in the embryo as outgrowths of the gut epithelium.

The liver is the largest of these glands. It develops at a site where a major vein runs close to the wall of the primitive gut tube, and the adult organ retains a special relationship with the blood. Cells in the liver that derive from the primitive gut epithelium—the **hepatocytes**—are arranged in interconnected sheets and cords, with blood-filled spaces called sinusoids running between them (**Figure 23–28**). The blood is separated from the surface of the hepatocytes by a single layer of flattened endothelial cells that covers the exposed faces of the hepatocytes. This structure facilitates the chief functions of the liver, which depend on the exchange of metabolites between hepatocytes and the blood.

The liver is the main site at which nutrients that have been absorbed from the gut and then transferred to the blood are processed for use by other cells of the body. It receives a major part of its blood supply directly from the intestinal tract (via the portal vein). Hepatocytes synthesize, degrade, and store a vast number of substances. They play a central part in the carbohydrate and lipid metabolism of the body as a whole, and they secrete most of the protein found in blood plasma. At the same time, the hepatocytes remain connected with the lumen of the gut via a system of minute channels (or canaliculi) and larger ducts



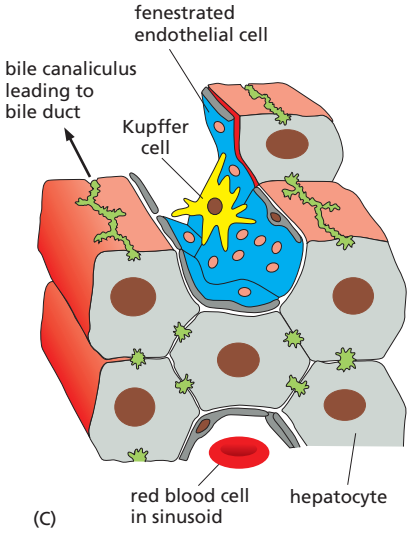




**Figure 23–28 The structure of the liver.** (A) A scanning electron micrograph of a portion of the liver, showing the irregular sheets and cords of hepatocytes and the many small channels, or sinusoids, for the flow of blood. The larger channels are vessels that distribute and collect the blood that flows through the sinusoids. (B) Detail of a sinusoid (enlargement of region similar to that marked by yellow rectangle at lower right in [A]). (C) Schematized diagram of the fine structure of the liver. A single thin sheet of endothelial cells with interspersed macrophagelike Kupffer cells separates the hepatocytes from the bloodstream. Small holes in the endothelial sheet, called fenestrae (Latin for "windows"), allow the exchange of molecules and small particles between the hepatocytes and the bloodstream. Besides exchanging materials with the blood, the hepatocytes form a system of tiny bile canaliculi into which they secrete bile, which is ultimately discharged into the gut via bile ducts. The real structure is less regular than this diagram suggests. (A and B, courtesy of Pietro M. Motta, University of Rome "La Sapienza.")

(see Figure 23–28B,C) and secrete into the gut by this route both waste products of their metabolism and an emulsifying agent, *bile*, which helps in the absorption of fats. Hepatocytes are big cells, and about 50% of them (in an adult human) are polyploid, with two, four, eight, or even more times the normal diploid quantity of DNA per cell.

In contrast to the rest of the digestive tract, there seems to be remarkably little division of labor within the population of hepatocytes. Each hepatocyte seems able to perform the same broad range of metabolic and secretory tasks. These fully differentiated cells can also divide repeatedly, when the need arises, as we explain next.

## Liver Cell Loss Stimulates Liver Cell Proliferation

The liver illustrates in a striking way one of the great unsolved problems of developmental and tissue biology: what determines the size of an organ of the body, or the quantity of one type of tissue relative to another? For different organs, the answers are almost certainly different, but there is scarcely any case in which the mechanism is well understood.

Hepatocytes normally live for a year or more and are renewed at a slow rate. Even in a slowly renewing tissue, however, a small but persistent imbalance between the rate of cell production and the rate of cell death would lead to disaster. If 2% of the hepatocytes in a human divided each week but only 1% died, the liver would grow to exceed the weight of the rest of the body within 8 years. Homeostatic mechanisms must operate to adjust the rate of cell proliferation or the rate of cell death, or both, so as to keep the organ at its normal size. This size, moreover, needs to be matched to the size of the rest of the body. Indeed, when the liver of a small dog is grafted into a large dog, it rapidly grows to almost the size appropriate to the host; conversely, when the liver is grafted from a large dog into a small one, it shrinks.

Direct evidence for the homeostatic control of liver cell proliferation comes from experiments in which large numbers of hepatocytes are removed surgically or are intentionally killed by poisoning with carbon tetrachloride. Within a day or so after either sort of damage, a surge of cell division occurs among the surviving hepatocytes, quickly replacing the lost tissue. (If the hepatocytes themselves are totally eliminated, another class of cells, located in the bile ducts, can serve as stem cells for the genesis of new hepatocytes, but usually there is no need for this.) If two-thirds of a rat's liver is removed, for example, a liver of nearly normal size can regenerate from the remainder by hepatocyte proliferation within about 2 weeks. Although many molecules have been implicated in the triggering of this reaction, one of the most important is a protein called *hepatocyte growth factor*. It stimulates hepatocytes to divide in culture, and its production increases steeply (by poorly understood mechanisms) in response to liver damage.

The balance between cell births and cell deaths in the adult liver (and other organs too) does not depend exclusively on the regulation of cell proliferation: cell survival controls also play a part. If an adult rat is treated with the drug phenobarbital, for example, hepatocytes are stimulated to divide, causing the liver to enlarge. When the phenobarbital treatment is stopped, hepatocyte cell death greatly increases until the liver returns to its original size, usually within a week or so. The mechanism of this type of cell survival control is unknown, but it has been suggested that hepatocytes, like most vertebrate cells, depend on signals from other cells for their survival and that the normal level of these signals can support only a certain standard number of hepatocytes. If the number of hepatocytes rises above this (as a result of phenobarbital treatment, for example), hepatocyte death will automatically increase to bring their number back down. It is not known how the appropriate levels of survival factors are maintained.

## Tissue Renewal Does Not Have to Depend on Stem Cells: Insulin-Secreting Cells in the Pancreas

Most of the organs of the respiratory and digestive tract, including the lungs, the stomach, and the pancreas, contain a subpopulation of endocrine cells similar to the enteroendocrine cells in the intestines and, like them, generated in the epithelium under the control of the Notch signaling pathway. The *insulin-secreting cells (β cells)* of the pancreas belong in this category. Their mode of renewal has a special importance, because it is the loss of these cells (through autoimmune attack) that is responsible for Type I (juvenile-onset) diabetes and a significant factor also in the Type II (adult-onset) form of the disease. In a normal pancreas, they are sequestered in cell clusters, called *islets of Langerhans* (**Figure 23–29**), where they are grouped with related enteroendocrine cells, secreting other hormones. The islets contain no obvious subset of cells specialized to act as stem cells, yet fresh β cells are continually generated within them. Where do these new cells come from?

The question has been answered by study of transgenic mice in which an ingenious variant of the Cre-Lox technique (described in Chapter 8) was used to produce a marker mutation just in those cells that were expressing the insulin gene at the time a drug was given to activate Cre. In this way, the only cells that became labeled and transmitted the label to their progeny were those that were already differentiated β cells at the time of the treatment. When the mice were analyzed as much as a year later, all the new β cells carried the label, implying that they were descendants of already-differentiated β cells, and not of some undifferentiated stem cell. As in the liver, it seems that the population of differentiated cells here is renewed and enlarged by simple duplication of existing differentiated cells, and not by means of stem cells.

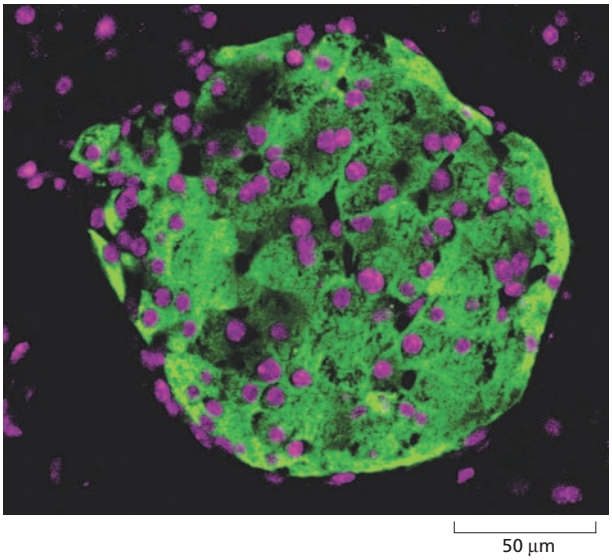



**Figure 23–29 An islet of Langerhans in the pancreas.** The insulin-secreting cells (β cells) are stained *green* by immunofluorescence. Cell nuclei are stained *purple* with a DNA dye. The surrounding pancreatic exocrine cells (secreting digestive enzymes and bicarbonate via ducts into the gut) are unstained, except for their nuclei. Within the islet, close to its surface, there are also small numbers of cells (unstained) secreting hormones such as glucagon. The insulin-secreting cells replace themselves by simple duplication, without need of specialized stem cells. (Adapted from a photograph courtesy of Yuval Dor. © 2004 Yuval Dor, The Hebrew University, Jerusalem.)

## Summary

*The lung performs a simple function—gas exchange—but its housekeeping systems are complex. Surfactant-secreting cells help to keep the alveoli from collapsing. Macrophages constantly scour the alveoli for dirt and microorganisms. A mucociliary escalator formed by mucus-secreting goblet cells and beating ciliated cells sweeps debris out of the airways.*

*In the gut, where more potentially damaging chemical processes occur, constant rapid cell renewal keeps the absorptive epithelium in good repair. In the small intestine, stem cells in the crypts generate new absorptive, goblet, enteroendocrine, and Paneth cells, replacing most of the epithelial lining of the intestine every week. Wnt signaling in the crypts maintains the stem-cell population, while Notch signaling drives diversification of the stem-cell progeny and limits the number that are consigned to a secretory fate. Cell–cell interactions within the epithelium mediated by ephrin–Eph signaling control the selective migration of cells from the crypts upward onto the villi. Interactions between the epithelium and the stroma, involving the Wnt, Hedgehog, PDGF, and BMP pathways organize the pattern of crypts and villi, thereby creating the niches that stem cells inhabit.*

*The liver is a more protected organ, but it too can rapidly adjust its size up or down by cell proliferation or cell death when the need arises. Differentiated hepatocytes remain able to divide throughout life, showing that a specialized class of stem cells is not always needed for tissue renewal. Similarly, the population of insulin-producing cells in the pancreas is enlarged and renewed by simple duplication of existing insulin-producing cells.*

# BLOOD VESSELS, LYMPHATICS, AND ENDOTHELIAL CELLS

From the tissues that derive from the embryonic ectoderm and endoderm, we turn now to those derived from *mesoderm*. This middle layer of cells, sandwiched between ectoderm and endoderm, grows and diversifies to provide many sorts of supportive functions. It gives rise to the body's connective tissues, blood cells, and blood and lymphatic vessels, as well as muscle, kidney, and many other structures and cell types. We begin with blood vessels.

Almost all tissues depend on a blood supply, and the blood supply depends on **endothelial cells**, which form the linings of the blood vessels. Endothelial cells have a remarkable capacity to adjust their number and arrangement to suit local requirements. They create an adaptable life-support system, extending by cell migration into almost every region of the body. If it were not for endothelial cells extending and remodeling the network of blood vessels, tissue growth and repair would be impossible. Cancerous tissue is as dependent on a blood supply as is normal tissue, and this has led to a surge of interest in endothelial cell biology. By blocking the formation of new blood vessels through drugs that act on endothelial cells, it may be possible to block the growth of tumors (discussed in Chapter 20).

## Endothelial Cells Line All Blood Vessels and Lymphatics

The largest blood vessels are arteries and veins, which have a thick, tough wall of connective tissue and many layers of smooth muscle cells (**Figure 23–30**). The wall is lined by an exceedingly thin single sheet of endothelial cells, the *endothelium,* separated from the surrounding outer layers by a basal lamina. The amounts of connective tissue and smooth muscle in the vessel wall vary according to the vessel's diameter and function, but the endothelial lining is always present. In the finest branches of the vascular tree—the capillaries and sinusoids—the walls consist of nothing but endothelial cells and a basal lamina (**Figure 23–31**), together with a few scattered—but functionally important—*pericytes*. These are cells of the connective-tissue family, related to vascular smooth muscle cells, that wrap themselves around the small vessels (**Figure 23–32**).

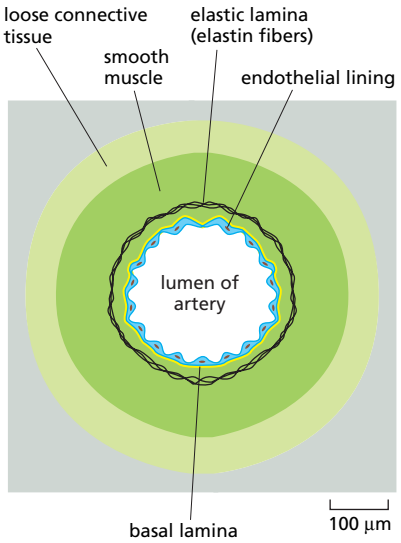



**Figure 23–30 Diagram of a small artery in cross section.** The endothelial cells, although inconspicuous, are the fundamental component. Compare with the capillary in Figure 23–31.

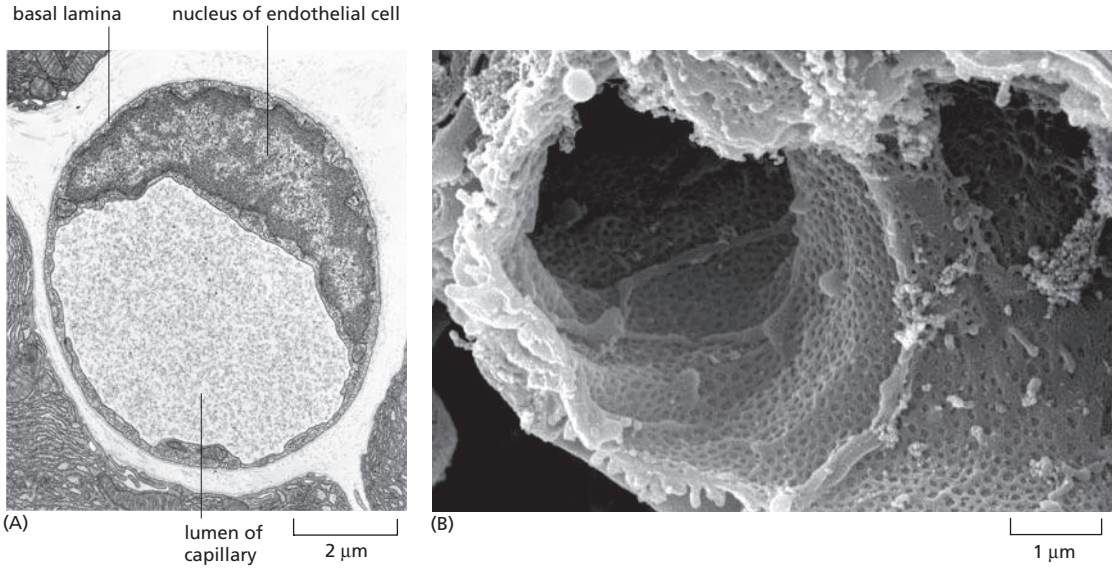



**Figure 23–31 Capillaries.** (A) Electron micrograph of a cross section of a small capillary in the pancreas. The wall is formed by a single endothelial cell surrounded by a basal lamina. (B) Scanning electron micrograph of the interior of a capillary in a glomerulus of the kidney, where blood filtration occurs to produce urine. Here, as in the liver (see Figure 23–28), the endothelial cells are specialized to form a sieve-like structure, with fenestrae, constructed rather like the pores in the nuclear envelope of eucaryotic cells, allowing water and most molecules to pass freely out of the bloodstream. (A, from R.P. Bolender, *J. Cell Biol.* 61:269–287, 1974. With permission from The Rockefeller University Press; B, courtesy of Steve Gschmeissner and David Shima.)

Less obvious than the blood vessels are the lymphatic vessels. These carry no blood and have much thinner and more permeable walls than the blood vessels. They provide a drainage system for the fluid (lymph) that seeps out of the blood vessels, as well as an exit route for white blood cells that have migrated from blood vessels into the tissues. Less happily, they often also provide the path by which cancer cells escape from a primary tumor to invade other tissues. The lymphatics form a branching system of tributaries all ultimately discharging into a single large lymphatic vessel, the thoracic duct, which opens into a large vein close to the heart. Like blood vessels, lymphatics are lined with endothelial cells.

Thus, endothelial cells line the entire blood and lymphatic vascular system, from the heart to the smallest capillary, and control the passage of materials—and the transit of white blood cells—into and out of the bloodstream. Arteries, veins, and lymphatics all develop from small vessels constructed primarily of endothelial cells and a basal lamina: connective tissue and smooth muscle are added later where required, under the influence of signals from the endothelial cells.

## Endothelial Tip Cells Pioneer Angiogenesis

To understand how the vascular system comes into being and how it adapts to the changing needs of tissues, we have to understand endothelial cells. How do they become so widely distributed, and how do they form channels that connect in just the right way for blood to circulate through the tissues and for lymph to drain back to the bloodstream?

Endothelial cells originate at specific sites in the early embryo from precursors that also give rise to blood cells. From these sites the early embryonic endothelial cells migrate, proliferate, and differentiate to form the first rudiments of blood vessels—a process called *vasculogenesis*. Subsequent growth and branching of the vessels throughout the body is mainly by proliferation and movement of the endothelial cells of these first vessels, in a process called **angiogenesis**.

Angiogenesis occurs in a broadly similar way in the young organism as it grows and in the adult during tissue repair and remodeling. We can watch the behavior of the cells in naturally transparent structures, such as the cornea of the eye or the fin of a tadpole, or in tissue culture, or in the embryo. The embryonic

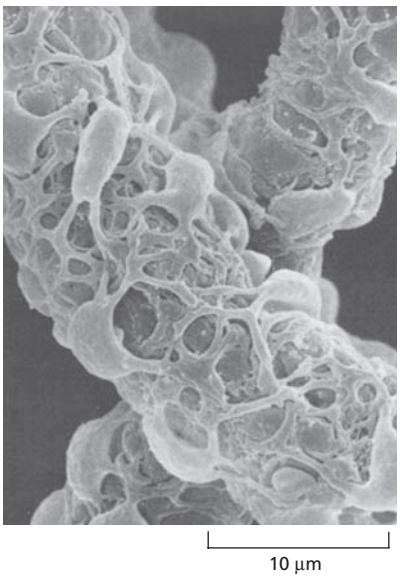



**Figure 23–32 Pericytes.** The scanning electron micrograph shows pericytes wrapping their processes around a small blood vessel (a post-capillary venule) in the mammary gland of a cat. Pericytes are present also around capillaries, but are much more sparsely distributed there. (From T. Fujiwara and Y. Uehara, *Am. J. Anat.* 170:39–54, 1984. With permission from Wiley-Liss.)

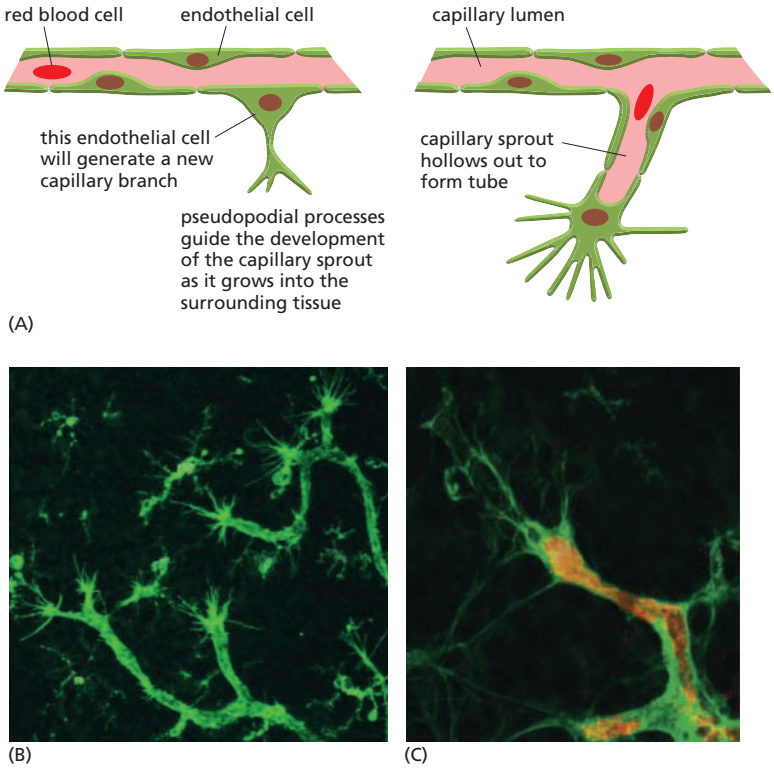



**Figure 23–33 Angiogenesis.** (A) A new blood capillary forms by the sprouting of an endothelial cell from the wall of an existing small vessel. An endothelial tip cell, with many filopodia, leads the advance of each capillary sprout. The endothelial stalk cells trailing behind the tip cell become hollowed out to form a lumen. (B) Blood capillaries sprouting in the retina of an embryonic mouse. (C) A similar specimen, but with a red dye injected into the bloodstream, revealing the capillary lumen opening up behind the tip cell. (B and C, from H. Gerhardt et al., *J. Cell Biol.* 161:1163–1177, 2003. With permission from The Rockefeller University Press.)

retina, which blood vessels invade according to a predictable timetable, is a convenient example for experimental study. Each new vessel originates as a capillary sprout from the side of an existing capillary or small venule (**Figure 23–33**A). At the tip of the sprout, leading the way, is an endothelial cell with a distinctive character. This *tip cell* has a pattern of gene expression somewhat different from that of the endothelial stalk cells following behind it, and while they divide, it does not; but the tip cell's most striking feature is that it puts out many long filopodia, resembling those of a neuronal growth cone (Figure 23–33B). The stalk cells, meanwhile, become hollowed out to form a lumen (see Figure 23–33A). One can watch this process in the transparent zebrafish embryo: the individual cells develop internal vacuoles that join up with those of their neighbors to create a continuous multicellular tube. <GTTG>

The endothelial tip cells that pioneer the growth of normal capillaries not only look like neuronal growth cones, but also respond similarly to signals in the environment. In fact, many of the same guidance molecules are involved, including semaphorins, netrins, slits, and ephrins, along with the corresponding receptors, which are expressed in the tip cells and guide the vascular sprouts along specific pathways in the embryo, often in parallel with nerves. Perhaps the most important of the guidance molecules for endothelial cells, however, is one that is specifically dedicated to the control of vascular development: *vascular endothelial growth factor*, or *VEGF*. We shall have more to say about it below.

## Different Types of Endothelial Cells Form Different Types of Vessel

To create a new circuit for blood flow, a vascular sprout must continue to grow out until it encounters another sprout or vessel with which it can connect. The rules of connection presumably have to be selective, to prevent the formation of undesirable short circuits and to keep the blood and lymphatic systems properly segregated. In fact, endothelial cells of developing arterial, venous, and lymphatic vessels express different genes and have different surface properties. These differences evidently help guide the various types of vessels along different paths, control the selective formation of connections, and govern the development of different types of wall as the vessel enlarges. Arterial endothelial cells, in the embryo at least, express the transmembrane protein ephrinB2,

for example, while the venous arterial cells express the corresponding receptor protein, EphB4 (discussed in Chapter 15). These molecules mediate signaling at sites of cell–cell contact, and they are essential for the development of a properly organized network of vessels.

Expression of the gene regulatory protein Prox1 distinguishes the endothelial cells of lymphatic vessels from arterial and venous endothelial cells. This gene switches on in a subset of endothelial cells in the wall of a large vein (the cardinal vein) in the embryo, converting them into lymphatic progenitors. From these, the whole of the lymphatic vasculature derives by sprouting as described above. Prox1 causes the lymphatic endothelial cells to express receptors for a different member of the VEGF family of guidance molecules, as well as proteins that prevent the lymphatic cells from forming connections with blood vessels.

## Tissues Requiring a Blood Supply Release VEGF; Notch Signaling Between Endothelial Cells Regulates the Response

Almost every cell, in almost every tissue of a vertebrate, is located within 50–100 μm of a blood capillary. What mechanism ensures that the system of blood vessels branches into every nook and cranny? How is it adjusted so perfectly to the local needs of the tissues, not only during normal development but also in pathological circumstances? Wounding, for example, induces a burst of capillary growth in the neighborhood of the damage, to satisfy the high metabolic requirements of the repair process (**Figure 23–34**). Local irritants and infections also cause a proliferation of new capillaries, most of which regress and disappear when the inflammation subsides. Less benignly, a small sample of tumor tissue implanted in the cornea, which normally lacks blood vessels, causes blood vessels to grow quickly toward the implant from the vascular margin of the cornea; the growth rate of the tumor increases abruptly as soon as the vessels reach it.

In all these cases, the invading endothelial cells respond to signals produced by the tissue that they invade. The signals are complex, but a key part is played by **vascular endothelial growth factor** (**VEGF**), a distant relative of platelet-derived growth factor (PDGF). The regulation of blood vessel growth to match the needs of the tissue depends on the control of VEGF production, through changes in the stability of its mRNA and in its rate of transcription. The latter control is relatively well understood. A shortage of oxygen, in practically any type of cell, causes an increase in the intracellular concentration of a gene regulatory protein called **hypoxia-inducible factor 1α** (**HIF1α**). HIF1α stimulates transcription of *Vegf* (and of other genes whose products are needed when oxygen is in short supply). The VEGF protein is secreted, diffuses through the tissue (with different isoforms of VEGF diffusing to different extents), and acts on nearby endothelial cells, stimulating them to proliferate, to produce proteases to help them digest their way through the basal lamina of the parent capillary or venule, and to form sprouts. The tip cells of the sprouts detect the VEGF

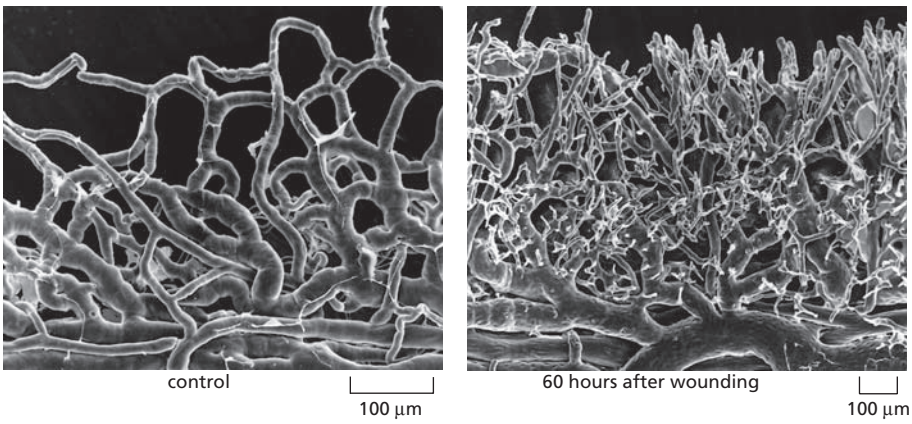



**Figure 23–34 New capillary formation in response to wounding.** Scanning electron micrographs of casts of the system of blood vessels surrounding the margin of the cornea show the reaction to wounding. The casts are made by injecting a resin into the vessels and letting the resin set; this reveals the shape of the lumen, as opposed to the shape of the cells. Sixty hours after wounding many new capillaries have begun to sprout toward the site of injury, which is just above the top of the picture. Their oriented outgrowth reflects a chemotactic response of the endothelial cells to an angiogenic factor released at the wound. (Courtesy of Peter C. Burger.)

gradient and move toward its source. (Other growth factors, including some members of the fibroblast growth factor family, can also stimulate angiogenesis, mediating reactions to other conditions such as inflammation.)

As the new vessels form, bringing blood to the tissue, the oxygen concentration rises, HIF1α activity declines, VEGF production is shut off, and angiogenesis comes to a halt (**Figure 23–35**). As in all signaling systems, it is as important to switch the signal off correctly as to switch it on. In normal well-oxygenated tissue, continual degradation of the HIF1α protein keeps the concentration of HIF1α low: in the presence of oxygen, an oxygen-requiring enzyme modifies HIF1α so as to target it for degradation. Degradation in turn requires the product of another gene, coding for an E3 ubiquitin ligase subunit, which is defective in a rare disorder called *von Hippel–Lindau (VHL) syndrome*. People with this condition are born with only one functional copy of the *Vhl* gene; mutations occurring at random in the body then give rise to cells with two defective gene copies. These cells contain large quantities of HIF1 regardless of oxygen availability, triggering the continual overproduction of VEGF. The result is development of *hemangioblastomas*, tumors that contain dense masses of blood vessels. The mutant cells that produce the VEGF are apparently themselves encouraged to proliferate by the over-rich nourishment provided by the excess blood vessels, creating a vicious cycle that promotes tumor growth. Loss of the VHL gene product also gives rise to other tumors as well as hemangioblastomas, by mechanisms that may be independent of effects on angiogenesis.

This is not the whole story of how angiogenesis is controlled, however. VEGF and related factors from the target tissue are essential to stimulate and guide angiogenesis, but interactions between one endothelial cell and another, mediated by the Notch signaling pathway, also have a critical role. These interactions control which cells will be singled out to behave as tip cells, extending filopodia and crawling forward to create new vascular sprouts, and they are required to bring this motile behavior to a halt when it is time to stop. Thus, when endothelial sprouts meet and join up to form a vascular circuit, they normally switch off to reduce their sprouting activities. The effect depends on a specific Notch ligand, called Delta4, which is expressed in tip cells and activates Notch in their neighbors; Notch activation leads to reduced expression of VEGF receptors, making the neighbors of the tip cell unresponsive to VEGF. In mutants where Notch signaling is defective, sprouting behavior continues inappropriately and fails to be confined to tip cells. The result is an excessively dense network of ill-organized, dysfunctional vessels that carry little or no blood.

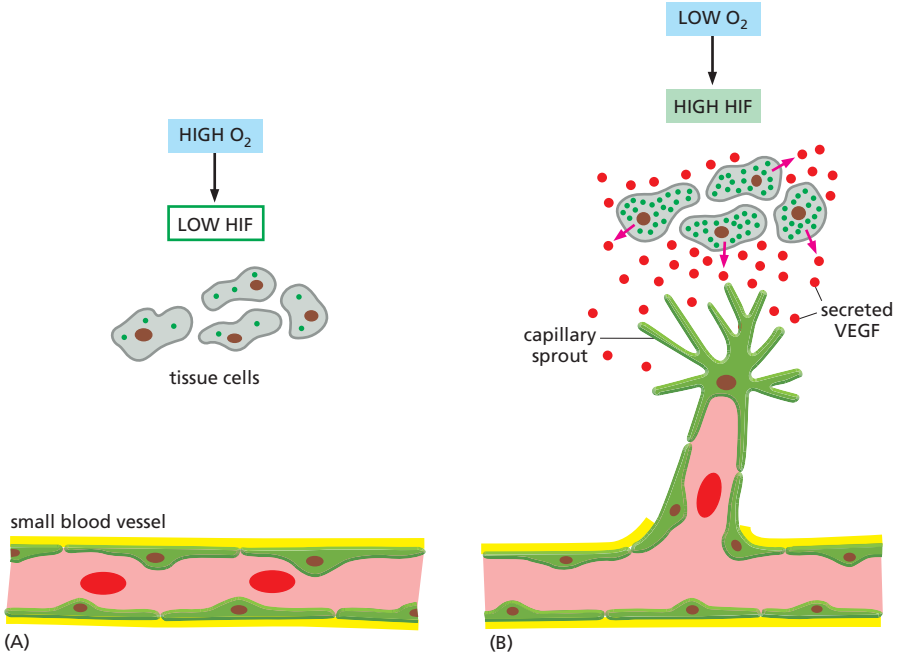



**Figure 23–35 The regulatory mechanism controlling blood vessel growth according to a tissue's need for oxygen.** Lack of oxygen triggers the secretion of VEGF, which stimulates angiogenesis.

### Signals from Endothelial Cells Control Recruitment of Pericytes and Smooth Muscle Cells to Form the Vessel Wall

The vascular network is continually remodeled as it grows and adapts. A newly formed vessel may enlarge; or it may sprout side branches; or it may regress. Smooth muscle and other connective-tissue cells that pack themselves around the endothelium (see Figure 23–32) help to stabilize vessels as they enlarge. This process of vessel wall formation begins with recruitment of pericytes. Small numbers of these cells travel outward in company with the stalk cells of each endothelial sprout. The recruitment and proliferation of pericytes and smooth muscle cells to form a vessel wall depend on PDGF-B secreted by the endothelial cells and on PDGF receptors in the pericytes and smooth muscle cells. In mutants lacking this signal protein or its receptor, these vessel wall cells in many regions are missing. As a result, the embryonic blood vessels develop microaneurysms—microscopic pathological dilatations—that eventually rupture, as well as other abnormalities, reflecting the importance of signals exchanged in both directions between the exterior cells of the wall and the endothelial cells.

Once a vessel has matured, signals from the endothelial cells to the surrounding connective tissue and smooth muscle continue to regulate the vessel's function and structure. For example, the endothelial cells have mechanoreceptors that allow them to sense the shear stress due to flow of blood over their surface. The cells react by generating and releasing the gas NO, thereby signaling to the surrounding cells and inducing changes in the vessel's diameter and wall thickness to accommodate the blood flow. Endothelial cells also mediate rapid responses to neural signals for blood vessel dilation, by releasing NO to make smooth muscle relax in the vessel wall, as discussed in Chapter 15.

### Summary

*Endothelial cells are the fundamental elements of the vascular system. They form a single cell layer that lines all blood vessels and lymphatics and regulates exchanges between the bloodstream and the surrounding tissues. New vessels originate as endothelial sprouts from the walls of existing small vessels. A specialized motile endothelial tip cell at the leading edge of each sprout puts out filopodia that respond to gradients of guidance molecules in the environment, leading the growth of the sprout like the growth cone of a neuron. The endothelial stalk cells following behind become hollowed out to form a capillary tube. Endothelial cells of developing arteries, veins, and lymphatics express different cell-surface proteins, which may control the way in which they link up to create the vascular networks. Signals from endothelial cells organize the growth and development of the connective-tissue cells that form the surrounding layers of the vessel wall.*

*A homeostatic mechanism ensures that blood vessels permeate every region of the body. Cells that are short of oxygen increase their concentration of hypoxia-inducible factor (HIF1α), which stimulates the production of vascular endothelial growth factor (VEGF). VEGF acts on endothelial cells, causing them to proliferate and invade the hypoxic tissue to supply it with new blood vessels. The endothelial cells also interact with one another via the Notch pathway. This exchange of Notch signals is necessary to limit the number of cells that behave as tip cells and to halt angiogenic behavior when tip cells meet.*

## RENEWAL BY MULTIPOTENT STEM CELLS: BLOOD CELL FORMATION

Blood contains many types of cells, with functions that range from the transport of oxygen to the production of antibodies. Some of these cells stay within the vascular system, while others use the vascular system only as a means of transport and perform their function elsewhere. All blood cells, however, have certain similarities in their life history. They all have limited life spans and are produced

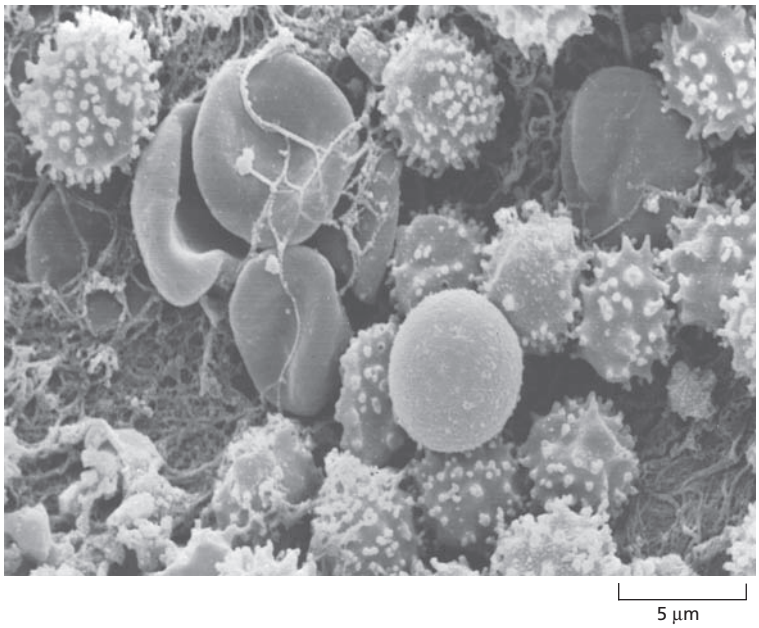



**Figure 23–36 Scanning electron micrograph of mammalian blood cells caught in a blood clot.** The larger, more spherical cells with a rough surface are white blood cells; the smoother, flattened cells are red blood cells. (Courtesy of Ray Moss.)

throughout the life of the animal. Most remarkably, they are all generated ultimately from a common stem cell in the bone marrow. This *hemopoietic* (blood-forming, also called *hematopoietic*) *stem cell* is thus multipotent, giving rise to all the types of terminally differentiated blood cells as well as some other types of cells, such as osteoclasts in bone, which we discuss later.

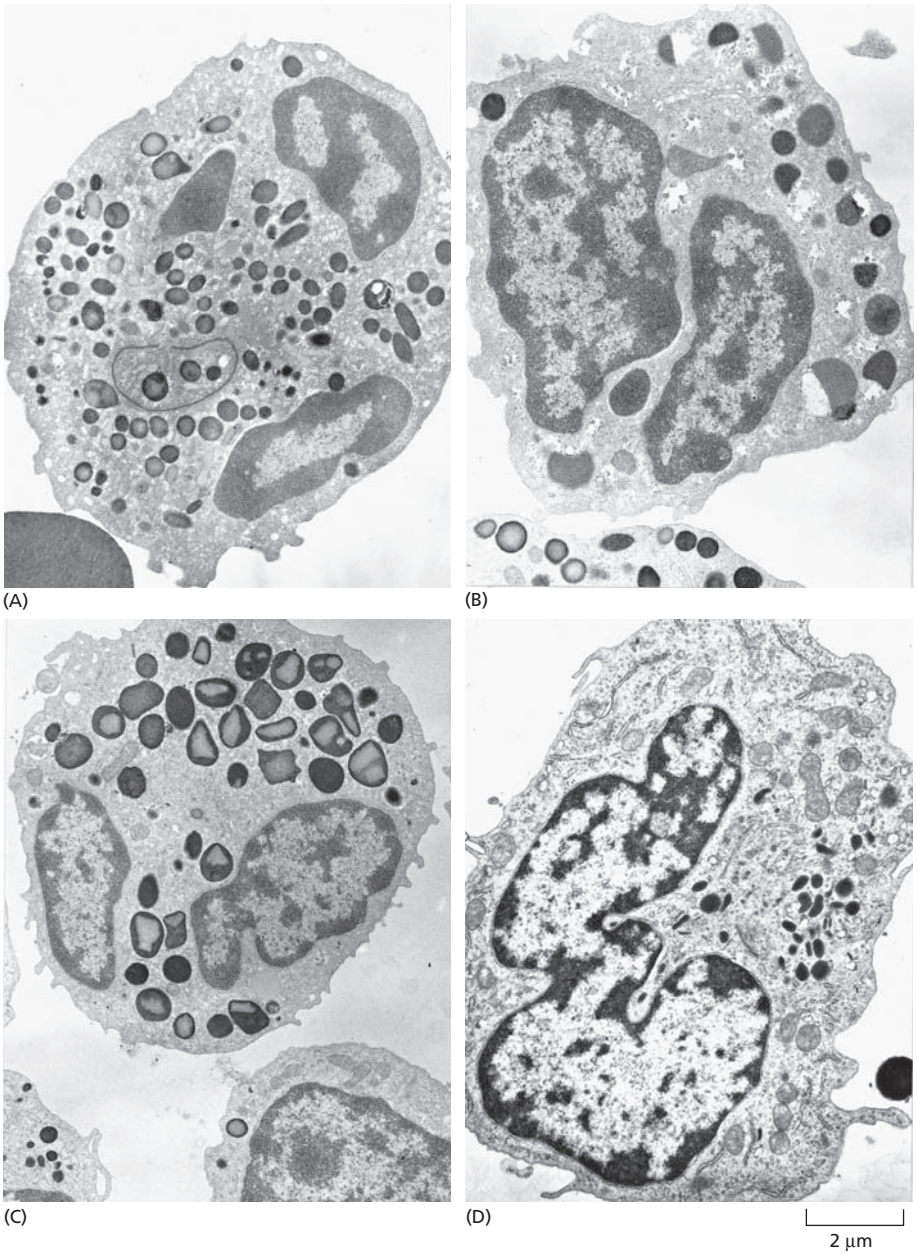
Blood cells can be classified as red or white (**Figure 23–36**). The **red blood cells**, or **erythrocytes**, remain within the blood vessels and transport $O_2$ and $CO_2$ bound to hemoglobin. The **white blood cells**, or **leucocytes**, combat infection and in some cases phagocytose and digest debris. Leucocytes, unlike erythrocytes, must make their way across the walls of small blood vessels and migrate into tissues to perform their tasks. In addition, the blood contains large numbers of **platelets**, which are not entire cells but small, detached cell fragments or "minicells" derived from the cortical cytoplasm of large cells called *megakaryocytes*. Platelets adhere specifically to the endothelial cell lining of damaged blood vessels, where they help to repair breaches and aid in blood clotting.

## The Three Main Categories of White Blood Cells Are Granulocytes, Monocytes, and Lymphocytes

All red blood cells belong in a single class, following the same developmental trajectory as they mature, and the same is true of platelets; but there are many distinct types of white blood cells. White blood cells are traditionally grouped into three major categories—granulocytes, monocytes, and lymphocytes—based on their appearance in the light microscope.

**Granulocytes** contain numerous lysosomes and secretory vesicles (or granules) and are subdivided into three classes according to the morphology and staining properties of these organelles (**Figure 23–37**). The differences in staining reflect major differences of chemistry and function. *Neutrophils* (also called *polymorphonuclear leucocytes* because of their multilobed nucleus) are the most common type of granulocyte; they phagocytose and destroy microorganisms, especially bacteria, and thus have a key role in innate immunity to bacterial infection, as discussed in Chapter 25. *Basophils* secrete histamine (and, in some species, serotonin) to help mediate inflammatory reactions; they are closely related to *mast cells*, which reside in connective tissues but are also generated from the hemopoietic stem cells. *Eosinophils* help to destroy parasites and modulate allergic inflammatory responses.

Once they leave the bloodstream, **monocytes** (see Figure 23–37D) mature into **macrophages**, which, together with neutrophils, are the main "professional phagocytes" in the body. As discussed in Chapter 13, both types of phagocytic



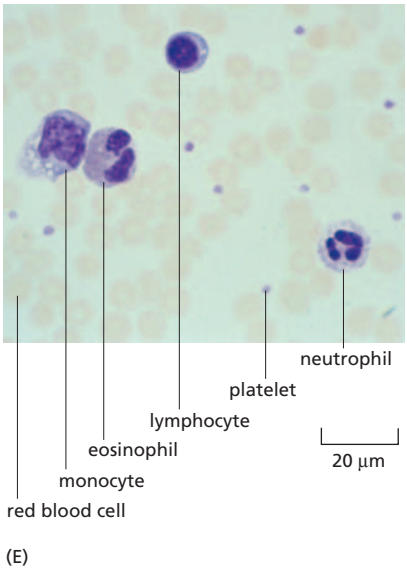




**Figure 23–37 White blood cells.** (A–D) These electron micrographs show (A) a neutrophil, (B) a basophil, (C) an eosinophil, and (D) a monocyte. Electron micrographs of lymphocytes are shown in Figure 25–7. Each of the cell types shown here has a different function, which is reflected in the distinctive types of secretory granules and lysosomes it contains. There is only one nucleus per cell, but it has an irregular lobed shape, and in (A), (B), and (C) the connections between the lobes are out of the plane of section. (E) A light micrograph of a blood smear stained with the Romanowsky stain, which colors the white blood cells strongly. (A–D, from B.A. Nichols et al., *J. Cell Biol.* 50:498–515, 1971. With permission from The Rockefeller University Press; E, courtesy of David Mason.)

cells contain specialized lysosomes that fuse with newly formed phagocytic vesicles (phagosomes), exposing phagocytosed microorganisms to a barrage of enzymatically produced, highly reactive molecules of superoxide ($O_2^-$) and hypochlorite (HOCl, the active ingredient in bleach), as well as to attack by a concentrated mixture of lysosomal hydrolases that become activated in the phagosome. Macrophages, however, are much larger and longer-lived than neutrophils. They recognize and remove senescent, dead, and damaged cells in many tissues, and they are unique in being able to ingest large microorganisms such as protozoa.

Monocytes also give rise to *dendritic cells*, such as the *Langerhans cells* scattered in the epidermis. Like macrophages, dendritic cells are migratory cells that can ingest foreign substances and organisms; but they do not have as active an appetite for phagocytosis and are instead specialized as presenters of foreign antigens to lymphocytes to trigger an immune response. Langerhans cells, for example, ingest foreign antigens in the epidermis and carry these trophies back to present to lymphocytes in lymph nodes.

There are two main classes of **lymphocytes**, both involved in immune responses: *B lymphocytes* make antibodies, while *T lymphocytes* kill virus-infected cells and regulate the activities of other white blood cells. In addition,

there are lymphocytelike cells called *natural killer (NK) cells*, which kill some types of tumor cells and virus-infected cells. The production of lymphocytes is a specialized topic discussed in detail in Chapter 25. Here we concentrate mainly on the development of the other blood cells, often referred to collectively as **myeloid cells**.

**Table 23–1** summarizes the various types of blood cells and their functions.

## The Production of Each Type of Blood Cell in the Bone Marrow Is Individually Controlled

Most white blood cells function in tissues other than the blood; blood simply transports them to where they are needed. A local infection or injury in any tissue rapidly attracts white blood cells into the affected region as part of the inflammatory response, which helps fight the infection or heal the wound.

The inflammatory response is complex and is governed by many different signal molecules produced locally by mast cells, nerve endings, platelets, and white blood cells, as well as by the activation of complement (discussed in Chapters 24 and 25). Some of these signal molecules act on nearby capillaries, causing the endothelial cells to adhere less tightly to one another but making their surfaces adhesive to passing white blood cells. The white blood cells are thus caught like flies on flypaper and then can escape from the vessel by squeezing between the endothelial cells and using digestive enzymes to crawl across the basal lamina. <ACCG> As discussed in Chapter 19, homing receptors called *selectins* mediate the initial binding to endothelial cells, while *integrins* mediate the stronger binding required for the white blood cells to crawl out of

**Table 23–1 Blood Cells**

| TYPE OF CELL | MAIN FUNCTIONS | TYPICAL CONCENTRATION IN HUMAN BLOOD (CELLS/LITER) |
|---|---|---|
| Red blood cells (erythrocytes) | transport $O_2$ and $CO_2$ | $5 \times 10^{12}$ |
| White blood cells (leucocytes) | | |
| *Granulocytes* | | |
| Neutrophils (polymorphonuclear leucocytes) | phagocytose and destroy invading bacteria | $5 \times 10^9$ |
| Eosinophils | destroy larger parasites and modulate allergic inflammatory responses | $2 \times 10^8$ |
| Basophils | release histamine (and in some species serotonin) in certain immune reactions | $4 \times 10^7$ |
| *Monocytes* | become tissue macrophages, which phagocytose and digest invading microorganisms and foreign bodies as well as damaged senescent cells | $4 \times 10^8$ |
| *Lymphocytes* | | |
| B cells | make antibodies | $2 \times 10^9$ |
| T cells | kill virus-infected cells and regulate activities of other leucocytes | $1 \times 10^9$ |
| *Natural killer (NK) cells* | kill virus-infected cells and some tumor cells | $1 \times 10^8$ |
| Platelets (cell fragments arising from *megakaryocytes* in bone marrow) | initiate blood clotting | $3 \times 10^{11}$ |

Humans contain about 5 liters of blood, accounting for 7% of body weight. Red blood cells constitute about 45% of this volume and white blood cells about 1%, the rest being the liquid blood plasma.

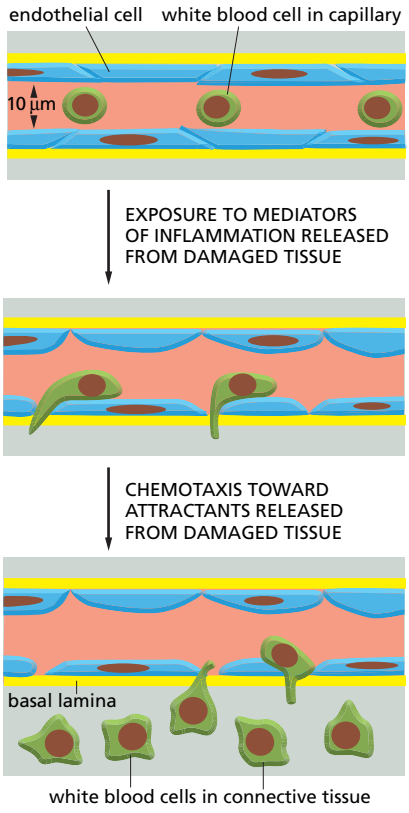



**Figure 23–38 The migration of white blood cells out of the bloodstream during an inflammatory response.** The response is initiated by signal molecules produced by cells in the neighborhood (mainly in the connective tissue) or by complement activation. Some of these mediators act on capillary endothelial cells, causing them to loosen their attachments to their neighbors so that the capillaries become more permeable. Endothelial cells are also stimulated to express selectins, cell-surface molecules that recognize specific carbohydrates that are present on the surface of leucocytes in the blood and cause them to stick to the endothelium. The inflamed tissues and local endothelial cells secrete other mediators called chemokines, and the chemokines act as chemoattractants, causing the bound leucocytes to crawl between the capillary endothelial cells into the tissue.

the blood vessel (see Figure 19–19). Damaged or inflamed tissues and local endothelial cells secrete other molecules called *chemokines*, which act as chemoattractants for specific types of white blood cells, causing them to become polarized and crawl toward the source of the attractant. As a result, large numbers of white blood cells enter the affected tissue (**Figure 23–38**).

Other signal molecules produced during an inflammatory response escape into the blood and stimulate the bone marrow to produce more leucocytes and release them into the bloodstream. The bone marrow is the key target for such regulation because, with the exception of lymphocytes and some macrophages, most types of blood cells in adult mammals are generated only in the bone marrow. The regulation tends to be cell-type-specific: some bacterial infections, for example, cause a selective increase in neutrophils, while infections with some protozoa and other parasites cause a selective increase in eosinophils. (For this reason, physicians routinely use differential white blood cell counts to aid in the diagnosis of infectious and other inflammatory diseases.)

In other circumstances erythrocyte production is selectively increased—for example, in the process of acclimatization when one goes to live at high altitude, where oxygen is scarce. Thus, blood cell formation, or *hemopoiesis* (also called *hematopoiesis*), necessarily involves complex controls, which regulate the production of each type of blood cell individually to meet changing needs. It is a problem of great medical importance to understand how these controls operate.

In intact animals, hemopoiesis is more difficult to analyze than is cell turnover in a tissue such as the epidermis or the lining of the gut, where a simple, regular spatial organization makes it easy to follow the process of renewal and to locate the stem cells. The hemopoietic tissues do not appear so orderly. However, hemopoietic cells have a nomadic lifestyle that makes them more accessible to experimental study in other ways. It is easy to obtain dispersed hemopoietic cells and to transfer them, without damage, from one animal to another. Moreover, the proliferation and differentiation of individual cells and their progeny can be observed and analyzed in culture, and numerous molecular markers distinguish the various stages of differentiation. Because of this, more is known about the molecules that control blood cell production than about those that control cell production in other mammalian tissues. Studies of hemopoiesis have strongly influenced current ideas about stem-cell systems in general.

## Bone Marrow Contains Hemopoietic Stem Cells

Routine staining methods allow us to recognize the different types of blood cells and their immediate precursors in the bone marrow (**Figure 23–39**). Here, these cells are intermingled with one another, as well as with fat cells and other stromal cells (connective-tissue cells), which produce a delicate supporting meshwork of collagen fibers and other extracellular matrix components. In addition, the whole tissue is richly supplied with thin-walled blood vessels, called *blood sinuses*, into which the new blood cells are discharged. **Megakaryocytes** are also present; these, unlike other blood cells, remain in the bone marrow when

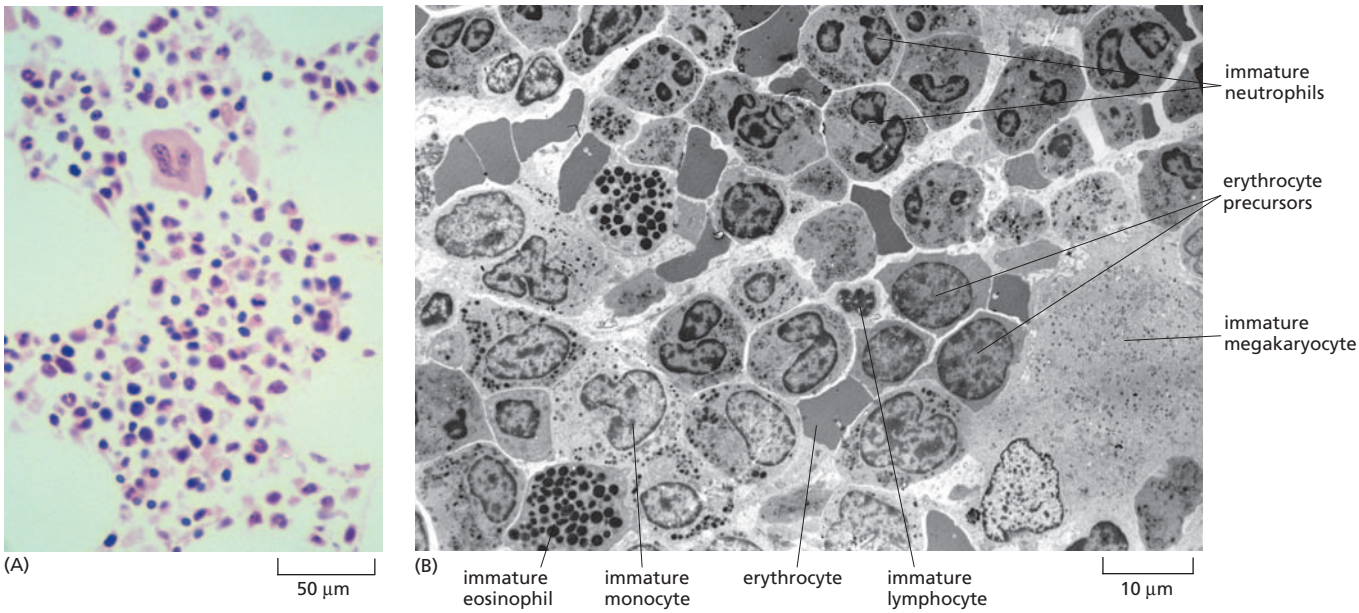



**Figure 23–39 Bone marrow.** (A) A light micrograph of a stained section. The large empty spaces correspond to fat cells, whose fatty contents have been dissolved away during specimen preparation. The giant cell with a lobed nucleus is a megakaryocyte. (B) A low-magnification electron micrograph. Bone marrow is the main source of new blood cells (except for T lymphocytes, which are produced in the thymus). Note that the immature blood cells of a particular type tend to cluster in "family groups." (A, courtesy of David Mason; B, from J.A.G. Rhodin, Histology: A Text and Atlas. New York: Oxford University Press, 1974.)

mature and are one of its most striking features, being extraordinarily large (diameter up to 60 μm), with a highly polyploid nucleus. They normally lie close beside blood sinuses, and they extend processes through holes in the endothelial lining of these vessels; platelets pinch off from the processes and are swept away into the blood (**Figure 23–40**). <GCAT>

Because of the complex arrangement of the cells in bone marrow, it is difficult to identify in ordinary tissue sections any but the immediate precursors of the mature blood cells. The corresponding cells at still earlier stages of development, before any overt differentiation has begun, look confusingly similar, and although the spatial distribution of cell types has some orderly features, there is no obvious visible characteristic by which we can recognize the ultimate stem cells. To identify and characterize the stem cells, we need a functional assay, which involves tracing the progeny of single cells. As we shall see, this can be done *in vitro* simply by examining the colonies that isolated cells produce in culture. The hemopoietic system, however, can also be manipulated so that such clones of cells can be recognized *in vivo* in the intact animal.

When an animal is exposed to a large dose of x-rays, most of the hemopoietic cells are destroyed and the animal dies within a few days as a result of its

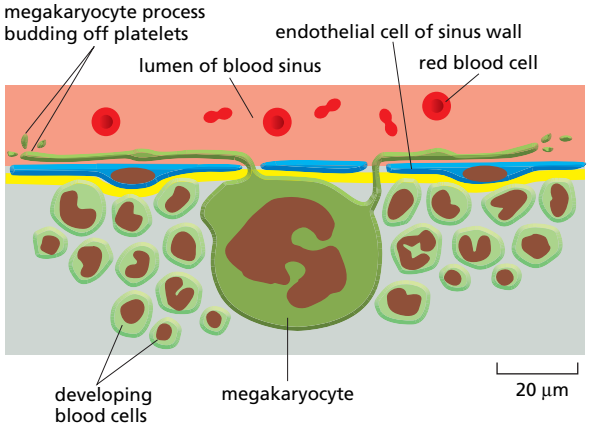



**Figure 23–40 A megakaryocyte among other cells in the bone marrow.** Its enormous size results from its having a highly polyploid nucleus. One megakaryocyte produces about 10,000 platelets, which split off from long processes that extend through holes in the walls of an adjacent blood sinus.

inability to manufacture new blood cells. The animal can be saved, however, by a transfusion of cells taken from the bone marrow of a healthy, immunologically compatible donor. Among these cells there are some that can colonize the irradiated host and permanently reequip it with hemopoietic tissue (**Figure 23–41**). Such experiments prove that the marrow contains hemopoietic stem cells. They also show how we can assay for the presence of hemopoietic stem cells and hence discover the molecular features that distinguish them from other cells.

For this purpose, cells taken from bone marrow are sorted (using a fluorescence-activated cell sorter) according to the surface antigens that they display, and the different fractions are transfused back into irradiated mice. If a fraction rescues an irradiated host mouse, it must contain hemopoietic stem cells. In this way, it has been possible to show that the hemopoietic stem cells are characterized by a specific combination of cell-surface proteins, and by appropriate sorting we can obtain virtually pure stem cell preparations. The stem cells turn out to be a tiny fraction of the bone marrow population—about 1 cell in 10,000; but this is enough. As few as five such cells injected into a host mouse with defective hemopoiesis are sufficient to reconstitute its entire hemopoietic system, generating a complete set of blood cell types, as well as fresh stem cells.

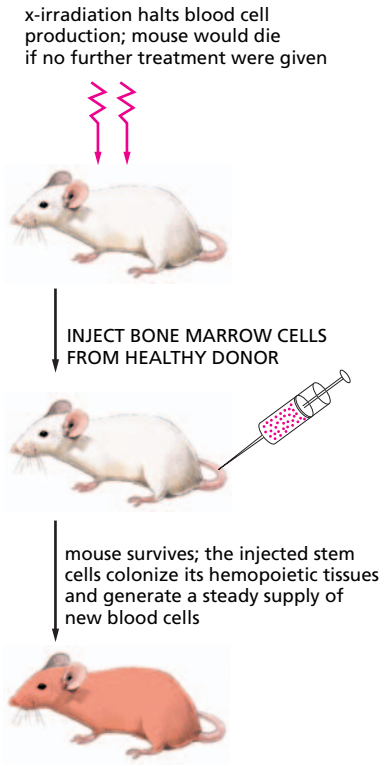



**Figure 23–41 Rescue of an irradiated mouse by a transfusion of bone marrow cells.** An essentially similar procedure is used in the treatment of leukemia in human patients by bone marrow transplantation.

## A Multipotent Stem Cell Gives Rise to All Classes of Blood Cells

To see what range of cell types a single **hemopoietic stem cell** can generate, we need a way to trace the fate of its progeny. This can be done by marking individual stem cells genetically, so that their progeny can be identified even after they have been released into the bloodstream. Although several methods have been used for this, a specially engineered retrovirus (a retroviral vector carrying a marker gene) serves the purpose particularly well. The marker virus, like other retroviruses, can insert its own genome into the chromosomes of the cell it infects, but the genes that would enable it to generate new infectious virus particles have been removed. The marker is therefore confined to the progeny of the cells that were originally infected, and the progeny of one such cell can be distinguished from the progeny of another because the chromosomal sites of insertion of the virus are different. To analyze hemopoietic cell lineages, bone marrow cells are first infected with the retroviral vector *in vitro* and then are transferred into a lethally irradiated recipient; DNA probes can then be used to trace the progeny of individual infected cells in the various hemopoietic and lymphoid tissues of the host. These experiments show that the individual hemopoietic stem cell is *multipotent* and can give rise to the complete range of blood cell types, both myeloid and lymphoid, as well as new stem cells like itself (**Figure 23–42**).

Later in this chapter, we explain how the same methods that were developed for experimentation in mice can now be used for treatment of disease in humans.

## Commitment Is a Stepwise Process

Hemopoietic stem cells do not jump directly from a multipotent state into a commitment to just one pathway of differentiation; instead, they go through a series of progressive restrictions. The first step, usually, is commitment to either a myeloid or a lymphoid fate. This is thought to give rise to two kinds of progenitor cells, one capable of generating large numbers of all the different types of myeloid cells, or perhaps of myeloid cells plus B lymphocytes, and the other giving rise to large numbers of all the different types of lymphoid cells, or at least T lymphocytes. Further steps give rise to progenitors committed to the production of just one cell type. The steps of commitment correlate with changes in the expression of specific gene regulatory proteins, needed for the production of different subsets of blood cells. These proteins seem to act in a complicated combinatorial fashion: the *GATA1 protein*, for example, is needed for the maturation of red blood cells, but is active also at much earlier steps in the hemopoietic pathway.

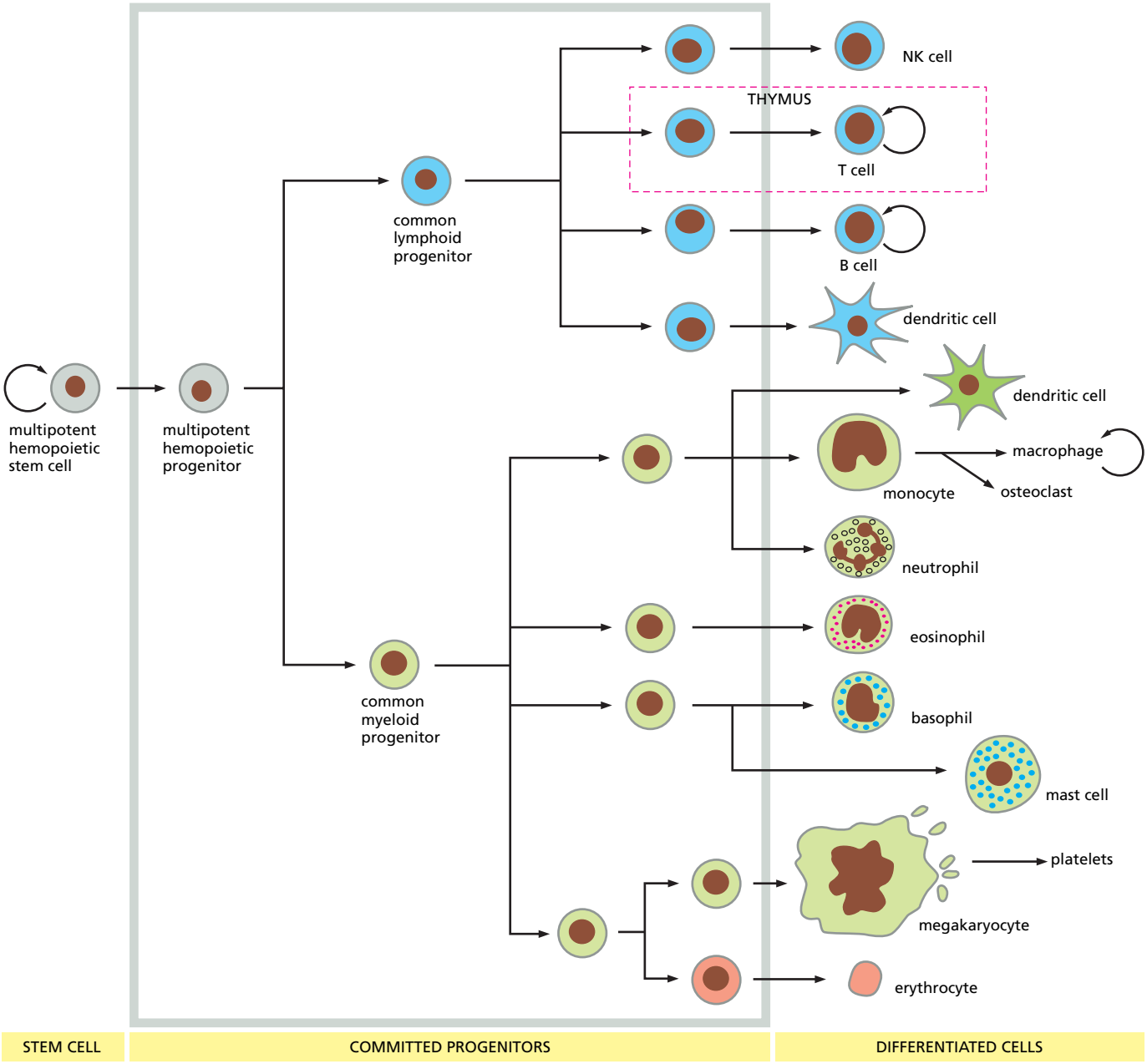



**Figure 23–42 A tentative scheme of hemopoiesis.** The multipotent stem cell normally divides infrequently to generate either more multipotent stem cells, which are self-renewing, or committed progenitor cells, which are limited in the number of times that they can divide before differentiating to form mature blood cells. As they go through their divisions, the progenitors become progressively more specialized in the range of cell types that they can give rise to, as indicated by the branching of the cell-lineage diagram in the region enclosed in the *gray* box. Many of the details of this part of the lineage diagram are still controversial, however. In adult mammals, all of the cells shown develop mainly in the bone marrow—except for T lymphocytes, which develop in the thymus, and macrophages and osteoclasts, which develop from blood monocytes. Some dendritic cells may also derive from monocytes.

## Divisions of Committed Progenitor Cells Amplify the Number of Specialized Blood Cells

Hemopoietic progenitor cells generally become committed to a particular pathway of differentiation long before they cease proliferating and terminally differentiate. The committed progenitors go through many rounds of cell division to amplify the ultimate number of cells of the given specialized type. In this way, a single stem-cell division can lead to the production of thousands of differentiated progeny, which explains why the number of stem cells is such a small fraction of the total population of hemopoietic cells. For the same reason, a high rate

of blood cell production can be maintained even though the stem-cell division rate is low. As noted earlier, infrequent division or quiescence is a common feature of stem cells in several tissues. By reducing the number of division cycles that the stem cells themselves have to undergo in the course of a lifetime, it lowers the risk of generating stem-cell mutations, which would give rise to persistent mutant clones of cells in the body. It also has another effect: it reduces the rate of replicative senescence (discussed in Chapter 17). In fact, hemopoietic stem cells that are forced to keep dividing rapidly (through knockout of a gene called *Gfi1* that restricts their proliferation rate, or by other means) fail to sustain hemopoiesis for a full normal lifespan.

The stepwise nature of commitment means that the hemopoietic system can be viewed as a hierarchical family tree of cells. Multipotent stem cells give rise to *committed progenitor cells*, which are specified to give rise to only one or a few blood cell types. The committed progenitors divide rapidly, but only a limited number of times, before they terminally differentiate into cells that divide no further and die after several days or weeks. Many cells normally die at the earlier steps in the pathway as well. Studies in culture provide a way to find out how the proliferation, differentiation, and death of the hemopoietic cells are regulated.

## Stem Cells Depend on Contact Signals From Stromal Cells

Hemopoietic cells can survive, proliferate, and differentiate in culture if, and only if, they are provided with specific signal proteins or are accompanied by cells that produce these proteins. If deprived of such proteins, the cells die. For long-term maintenance, contact with appropriate supporting cells also seems to be necessary: hemopoiesis can be kept going for months or even years *in vitro* by culturing dispersed bone marrow hemopoietic cells on top of a layer of bone-marrow stromal cells, which mimic the environment in intact bone marrow. Such cultures can generate all the types of myeloid cells, and their long-term continuation implies that stem cells, as well as differentiated progeny, are being continually produced.

In the bone marrow, where they normally live, the hemopoietic stem cells are mostly located in close contact with the *osteoblasts* that line the bony surfaces of the marrow cavity—the cells that produce the bone matrix. Treatments and mutations that increase or decrease the number of osteoblasts cause corresponding changes in the numbers of hemopoietic stem cells. This suggests that the osteoblasts provide the signals that the hemopoietic stem cells need to keep them in their uncommitted stem-cell state, just as the intestinal crypt provides the signals needed to maintain stem cells of the gut epithelium. In both systems, stem cells are normally confined to a particular niche, and when they leave this niche they tend to lose their stem-cell potential (**Figure 23–43**). Hemopoietic stem cells in the bone marrow and elsewhere are also often associated with a specialized class of endothelial cells, which may provide them with an alternative niche.

A key feature of the stem-cell niche in the bone marrow, as in the gut, is that it provides stimulation of the Wnt signaling pathway. Artificial activation of this pathway in cultured hemopoietic stem cells helps them to survive, proliferate, and keep their character as stem cells, while blocking Wnt signaling does the opposite. Another interaction that is important for the maintenance of hemopoiesis came to light through the analysis of mouse mutants with a curious combination of defects: a shortage of red blood cells (anemia), of germ cells (sterility), and of pigment cells (white spotting of the skin; see Figure 22–86). As discussed in Chapter 22, this syndrome results from mutations in either of two genes: one, called *Kit*, codes for a receptor tyrosine kinase; the other codes for its ligand. The cell types affected by the mutations all derive from migratory precursors, and it seems that these precursors in each case must express the receptor and be provided with the ligand by their environment if they are to survive and produce progeny in normal numbers. Studies in mutant mice suggest that Kit ligand must be membrane-bound to be fully effective, implying that normal hemopoiesis requires direct cell–cell contact between the hemopoietic

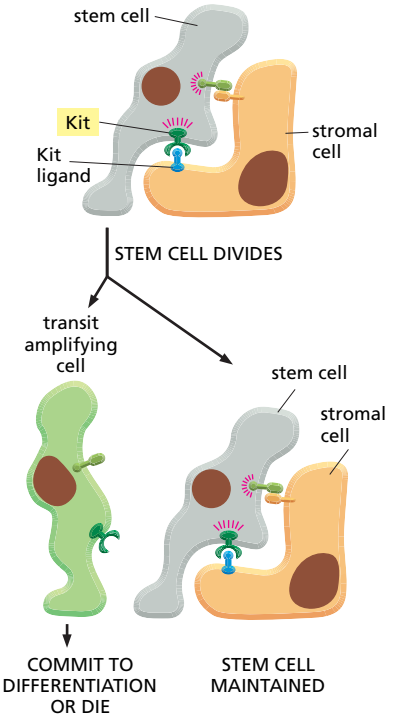



**Figure 23–43 Dependence of hemopoietic stem cells on contact with stromal cells.** The contact-dependent interaction between Kit and its ligand is one of several signaling mechanisms thought to be involved in hemopoietic stem-cell maintenance. The real system is certainly more complex; the dependence of hemopoietic cells on contact with stromal cells cannot be absolute, since small numbers of the functional stem cells can be found free in the circulation.

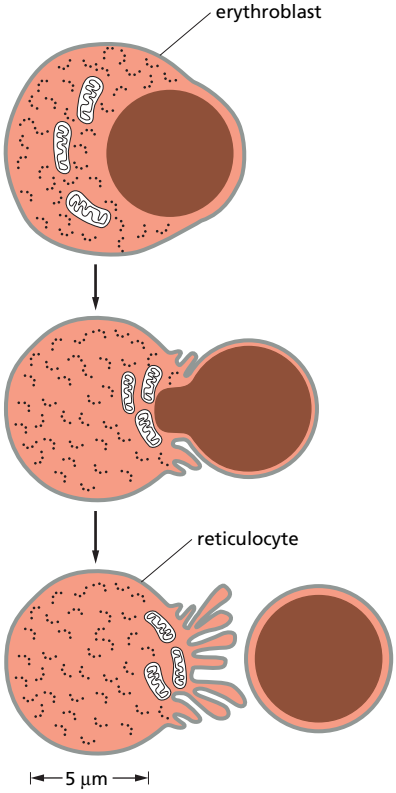



**Figure 23–44 A developing red blood cell (erythroblast).** The cell is shown extruding its nucleus to become an immature erythrocyte (a reticulocyte), which then leaves the bone marrow and passes into the bloodstream. The reticulocyte will lose its mitochondria and ribosomes within a day or two to become a mature erythrocyte. Erythrocyte clones develop in the bone marrow on the surface of a macrophage, which phagocytoses and digests the nuclei discarded by the erythroblasts.

cells that express Kit receptor protein, and stromal cells (osteoblasts among them) that express Kit ligand.

## Factors That Regulate Hemopoiesis Can Be Analyzed in Culture

While stem cells depend on contact with stromal cells for long-term maintenance, their committed progeny do not, or at least not to the same degree. Thus, dispersed bone marrow hemopoietic cells can be cultured in a semisolid matrix of dilute agar or methylcellulose, and factors derived from other cells can be added artificially to the medium. Because cells in the semisolid matrix cannot migrate, the progeny of each isolated precursor cell remain together as an easily distinguishable colony. A single committed neutrophil progenitor, for example, may give rise to a clone of thousands of neutrophils. Such culture systems have provided a way to assay for the factors that support hemopoiesis and hence to purify them and explore their actions. These substances are glycoproteins and are usually called **colony-stimulating factors** (**CSFs**). Of the growing number of CSFs that have been defined and purified, some circulate in the blood and act as hormones, while others act in the bone marrow either as secreted local mediators or, like Kit ligand, as membrane-bound signals that act through cell–cell contact. The best understood of the CSFs that act as hormones is the glycoprotein erythropoietin, which is produced in the kidneys and regulates *erythropoiesis*, the formation of red blood cells.

## Erythropoiesis Depends on the Hormone Erythropoietin

The erythrocyte is by far the most common type of cell in the blood (see Table 23–1). When mature, it is packed full of hemoglobin and contains hardly any of the usual cell organelles. In an erythrocyte of an adult mammal, even the nucleus, endoplasmic reticulum, mitochondria, and ribosomes are absent, having been extruded from the cell in the course of its development (**Figure 23–44**). The erythrocyte therefore cannot grow or divide; the only possible way of making more erythrocytes is by means of stem cells. Furthermore, erythrocytes have a limited life-span—about 120 days in humans or 55 days in mice. Worn-out erythrocytes are phagocytosed and digested by macrophages in the liver and spleen, which remove more than $10^{11}$ senescent erythrocytes in each of us each day. Young erythrocytes actively protect themselves from this fate: they have a protein on their surface that binds to an inhibitory receptor on macrophages and thereby prevents their phagocytosis.

A lack of oxygen or a shortage of erythrocytes stimulates specialized cells in the kidney to synthesize and secrete increased amounts of **erythropoietin** into the bloodstream. The erythropoietin, in turn, stimulates the production of more erythrocytes. Since a change in the rate of release of new erythrocytes into the bloodstream is observed as early as 1–2 days after an increase in erythropoietin levels in the bloodstream, the hormone must act on cells that are very close precursors of the mature erythrocytes.

The cells that respond to erythropoietin can be identified by culturing bone marrow cells in a semisolid matrix in the presence of erythropoietin. In a few days, colonies of about 60 erythrocytes appear, each founded by a single committed erythroid progenitor cell. This progenitor depends on erythropoietin for its survival as well as its proliferation. It does not yet contain hemoglobin, and it is derived from an earlier type of committed erythroid progenitor that does not depend on erythropoietin.

**Table 23–2 Some Colony-stimulating Factors (CSFs) That Influence Blood Cell Formation**

| FACTOR | TARGET CELLS | PRODUCING CELLS | RECEPTORS |
|---|---|---|---|
| Erythropoietin | CFC-E | kidney cells | cytokine family |
| Interleukin 3 (IL3) | multipotent stem cell, most progenitor cells, many terminally differentiated cells | T lymphocytes, epidermal cells | cytokine family |
| Granulocyte/macrophage CSF (GMCSF) | GM progenitor cells | T lymphocytes, endothelial cells, fibroblasts | cytokine family |
| Granulocyte CSF (GCSF) | GM progenitor cells and neutrophils | macrophages, fibroblasts | cytokine family |
| Macrophage CSF (MCSF) | GM progenitor cells and macrophages | fibroblasts, macrophages, endothelial cells | receptor tyrosine kinase family |
| Kit ligand | hemopoietic stem cells | stromal cells in bone marrow and many other cells | receptor tyrosine kinase family |

A second CSF, called **interleukin-3** (**IL3**), promotes the survival and proliferation of the earlier erythroid progenitor cells. In its presence, much larger erythroid colonies, each comprising up to 5000 erythrocytes, develop from cultured bone marrow cells in a process requiring a week or 10 days. Evidently the descendants of the hemopoietic stem cells, after they have become committed to an erythroid fate, have to step their way through a further long program of cell divisions, changing their character and their dependence on environmental signals as they progress toward the final differentiated state.

## Multiple CSFs Influence Neutrophil and Macrophage Production

The two classes of cells dedicated to phagocytosis, neutrophils and macrophages, develop from a common progenitor cell called a **granulocyte/macrophage** (**GM**) **progenitor cell**. Like the other granulocytes (eosinophils and basophils), neutrophils circulate in the blood for only a few hours before migrating out of capillaries into the connective tissues or other specific sites, where they survive for only a few days. They then die by apoptosis and are phagocytosed by macrophages. Macrophages, in contrast, can persist for months or perhaps even years outside the bloodstream, where they can be activated by local signals to resume proliferation.

At least seven distinct CSFs that stimulate neutrophil and macrophage colony formation in culture have been defined, and some or all of these are thought to act in different combinations to regulate the selective production of these cells *in vivo*. These CSFs are synthesized by various cell types—including endothelial cells, fibroblasts, macrophages, and lymphocytes—and their concentration in the blood typically increases rapidly in response to bacterial infection in a tissue, thereby increasing the number of phagocytic cells released from the bone marrow into the bloodstream. IL3 is one of the least specific of the factors, acting on multipotent stem cells as well as on most classes of committed progenitor cells, including GM progenitor cells. Various other factors act more selectively on committed GM progenitor cells and their differentiated progeny (**Table 23–2**), although in many cases they act on certain other branches of the hemopoietic family tree as well.

All of these CSFs, like erythropoietin, are glycoproteins that act at low concentrations (about $10^{-12}$ M) by binding to specific cell-surface receptors, as discussed in Chapter 15. A few of these receptors are transmembrane tyrosine kinases but most belong to the large cytokine receptor family, whose members are usually composed of two or more subunits, one of which is frequently shared among several receptor types (**Figure 23–45**). The CSFs not only operate on the precursor cells to promote the production of differentiated progeny, they also activate the specialized functions (such as phagocytosis and target-cell killing) of the terminally differentiated cells. Proteins produced artificially from the cloned genes for these factors are strong stimulators of hemopoiesis in experimental animals. They are now widely used in human patients to stimulate the

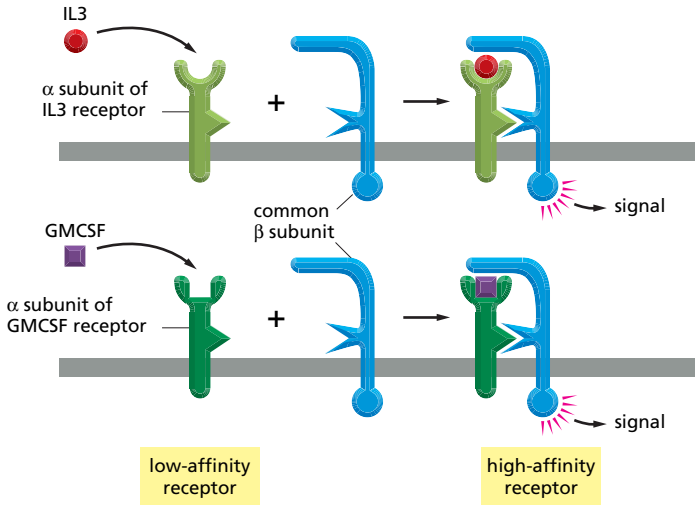



**Figure 23–45 Sharing of subunits among CSF receptors.** Human IL3 receptors and GMCSF receptors have different α subunits and a common β subunit. Their ligands are thought to bind to the free α subunit with low affinity, and this triggers the assembly of the heterodimer that binds the ligand with high affinity.

regeneration of hemopoietic tissue and to boost resistance to infection—an impressive demonstration of how basic cell biological research and animal experiments can lead to better medical treatment.

## The Behavior of a Hemopoietic Cell Depends Partly on Chance

CSFs are defined as factors that promote the production of colonies of differentiated blood cells. But precisely what effect does a CSF have on an individual hemopoietic cell? The factor might control the rate of cell division or the number of division cycles that the progenitor cell undergoes before differentiating; it might act late in the hemopoietic lineage to facilitate differentiation; it might act early to influence commitment; or it might simply increase the probability of cell survival (**Figure 23–46**). By monitoring the fate of isolated individual hemopoietic cells in culture, it has been possible to show that a single CSF, such as GMCSF, can exert all these effects, although it is still not clear which are most important *in vivo*.

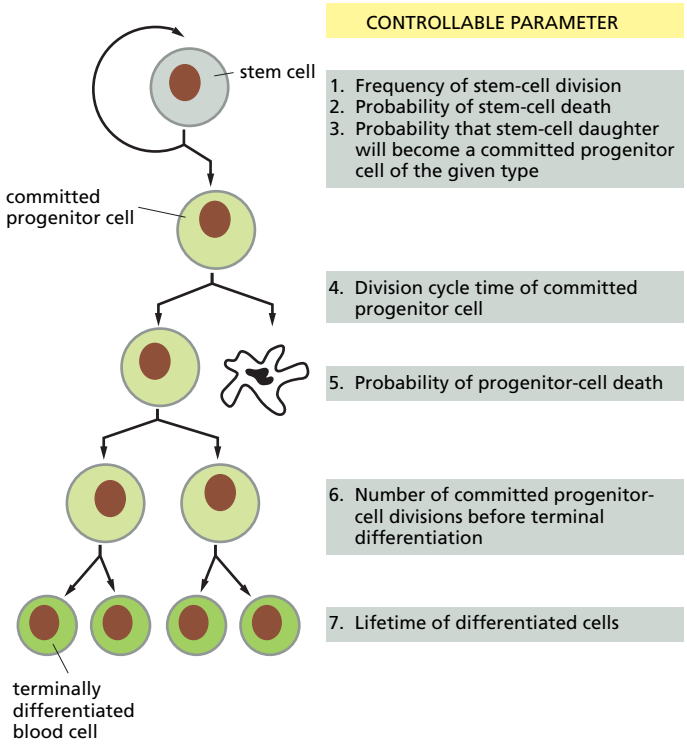



**Figure 23–46 Some of the parameters through which the production of blood cells of a specific type might be regulated.** Studies in culture suggest that colony-stimulating factors (CSFs) can affect all of these aspects of hemopoiesis.

Studies *in vitro* indicate, moreover, that there is a large element of chance in the way a hemopoietic cell behaves—a reflection, presumably, of "noise" in the genetic control system, as discussed in Chapter 7. At least some of the CSFs seem to act by regulating probabilities, not by dictating directly what the cell shall do. In hemopoietic cell cultures, even if the cells have been selected to be as homogeneous a population as possible, there is a remarkable variability in the sizes and often in the characters of the colonies that develop. And if two sister cells are taken immediately after a cell division and cultured apart under identical conditions, they frequently give rise to colonies that contain different types of blood cells or the same types of blood cells in different numbers. Thus, both the programming of cell division and the process of commitment to a particular path of differentiation seem to involve random events at the level of the individual cell, even though the behavior of the multicellular system as a whole is regulated in a reliable way. The sequence of cell fate restrictions shown in Figure 23–42 conveys the impression of a program executed with computer-like logic and precision. Individual cells may be more quirky and erratic, and may sometimes progress by other decision pathways from the stem-cell state toward terminal differentiation.

## Regulation of Cell Survival Is as Important as Regulation of Cell Proliferation

The default behavior of hemopoietic cells in the absence of CSFs is death by apoptosis (discussed in Chapter 18). Thus, in principle, the CSFs could regulate the numbers of the various types of blood cells entirely through selective control of cell survival in this way. There is evidence that the control of cell survival does indeed play a central part in regulating the numbers of blood cells, just as it does for hepatocytes and many other cell types, as we have already seen. The amount of apoptosis in the vertebrate hemopoietic system is enormous: billions of neutrophils die in this way each day in an adult human, for example. In fact, most neutrophils produced in the bone marrow die there without ever functioning. This futile cycle of production and destruction presumably serves to maintain a reserve supply of cells that can be promptly mobilized to fight infection whenever it flares up, or phagocytosed and digested for recycling when all is quiet. Compared with the life of the organism, the lives of cells are cheap.

Too little cell death can be as dangerous to the health of a multicellular organism as too much proliferation. In the hemopoietic system, mutations that inhibit cell death by causing excessive production of the intracellular apoptosis inhibitor Bcl2 promote the development of cancer in B lymphocytes. Indeed, the capacity for unlimited self-renewal is a dangerous property for any cell to possess, and many cases of leukemia arise through mutations that confer this capacity on committed hemopoietic precursor cells that would normally be fated to differentiate and die after a limited number of division cycles.

## Summary

*The many types of blood cells, including erythrocytes, lymphocytes, granulocytes, and macrophages, all derive from a common multipotent stem cell. In the adult, hemopoietic stem cells are found mainly in bone marrow, and they depend on signals from the marrow stromal (connective-tissue) cells, especially osteoblasts, to maintain their stem-cell character. As in some other stem-cell systems, the Wnt signaling pathway appears to be critical for stem-cell maintenance, though it is not the only one involved. The stem cells normally divide infrequently to produce more stem cells (self-renewal) and various committed progenitor cells (transit amplifying cells), each able to give rise to only one or a few types of blood cells. The committed progenitor cells divide extensively under the influence of various protein signal molecules (colony-stimulating factors, or CSFs) and then terminally differentiate into mature blood cells, which usually die after several days or weeks.*

*Studies of hemopoiesis have been greatly aided by* in vitro *assays in which stem cells or committed progenitor cells form clonal colonies when cultured in a semisolid*

*matrix. The progeny of stem cells seem to make their choices between alternative developmental pathways in a partly random manner. Cell death by apoptosis,controlled by the availability of CSFs, also plays a central part in regulating the numbers of mature differentiated blood cells.*

## GENESIS, MODULATION, AND REGENERATION OF SKELETAL MUSCLE

The term "muscle" includes many cell types, all specialized for contraction but in other respects dissimilar. As noted in Chapter 16, all eucaryotic cells possess a contractile system involving actin and myosin, but muscle cells have developed this apparatus to a high degree. Mammals possess four main categories of cells specialized for contraction: skeletal muscle cells, heart (cardiac) muscle cells, smooth muscle cells, and myoepithelial cells (**Figure 23–47**). These differ in function, structure, and development. Although all of them generate contractile forces by using organized filament systems based on actin and myosin, the actin and myosin molecules employed have somewhat different amino acid sequences, are differently arranged in the cell, and are associated with different sets of proteins to control contraction.

**Skeletal muscle cells** are responsible for practically all movements that are under voluntary control. These cells can be very large (2–3 cm long and 100 μm

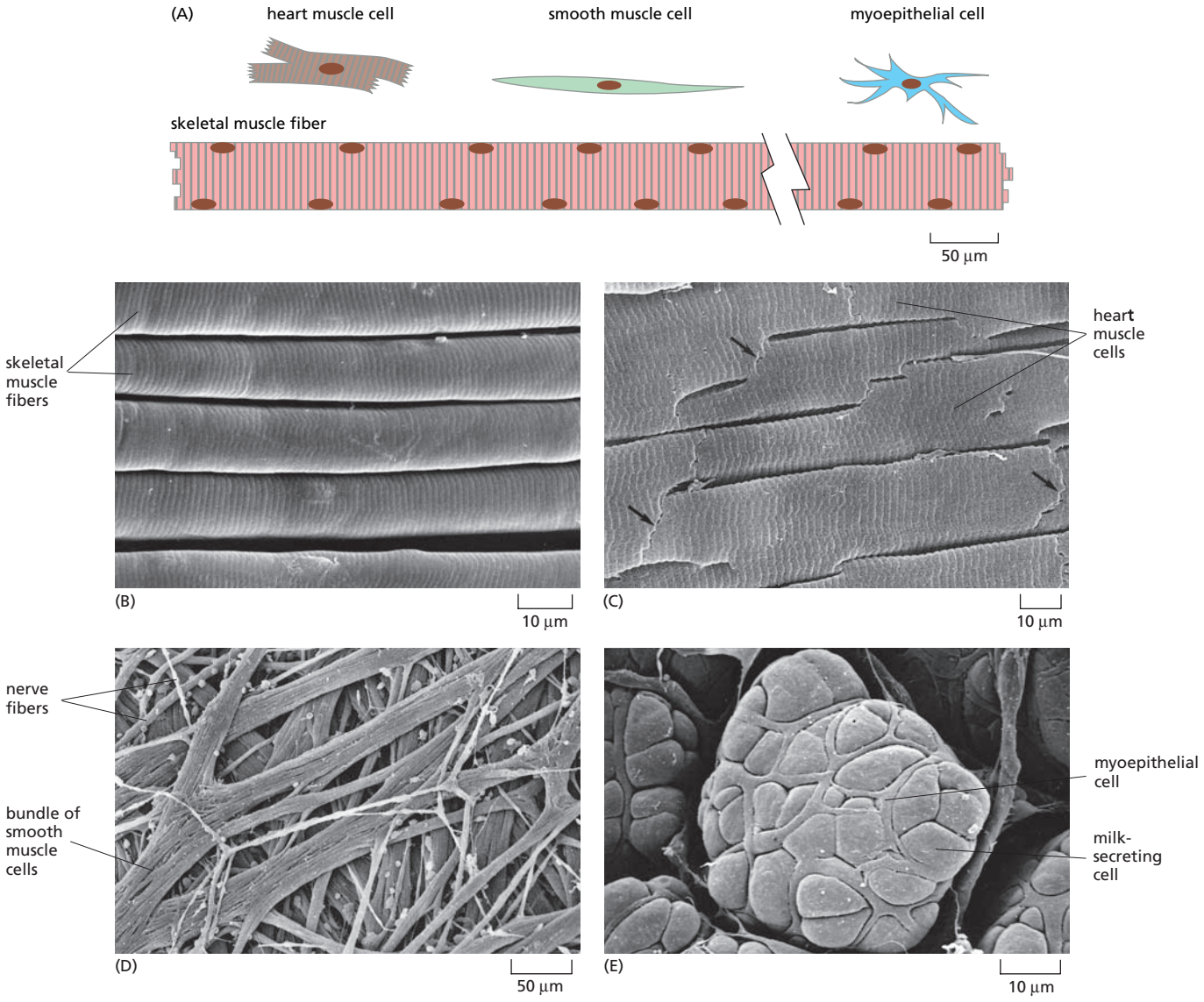



**Figure 23–47 The four classes of muscle cells of a mammal.** (A) Schematic drawings (to scale). (B–E) Scanning electron micrographs, showing (B) skeletal muscle from the neck of a hamster, (C) heart muscle from a rat, (D) smooth muscle from the urinary bladder of a guinea pig, and (E) myoepithelial cells in a secretory alveolus from a lactating rat mammary gland. The *arrows* in (C) point to intercalated discs—end-to-end junctions between the heart muscle cells; skeletal muscle cells in long muscles are joined end to end in a similar way. Note that the smooth muscle is shown at a lower magnification than the others. (B, courtesy of Junzo Desaki; C, from T. Fujiwara, in Cardiac Muscle in Handbook of Microscopic Anatomy [E.D. Canal, ed.]. Berlin: Springer-Verlag, 1986; D, courtesy of Satoshi Nakasiro; E, from T. Nagato et al., *Cell Tiss. Res.* 209:1–10, 1980. With permission from Springer-Verlag.)

in diameter in an adult human) and are often called *muscle fibers* because of their highly elongated shape. Each one is a syncytium, containing many nuclei within a common cytoplasm. The other types of muscle cells are more conventional, generally having only a single nucleus. **Heart muscle cells** resemble skeletal muscle fibers in that their actin and myosin filaments are aligned in very orderly arrays to form a series of contractile units called *sarcomeres*, so that the cells have a striated (striped) appearance. **Smooth muscle cells** are so named because they do not appear striated. The functions of smooth muscle vary greatly, from propelling food along the digestive tract to erecting hairs in response to cold or fear. **Myoepithelial cells** also have no striations, but unlike all other muscle cells they lie in epithelia and are derived from the ectoderm. They form the dilator muscle of the eye's iris and serve to expel saliva, sweat, and milk from the corresponding glands, as discussed earlier (see Figure 23–11). The four main categories of muscle cells can be further divided into distinctive subtypes, each with its own characteristic features.

The mechanisms of muscle contraction are discussed in Chapter 16. Here we consider how muscle tissue is generated and maintained. We focus on the skeletal muscle fiber, which has a curious mode of development, a striking ability to modulate its differentiated character, and an unusual strategy for repair.

## Myoblasts Fuse to Form New Skeletal Muscle Fibers

Chapter 22 described how certain cells, originating from the somites of a vertebrate embryo at a very early stage, become determined as **myoblasts**, the precursors of skeletal muscle fibers. The commitment to be a myoblast depends on gene regulatory proteins of at least two families—a pair of homeodomain proteins called Pax3 and Pax7, and the *MyoD family* of basic helix–loop–helix proteins (discussed in Chapter 7). These act in combination to give the myoblast a memory of its committed state, and, eventually, to regulate the expression of other genes that give the mature muscle cell its specialized character (see Figure 7–75). After a period of proliferation, the myoblasts undergo a dramatic change of state: they stop dividing, switch on the expression of a whole battery of muscle-specific genes required for terminal differentiation, and fuse with one another to form multinucleate skeletal muscle fibers (**Figure 23–48**). Fusion involves specific cell–cell adhesion molecules that mediate recognition between newly differentiating myoblasts and fibers. Once differentiation has occurred, the cells do not divide and the nuclei never again replicate their DNA.

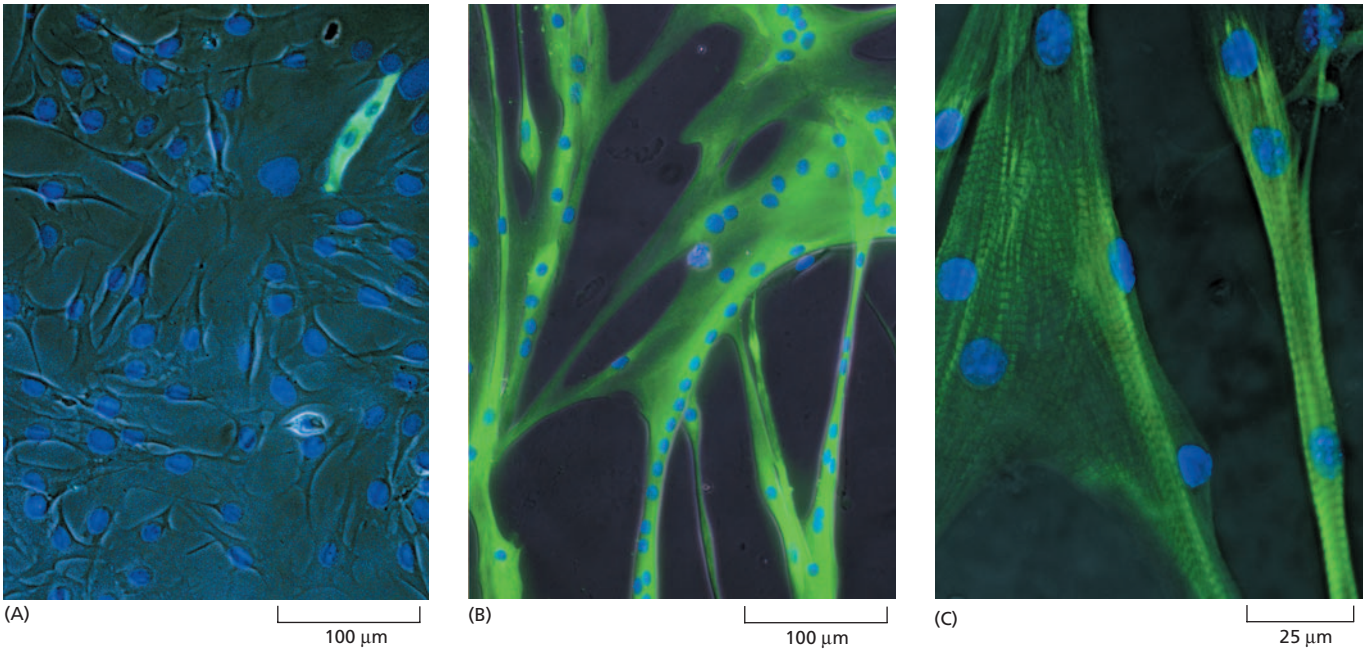



**Figure 23–48 Myoblast fusion in culture.** The culture is stained with a fluorescent antibody *(green)* against skeletal muscle myosin, which marks differentiated muscle cells, and with a DNA-specific dye *(blue)* to show cell nuclei. (A) A short time after a change to a culture medium that favors differentiation, just two of the many myoblasts in the field of view have switched on myosin production and have fused to form a muscle cell with two nuclei *(upper right)*. (B) Somewhat later, almost all the cells have differentiated and fused. (C) High-magnification view, showing characteristic striations (fine transverse stripes) in two of the multinucleate muscle cells. (Courtesy of Jacqueline Gross and Terence Partridge.)

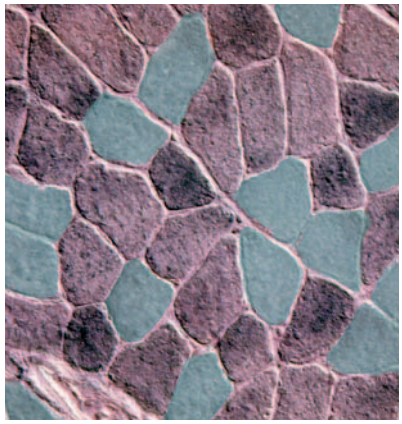



**Figure 23–49 Fast and slow muscle fibers.** Two consecutive cross sections of the same piece of adult mouse leg muscle were stained with different antibodies, each specific for a different isoform of myosin heavy chain protein, and images of the two sections were overlaid in false color to show the pattern of muscle fiber types. Fibers stained with antibodies against "fast" myosin *(gray)* are specialized to produce fast-twitch contractions; fibers stained with antibodies against "slow" myosin *(pink)* are specialized to produce slow, sustained contractions. The fast-twitch fibers are known as white muscle fibers because they contain relatively little of the colored oxygen-binding protein myoglobin. The slow muscle fibers are called red muscle fibers because they contain much more of it. (Courtesy of Simon Hughes.)

Myoblasts that have been kept proliferating in culture for as long as two years still retain the ability to differentiate and can fuse to form muscle cells in response to a suitable change in culture conditions. Appropriate signal proteins such as fibroblast or hepatocyte growth factor (FGF or HGF) in the culture medium can maintain myoblasts in the proliferative, undifferentiated state: if these soluble factors are removed, the cells rapidly stop dividing, differentiate, and fuse. The system of controls is complex, however, and attachment to the extracellular matrix is also important for myoblast differentiation. Moreover, the process of differentiation is cooperative: differentiating myoblasts secrete factors that apparently encourage other myoblasts to differentiate.

## Muscle Cells Can Vary Their Properties by Changing the Protein Isoforms They Contain

Once formed, a skeletal muscle fiber grows, matures, and modulates its character. The genome contains multiple variant copies of the genes encoding many of the characteristic proteins of the skeletal muscle cell, and the RNA transcripts of many of these genes can be spliced in several ways. As a result, muscle fibers produce many variant forms (isoforms) of the proteins of the contractile apparatus. As the muscle fiber matures, it synthesizes different isoforms, satisfying the changing demands for speed, strength, and endurance in the fetus, the newborn, and the adult. Within a single adult muscle, several distinct types of skeletal muscle fibers, each with different sets of protein isoforms and different functional properties, can be found side by side (**Figure 23–49**). The characteristics of the different fiber types are determined partly before birth by the genetic program of development, partly in later life by activity and training. Different classes of motor neurons innervate slow muscle fibers (for sustained contraction) and fast muscle fibers (for rapid twitch), and the innervation can regulate muscle-fiber gene expression and size through the different patterns of electrical stimulation that these neurons deliver.

## Skeletal Muscle Fibers Secrete Myostatin to Limit Their Own Growth

A muscle can grow in three ways: its fibers can increase in number, in length, or in girth. Because skeletal muscle fibers are unable to divide, more of them can be made only by the fusion of myoblasts, and the adult number of multinucleated skeletal muscle fibers is in fact attained early—before birth, in humans. Once formed, a skeletal muscle fiber generally survives for the entire lifetime of the animal. However, individual muscle nuclei can be added or lost. The enormous postnatal increase in muscle bulk is achieved by cell enlargement. Growth in length depends on recruitment of more myoblasts into the existing multinucleated fibers, which increases the number of nuclei in each cell. Growth in girth, such as occurs in the muscles of weightlifters, involves both myoblast recruitment and an increase in the size and numbers of the contractile myofibrils that each muscle fiber nucleus supports.

What, then, are the mechanisms that control muscle cell numbers and muscle cell size? One part of the answer lies in an extracellular signal protein called *myostatin*. Mice with a loss-of-function mutation in the myostatin gene have enormous muscles—two to three times larger than normal (**Figure 23–50**). Both the numbers and the size of the muscle cells seem to be increased. Mutations in the same gene are present in so-called "double-muscled" breeds of cattle (see Figure 17–69): in selecting for big muscles, cattle breeders have unwittingly selected for myostatin deficiency. Myostatin belongs to the TGFβ superfamily of signal proteins. It is normally made and secreted by skeletal muscle cells, and it acts powerfully on myoblasts, inhibiting both proliferation and differentiation. Its function, evidently, is to provide negative feedback to limit muscle growth, in adult life as well as during development. The growth of some other organs is similarly controlled by a negative-feedback action of a factor that they themselves produce. We shall encounter another example in a later section.

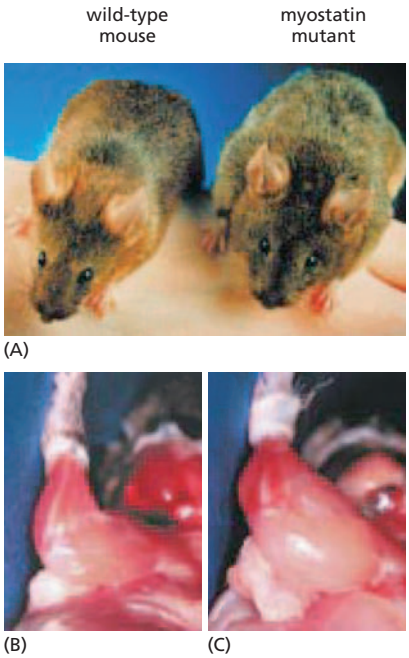



**Figure 23–50 Regulation of muscle size by myostatin.** (A) A normal mouse compared with a mutant mouse deficient in myostatin. (B) Leg of a normal and (C) of a myostatin-deficient mouse, with skin removed to show the massive enlargement of the musculature in the mutant. (From S.J. Lee and A.C. McPherron, *Curr. Opin. Genet. Devel.* 9:604–607, 1999. With permission from Elsevier.)

## Some Myoblasts Persist as Quiescent Stem Cells in the Adult

Even though humans do not normally generate new skeletal muscle fibers in adult life, they still have the capacity to do so, and existing muscle fibers can resume growth when the need arises. Cells capable of serving as myoblasts are retained as small, flattened, and inactive cells lying in close contact with the mature muscle cell and contained within its sheath of basal lamina (**Figure 23–51**). If the muscle is damaged or stimulated to grow, these *satellite cells* are activated to proliferate, and their progeny can fuse to repair the damaged muscle or to allow muscle growth. Like myoblasts, they are regulated by myostatin. Satellite cells, or some subset of the satellite cells, are thus the stem cells of adult skeletal muscle, normally held in reserve in a quiescent state but available when needed as a self-renewing source of terminally differentiated cells. Studies of these cells have provided some of the clearest evidence for the immortal strand hypothesis of asymmetric stem-cell division, as illustrated earlier in Figure 23–10).

The process of muscle repair by means of satellite cells is, nevertheless, limited in what it can achieve. In one form of *muscular dystrophy*, for example, a genetic defect in the cytoskeletal protein dystrophin damages differentiated skeletal muscle cells. As a result, satellite cells proliferate to repair the damaged muscle fibers. This regenerative response is, however, unable to keep pace with the damage, and connective tissue eventually replaces the muscle cells, blocking any further possibility of regeneration. A similar loss of capacity for repair seems to contribute to the weakening of muscle in the elderly.

In muscular dystrophy, where the satellite cells are constantly called upon to proliferate, their capacity to divide may become exhausted as a result of progressive shortening of their telomeres in the course of each cell cycle (discussed in Chapter 17). Stem cells of other tissues seem to be limited in the same way, as we noted earlier in the case of hemopoietic stem cells: they normally divide only at a slow rate, and mutations or exceptional circumstances that cause them to divide more rapidly can lead to premature exhaustion of the stem-cell supply.

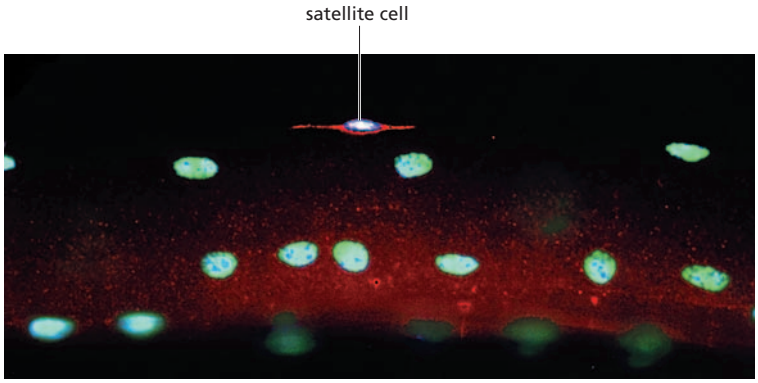



**Figure 23–51 A satellite cell on a skeletal muscle fiber.** The specimen is stained with an antibody *(red)* against a muscle cadherin, M-cadherin, which is present on both the satellite cell and the muscle fiber and is concentrated at the site where their membranes are in contact. The nuclei of the muscle fiber are stained *green,* and the nucleus of the satellite cell is stained *blue.* (Courtesy of Terence Partridge.)

## Summary

*Skeletal muscle fibers are one of four main categories of vertebrate cells specialized for contraction, and they are responsible for all voluntary movement. Each skeletal muscle fiber is a syncytium and develops by the fusion of many myoblasts. Myoblasts proliferate extensively, but once they have fused, they can no longer divide. Fusion generally follows the onset of myoblast differentiation, in which many genes encoding muscle-specific proteins are switched on coordinately. Some myoblasts persist in a quiescent state as satellite cells in adult muscle; when a muscle is damaged, these cells are reactivated to proliferate and to fuse to replace the muscle cells that have been lost. They are the stem cells of skeletal muscle. Muscle bulk is regulated homeostatically by a negative-feedback mechanism, in which existing muscle secretes myostatin, which inhibits further muscle growth.*


# FIBROBLASTS AND THEIR TRANSFORMATIONS: THE CONNECTIVE-TISSUE CELL FAMILY

Many of the differentiated cells in the adult body can be grouped into families whose members are closely related by origin and by character. An important example is the family of **connective-tissue cells**, whose members are not only related but also unusually interconvertible. The family includes *fibroblasts*, *cartilage cells*, and *bone cells*, all of which are specialized for the secretion of collagenous extracellular matrix and are jointly responsible for the architectural framework of the body. The connective-tissue family also includes *fat cells* and *smooth muscle cells*. **Figure 23–52** illustrates these cell types and the interconversions that are thought to occur between them. Connective-tissue cells contribute to the support and repair of almost every tissue and organ, and the adaptability of their differentiated character is an important feature of the responses to many types of damage.

## Fibroblasts Change Their Character in Response to Chemical Signals

Fibroblasts seem to be the least specialized cells in the connective-tissue family. They are dispersed in connective tissue throughout the body, where they secrete a nonrigid extracellular matrix that is rich in type I or type III collagen, or both, as discussed in Chapter 19. When a tissue is injured, the fibroblasts nearby proliferate, migrate into the wound <TGAT>, and produce large amounts of collagenous matrix, which helps to isolate and repair the damaged tissue. Their ability to thrive in the face of injury, together with their solitary lifestyle, may explain why fibroblasts are the easiest of cells to grow in culture—a feature that has made them a favorite subject for cell biological studies (**Figure 23–53**).

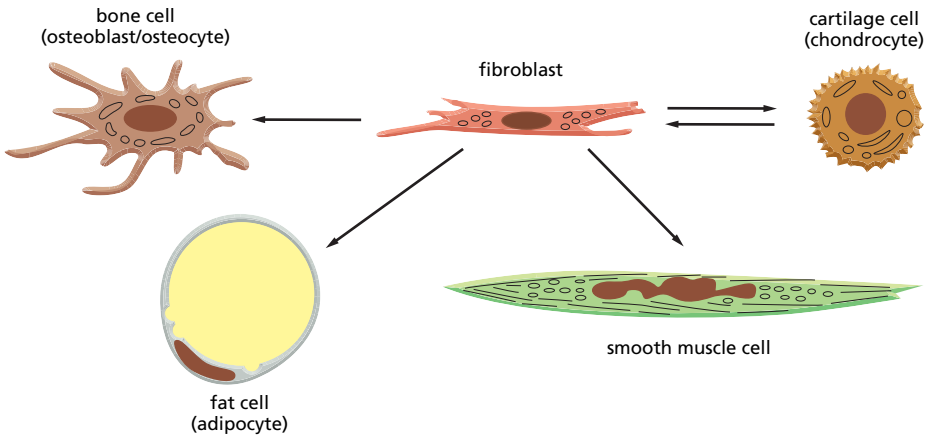



**Figure 23–52 The family of connective-tissue cells.** *Arrows* show the interconversions that are thought to occur within the family. For simplicity, the fibroblast is shown as a single cell type, but it is uncertain how many types of fibroblasts exist in fact and whether the differentiation potential of different types is restricted in different ways.

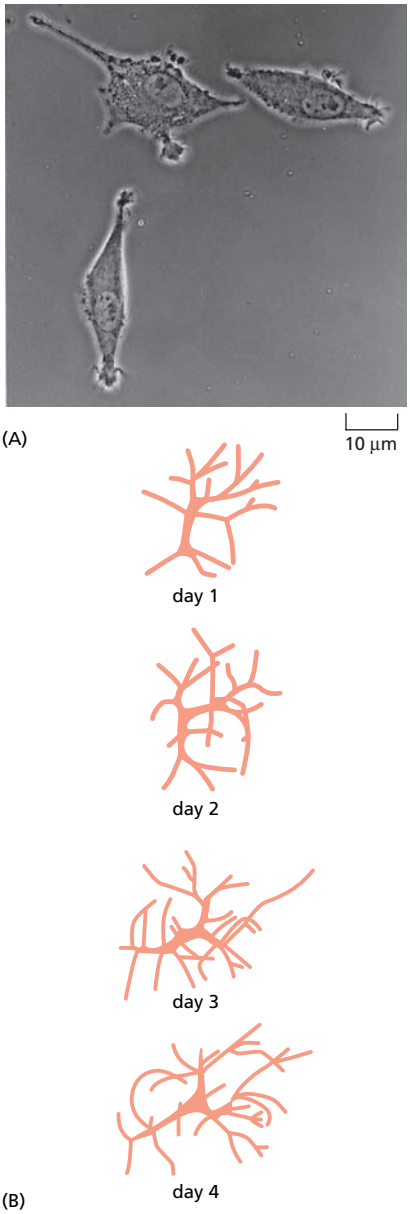



**Figure 23–53 The fibroblast.** (A) A phase-contrast micrograph of fibroblasts in culture. (B) These drawings of a living fibroblastlike cell in the transparent tail of a tadpole show the changes in its shape and position on successive days. Note that while fibroblasts flatten out in culture, they can have more complex, process-bearing morphologies in tissues. See also Figure 19–54. (A, from E. Pokorna et al., *Cell Motil. Cytoskeleton* 28:25–33, 1994; B, redrawn from E. Clark, *Am. J. Anat.* 13:351–379, 1912. Both with permission from Wiley-Liss.)

As indicated in Figure 23–52, fibroblasts also seem to be the most versatile of connective-tissue cells, displaying a remarkable capacity to differentiate into other members of the family. There are uncertainties about their interconversions, however. Fibroblasts in different parts of the body are intrinsically different, and there may be differences between them even in a single region. "Mature" fibroblasts with a lesser capacity for transformation may, for example, exist side by side with "immature" fibroblasts (often called mesenchymal cells) that can develop into a variety of mature cell types.

The stromal cells of bone marrow, mentioned earlier, provide a good example of connective-tissue versatility. These cells, which can be regarded as a kind of fibroblast, can be isolated from the bone marrow and propagated in culture. Large clones of progeny can be generated in this way from single ancestral stromal cells. According to the signal proteins that are added to the culture medium, the members of such a clone can either continue proliferating to produce more cells of the same type, or can differentiate as fat cells, cartilage cells, or bone cells. Because of their self-renewing, multipotent character, they are referred to as *mesenchymal stem cells*.

Fibroblasts from the dermal layer of the skin are different. When placed in the same culture conditions, they do not show the same plasticity. Yet they, too, can be induced to change their character. At a healing wound, for example, they change their actin gene expression and take on some of the contractile properties of smooth muscle cells, thereby helping to pull the wound margins together; such cells are called *myofibroblasts*. More dramatically, if a preparation of bone matrix, made by grinding bone into a fine powder and dissolving away the hard mineral component, is implanted in the dermal layer of the skin, some of the cells there (probably fibroblasts) become transformed into cartilage cells, and a little later, others transform into bone cells, thereby creating a small lump of bone. These experiments suggest that components in the extracellular matrix can dramatically influence the differentiation of connective-tissue cells.

We shall see that similar cell transformations occur in the natural repair of broken bones. In fact, bone matrix contains high concentrations of several signal proteins that can affect the behavior of connective-tissue cells. These include members of the TGFβ superfamily, including BMPs and TGFβ itself. These factors regulate growth, differentiation, and matrix synthesis by connective-tissue cells, exerting a variety of actions depending on the target cell type and the combination of other factors and matrix components that are present. When injected into a living animal, they can induce the formation of cartilage, bone, or fibrous matrix, according to the site and circumstances of injection. TGFβ is especially important in wound healing, where it stimulates the conversion of fibroblasts into myofibroblasts and promotes the formation of the collagen-rich scar tissue that gives a healed wound its strength.

## The Extracellular Matrix May Influence Connective-Tissue Cell Differentiation by Affecting Cell Shape and Attachment

The extracellular matrix may influence the differentiated state of connective-tissue cells through physical as well as chemical effects. This has been shown in studies on cultured cartilage cells, or **chondrocytes**. Under appropriate culture conditions, these cells proliferate and maintain their differentiated character, continuing for many cell generations to synthesize large quantities of highly distinctive cartilage matrix, with which they surround themselves. If, however, the cells are kept at relatively low density and remain as a monolayer on the culture

dish, a transformation occurs. They lose their characteristic rounded shape, flatten down on the substratum, and stop making cartilage matrix: they stop producing type II collagen, which is characteristic of cartilage, and start producing type I collagen, which is characteristic of fibroblasts. By the end of a month in culture, almost all the cartilage cells have switched their collagen gene expression and taken on the appearance of fibroblasts. The biochemical change must occur abruptly, since very few cells are observed to make both types of collagen simultaneously.

The biochemical change seems to be induced, at least in part, by the change in cell shape and attachment. Cartilage cells that have made the transition to a fibroblast-like character, for example, can be gently detached from the culture dish and transferred to a dish of agarose. By forming a gel around them, the agarose holds the cells suspended without any attachment to a substratum, forcing them to adopt a rounded shape. In these circumstances, the cells promptly revert to the character of chondrocytes and start making type II collagen again. Cell shape and anchorage may control gene expression through intracellular signals generated at focal contacts by integrins acting as matrix receptors, as discussed in Chapter 19.

For most types of cells, and especially for a connective-tissue cell, the opportunities for anchorage and attachment depend on the surrounding matrix, which is usually made by the cell itself. Thus, a cell can create an environment that then acts back on the cell to reinforce its differentiated state. Furthermore, the extracellular matrix that a cell secretes forms part of the environment for its neighbors as well as for the cell itself, and thus tends to make neighboring cells differentiate in the same way. A group of chondrocytes forming a nodule of cartilage, for example, either in the developing body or in a culture dish, can be seen to enlarge by the conversion of neighboring fibroblasts into chondrocytes.

## Osteoblasts Make Bone Matrix

Cartilage and bone are tissues of very different character; but they are closely related in origin, and the formation of the skeleton depends on an intimate partnership between them.

**Cartilage** tissue is structurally simple, consisting of cells of a single type—chondrocytes—embedded in a more or less uniform highly hydrated matrix consisting of proteoglycans and type II collagen, whose remarkable properties we have already discussed in Chapter 19. The cartilage matrix is deformable, and the tissue grows by expanding as the chondrocytes divide and secrete more matrix (**Figure 23–54**). **Bone**, by contrast, is dense and rigid; it grows by apposition—that is, by deposition of additional matrix on free surfaces. Like reinforced concrete, the bone matrix is predominantly a mixture of tough fibers (type I collagen fibrils), which resist pulling forces, and solid particles (calcium phosphate as *hydroxylapatite* crystals), which resist compression. The collagen fibrils in adult bone are arranged in regular plywoodlike layers, with the fibrils in each layer lying parallel to one another but at right angles to the fibrils in the layers on either side. They occupy a volume nearly equal to that occupied by the calcium phosphate. The bone matrix is secreted by **osteoblasts** that lie at the surface of the existing matrix and deposit fresh layers of bone onto it. Some of the osteoblasts remain free at the surface, while others gradually become embedded in their own secretion. This freshly formed material (consisting chiefly of type I collagen) is

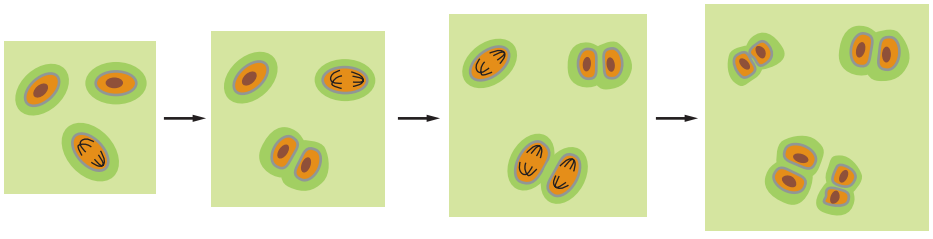


**Figure 23–54 The growth of cartilage.** The tissue expands as the chondrocytes divide and make more matrix. The freshly synthesized matrix with which each cell surrounds itself is shaded *dark green*. Cartilage may also grow by recruiting fibroblasts from the surrounding tissue and converting them into chondrocytes.

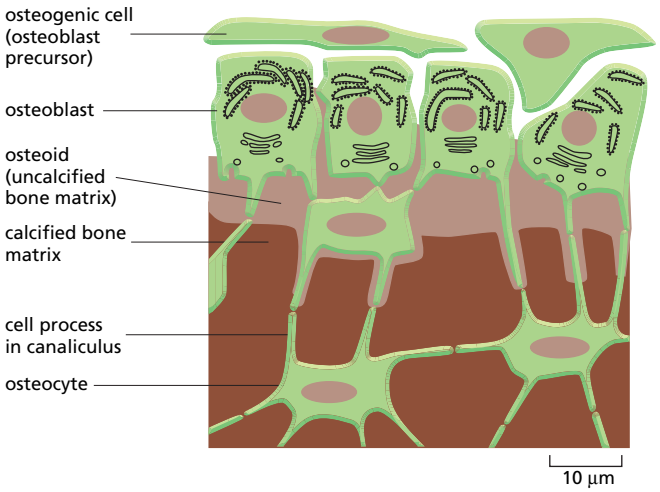



**Figure 23–55 Deposition of bone matrix by osteoblasts.** Osteoblasts lining the surface of bone secrete the organic matrix of bone (osteoid) and are converted into osteocytes as they become embedded in this matrix. The matrix calcifies soon after it has been deposited. The osteoblasts themselves are thought to derive from osteogenic stem cells that are closely related to fibroblasts.

called *osteoid*. It is rapidly converted into hard bone matrix by the deposition of calcium phosphate crystals in it. Once imprisoned in hard matrix, the original bone-forming cell, now called an **osteocyte**, has no opportunity to divide, although it continues to secrete further matrix in small quantities around itself. The osteocyte, like the chondrocyte, occupies a small cavity, or *lacuna*, in the matrix, but unlike the chondrocyte it is not isolated from its fellows. Tiny channels, or *canaliculi*, radiate from each lacuna and contain cell processes from the resident osteocyte, enabling it to form gap junctions with adjacent osteocytes (**Figure 23–55**). Although the networks of osteocytes do not themselves secrete or erode substantial quantities of matrix, they probably play a part in controlling the activities of the cells that do. Blood vessels and nerves run through the tissue, keeping the bone cells alive and reacting when the bone is damaged.

A mature bone has a complex and beautiful architecture, in which dense plates of *compact bone* tissue enclose spaces spanned by light frameworks of *trabecular bone*—a filigree of delicate shafts and flying buttresses of bone tissue, with soft marrow in the interstices (**Figure 23–56**). The creation, maintenance, and repair of this structure depend not only on the cells of the connective-tissue family that synthesize matrix, but also on a separate class of cells called *osteoclasts* that degrade it, as we shall discuss below.

## Most Bones Are Built Around Cartilage Models

Most bones, and in particular the long bones of the limbs and trunk, originate from minute "scale models" formed out of cartilage in the embryo. Each scale model grows, and as new cartilage forms, the older cartilage is replaced by bone. The process is known as *endochondral* bone formation. Cartilage growth and erosion and bone deposition are so ingeniously coordinated that the adult bone, though it may be half a meter long, is almost the same shape as the initial cartilaginous model, which was no more than a few millimeters long.

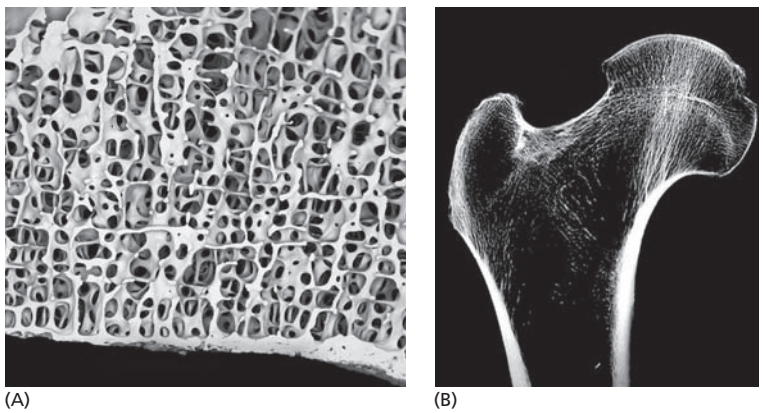

(A) (B)

**Figure 23–56 Trabecular and compact bone.** (A) Low-magnification scanning electron micrograph of trabecular bone in a vertebra of an adult man. The soft marrow tissue has been dissolved away. (B) A slice through the head of the femur, with bone marrow and other soft tissue likewise dissolved away, reveals the compact bone of the shaft and the trabecular bone in the interior. Because of the way in which bone tissue remodels itself in response to mechanical load, the trabeculae become oriented along the principle axes of stress within the bone. (A, courtesy of Alan Boyde; B, from J.B. Kerr, Atlas of Functional Histology. Mosby, 1999.)

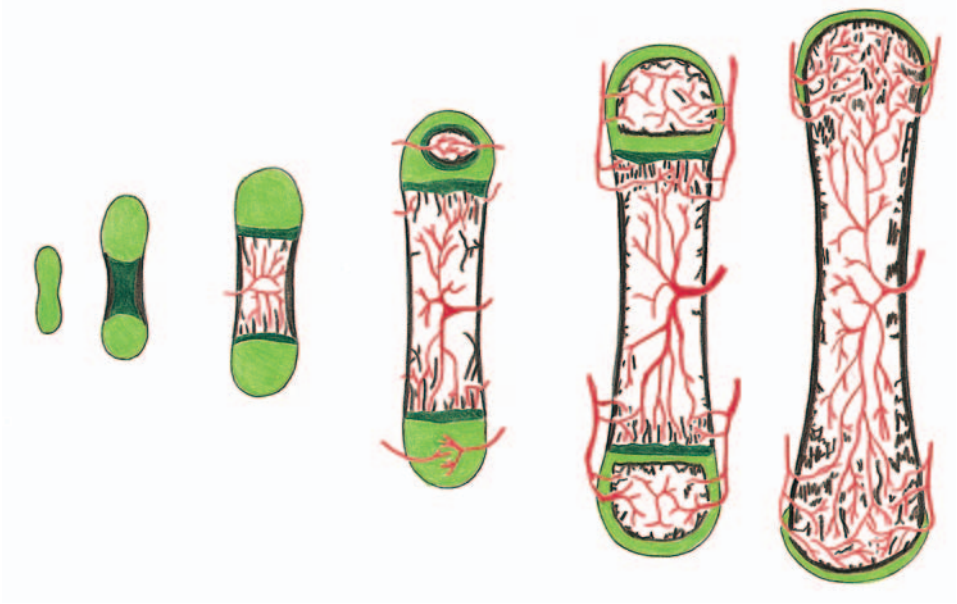


**Figure 23–57 The development of a long bone.** Long bones, such as the femur or the humerus, develop from a miniature cartilage model. Uncalcified cartilage is shown in *light green,* calcified cartilage in *dark green,* bone in *black,* and blood vessels in *red*. The cartilage is not converted to bone but is gradually replaced by it through the action of osteoclasts and osteoblasts, which invade the cartilage in association with blood vessels. Osteoclasts erode cartilage and bone matrix, while osteoblasts secrete bone matrix. The process of ossification begins in the embryo and is not completed until the end of puberty. The resulting bone consists of a thick-walled hollow cylinder of compact bone enclosing a large central cavity occupied by the bone marrow. Note that not all bones develop in this way. The membrane bones of the skull, for example, are formed directly as bony plates, not from a prior cartilage model. (Adapted from D.W. Fawcett, A Textbook of Histology, 12th ed. New York: Chapman and Hall, 1994.)

The process begins in the embryo with the appearance of hazily defined "condensations"—groups of embryonic connective tissue cells that become more closely packed than their neighbors and begin to express a characteristic set of genes—including, in particular, *Sox9* and, after a slight delay, *Runx2*. These two genes code for gene regulatory proteins that are critical for cartilage and bone development, respectively. Mutant cells lacking *Sox9* are unable to differentiate as cartilage but can form bone (and in some parts of the body will make bone where cartilage should be). Conversely, animals lacking functional *Runx2* make no bone and are born with a skeleton consisting solely of cartilage.

Soon after expression of *Sox9* has begun, the cells in the core of the condensation begin to secrete cartilage matrix, dividing and enlarging individually as they do so. In this way, they form an expanding rod of cartilage surrounded by more densely packed non-cartilage cells. The cartilage cells in the middle segment of the rod become hypertrophied (grossly enlarged) and cease dividing; and at the same time, they start to secrete Indian Hedgehog—a signal molecule of the Hedgehog family. This in turn provokes increased production of certain Wnt proteins, which activate the Wnt pathway in cells surrounding the cartilage rod. As a result, they switch off expression of *Sox9,* maintain expression of *Runx2*, and begin to differentiate as osteoblasts, creating a collar of bone around the shaft of the cartilage model. Artificial overactivation of the Wnt pathway tips a larger proportion of cells into making bone rather than cartilage; an artificial block in the Wnt signaling pathway does the opposite. In this system, therefore, Wnt signaling controls the choice between alternative paths of differentiation, with *Sox9* expression leading the way toward cartilage, and *Runx2* expression leading the way toward bone.

The hypertrophied cartilage cells in the shaft of the cartilage model soon die, leaving large cavities in the matrix, and the matrix itself becomes mineralized, like bone, by the deposition of calcium phosphate crystals. Osteoclasts and blood vessels invade the cavities and erode the residual cartilage matrix, creating a space for bone marrow, and osteoblasts following in their wake begin to deposit trabecular bone in parts of the cavity where strands of cartilage matrix remain as a template. The cartilage tissue at the ends of the bone is replaced by bone tissue at a much later stage, by a somewhat similar process, as shown in **Figure 23–57**. Continuing elongation of the bone, up to the time of puberty, depends on a plate of growing cartilage between the shaft and the head of the bone. Defective growth of the cartilage in this plate, as a result of a dominant mutation in the gene that codes for an FGF receptor (FGFR3), is responsible for the commonest form of dwarfism, known as *achondroplasia* (**Figure 23–58**).

The cartilage growth plate is eventually replaced by bone and disappears. The only surviving remnant of cartilage in the adult long bone is a thin but

**Figure 23–58 Achondroplasia.** This type of dwarfism occurs in one of 10,000–100,000 births; in more than 99% of cases it results from a mutation at an identical site in the genome, corresponding to amino acid 380 in the FGF receptor FGFR3 (a glycine in the transmembrane domain). The mutation is dominant, and almost all cases are due to new, independently occurring mutations, implying an extraordinarily high mutation rate at this particular site in the genome. The defect in FGF signaling causes dwarfism by interfering with the growth of cartilage in developing long bones. (From Velasquez's painting of Sebastian de Morra. © Museo del Prado, Madrid.)

important layer that forms a smooth, slippery covering on the bone surfaces at joints, where one bone articulates with another (see Figure 23–57). Erosion of this layer of cartilage, through aging, mechanical damage, or autoimmune attack, leads to *arthritis*, one of the commonest and most painful afflictions of old age.

## Bone Is Continually Remodeled by the Cells Within It

For all its rigidity, bone is by no means a permanent and immutable tissue. Running through the hard extracellular matrix are channels and cavities occupied by living cells, which account for about 15% of the weight of compact bone. These cells are engaged in an unceasing process of remodeling: while osteoblasts deposit new bone matrix, osteoclasts demolish old bone matrix. This mechanism provides for continuous turnover and replacement of the matrix in the interior of the bone.

**Osteoclasts** (**Figure 23–59**) are large multinucleated cells that originate, like macrophages, from hemopoietic stem cells in the bone marrow. The precursor cells are released as monocytes into the bloodstream and collect at sites of bone resorption, where they fuse to form the multinucleated osteoclasts, which cling to surfaces of the bone matrix and eat it away. Osteoclasts are capable of tunneling deep into the substance of compact bone, forming cavities that are then invaded by other cells. A blood capillary grows down the center of such a tunnel,

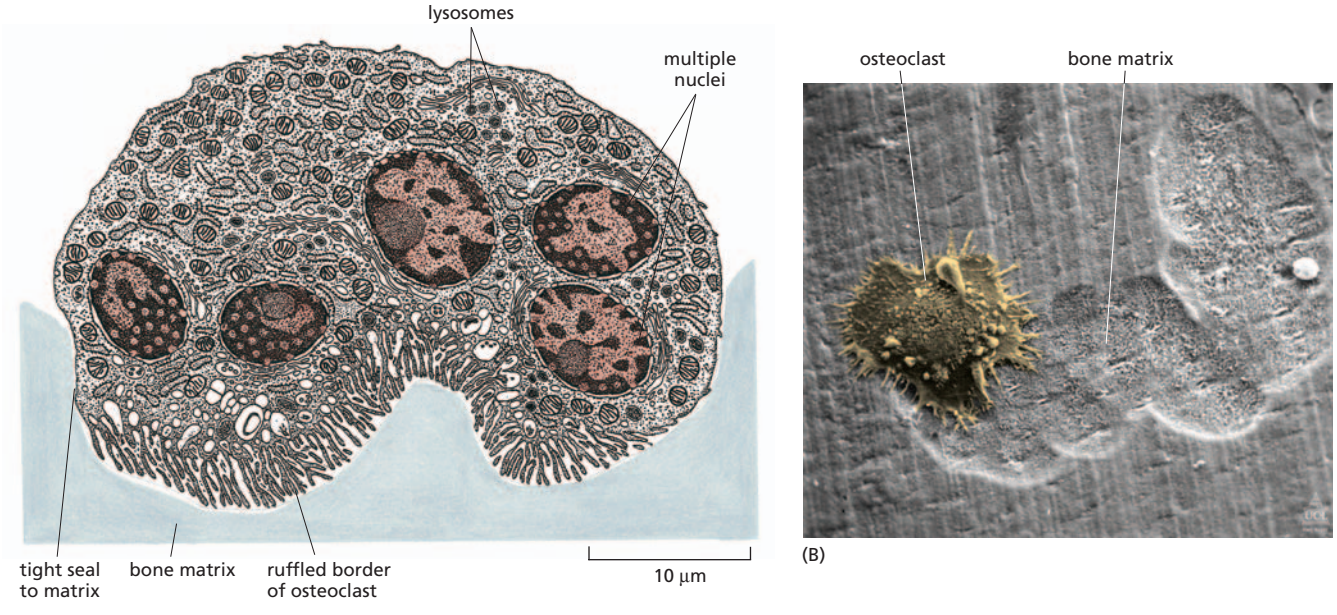



**Figure 23–59 Osteoclasts.** (A) Drawing of an osteoclast in cross section. This giant, multinucleated cell erodes bone matrix. The "ruffled border" is a site of secretion of acids (to dissolve the bone minerals) and hydrolases (to digest the organic components of the matrix). Osteoclasts vary in shape, are motile, and often send out processes to resorb bone at multiple sites. They develop from monocytes and can be viewed as specialized macrophages. (B) An osteoclast on bone matrix, seen by scanning electron microscopy. The osteoclast has been crawling over the matrix, eating it away, and leaving a trail of pits where it has done so. (A, from R.V. Krstić, Ultrastructure of the Mammalian Cell: An Atlas. Berlin: Springer-Verlag, 1979; B, courtesy of Alan Boyde.)

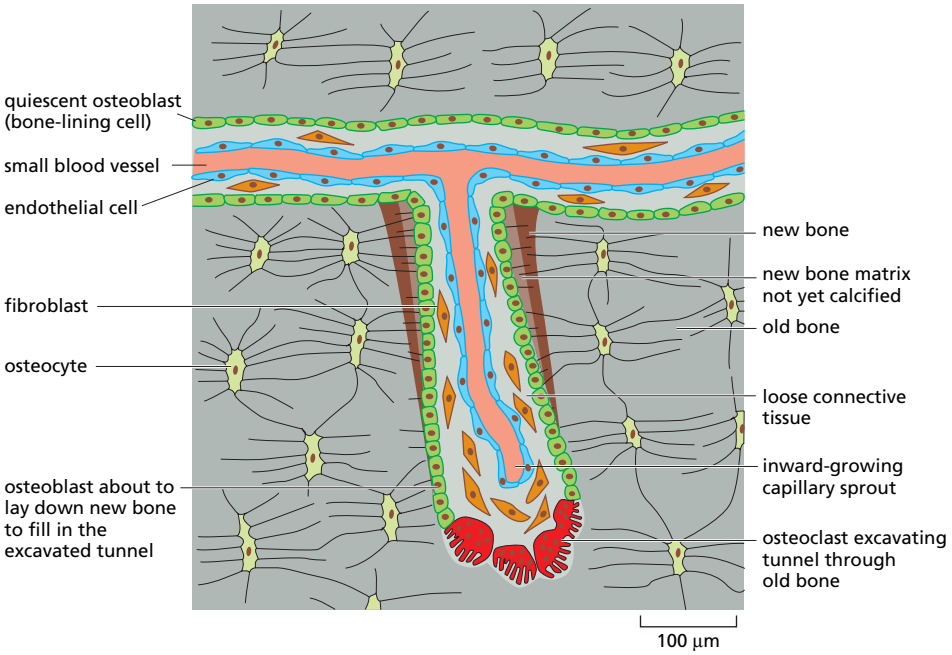



**Figure 23–60 The remodeling of compact bone.** Osteoclasts acting together in a small group excavate a tunnel through the old bone, advancing at a rate of about 50 μm per day. Osteoblasts enter the tunnel behind them, line its walls, and begin to form new bone, depositing layers of matrix at a rate of 1–2 μm per day. At the same time, a capillary sprouts down the center of the tunnel. The tunnel eventually becomes filled with concentric layers of new bone, with only a narrow central canal remaining. Each such canal, besides providing a route of access for osteoclasts and osteoblasts, contains one or more blood vessels that transport the nutrients the bone cells require for survival. Typically, about 5–10% of the bone in a healthy adult mammal is replaced in this way each year. (After Z.F.G. Jaworski, B. Duck and G. Sekaly, *J. Anat.* 133:397–405, 1981. With permission from Blackwell Publishing.)

and the walls of the tunnel become lined with a layer of osteoblasts (**Figure 23–60**). To produce the plywoodlike structure of compact bone, these osteoblasts lay down concentric layers of new matrix, which gradually fill the cavity, leaving only a narrow canal surrounding the new blood vessel. Many of the osteoblasts become trapped in the bone matrix and survive as concentric rings of osteocytes. At the same time as some tunnels are filling up with bone, others are being bored by osteoclasts, cutting through older concentric systems. The consequences of this perpetual remodeling are beautifully displayed in the layered patterns of matrix observed in compact bone (**Figure 23–61**).

## Osteoclasts Are Controlled by Signals From Osteoblasts

The osteoblasts that make the matrix also produce the signals that recruit and activate the osteoclasts to degrade it. Two proteins appear to have this role: one is Macrophage-CSF (MCSF), which we already encountered in our account of hemopoiesis (see Table 23–2); the other is TNF11, a member of the TNF family

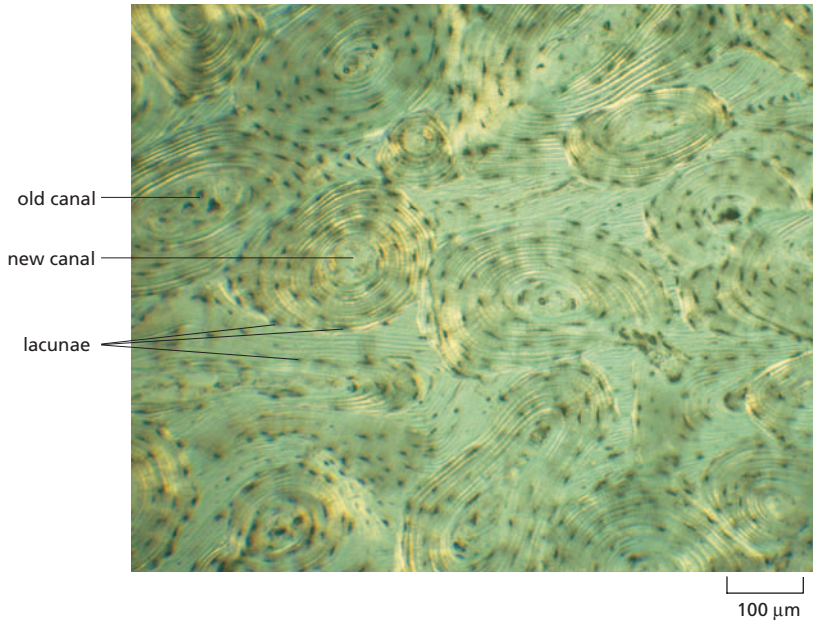



**Figure 23–61 A transverse section through a compact outer portion of a long bone.** The micrograph shows the outlines of tunnels that have been formed by osteoclasts and then filled in by osteoblasts during successive rounds of bone remodeling. The section has been prepared by grinding. The hard matrix has been preserved, but not the cells. Lacunae and canaliculi that were occupied by osteocytes are clearly visible, however. The alternating bright and dark concentric rings correspond to an alternating orientation of the collagen fibers in the successive layers of bone matrix laid down by the osteoblasts that lined the wall of the canal during life. (This pattern is revealed here by viewing the specimen between partly crossed polarizing filters.) Note how older systems of concentric layers of bone have been partly cut through and replaced by newer systems.

(also called RANKL). The behavior of the osteoblasts in attracting their opponents may seem self-defeating, but it has the useful function of localizing osteoclasts in the tissue where they are needed.

To prevent excessive degradation of matrix, the osteoblasts secrete, along with MCSF and TNF11, another protein, *osteoprotegerin*, that tends to block the action of TNF11. The higher the level of Wnt activation in the osteoblasts, the more osteoproteregin they secrete and, consequently, the lower the level of osteoclast activation and the lower the rate of bone matrix degradation. The Wnt signaling pathway thus seems to have two distinct functions in bone formation: at early stages, it controls the initial commitment of cells to an osteoblast fate; later, it acts in the differentiated osteoblasts to help govern the balance between matrix deposition and matrix erosion.

Disturbance of this balance can lead to *osteoporosis,* where there is excessive erosion of the bone matrix and weakening of the bone, or to the opposite condition, *osteopetrosis,* where the bone becomes excessively thick and dense. Hormonal signals, including estrogen, androgens, and the peptide hormone *leptin*, famous for its role in the control of appetite (discussed below), have powerful effects on this balance. At least some of these effects are mediated through influences on the osteoblasts' production of TNF11 and osteoprotegerin.

Circulating hormones affect bones throughout the body. No less important are local controls that allow bone to be deposited in one place while it is resorbed in another. Through such controls over the process of remodeling, bones are endowed with a remarkable ability to adjust their structure in response to long-term variations in the load imposed on them. It is this that makes orthodontics possible, for example: a steady force applied to a tooth with a brace will cause it to move gradually, over many months, through the bone of the jaw, through remodeling of the bone tissue ahead of it and behind it. The adaptive behavior of bone implies that the deposition and erosion of the matrix are in some way governed by local mechanical stresses (see Figure 23–56). Some evidence suggests that this is because mechanical stress on the bone tissue activates the Wnt pathway in the osteoblasts or osteocytes, thereby regulating their production of the signals that regulate osteoclast activity.

Bone can also undergo much more rapid and dramatic reconstruction when the need arises. Some cells capable of forming new cartilage persist in the connective tissue that surrounds a bone. If the bone is broken, the cells in the neighborhood of the fracture repair it by a sort of recapitulation of the original embryonic process: cartilage is first laid down to bridge the gap and is then replaced by bone. The capacity for self-repair, so strikingly illustrated by the tissues of the skeleton, is a property of living structures that has no parallel among present-day man-made objects.

## Fat Cells Can Develop From Fibroblasts

**Fat cells**, or **adipocytes**, also derive from fibroblastlike cells, both during normal mammalian development and in various pathological circumstances. In muscular dystrophy, for example, where the muscle cells die, they are gradually replaced by fatty connective tissue, probably by conversion of local fibroblasts. Fat-cell differentiation (whether normal or pathological) begins with the expression of two families of gene regulatory proteins: the *CEBP* (CCAAT/enhancer binding protein) family and the *PPAR* (peroxisome proliferator-activated receptor) family, especially PPARγ. Like the MyoD and MEF2 families in skeletal muscle development, the CEBP and PPARγ proteins drive and maintain one another's expression, through various cross-regulatory and autoregulatory control loops. They work together to control the expression of the other genes characteristic of adipocytes.

The production of enzymes for import of fatty acids and glucose and for fat synthesis leads to an accumulation of fat droplets, consisting mainly of triacylglycerol (see Figure 2–81). These then coalesce and enlarge until the cell is hugely distended (up to 120 μm in diameter), with only a thin rim of cytoplasm around the mass of lipid (**Figure 23–62** and **Figure 23–63**). Lipases are also made

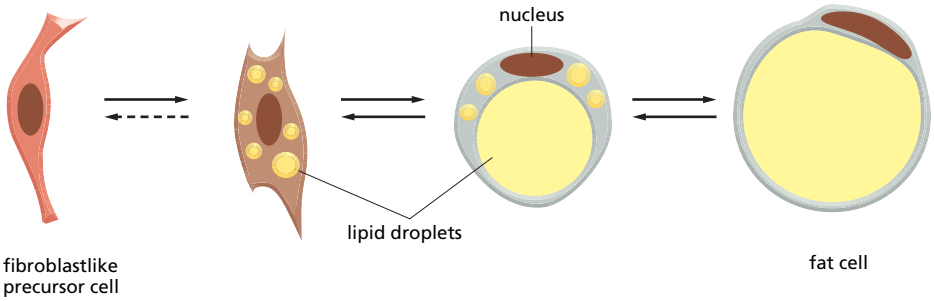



**Figure 23–62 The development of a fat cell.** A fibroblastlike precursor cell is converted into a mature fat cell by the accumulation and coalescence of lipid droplets. The process is at least partly reversible, as indicated by the *arrows;* the *dashed arrow* indicates uncertainty as to whether a differentiated fat cell can ever revert to the state of a pluripotent fibroblast. The cells in the early and intermediate stages can divide, but the mature fat cell cannot.

in the fat cell, giving it the capacity to reverse the process of lipid accumulation, by breaking down the triacylglycerols into fatty acids that can be secreted for consumption by other cells. The fat cell can change its volume by a factor of a thousand as it accumulates and releases lipid.

## Leptin Secreted by Fat Cells Provides Feedback to Regulate Eating

Almost all animals under natural circumstances have to cope with food supplies that are are variable and unpredictable. Fat cells have the vital role of storing reserves of nourishment in times of plenty and releasing them in times of dearth. It is thus essential to the function of adipose tissue that its quantity should be adjustable throughout life, according to the supply of nutrients. For our ancestors, this was a blessing; in the well-fed half of the modern world, it has become also a curse. In the United States, for example, approximately 30% of the population suffers from obesity, defined as a body mass index (weight/height$^2$) more than 30 kg/m$^2$, equivalent to about 30% above ideal weight.

It is not easy to determine to what extent the changes in the quantity of adipose tissue depend on changes in the numbers of fat cells, as opposed to changes in fat-cell size. Changes in cell size are probably the main factor in normal nonobese adults, but in severe obesity, at least, the number of fat cells also increases. The factors that drive the recruitment of new fat cells are not well understood, although they are thought to include growth hormone and IGF1 (insulinlike growth factor-1). It is clear, however, that the increase or decrease of fat cell size is regulated directly by levels of circulating nutrients and by hormones, such as insulin, that reflect nutrient levels. The surplus of food intake over energy expenditure thus directly governs the accumulation of adipose tissue.

But how are food intake and energy expenditure themselves regulated? Factors such as cholecystokinin, secreted by gut cells in response to food in the gut lumen as discussed earlier, are responsible for short-term control, over the course of a meal or a day. But we also need long-term controls, if we are not to get steadily fatter and fatter or thinner and thinner over the course of a lifetime. Most important, from an evolutionary point of view and for our ancestors coping with food supplies that were often scanty and uncertain, starvation must provoke hunger and the pursuit of food. Those who have known real prolonged hunger testify to the overwhelming force of this compulsion. The key signal appears to be a protein hormone called **leptin**, which normally circulates in the bloodstream when fat reserves are adequate, and disappears, producing chronic hunger, when they are not. Mutant mice that lack leptin or the appropriate leptin receptor are extremely fat (**Figure 23–64**). Mutations in the same genes sometimes occur in humans, although very rarely. The consequences are similar: constant hunger, overeating, and crippling obesity.

Leptin is normally made by fat cells; the bigger they are, the more they make. Leptin acts on many tissues, and in particular in the brain, on cells in those regions of the hypothalamus that regulate eating behavior. Absence of leptin is a signal of starvation, driving the behavior that will restore fat reserves to their proper level. Thus, leptin, like myostatin released from muscle cells, provides a feedback mechanism to regulate the growth of the tissue that secretes it.

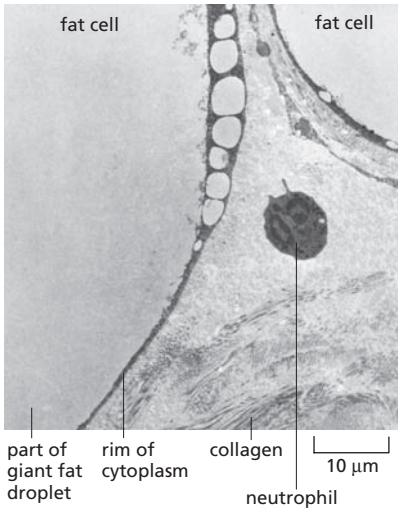



**Figure 23–63 Fat cells.** This low-magnification electron micrograph shows parts of two fat cells. A neutrophil cell that happens to be present in the adjacent connective tissue provides a sense of scale; each of the fat cells is more than 10 times larger than the neutrophil in diameter and is almost entirely filled with a single large fat droplet. The small fat droplets *(pale oval shapes)* in the remaining rim of cytoplasm are destined to fuse with the central droplet. The nucleus is not visible in either of the fat cells in the picture. (Courtesy of Don Fawcett, from D.W. Fawcett, A Textbook of Histology, 12th ed. New York: Chapman and Hall, 1994.)

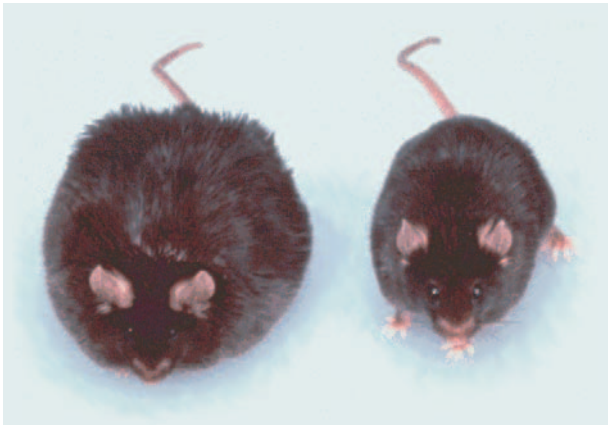


**Figure 23–64 Effects of leptin deficiency.** A normal mouse *(right)* compared with a mouse that has a mutation in the *Obese* gene, which codes for leptin *(left)*. The leptin-deficient mutant fails to limit its eating and becomes grotesquely fat (three times the weight of a normal mouse). (Courtesy of Jeffrey M. Friedman.)

In most obese people, leptin levels in the bloodstream are persistently high, and yet appetite is not suppressed, even though leptin receptors are also present and functional. The leptin feedback control evolved, it seems, to save us from death by starvation, rather than from obesity through overeating. In the well-fed regions of the world, we depend on a complex of other mechanisms, many of them still poorly understood, to keep us from getting too fat.

### Summary

*The family of connective-tissue cells includes fibroblasts, cartilage cells, bone cells, fat cells, and smooth muscle cells. Some classes of fibroblasts, such as the mesenchymal stem cells of bone marrow, seem to be able to transform into any of the other members of the family. These transformations of connective-tissue cell type are regulated by the composition of the surrounding extracellular matrix, by cell shape, and by hormones and growth factors.*

*Cartilage and bone both consist of cells and solid matrix that the cells secrete around themselves—chondrocytes in cartilage, osteoblasts in bone (osteocytes being osteoblasts that have become trapped within the bone matrix). The matrix of cartilage is deformable so that the tissue can grow by swelling, whereas bone is rigid and can grow only by apposition. The two tissues have related origins and collaborate closely. Thus, most long bones develop from miniature cartilage "models," which, as they grow, serve as templates for the deposition of bone. Wnt signaling regulates the choice between the two pathways of cell differentiation—as chondrocyte (requiring Sox9 expression) or as osteoblast (requiring Runx2 expression). While osteoblasts secrete bone matrix, they also produce signals that recruit monocytes from the circulation to become osteoclasts, which degrade bone matrix. Osteoblasts and osteocytes control the balance of deposition and degradation of matrix by adjusting the signals they send to the osteoclasts. Through the activities of these antagonistic classes of cells, bone undergoes perpetual remodeling through which it can adapt to the load it bears and alter its density in response to hormonal signals. Moreover, adult bone retains an ability to repair itself if fractured, by reactivation of the mechanisms that governed its embryonic development: cells in the neighborhood of the break convert into cartilage, which is later replaced by bone.*

*While the chief function of most members of the connective-tissue family is to secrete extracellular matrix, fat cells serve as storage sites for fat. Feedback control keeps the quantity of fat tissue from falling too low: fat cells release a hormone, leptin, which acts in the brain, and disappearance of leptin acts as a starvation danger signal, driving the behavior that will restore fat reserves to an adequate level.*

## STEM-CELL ENGINEERING

As we have seen, many of the tissues of the body are not only self-renewing but also self-repairing, and this is largely thanks to stem cells and the feedback

controls that regulate their behavior. But where Nature's own mechanisms fail, can we intervene and do better? Can we find ways of getting cells to reconstruct living tissues that have been lost or damaged by disease or injury and are incapable of spontaneous repair? An obvious strategy is to exploit the special developmental capabilities of the stem cells or progenitors from which the missing tissue components normally derive. But how are such cells to be obtained, and how can we put them to use? That is the topic of this final section.

## Hemopoietic Stem Cells Can Be Used to Replace Diseased Blood Cells with Healthy Ones

Earlier in this chapter, we saw how mice can be irradiated to kill off their hemopoietic cells, and then rescued by a transfusion of new stem cells, which repopulate the bone marrow and restore blood-cell production. In the same way, patients with leukemia, for example, can be irradiated or chemically treated to destroy their cancerous cells along with the rest of their hemopoietic tissue, and then can be rescued by a transfusion of healthy, non-cancerous hemopoietic stem cells, which can be harvested from the bone marrow of a suitable donor. This creates problems of immune rejection if the bone marrow donor and the recipient differ genetically, but careful tissue matching and the use of immunosuppressive drugs can reduce these difficulties to a tolerable level. In some cases, where the leukemia arises from a mutation in a specialized type of blood cell progenitor rather than in the hemopoietic stem cell itself, it is possible to rescue the patient with his or her own cells. A sample of bone marrow is taken before the irradiation and sorted to obtain a preparation of hemopoietic stem cells that is free from leukemic cells. This purified preparation is then transfused back into the patient after the irradiation.

The same technology also opens the way, in principle, to one form of gene therapy: hemopoietic stem cells can be isolated in culture, genetically modified by DNA transfection or some other technique to introduce a desired gene, and then transfused back into a patient in whom the gene was lacking, to provide a self-renewing source of the missing genetic component. A version of this approach is under trial for the treatment of AIDS. Hemopoietic stem cells can be taken from the patient infected with HIV, genetically modified by transfection with genetic material that makes the stem cells and their progeny resistant to HIV infection, and transfused back into the same patient.

## Epidermal Stem-Cell Populations Can Be Expanded in Culture for Tissue Repair

Another simple example of the use of stem cells is in the repair of the skin after extensive burns. By culturing cells from undamaged regions of the burned patient's skin, it is possible to obtain epidermal stem cells quite rapidly in large numbers. These can then be used to repopulate the damaged body surface. For good results after a third-degree burn, however, it is essential to provide first an immediate replacement for the lost dermis. For this, dermis taken from a human cadaver can be used, or an artificial dermis substitute. This is still an area of active experimentation. In one technique, an artificial matrix of collagen mixed with a glycosaminoglycan is formed into a sheet, with a thin membrane of silicone rubber covering its external surface as a barrier to water loss, and this skin substitute (called Integra) is laid on the burned body surface after the damaged tissue has been cleaned away. Fibroblasts and blood capillaries from the patient's surviving deep tissues migrate into the artificial matrix and gradually replace it with new connective tissue. Meanwhile, the epidermal cells are cultivated until there are enough to form a thin sheet of adequate extent. Two or more weeks after the original operation, the silicone rubber membrane is carefully removed and replaced with this cultured epidermis, so as to reconstruct a complete skin.

## Neural Stem Cells Can Be Manipulated in Culture

While the epidermis is one of the simplest and most easily regenerated tissues, the central nervous system (the CNS) is the most complex and seems the most difficult to reconstruct in adult life. The adult mammalian brain and spinal cord have very little capacity for self-repair. Stem cells capable of generating new neurons are hard to find in adult mammals—so hard to find, indeed, that until recently they were thought to be absent.

We now know, however, that CNS neural stem cells capable of giving rise to both neurons and glial cells do persist in the adult mammalian brain. Moreover, in certain parts of the brain they continually produce new neurons to replace those that die (**Figure 23–65**). Neuronal turnover occurs on a more dramatic scale in certain songbirds, where large numbers of neurons die each year and are replaced by newborn neurons as part of the process by which the bird learns a new song in each breeding season.

The proof that the adult mammalian brain contains neural stem cells came from experiments in which pieces of brain tissue were dissociated and used to establish cell cultures. In suitable culture conditions, cells derived from an appropriate region of the brain will form floating "neurospheres"—clusters consisting of a mixture of neural stem cells with neurons and glial cells derived from the stem cells. These neurospheres can be propagated through many cell generations, or their cells can be taken at any time and implanted back into the brain of an intact animal. Here they will produce differentiated progeny, in the form of neurons and glial cells.

Using slightly different culture conditions, with the right combination of growth factors in the medium, the neural stem cells can be grown as a monolayer and induced to proliferate as an almost pure stem-cell population without attendant differentiated progeny. By a further change in the culture conditions, these cells can be induced at any time to differentiate to give a mixture of neurons and glial cells (**Figure 23–66**), or just one of these two cell types, according to the composition of the culture medium.

The pure cultures of neural stem cells, dividing to produce more neural stem cells, are valuable as more than just a source of cells for transplantation. They should help in the analysis of the factors that define the stem-cell state and control the switch to differentiation. Since the cells can be manipulated genetically by DNA transfection and other means, they open up new ways to investigate the role of specific genes in these processes and in genetic diseases of the nervous system, such as neurodegenerative diseases. They also create opportunities, in principle at least, for genetic engineering of neural cells to treat disease.

## Neural Stem Cells Can Repopulate the Central Nervous System

Neural stem cells grafted into an adult brain show a remarkable ability to adjust their behavior to match their new location. Stem cells from the mouse hippocampus, for example, implanted in the mouse olfactory-bulb-precursor pathway (see Figure 23–65) give rise to neurons that become correctly incorporated into the olfactory bulb. This capacity of neural stem cells and their progeny to

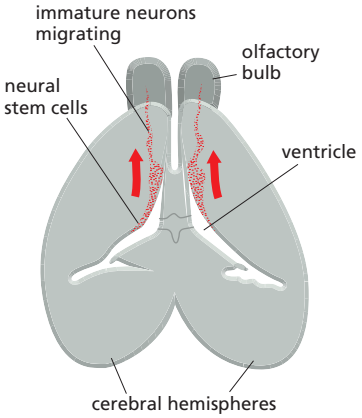



**Figure 23–65 The continuing production of neurons in an adult mouse brain.** The brain is viewed from above, in a cut-away section, to show the region lining the ventricles of the forebrain where neural stem cells are found. These cells continually produce progeny that migrate to the olfactory bulb, where they differentiate as neurons. The constant turnover of neurons in the olfactory bulb is presumably linked in some way to the turnover of the olfactory receptor neurons that project to it from the olfactory epithelium, as discussed earlier. There is also a continuing turnover of neurons in the adult hippocampus, a region specially concerned with learning and memory, where plasticity of adult function seems to be associated with turnover of a specific subset of neurons. (Adapted from B. Barres, *Cell* 97:667–670, 1999. With permission from Elsevier.)

adapt to a new environment promises to have important clinical applications in the treatment of diseases where neurons degenerate or lose their myelin sheaths, and in injuries of the central nervous system. Thus, neural stem cells (derived from fetal human tissue) have been grafted into the spinal cord of mice that are crippled by a spinal cord injury or by a mutation that leads to defective myelination; the mice chosen were of an immunodeficient strain, and so did not reject the grafted cells. The grafted cells then gave rise both to neurons that connected with the host neurons and to oligodendrocytes that formed new myelin sheaths around demyelinated host axons. As a result, the host mice recovered some of their control over their limbs.

Such findings hold out the hope that, in spite of the extraordinary complexity of nerve cell types and neuronal connections, it may be possible to use neural stem cells to repair at least some types of damage and disease in the central nervous system.

## Stem Cells in the Adult Body Are Tissue-Specific

When cells are removed from the body and maintained in culture or are transplanted from one site in the body to another, as in the procedures we have just described, they generally remain broadly faithful to their origins. Keratinocytes continue to behave as keratinocytes, hemopoietic cells as hemopoietic cells, neural cells as neural cells, and so on. Placed in an abnormal environment, differentiated cells may, it is true, cease to display the full normal set of differentiated features, and stem cells may lose their stem-cell character and differentiate; but they do not switch to expressing the characteristics of another radically different cell type. Thus, each type of specialized cell has a memory of its developmental history and seems fixed in its specialized fate. Some limited transformations can certainly occur, as we saw in our account of the connective-tissue cell family, and some stem cells can generate a variety of differentiated cell types, but the possibilities are restricted. Each type of stem cell serves for the renewal of one particular type of tissue.

Obviously, the practical opportunities would be much greater if stem cells were more versatile and not so specialized—if we could take them from one type of tissue where they are easily available, and use them to repair a different tissue

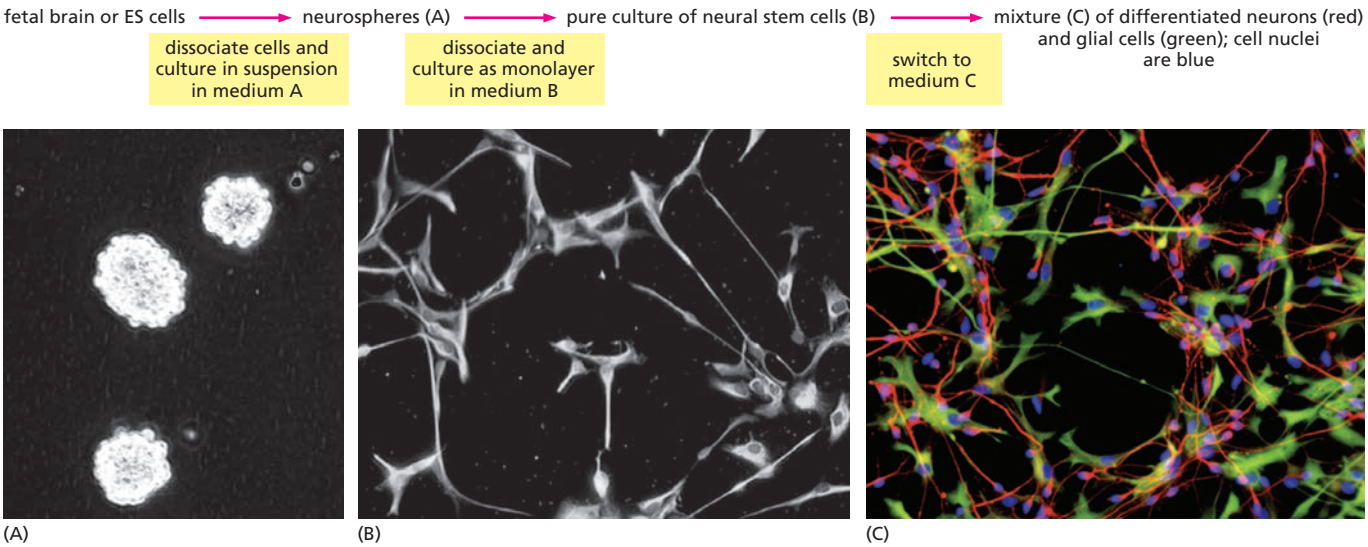



**Figure 23–66 Neural stem cells.** The photographs show the steps leading from fetal brain tissue, via neurospheres (A), to a pure culture of neural stem cells (B). These stem cells can be kept proliferating as such indefinitely, or, through a change of medium, can be caused to differentiate (C) into neurons *(red)* and glial cells *(green)*. Neural stem cells with the same properties can also be derived, via a similar series of steps, from ES cells. (Micrographs from L. Conti et al., *PLoS* 3:1594–1606, 2005. With permission from Public Library of Science.)

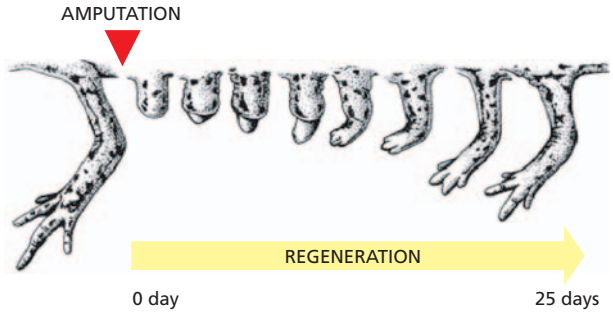



**Figure 23–67 Newt limb regeneration.** A time-lapse sequence showing the progress of limb regeneration in an axolotl from amputation at the level of the humerus. The sequence show the wound-healing, dedifferentiation, blastema, and redifferentiation stages of regeneration. Total time shown is approximately 20–30 days. (Courtesy of Susan Bryant and David Gardiner.)

where they are needed. Thus, there has been great excitement in the past decade over reports that stem cells of various specialized tissues can, in certain circumstances, show astonishing developmental plasticity, giving rise to cells of radically different types—hemopoietic stem cells to neurons, for example, or neural stem cells to muscle. The validity of these findings is hotly debated, however, and faults have been found in some of the key evidence. For example, many apparent cases of such switches of cell fate are now thought to be actually the result of cell fusion events, through which nuclei from one type of specialized cell are exposed to cytoplasm of another cell type and consequently switch on an altered set of genes. In any case, most reports of interconversions between radically different adult cell lineages agree that these are rare events. While research continues into these extreme forms of stem-cell plasticity, we do not yet know how to make such direct interconversions happen on a large enough scale or reliably enough, if at all, for practical medical application.

This is not to say that the radical transformation of cells from one differentiated character to another is an impossible dream or that efficient ways of bringing it about will never be found. In fact, some non-mammalian species can regenerate lost tissues and organs by just such interconversions. A newt, for example, can regenerate an amputated limb through a process in which differentiated cells seem to revert to an embryonic character and recapitulate embryonic development. Differentiated multinucleate muscle cells in the remaining limb stump reenter the cell cycle, dedifferentiate, and break up into mononucleated cells; these then proliferate to form a bud similar to the limb bud of an embryo, and eventually redifferentiate into the range of cell types needed to reconstruct the missing part of the limb (**Figure 23–67**). Why a newt can manage this—as well as many other extraordinary feats of regeneration—but a mammal cannot is still a profound mystery.

## ES Cells Can Make Any Part of the Body

While stem cells of adult mammalian tissues seem to be quite restricted in what they can do, another type of mammalian stem cell is extraordinarily versatile. As described in Chapters 8 and 22, it is possible to take an early mouse embryo, at the blastocyst stage, and through cell culture to derive from it a class of stem cells called **embryonic stem cells**, or **ES cells**. ES cells can be kept proliferating indefinitely in culture and yet retain an unrestricted developmental potential. If ES cells are put back into a blastocyst, they become incorporated into the embryo and can give rise to all the tissues and cell types in the body, including germ cells, integrating perfectly into whatever site they may come to occupy, and adopting the character and behavior that normal cells would show at that site. We can think of development in terms of a series of choices presented to cells as they follow a road that leads from the fertilized egg to terminal differentiation. After their long sojourn in culture, the ES cell and its progeny can evidently still read the signs at each branch in the highway and respond as normal embryonic cells would. If ES cells are implanted directly into an embryo at a later stage or into an adult tissue, however, they fail to receive the appropriate sequence of cues; their differentiation then is not properly controlled, and they will often give rise to a tumor.

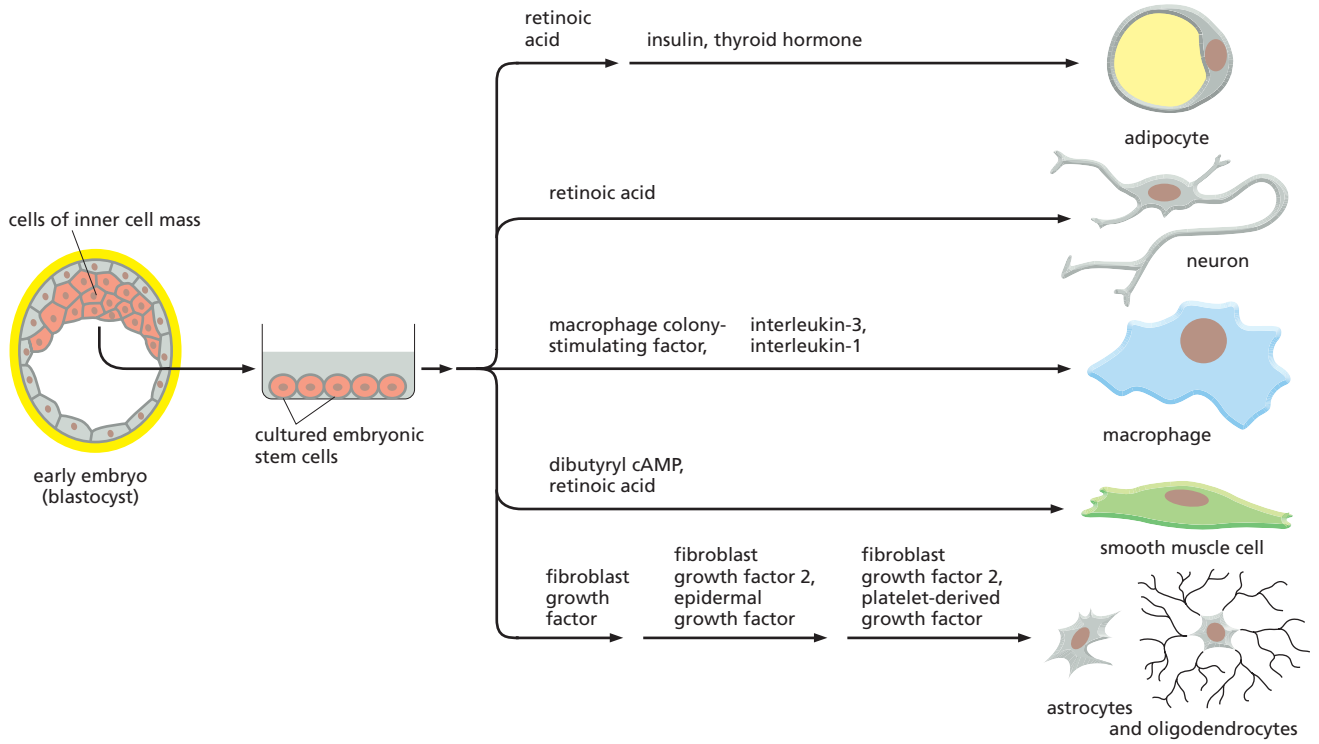



**Figure 23–68 Production of differentiated cells from mouse ES cells in culture.** ES cells derived from an early mouse embryo can be cultured indefinitely as a monolayer, or allowed to form aggregates called embryoid bodies, in which the cells begin to specialize. Cells from embryoid bodies, cultured in media with different factors added, can then be driven to differentiate in various ways. (Based on E. Fuchs and J.A. Segre, *Cell* 100:143–155, 2000. With permission from Elsevier.)

Cells with properties similar to those of mouse ES cells can now be derived from early human embryos and from human fetal germ cells, creating a potentially inexhaustible supply of cells that might be used for the replacement and repair of mature human tissues that are damaged. Although one may have ethical objections to such use of human embryos, it is worth considering the possibilities that are opened up. Setting aside the dream of growing entire organs from ES cells by a recapitulation of embryonic development, experiments in mice suggest that it should be possible in the future to use ES cells to replace the skeletal muscle fibers that degenerate in victims of muscular dystrophy, the nerve cells that die in patients with Parkinson's disease, the insulin-secreting cells that are lacking in type I diabetics, the heart muscle cells that die in a heart attack, and so on.

If ES cells are to be used for this sort of tissue repair, they first have to be coaxed along the desired pathway of development. ES cells can, in fact, be induced to differentiate into a wide variety of cell types in culture (**Figure 23–68**), by treatment with appropriate combinations of signal proteins and growth factors. <GGAA> They can, for example, be used to generate neurospheres and neural stem cells. Neural stem cells derived from mouse ES cells, like those derived from brain tissue, can be grafted into the brain of an adult host mouse, where they will differentiate to give neurons and glial cells. If the host is deficient in myelin-forming oligodendrocytes, a graft of ES-derived oligodendrocyte precursors can correct the deficiency and supply myelin sheaths for axons that lack them.

## Patient-Specific ES Cells Could Solve the Problem of Immune Rejection

There are many problems to be solved before ES cells can be used effectively for tissue repair in human patients. One of the most severe, limiting the use of adult

stem cells also, is immune rejection. If ES-derived cells of a given genotype are grafted into a genetically different individual, the grafted cells are likely to be rejected by the immune system as foreign. Ways of dealing with this problem using immunosuppressive drugs have been developed for the transplantation of organs such as kidneys and hearts, but they are far from perfect.

To avoid immunological problems altogether, we need grafted cells that are genetically identical to those of the host. How, then, can ES cells be produced to order, with the same genotype as an adult human patient who needs a transplant? As discussed in Chapter 8, one possible route is via *somatic cell nuclear transfer*. In this procedure—not yet achieved with human cells, despite some false hopes—the nucleus would be taken from a somatic cell of the patient, and injected into an oocyte provided by a donor (in general, a woman other than the patient), replacing the original oocyte nucleus. From this hybrid oocyte, a blastocyst could be obtained, and from the blastocyst, ES cells. These and their progeny would contain the nuclear genome of the patient, and should in principle be transplantable without risk of immune rejection. But the whole procedure involves many difficulties, and is a long way from the stage where it could be used for treatment.

It would be far preferable if we could take cells from the adult patient and convert them to an ES-like character by manipulating gene expression more directly. A first step along this road is to identify the key determinants of ES cell character—the master regulatory proteins that specify that character, if they exist. Biochemical comparisons of ES cells with other cell types suggest a set of candidates for this role. These candidates can be tested by introducing the appropriate DNA expression constructs into differentiated cells, such as fibroblasts, that can be grown in culture. A combination of such transgenes, coding for a set of four gene regulatory proteins (Oct3/4, Sox2, Myc, and Klf4), seems in fact to be able to convert fibroblasts into cells with ES-like properties, including the ability to differentiate in diverse ways. The conversion rate is low—only a small proportion of fibroblasts containing the transgenes make the switch—and the converted cells are different from true ES cells in significant respects. Nevertheless, these experiments show a possible way toward the production of cells with ES-like versatility from adult somatic cells.

## ES Cells Are Useful for Drug Discovery and Analysis of Disease

Although transplantation of ES-derived cells for the treatment of human diseases still seems to be far in the future, there are other ways in which ES cells promise to be more immediately valuable. They can be used to generate large homogeneous populations of differentiated cells of a specific type in culture; and these can serve for testing the effects of large numbers of chemical compounds in the search for new drugs with useful actions on a given human cell type. By techniques such as those we have just described, it may be possible, furthermore, to create ES-like cells containing the genomes of patients who suffer from a given genetic disease, and to use these patient-specific stem cells for the discovery of drugs useful in the treatment of that disease. Such cells should be valuable also for analysis of the disease mechanism. And at a basic level, manipulations of ES cells in culture should help us to fathom some of the many unsolved mysteries of stem-cell biology.

Serious ethical issues to need be resolved and enormous technical problems overcome before stem-cell technology can yield all the benefits that we dream of. But by one route or another, it seems that cell biology is beginning to open up new opportunities for improving on Nature's mechanisms of tissue repair, remarkable as those mechanisms are.

## Summary

*Stem cells can be manipulated artificially and used both for the treatment of disease and for other purposes such as drug discovery. Hemopoietic stem cells, for example,*

*can be transfused into leukemia patients to replace a diseased hemopoietic system, and epidermal stem cells taken from undamaged skin of a badly burned patient can be rapidly grown in large numbers in culture and grafted back to reconstruct an epidermis to cover the burns. Neural stem cells can be derived from some regions of the fetal or adult brain, and when grafted into a brain that is damaged can differentiate into neurons and glial cells that become integrated into the host tissue and may help to bring about a partial repair, at least in experimental studies in animals.*

*In the normal adult body, each type of stem cell gives rise to a restricted range of differentiated cell types. Although there have been many reports of stem-cell plasticity that violates these restrictions, the evidence is still contentious. Embryonic stem cells (ES cells), however, are able to differentiate into any cell type in the body, and they can be induced to differentiate into many different cell types in culture. From ES cells it is possible, for example, to generate neural stem cell lines that will proliferate indefinitely as pure stem-cell cultures but can respond to an appropriate change of culture conditions at any time by differentiating into neurons and glia. Methods to derive ES-like cells from cells of adult tissues are under development. In principle, such ES-like cells, carrying the genome of a specific patient, could be used for tissue repair, avoiding the problem of immune rejection. More immediately, they provide an in vitro testing ground for the investigation of the physiology and pharmacology of cells of any normal or pathological genotype, and for the discovery of drugs with useful effects on these cells.*

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