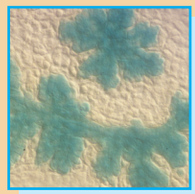
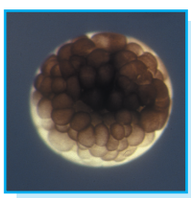
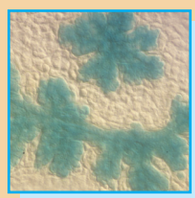
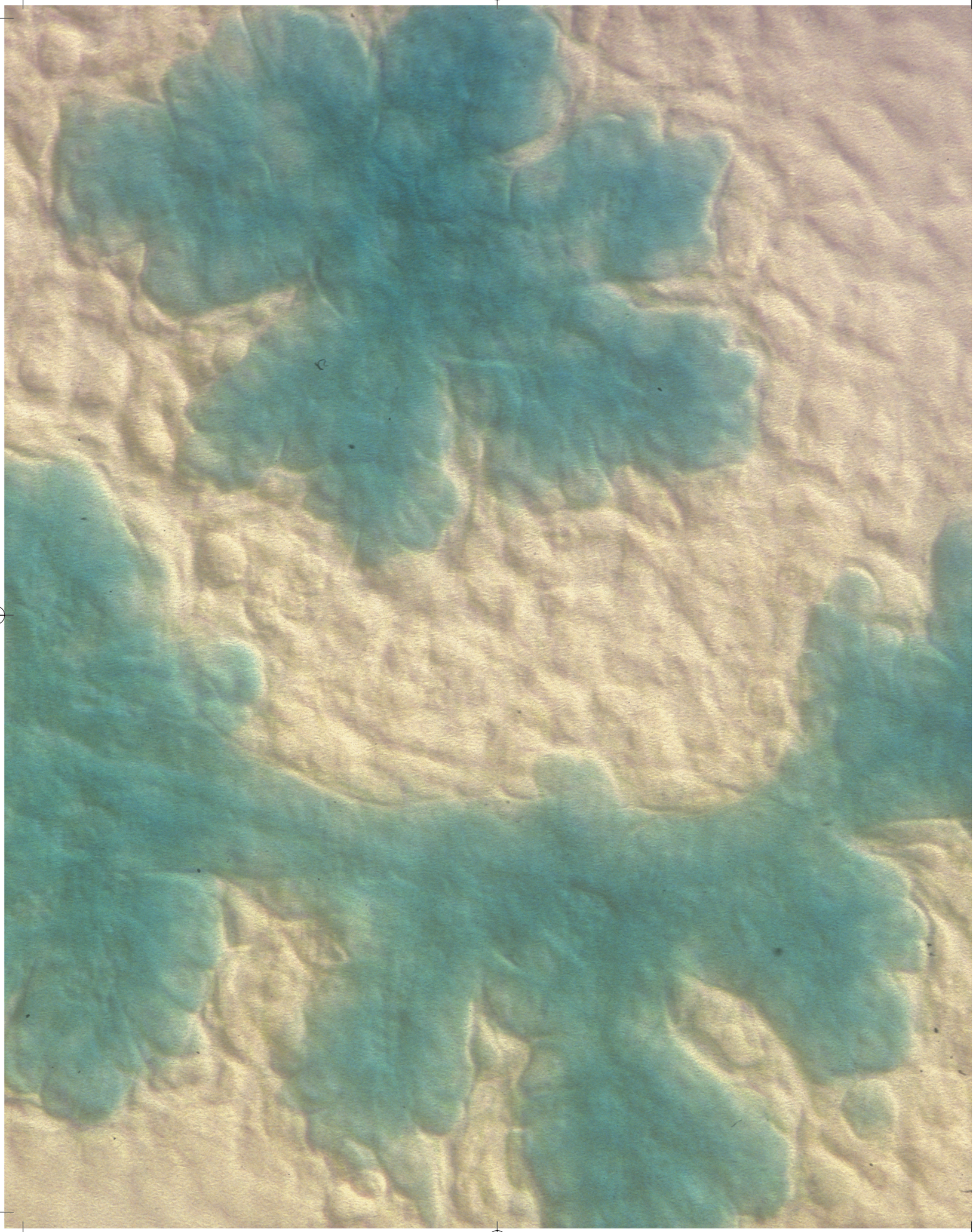
Section 3



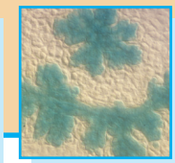
Organogenesis







Chapter 13



Tissue organization and stem cells

The chapters of this section will describe the development of various selected organ systems, mostly in higher vertebrates, although certain aspects are illuminated by studies on the lower vertebrate and invertebrate models.

The techniques used in organogenesis research are essentially similar to those used in early development. In addition, much use is made of *in vitro* **organ cultures** of rudiments from mouse and chick embryos as these are more accessible than the same stage organ *in vivo*. **Knockout** mouse strains provide most of the **loss-of-function** data, and it is often possible to grow an organ culture from a knockout embryo beyond the stages at which the whole embryo remains viable. Expression studies are done by ***in situ*** **hybridization** and **immunostaining**. Biological activity data are acquired from addition of proteins to the organ cultures, or introduction of the genes encoding them by **electroporation** or viral infection.

This chapter deals with the chief tissue types found in the vertebrate body with special attention to their cellular renewal. On the basis of light microscopy there are about 200 types of differentiated cell, although *in situ* hybridization and immunostaining reveal many more. They are arranged in tissues, each of which contains several different cell types. An organ or body part contains several tissue types arranged to fulfill a common function, and they are usually derived from more than one embryonic cell lineage.

Types of tissue

Epithelia

An **epithelium** (plural **epithelia**) is a sheet of cells, arranged on a **basement membrane** with each cell joined to its neighbors by specialized junctions. About 60% of visible cell types are constituents of epithelia. The cells show a distinct apical–basal polarity, where the basal surface is that next to the basement membrane and the apical surface is on the opposite side, often facing a fluid-filled lumen. The basement membrane consists of a basal lamina secreted by the epithelium itself, together with some additional extracellular material from the underlying connective tissue. It is composed of laminin, type IV collagen, entactin, and heparan sulfate proteoglycan. The junctional complexes consist of three components: **tight junctions**, adherens junctions, and desmosomes (Fig. 13.1). The tight junction belt prevents liquid leaking between the cells and also isolates the components of the apical and basolateral membranes; adherens junctions are attachment points joining the microfilament networks of the cells; and desmosomes are point contacts joining bundles of cytokeratin filaments. Cell–cell contacts through adherens junctions and desmosomes are made by cadherins, with their homophilic calcium-dependent binding. The cells are anchored to the basement membrane by cell–matrix adherens junctions and by hemidesmosomes. These are similar to the cell–cell junctions but utilize integrins for attaching the cell to the matrix components. Apical surfaces often bear cilia, and may bear **microvilli** if the epithelium has an absorptive function (see Appendix for some further notes about these various cell components).

Epithelia may be simple, with one layer of cells, stratified, with many layers of cells, or pseudostratified, meaning that they look stratified but in fact all cells contact the apical and basal surfaces. They may be **squamous**, with flattened cells, cuboidal, or columnar. Many epithelia are glandular and secrete materials into their surroundings. Glands may be simple or branched, and tubular or acinar (Fig. 13.2). The duct of a gland represents its original site of invagination during development. **Exocrine** glands secrete into the duct, **endocrine** glands have lost their ducts and secrete into the bloodstream. **Myoepithelial cells** are often found surrounding the **acini**, and their contraction helps drive the secretion down the duct. The terms mucous membrane or **mucosa** are often used to refer to a moist internal epithelium together with the immediately underlying connective tissue layer.

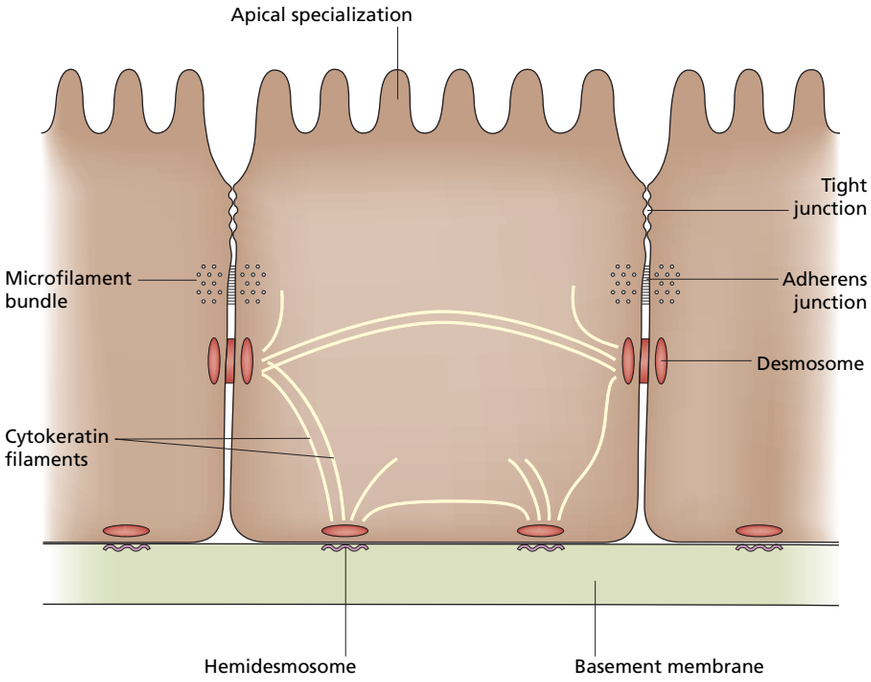


Fig. 13.1 Diagram of an epithelial cell showing the types of cell junction.

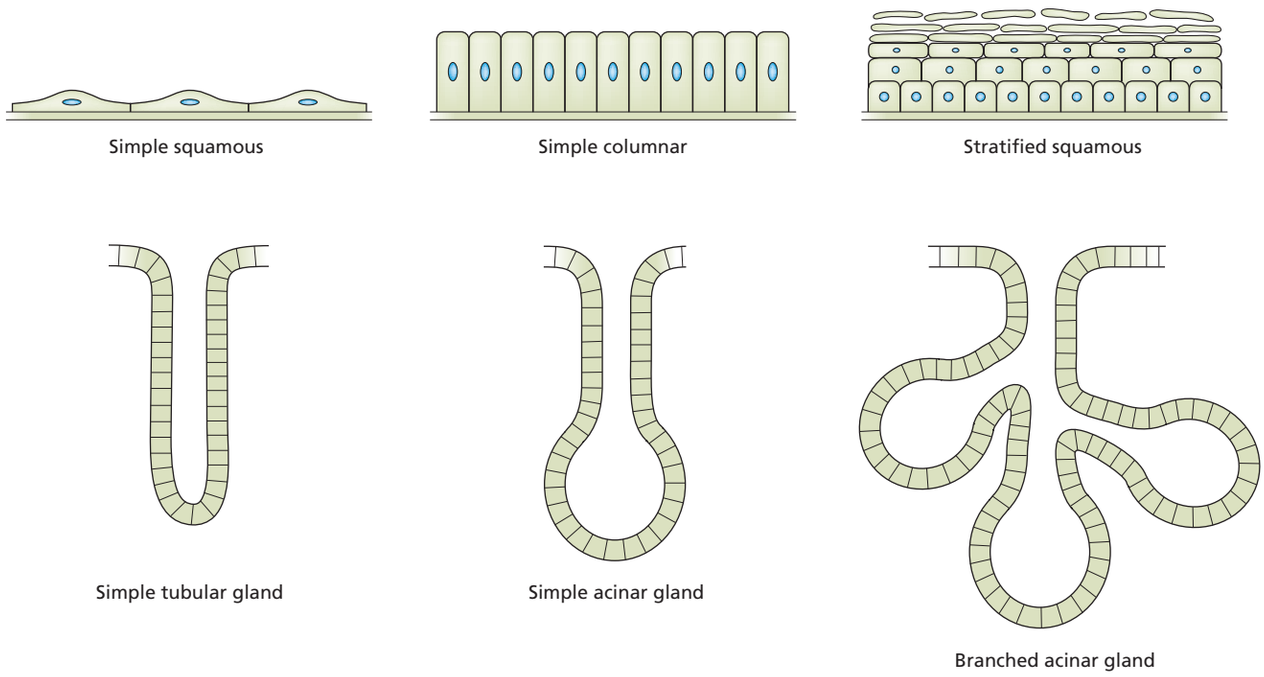


Fig. 13.2 Types of epithelium.

Although commonly thought to be ectodermal in origin, epithelia are, in fact, derived from all three of the germ layers of the embryo. The organization and cell renewal in epidermis and intestinal epithelium are described below, and of neuroepithelium in Chapter 14.

Connective tissues

The term **connective tissue** refers to those tissues dominated by fibroblasts, such as the dermis of the skin and the fibrous capsules surrounding most organs. In some histology or biology

textbooks it may be used in a wider sense to include the skeletal tissues, muscle, and even the cells of the blood.

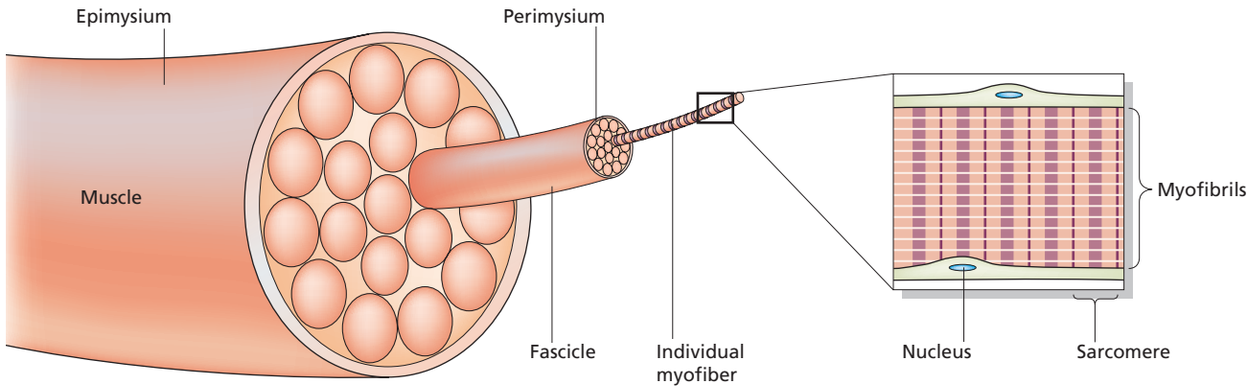
Much of the connective tissue is derived from the mesoderm of the embryo, although some is also formed by the **neural crest**. Mature connective tissue consists of **fibroblasts** embedded in an extracellular matrix. Fibroblasts are cells specialized to secrete the matrix components which include hyaluronan, proteoglycans, fibronectin, type I collagen, type III collagen (reticulin), and elastin. Also found in connective tissue are histiocytes, which are macrophages resident in the tissue, and mast cells, which are histamine-secreting cells similar to the basophils of the blood but also resident in the tissues. Both these types originate from the bone marrow. **Adipose tissue** is closely related to loose connective tissue, as fibroblasts can become adipocytes under appropriate conditions.

The skeletal tissues are composed of cartilage and bone and arise both from embryonic mesoderm and neural crest. Much of the skeleton is formed initially as cartilage which is then gradually replaced by bone. Skeletal parts formed in cartilage are known as **cartilage models**. Some parts, particularly in the skull, differentiate directly from mesenchyme into bone, and these are known as **membrane bones**. Skeletal tissues are discussed further in Chapter 18.

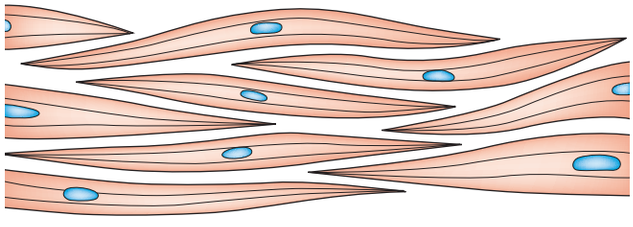
A term causing much confusion is **mesenchyme**. This is not a synonym for connective tissue nor for mesoderm. It is a descriptive term for scattered stellate cells embedded in a loose **extracellular matrix** (see also Chapter 2). Mesenchyme, derived either from mesoderm or from neural crest, fills up much of the embryo and forms fibroblasts, adipose tissue, smooth muscle, and skeletal tissues, however these tissues should not be referred to as "mesenchymal" once they are differentiated.

Muscle

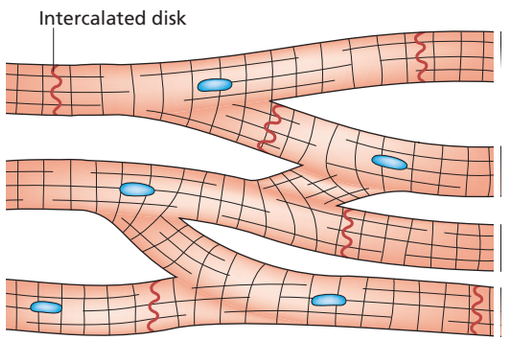
There are three main types of muscle (Fig. 13.3): skeletal muscle is composed of elongated multinucleate cells called **myofibers**. Bundles of myofibers are gathered together in fascicles surrounded by a fibrous sheath, the perimysium, and the whole muscle is surrounded by another sheath called the epimysium. Skeletal muscle is derived from the **myotomes** of the **somites** and its development is further described in Chapter 15. Smooth (= visceral) muscle exists as bundles of individual spindle-shaped mononuclear cells. These contain a similar contractile apparatus to skeletal muscle but it is not arranged as visible sarcomeres. Smooth muscle is derived from the lateral plate of the embryo



(a) Striated muscle



(b) Smooth muscle



(c) Cardiac muscle

Fig. 13.3 Types of muscle.

and is found mainly around the gut, blood vessels, and the ducts of glands, where inherent rhythmic contraction is required. Smooth muscle is usually mitotically quiescent but can be stimulated to grow following tissue damage. Cardiac muscle occurs only in the heart. It derives from the anteroventral margin of the lateral plate mesoderm of the embryo. Like skeletal muscle it has visible myofibrils, but like smooth muscle it remains as individual cells. The cells are joined end to end by intercalated discs which contain structural junctions (adherens and desmosomes), together with gap junctions that allow rapid spread of electrical signals through the myocardium. Cardiac muscle, like skeletal muscle, is postmitotic, although some growth can occur by cell enlargement. Development of the skeletal muscle and heart are described in Chapter 15.

Neural tissues

Neural tissues comprise those cell types formed from the **neural tube** and some of those formed from the **neural crest**. The neural tube is composed of a specialized epithelium, the **neuroepithelium**, and produces both central neurons and glial cells, while the neural crest produces autonomic neurons of the peripheral nervous system, together with **Schwann cells** and pigment cells. Development of neural tissues is described in Chapter 14.

Blood and blood vessels

Blood contains a variety of cell types. In addition to the red cells, there are **granulocytes**, **monocytes**, and lymphocytes. All these, as well as other cells such as histiocytes, osteoclasts, and Langerhans cells of the skin, arise from **hematopoietic** tissue in the bone marrow. The hematopoietic system is a state of continuous cell production and renewal throughout life, and is further described below.

The circulatory system consists of arteries taking blood from the heart to the tissues, capillaries supplying the tissues, and veins returning blood to the heart (Fig. 13.4). All blood vessels have three layers. The inner layer is composed of a single layer of **endothelial cells** sometimes with a little underlying connective tissue. The middle layer is composed of smooth muscle, which may be very thick in arteries and thinner in veins, and the outer layer is composed of fibrous connective tissue.

The capillaries consist of a single layer of endothelial cells with a basal lamina on the exterior surface. There is no smooth muscle but there may be some associated contractile cells called pericytes. Usually the capillary wall is continuous but sometimes, as in the sinusoids of the liver, it contains gaps. Endothelial cells can divide throughout life and there is usually a low level of growth associated with tissue remodeling. The formation of new capillaries by endothelial cell division and cell movement is known as **angiogenesis**. A number of growth factors are active in promoting angiogenesis, particularly vascular endothelial cell growth factor (VEGF) and the fibroblast growth factors (FGFs). The embryonic development of blood vessels is considered in Chapter 15.

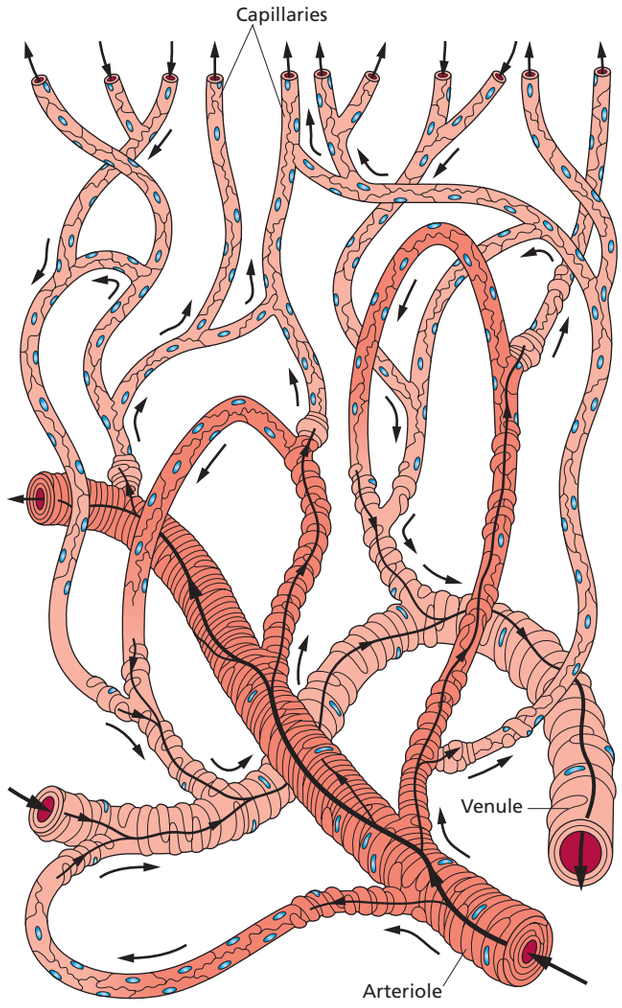


Fig. 13.4 A microcirculatory unit, showing joining of terminal arteriole and venule by capillaries. (After *Gray's Anatomy*, 35th edn, 1973. Longman, figure 6.10, p. 595.)

Tissue renewal

Measurement of cell turnover

The brief sketch above focused on the visible appearance of tissues. But overall morphology tells us little about the cell turnover which is so critical to their maintenance. Although tissue culture cells in optimal medium may grow exponentially, this is rarely true of cells within the body. Usually cell turnover is slow, and particularly in epithelia it is often compartmentalized with separate proliferative and differentiating zones.

Classic Experiments

The hematopoietic stem cell and its cell lineage

In the 1940s it was known that irradiated mice could be rescued by a bone marrow graft. But it was thought that some substance or hormone present in the marrow was responsible. The paper by Ford et al. showed by identification of a chromosomal marker that the rescue activity of the marrow graft was actually due to colonization by blood-forming cells.

The second paper describes the ability of hematopoietic cells to form monoclonal colonies in the spleen of irradiated animals. This provided a method for quantifying numbers of particular types of progenitor and showed the existence of multipotent cells forming clones of mixed cell type.

The third paper established an *in vitro* assay for colony formation which resulted in the characterization of further multipotent cell types and was used to purify the hematopoietic growth factors.

Finally, the paper of Spangrude et al. describes the isolation of pure HSCs from mouse bone marrow by cell sorting, using the criterion of $Sca1^{+}Lin^{-}Thy1^{lo}$.

Ford, C.E., Hamerton, J.L., Barnes, D.W.H. & Loutit, J.F. (1956) Cytological identification of radiation chimaeras. *Nature* **177**, 452–454.

Till, J.E. & McCulloch, E.A. (1961) A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiation Research* **14**, 213–222.

Bradley, T.R. & Metcalf, D. (1966) The growth of mouse bone marrow cells in vitro. *Australian Journal of Experimental Biology and Medical Science* **44**, 287–300.

Spangrude, G.J., Heimfeld, S. & Weissman, I.L. (1988) Purification and characterization of mouse hematopoietic stem-cells. *Science* **241**, 58–62.

The multiplication of cell populations can be estimated by counting the proportion of visible mitoses to obtain a **mitotic index**. However, mitosis usually occupies a short period within the cell cycle, and a population has to be growing fast to show a significant mitotic index. More sensitive are methods that identify cells in **S phase**, which represents a longer fraction of the cell cycle and hence enables more cycling cells to be observed. One simple method is immunostaining for a protein associated with DNA replication: proliferating cell nuclear antigen (PCNA). This will give an estimate of the proportion of cells in S phase at the time of fixation. Alternatively, cells, tissues, or whole animals can be labeled by administration of a DNA precursor, usually **bromodeoxyuridine (BrdU)**, a thymidine analog that is incorporated into DNA and can be detected with a specific antibody (see Chapter 5). This will reveal which cells were in S phase at the time that the label was administered. It makes it possible to label at one time and then to trace the subsequent position and differentiation class of the cells that were labeled. If the cells continue dividing, then the incorporated BrdU will be diluted out after a few rounds of division and will no longer be detectable, therefore long-term retention of the label is taken to indicate that the cells underwent their final S phase at the time of administration, and divided only once before becoming postmitotic. This final division is sometimes called the cell's "birthday." Before BrdU became available, many similar studies were conducted using **^{3}H-thymidine** (**^{3}HTdR**), which is also incorporated into DNA in S phase and can subsequently be localized by **autoradiography** (see Chapter 5).

Tissue types in the body can be classified on the basis of their proliferative behavior, visualized with BrdU or ^{3}H-thymidine:

1 Postmitotic, such as neurons and skeletal or cardiac muscle. Once formed these cells do not divide again, although it is now known that their numbers can be replaced to a small extent from undifferentiated progenitors. There is a limited new formation of neurons of the olfactory bulbs and the hippocampus from neuronal stem cells in the ependyma. There is also some new formation of myofibers from satellite cells.

2 "Expanding." These tissues grow while the animal is growing and stop when adult size is attained. In the adult such tissues are mostly quiescent although there may be a slow turnover of cells. In addition they remain capable of growth to a greater or lesser degree when stimulated by wounding. In this category are connective tissue, smooth muscle, and liver.

3 Renewal. Here the tissue is in a constant state of cell turnover. There is an active proliferative zone containing stem cells (see below) and this feeds a population of differentiated cells, which itself has a finite lifetime and is constantly dying and being repopulated. Examples are the hematopoietic system, the epidermis of the skin, and the epithelium of the gut.

Much of the developmental biology of postnatal life concerns the behavior of the **renewal tissues**, some of which are described below. Of course renewal involves cell death as well as cell birth. The index of apoptotic cell death can be measured by several methods. The most popular are immunostaining for the presence of one of the apoptosis-associated proteins such as the caspase enzymes, and the detection of DNA breaks by a method called TUNEL (TdT-mediated dUTP nick end labeling). Here the enzyme terminal nucleotidyl transferase is used to add a modified nucleotide, usually biotin-labeled, to the fragemented DNA of the dying cell. This is then detected with a fluorescent or enzyme-linked streptavidin.

Although most measurements of cell turnover look at the proportion of cells in cycle, or the proportion of cells in apoptosis, what is really required to understand the situation is a measure of cell production rate and cell removal rate. To obtain a cell production rate it is necessary to know, as well as the S-phase labeling index, the duration of the cell cycle and the proportion of the cycle spent in S phase. For example if cells divide on average once per 24 hours and the S phase lasts 6 hours, then a short pulse of BrdU would enable the observation of about 6/24, or one quarter, of the cells in cycle. So in this case the cell production rate is about 4× the cell labeling index. The cell removal rate

is rarely calculated, and often underestimated. Because the duration of apotosis is quite short (1–4 hours) the flux to cell death per 24 hours is a high multiple of the apoptotic labeling index. For example, if the apoptotic index is 1% and the dying cells are observable for only 2 hours, then the flux to cell death is actually about 1× (24/2) or 12% per day.

Stem cells

Stem cells (Fig. 13.5) are defined as cells:

1 that can divide without limit;
2 that are visibly undifferentiated;
3 whose progeny include both further stem cells and cells destined to differentiate.

In addition, there are other properties characteristic of some but not all types of stem cell:

1 some can give rise to more than one type of differentiated progeny (**pluripotent**);
2 some undergo obligatory **asymmetrical division** to yield one stem cell daughter and one daughter destined to differentiate.

Embryonic stem cells (**ES cells**, see Chapter 10) are usually considered to be similar to the cells of the mammalian embryonic epiblast or inner cell mass and are capable of forming any cell type in the body if reimplanted into an embryo. **Tissue stem cells**, sometimes called **adult stem cells,** are found in renewal tissues of the postnatal animal and are normally thought to be committed to form one particular tissue type. Thus, an intestinal stem cell can form only intestinal types and an epidermal stem cell can form only keratinocytes. This corresponds to the idea that embryonic development is hierarchical, with the cells of the early blastula or epiblast being able to form anything, and then being progressively restricted in their potency by a succession of inductive signals. For example, in the course of development the precursors of an intestinal stem cell would have been committed to endoderm and then to intestine, while the embryonic precursors of a hematopoietic stem cell would have been committed to mesoderm and then to the blood-forming tissue. According to this view, the stem cell for each tissue type may be quite similar to the cells of the appropriate organ rudiment at the **phylotypic stage**. This conception of stem cells resembling embryonic tissue rudiments has recently been challenged by experiments showing repopulation of many tissues by a single type of stem cell, but this area remains controversial (see below).

By definition, stem cells are capable of unlimited division. However, they are by no means the only dividing cells in the tissue because their direct progeny are usually **transit amplifying cells** capable of dividing only a few times but whose division can be regulated to correspond to the demand for new differentiated cells. The stem cells are usually a minority among the dividing population and generally grow more slowly than the transit amplifying cells. Although they must repopulate the stem-cell compartment as well as feed cells to the transit amplifying and differentiated compartments, this does not necessarily mean that every individual cell division need be an asymmetrical one. It is simply required that, on average, the progeny of the stem cell consists of 50% stem cells and 50% cells destined to differentiate (Fig. 13.6). This is important, for example, when considering the acquisition of monoclonality in intestinal crypts (see below).

In the adult body, cell growth is mainly confined to the regions of the renewal tissues containing the stem cells and the transit amplifying cells, and elsewhere most cells are quiescent. It is to be expected that cell division should be under strict inhibitory control, as otherwise the unregulated growth of even one single cell could easily become a macroscopic cancer and destroy the organism in a few months.

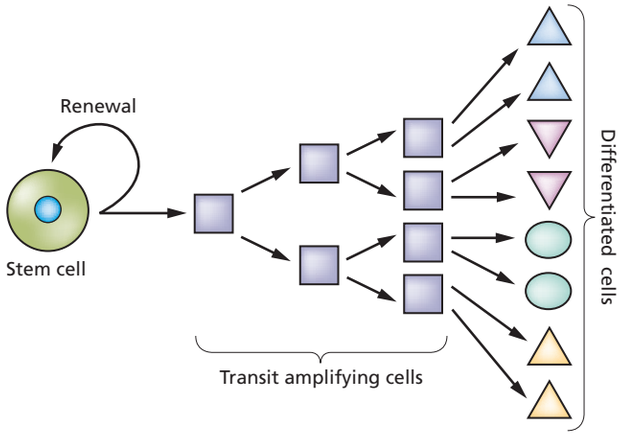


Fig. 13.5 Cell lineage in a renewal tissue, showing stem cells, transit amplifying cells, and differentiated cells.

Stem cell niches

Many tissues have a histological substructure such that they consist of many small repeating modules or units, for example glandular acini or intestinal crypts. These units are not only the functional units of the tissue, but are also often the units within which cell proliferation and turnover are organized. The places where stem cells are found contain a specific microenvironment known as the **stem cell niche** suitable for their persistence and growth. If the stem cells are removed from this microenvironment they will grow no more. Conversely if they are reintroduced to the niche then they will grow again.

The molecular identity of stem cell niches is now becoming known. One well characterized example lies in the germarium regions of the *Drosophila* ovary (Fig. 13.7). The ovary comprises a set of ovarioles, each of which contains a string of egg chambers with the germarium at the proximal end. The egg chambers consist of one oocyte and 15 nurse cells surrounded by follicle cells, as previously described in Chapter 11. The oocyte and nurse cells of each egg chamber are formed from four divisions of a single germ cell in the germarium region. This cell is known

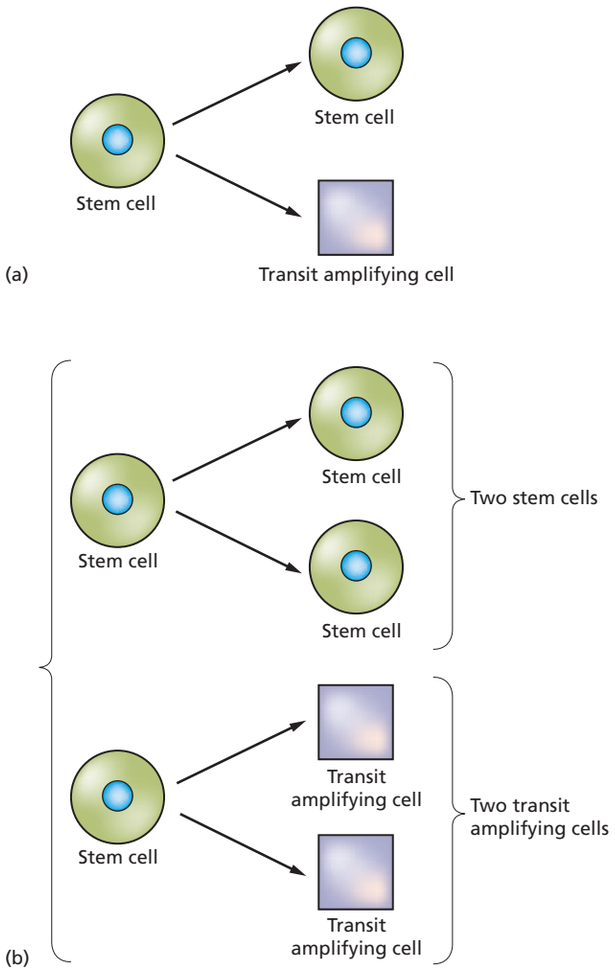


Fig. 13.6 Stem cells can maintain themselves either (a) by repeated asymmetrical division or (b) by generating stem cell and transit amplifying daughters with equal frequency but at separate divisions.

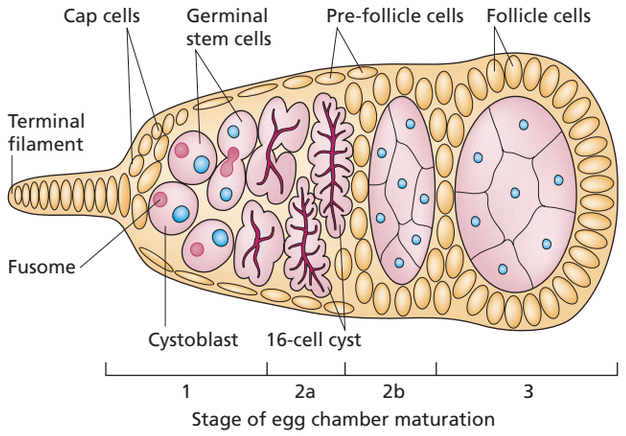


Fig. 13.7 The stem cell niche in the *Drosophila* ovary germarium.

absence of *bam* the the germinal stem cells do not differentiate but continue to proliferate, forming a germ cell tumor. Conversely the overexpression of *bam* under control of a heat shock promoter causes premature cystoblast formation in all the germinal stem cells. Evidence that the repression of *bam* depends on the dpp signal from the somatic cap cells comes from experiments showing that overexpression of *dpp* has a similar effect to loss of function of *bam*, and loss of function of *dpp* has the same effect as overexpression of *bam*. Normally the germinal stem cell divisions are asymmetrical, because the daughter not contacting the cap cells turns on *bam* and becomes a cystoblast. But one of the properties of the niche is that if some stem cells are removed by genetic means then this creates some space adjacent to the cap cells. This space can become occupied by progeny from the surviving stem cell(s) that would normally have become cystoblasts, and thus the production of cystoblasts is suspended while the normal number of stem cells is restored. This behavior resembles the repopulation of mammalian bone marrow, following irradiation and grafting of marrow from a healthy donor (see below).

as a **cystoblast**, and its precursors are the germinal stem cells (= **oogonia**) which divide mitotically about once per day. Each germarium contains two or three germinal stem cells, adjacent to five or six somatic cap cells and it is these somatic cap cells that define the niche. They do so by secretion of the decapentaplegic (dpp) protein. This represses expression of the *bag of marbles* (*bam*) gene in the adjacent cells. *bam* encodes a cytoplasmic protein which interacts with a germ cell-specific organelle called the fusome and is needed for the cystoblast maturation. In the

Classic Experiments

Cell turnover in tissues

The first paper is a study of mitoses in the epithelium of the small intestine and arrives at the conclusion that cells must be being continuously produced in the crypts and shed from the villi. The second paper uses the incorporation of radioactive debris from ^{3}H-thymidine-labeled cells that have died as a cell marker for neighboring cells. From studying the subsequent distribution of these radioactive debris it is postulated that a single type of stem cell produces all four cell types of the small intestinal epithelium.

Leblond, C.P. & Stevens, C.E. (1948) The constant renewal of the intestinal epithelium in the albino rat. *Anatomical Record* **100**, 357–377.

Cheng, H. & Leblond, C.P. (1974) Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. V. Unitarian theory of the origin of the four epithelial cell types. *American Journal of Anatomy* **141**, 537–562.

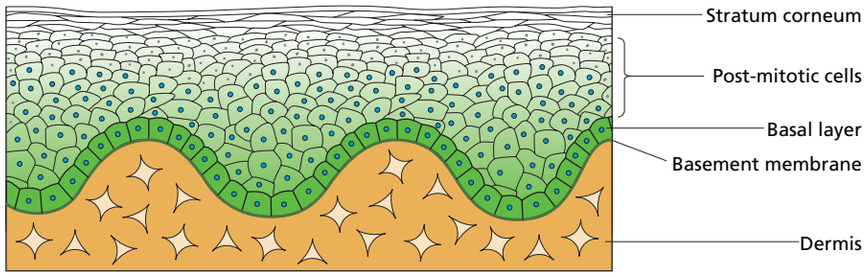


Fig. 13.8 Organization of the epidermis. All keratinocytes are born in the basal layer and differentiate progressively as they move up to the surface.

Skin

Skin consists of a squamous stratified epithelium, the **epidermis**, on top of a connective tissue, the **dermis** (Fig. 13.8). The main cell type in the epidermis is the **keratinocyte**. Cell division is confined to the basal layer. This contains **stem cells**, which can both renew themselves and generate keratinocytes, and also **transit amplifying cells**, which are formed from the stem cells and have a finite division potential before they differentiate. In humans the epidermis is renewed from the basal layer about every 2 weeks. The entire basal layer of the epidermis, and of other squamous epithelia such as those of the esophagus or vagina, depends on the activity of a transcription factor called p63. This is expressed throughout the basal layer and is switched off when cells migrate upwards. The knockout mouse lacking p63 is unable to form any squamous epithelia and dies soon after birth.

The dermis is a dense fibroelastic connective tissue derived from the dermatome and neural crest of the embryo. At deeper levels it is largely adipose tissue. The junction between dermis and epidermis is marked by a basement membrane and, in humans, this is wrinkled with epidermal ridges and corresponding dermal papillae (note that the specialized dermal core of the hair bulb is also called a dermal papilla) . The dermis contains the usual nerves and blood vessels, and pressure receptors called Pacinian corpuscles, as well as the epidermis-derived specializations.

The factors required for proliferation of the epidermal basal layer include both factors produced by the epidermis itself, such as TGFα, and factors secreted by the underlying dermis, including keratinocyte growth factor (KGF), a member of the FGF family. Keratinocyte cultures can be grown *in vitro* and form the same stratified arrangement as the natural skin. There is no need for the dermis in such cultures as the growth factors which it normally supplies are present in the medium. Labeling studies show that about 60% of basal layer cells are in cycle, but only a fraction of these are stem cells. The functional test for a stem cell is that it can form a large self-sustaining epidermal colony either in culture, or after grafting to a **nude mouse** (a type of mouse that cannot reject tissue grafts). Transit amplifying cells, by contrast, can only form small colonies of a few cells and then stop growing. By this criterion about 10% of basal layer cells are thought to be stem cells. A similar proportion, presumably the same stem cells, are capable of forming large colonies that can repopulate the epidermis following severe radiation damage.

The stem cells defined by these criteria are characterized by a higher level of β-1 integrin than the transit amplifying cells. This is a cell adhesion molecule involved in the recognition of collagen, laminin, and fibronectin. *In vivo*, in human foreskin, the high-integrin cell clusters are found at the tips of the dermal papillae, suggesting that this may define the stem cell niche for the epidermis. The stem cells also show an enhanced level of nuclear β-catenin, indicating a possible role for Wnt signaling in the maintenance of the niche. If either β-1 integrin or β-catenin are introduced into keratinocyte cultures by retroviral infection, then the cells receiving the gene will acquire stem cell properties. The stem cells, both *in vivo* and *in vitro*, also contain an elevated level of the Notch ligand delta-1. If delta-1 is introduced into cells of a keratinocyte culture by retroviral infection, then the high delta-expressing cells tend to cluster and to be inhibited from differentiation, while their neighbors are stimulated to differentiate. This suggests that the distinction between stem cell clusters and the surrounding transit cells may be maintained by a **lateral inhibition** mechanism similar to that involved in neurogenesis and pancreatic differentiation (see Chapters 4, 14, and 16). Although this picture is incomplete, it seems likely that the dermal papillae emit a Wnt signal, and that the adjacent basal layer cells respond by increasing synthesis of β-1 integrin. This helps maintain the stem cells as a cluster and also has additional intracellular signaling effects leading to the increase of delta-1 expression and the spatial segregation of the basal layer into stem cell and transit amplifying cell zones (Fig. 13.9).

Once cells leave the basal layer they stop dividing and enter a program of further differentiation. The progress of maturation is reflected by the names given to successive layers of the epidermis: stratum germinativum (the basal layer), stratum spinosum (the "prickle" layer – the apparent prickles are abundant desmosomes), stratum granulosum (with granules), and stratum corneum (cells have lost nuclei and have become flat sacs of keratin). Keratin is a generic name for the large family of fibrous proteins forming the cytokeratin **intermediate filament** family and found in all epithelial cells. There are many different keratins coded by different genes, and the repertoire expressed changes as cells move up from the basal layer. In the granular and cornified layers the cells also contain a tough internal sheath of an insoluble protein called involucrin.

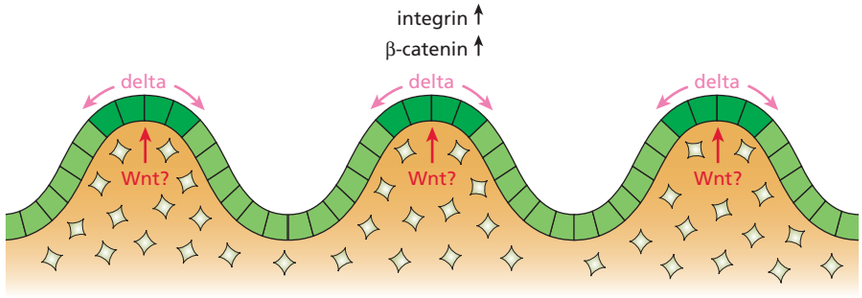


Fig. 13.9 The stem cell niche in the epidermis. Signals from the dermis, probably including Wnt, maintain groups of stem cells. The signals increase the level of β1-integrin, which causes the stem cells to remain as a small cluster. The stem cells display delta-1 on their surfaces, thereby repressing the surrounding cells from stem cell behavior.

Hair follicles

The structure of the hair follicle is shown in Fig. 13.10a. The hair shaft is composed of dead keratinocytes, which are produced in the epidermal **matrix** region at the base. This lies in close proximity to the **dermal papilla**, a projecting bud of fibroblastic cells, and also contains **melanocytes** which transfer pigment granules to the keratinocytes of the hair. Surrounding the whole is a layer of cells continuous with the surface epidermis called the outer root sheath. High up, near the junction with the surface epidermis, lies a sebaceous gland. The entire region at the base of the follicle comprising the dermal papilla, the proliferative epidermal zone, and the outer layers, is known as the hair bulb. Hair does not grow continuously but in a cycle. The active growth phase is known as anagen, which lasts about 3 weeks in mice but can be much longer in humans. The period of regression of the follicle is called catagen, and the period of quiescence is called telogen (Fig. 13.10b).

Hair follicles start life in the late mammalian embryo as epidermal invaginations. Their formation requires both Wnt signaling and also inhibition of BMP signaling from the dermis. BMPs are produced by the epidermis, and noggin by the dermis. A combination of Wnt3A and noggin will induce new follicle buds. The BMP inhibition causes transcription of Lef1 while the Wnt signal stabilizes β-catenin and the combination carries the Lef1 into the nucleus to control target genes (see Appendix). One target is E-cadherin, whose expression becomes repressed, thus reducing the mutual adhesion of the cells and leading to the invagination behavior to form the bud. Evidence for this mechanism is based on the the following:

1 Invaginating buds are produced by adding Wnt3A + noggin to keratinocyte cultures, or in transfecting in *Lef1* plus a consititutive form of *β-catenin*.

2 Knockout mice lacking either *noggin* or *Lef1* have few hair follicles.

3 The Wnt signaling pathway is active in the early buds. This may be seen in a reporter strain of transgenic mice which contains *lacZ* driven by a Wnt-sensitive promoter.

4 Ectopic hair follicles are formed in mice transgenic for constitutive *β*-catenin, driven by the *keratin 14* promoter which is active only in the basal layer of the epidermis.

5 Bud formation is suppressed in mice transgenic for production of the Wnt-inhibitor Dickkopf, also driven in the basal layer by the *keratin 14* promoter.

The understanding of hair follicle initiation makes it possible to contemplate a possible "cure" for human baldness. However the overexpression of Wnt pathway components in human patients would probably not be acceptable because of the risk of inducing cancers.

Although the initial signal for bud formation comes from the dermis, the formation of the **dermal papilla** depends on a second signal from the invaginating bud to the dermis (Fig. 13.11). The dermal papilla secretes growth factors needed by the proliferative zone of the epidermal matrix region. Isolated papillae will induce new epidermal proliferative zones from the upper halves of follicles, and in some situations can induce complete new follicles from epidermis. Examination of mouse **aggregation chimeras** in which one component is labeled and the other unlabeled suggests that each follicle contains about four epidermal stem cells, all of which contribute to all the layers of the hair shaft. In aggregation chimeras the cells derived from the two embryos are intimately mixed and so, allowing for similar adjacent clones, the number of labeled and unlabeled patches in a small structure like a hair approximate to the number of clones, and therefore the number of stem cells (see also Chapter 10).

As far as stem cells are concerned, attention in the past concentrated on the epidermal matrix region and the dermal papilla because of their obvious role in hair shaft formation. However it is now thought that the real epidermal stem cell population of the hair follicle lies not in the bulb but in a lateral bulge half way up the outer root sheath. The evidence for this is as follows:

1 A 3-day label with BrdU will label many cells in the epidermis of the hair follicle. But a long chase period shows that the cells that retain the label, and are thus very slowly dividing, are concentrated in the bulge region rather than in the bulb. In the next active growth cycle (anagen) some of these labeled cells are seen to have migrated into the bulb.

2 If a bulge region from a *lacZ* positive (**Rosa26**) mouse is grafted to a large vibrissal follicle and then cultured under the kidney capsule of a **nude mouse**, the β-galactoside expressing cells are seen to populate the entire follicle.

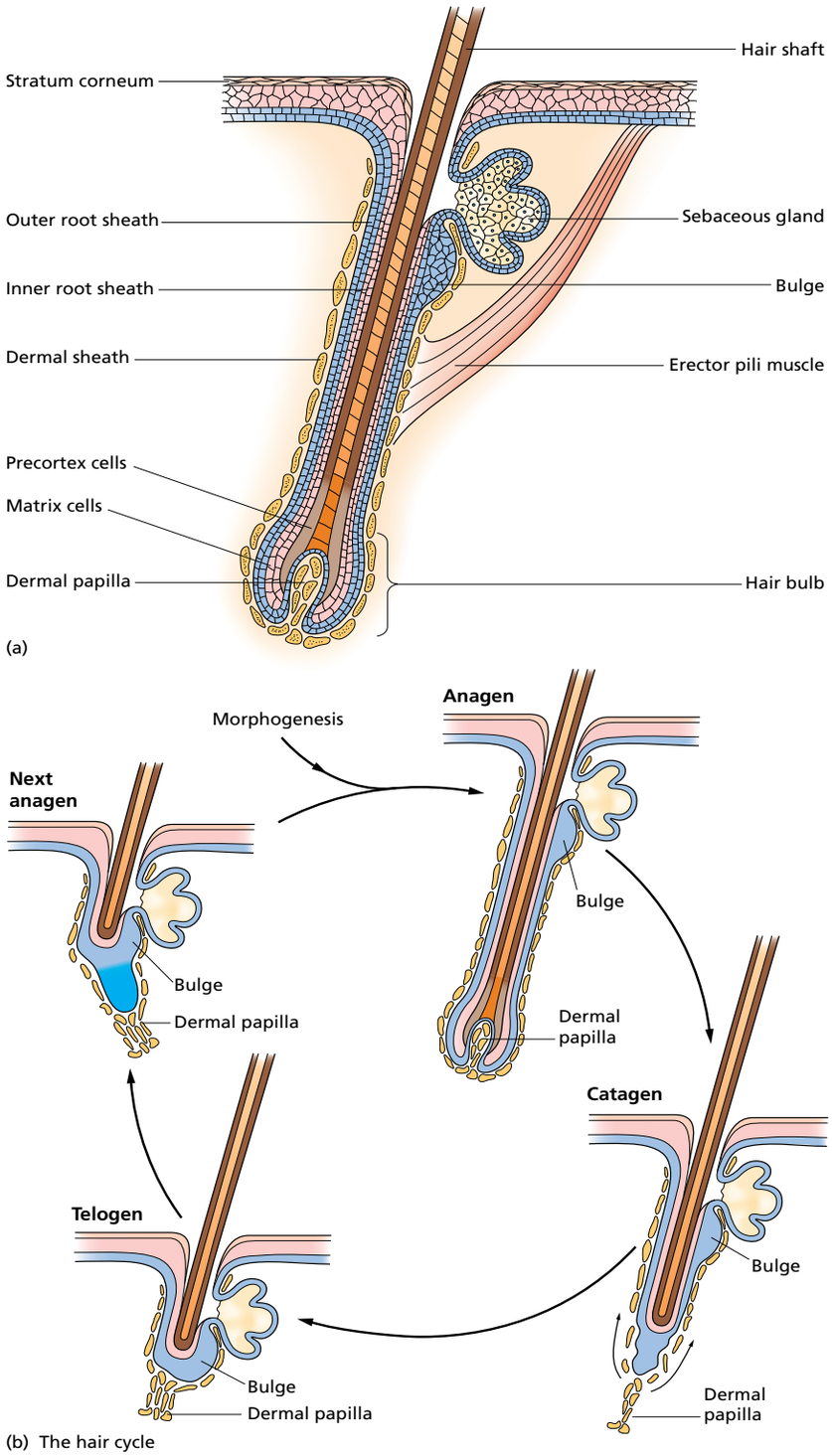


Fig. 13.10 (a) Structure of the hair follicle. (b) The hair growth cycle.

3 Individual *lacZ* positive bulge regions grafted to the back of unlabeled late embryos will generate labeled surface epidermis, hair follicles, and sebaceous glands (Fig. 13.12).

4 If the surface epidermis is wounded, it can be repopulated by label-retaining cells from the bulge.

The hair follicle bulge is certainly a stem cell niche. Although it seems that the stem cells of the bulge are "more primitive" than those of the basal layer of the surface epidermis, it is not clear whether there is a slow continuous repopulation of the surface epidermis from the bulge, or whether this occurs only as

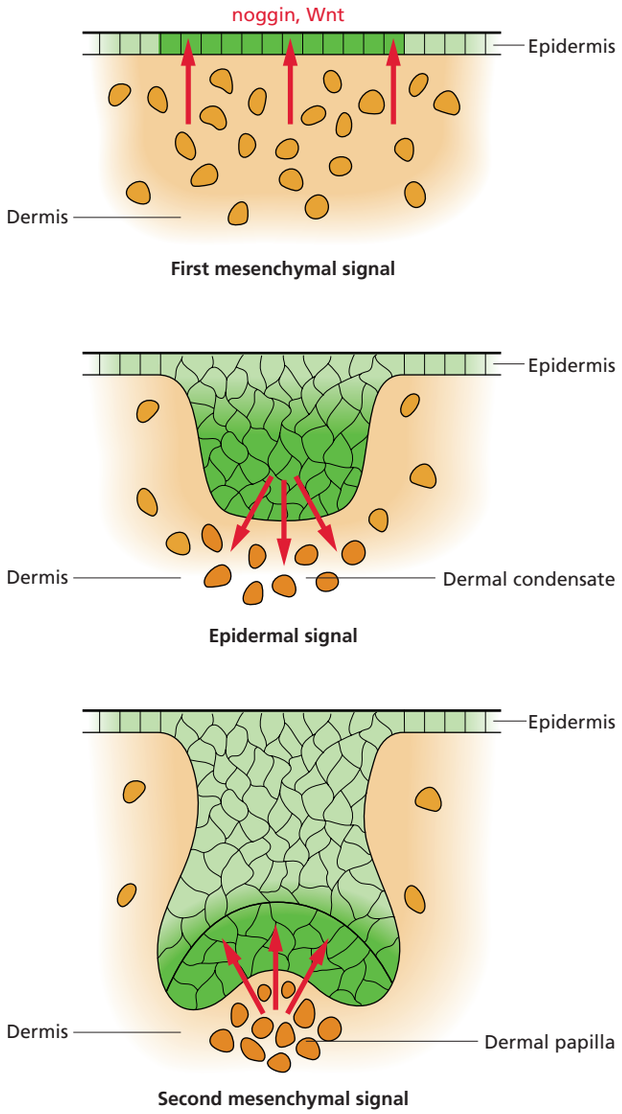


Fig. 13.11 Initial formation of hair follicles. The first phase involves induction by noggin and Wnt from the dermis. This is followed by a signal from the epidermal bud inducing a specialized dermal papilla.

a specific response to wounding. The bulge region does, in a sense, represent the most secluded part of the epidermis as it is continuous with the surface epidermis and is the lowest level of the follicle that persists throughout the hair cycle. In the human there is no visible bulge, but the stem cells also reside in the lowest permanent part of the outer root sheath.

Intestine

The gastrointestinal tract of vertebrates consists of a muscular tube running from the mouth to the anus. It is lined by a number of different epithelia: pharyngeal, esophageal, gastric, small intestinal, and colonic, separated by abrupt discontinuities of cell type. This **epithelium** is derived from the **endoderm** of the early embryo, while the other cell layers of the gut are derived from the **splanchnic mesoderm**. The epithelium, together with underlying connective tissue called the lamina propria and a thin muscle layer called the muscularis mucosa, is often known as the **mucosa**. Outside the mucosa lie further thick layers of connective tissue and smooth muscle.

The program of cell renewal is best understood for the small intestine. This contains regions called the duodenum, jejunum, and ileum, although the difference of cell type between them is not great. On a microscopic scale the intestinal epithelium is arranged on finger-like villi projecting into the lumen, and between the villi lie crypts of Lieberkuhn sunk below the surface (Fig. 13.13a,b). Cell proliferation takes place only in the crypts, and differentiated cells are continuously moving out from the crypts, moving up the villi, then dropping off into the gut lumen. The main cell types are **enterocytes** (absorptive cells) and **goblet cells**. The absorptive cells are characterized by a brush border at their apical surface, consisting of numerous close-packed microvilli. Goblet cells contain a large vesicle filled with mucins. In addition there are **Paneth cells** located at the base of the crypts, which secrete antibacterial substances, and several types of **enteroendocrine cells** each secreting a particular peptide hormone. The crypts themselves are set up shortly before birth by folding of the endodermal epithelium which before this stage is a simple columnar epithelium. During the growth of the animal they can divide by budding, starting at the base (Fig. 13.13c). The signal for crypt division is not known but may be an increase in the number of stem cells. Some experimental studies have also been performed on the colon because of its importance in terms of colonic cancer. Its structure is similar to that of the small intestine, but without villi and without Paneth cells.

Cell division in the crypts is rapid. The stem cells are located near the crypt base, above the Paneth cells, with several layers of transit amplifying cells lying above them. A mouse crypt contains about 250 cells of which roughly 160 are dividing, with a cycle time of about 13 hours. The progeny move up and out of the crypts and the tissue is arranged such that each crypt feeds more than one villus, and each villus draws cells from several crypts. A small proportion of cells also move down the crypt to replenish the Paneth cells at the crypt base.

It has been possible to examine the clonal composition of the crypts by making aggregation chimeras between mouse strains that differ in the expression of a marker, *Dolichos* lectin receptor (Fig. 13.14a). This carbohydrate is expressed by intestinal cells in some mouse strains and is absent in others. In an aggregation chimera the cells of the two donor embryos are intimately mixed, so at the time of crypt formation most crypts will receive cells of both donor types. However, over the first 1–2 weeks of postnatal life the crypts lose one of the two types, such that all cells in each crypt are either one type or the other. In other words, the crypts become **monoclonal**. Initially it was thought that this meant there was only one stem cell per crypt. However,

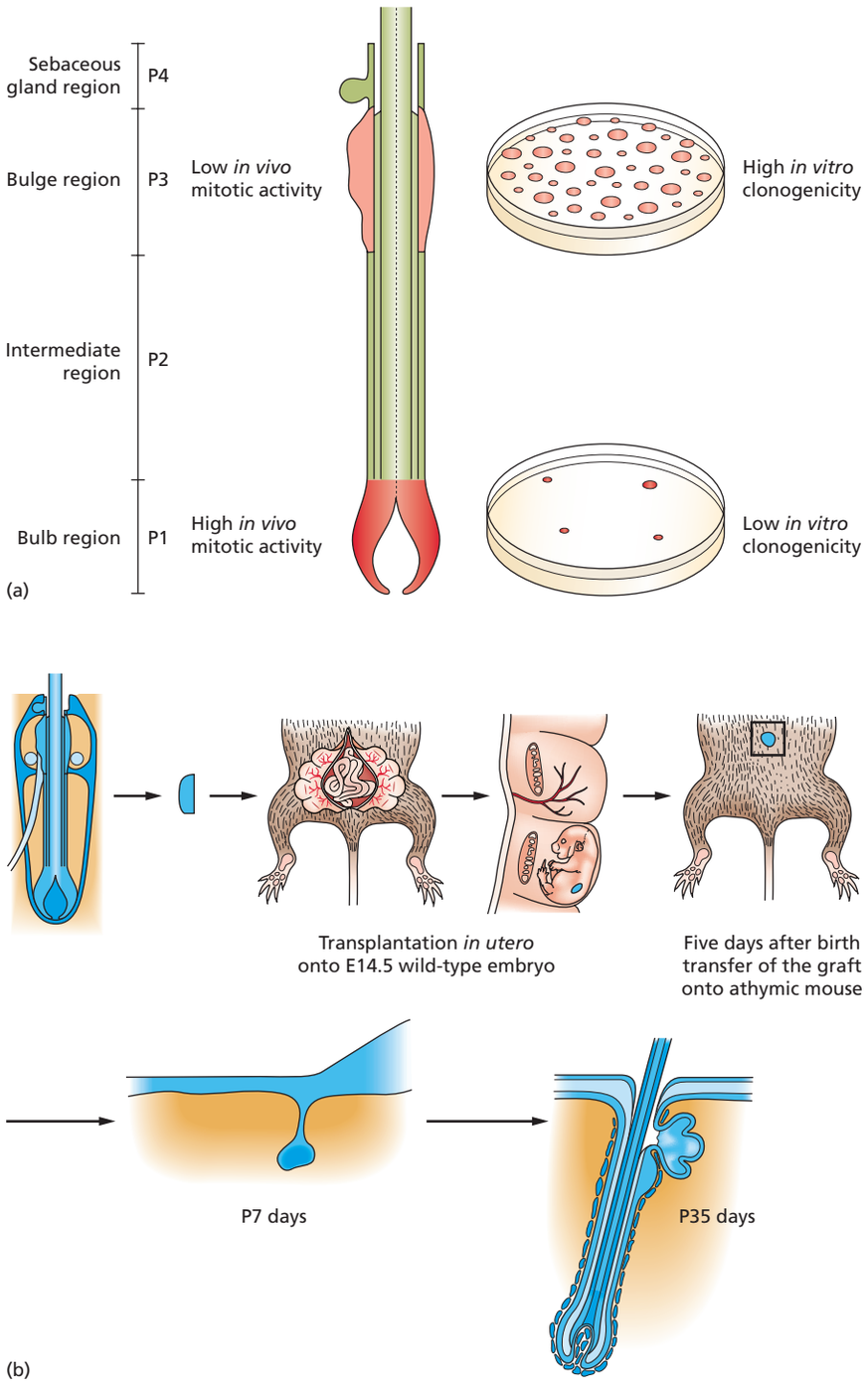


Fig. 13.12 Evidence that the bulge region of the hair follicle contains the stem cells. (a) The bulb region contains more cells in cycle but the bulge region contains more clonogenic cells. (b) A β-galactosidease labeled graft from the bulge region can repopulate the surface epidermis and other epidermal sturctures as well as the entire hair follicle.

it now seems that there are about six stem cells per crypt and that the monoclonality arises by a random cell selection process. This probably arises because the stem cells, on division, are able to produce either two stem cells or one stem and one transit amplifying, or two transit cells. Over several cell cycles the genetic diversity of the stem cells within a crypt will become progressively reduced because any stem cell that produces two transit amplifying cells will be lost to the stem-cell pool. Eventually this random loss of stem-cell diversity will result in monoclonality. An additional reason for the acquisition of monoclonality is the fact that the crypts themselves multiply by budding, sharing their stem cells between the two daughter crypts. By this process it is possible to lose all the stem cells of one genotype in one or both of the daughter crypts.

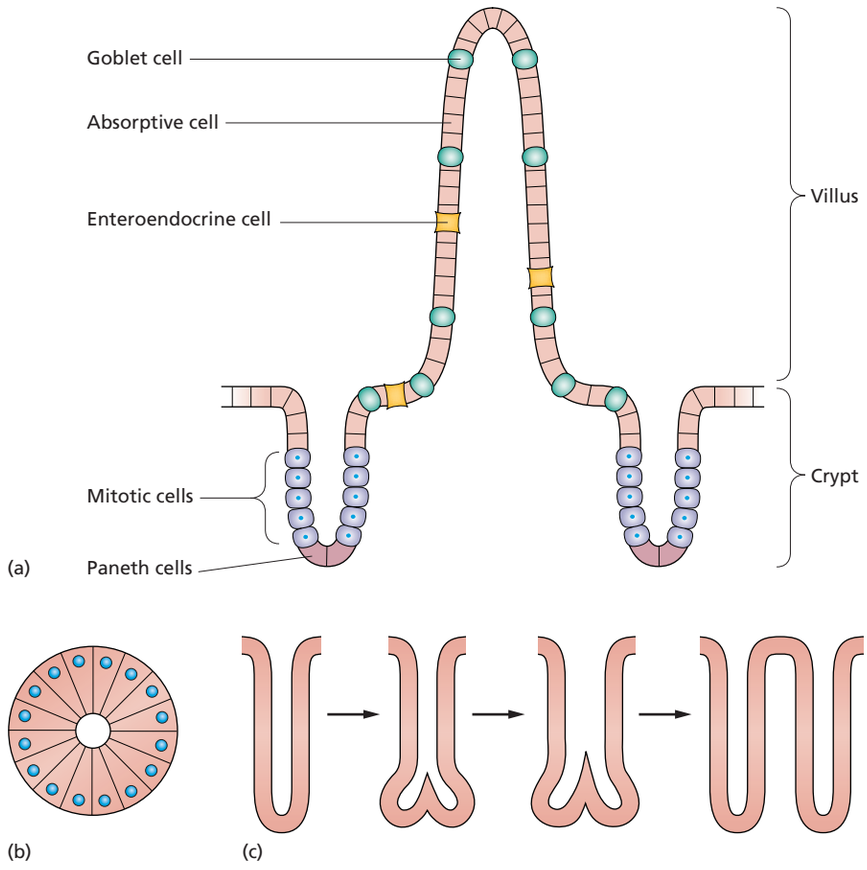


Fig. 13.13 Diagrammatic organization of the small intestinal epithelium. (a) Longitudinal section of two crypts and a villus. (b) Transverse section of a crypt. (c) Multiplication of crypts by budding from the base.

Estimates of the proportion of stem cells among the dividing cells of the crypts have been made by several methods including modeling of the cell-cycle kinetics of the whole crypt. The estimate of six is based mainly on mutagenesis studies (Fig. 13.14b). If a mouse of a suitable strain is mutagenized, then a proportion of cells will acquire reactivity for *Dolichos* lectin. Shortly after the mutagenesis, many crypts containing mutant clones are seen. Most of the clones arise in transit amplifying cells and are soon lost. But if they arise in stem cells they are retained in the long term. In principle, if there are n stem cells per crypt, then a mutation in a stem cell should appear as a labeled sector within the crypt occupying about $1/n$ of its circumference. In fact, after a few weeks many mutant crypts are uniformly composed of mutant cells, showing the same drift to monoclonality as aggregation chimeras, probably for the same reasons. Mutagenesis also enables the study of the contribution of each crypt to the villi, as a labeled crypt will emit a stream of labeled cells that form a strip running up each of the villi to which it contributes (Fig. 13.14b).

A different type of estimate of stem-cell number can be made based on radiation toxicity (Fig. 13.14c). A given dose of X- or gamma rays will sterilize a proportion of cells in the epithelium. Cells that are capable of growing and repopulating the tissue are known as **clonogenic** cells. It is assumed that a crypt can only regenerate if it includes at least one clonogenic cell that survived the radiation. Measurements of crypt survival following various dose regimes suggest that there are about 80 clonogenic cells per crypt. This is substantially greater than the likely number of stem cells per crypt calculated from mutagenesis, and the difference in the two estimates suggests that a proportion of the transit amplifying cells are capable of becoming stem cells again under conditions of severe tissue damage. This makes sense if it assumed that a transit amplifying cell will become promoted to stem cell status if it can enter the stem cell niche. The mutagenesis and radiation experiments also provide data to support the idea that the stem cells of the small intestine are **pluripotent**, being able to generate all four of the usual cell types: columnar, goblet, Paneth, and enteroendocrine. Crypts entirely populated by one mutant clone contain all four cell types, so one cell must have generated them all. Similarly, after high doses of radiation from which only a minority of crypts survive, these will mostly have regenerated from a single clonogenic cell, but will nevertheless all acquire the four cell types.

Knowledge of the molecular characteristics of intestinal stem cells remains limited. An RNA-binding protein called Musashi-1 is expressed in a few cells between and just above the Paneth cells, and may be a stem cell marker. There is an increase in the number of Musashi-1 positive cells during the recovery

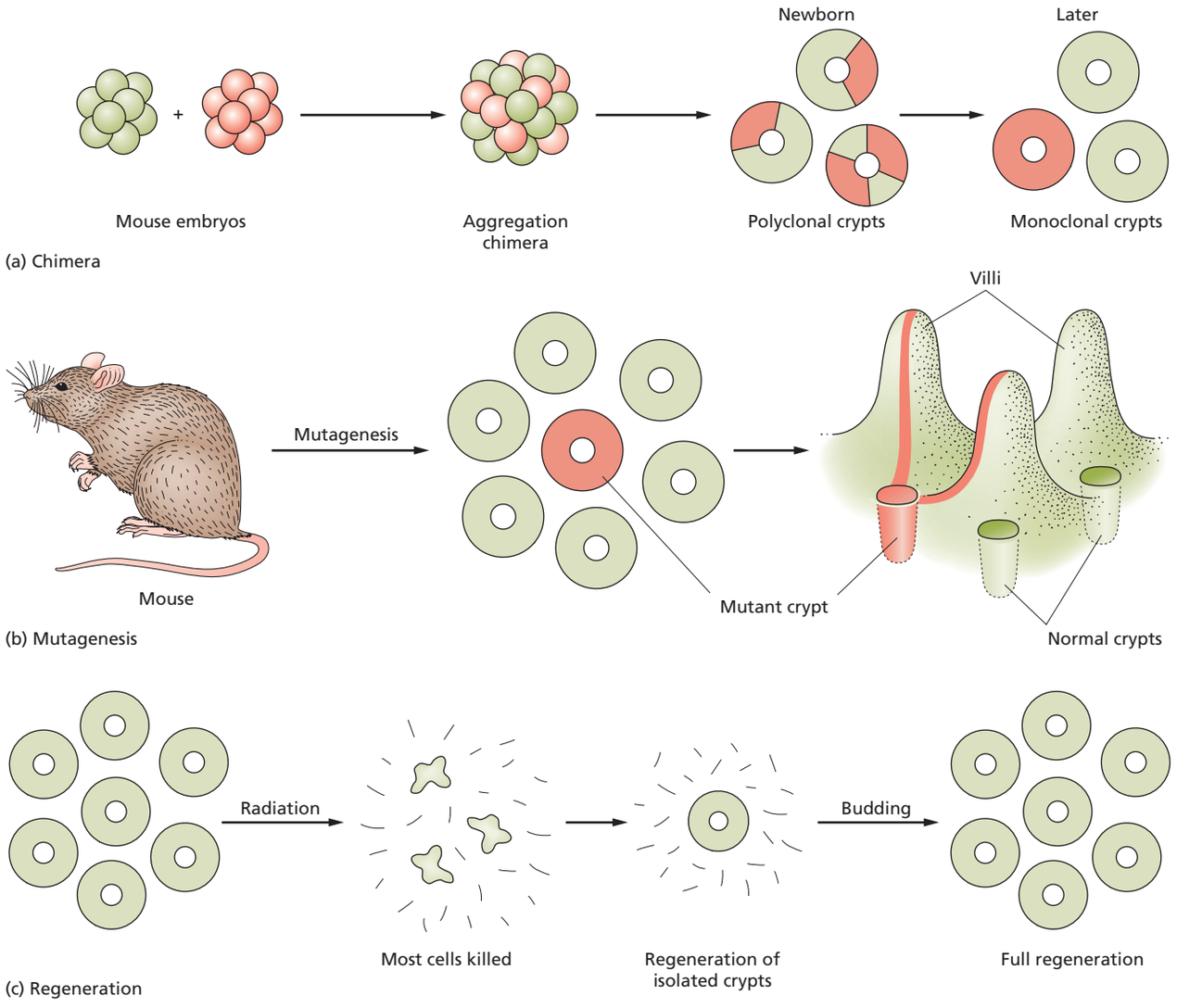


Fig. 13.14 Methods for studying intestinal crypt organization. (a) Aggregation chimeras. Early crypts are polyclonal but later become monoclonal. (b) Mutagenesis produces occasional cells that can be visualized by binding of Dolichos lectin. One mutant stem cell may often come to populate an entire crypt, and its progeny form streams up to the tips of the adjacent villi. (c) A dose of X-radiation that destroys most cells leads to regeneration of whole crypts from individual clonogenic survivors.

from radiation damage, which is consistent with the increase in the number of clonogenic cells in this situation. As in the epidermis, there is good evidence that the intestinal proliferative compartment depends on the Wnt signal transduction pathway (Fig. 13.15). It is known that the mouse knockout of the transcription factor gene *tcf4* fails to form any proliferative compartment. TCF4 is one of the HMG-type transcription factors that is activated by β-catenin and nuclear β-catenin is normally found in the cells of the bottom third of the crypt, which represents the proliferative compartment. So it is likely that Wnt signaling from the lamina propria is needed to maintain proliferation in the epithelium, and something additional is needed to define the much smaller stem cell niche.

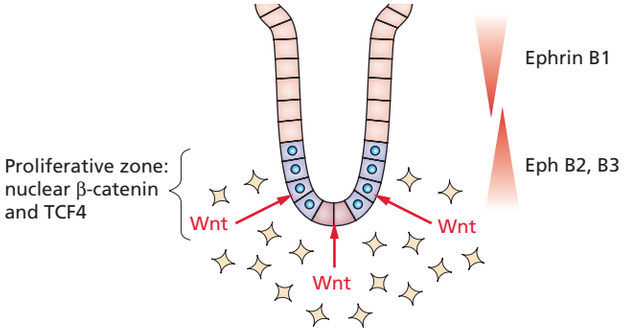


Fig. 13.15 Role of the Wnt pathway in controlling the proliferative structure of the intestine.

New Directions in Research

Stem cell research is thought to be rich in practical applications, mostly having the aim of repairing damaged tissues and organs by grafts of stem cells. In recent years many biotech companies have been founded on the basis of these opportunities.

In terms of basic knowledge it is important to establish exactly how tissue stem cells arise during development and how similar they really are to the whole embryonic rudiment from which the tissue develops.

We need to know if Wnt signaling really controls stem cell behavior, or just proliferative behavior generally.

We also need to understand whether the Notch lateral inhibition system controls the differentiation of multiple cell in all cases, or whether there are other similar systems.

Ultimately, understanding the signals that control the self-renewal and differentiation of the stem cells should enable the design of culture conditions for growing tissue stem cells without limit *in vitro*.

There is also an intimate connection between the control of proliferation and the actual structure of the epithelium, because the crypts are characterized by expression of the adhesion molecules Eph B2 and B3, while the villi are characterized by expression of their ligand ephrin B1. Transcription of these molecules is, respectively, activated and repressed by β-catenin. If both Eph B2 and B3 are removed by targeted mutagenesis then the intestine loses its structure and both dividing and differentiated cells are found mixed together. If only EphB3 is removed then the structure is normal but Paneth cells are found all over the epithelium instead of being confined to the crypt bases. APC, the product of the *adenomatous polyposis coli* gene, is a cytoplasmic protein required to enable the phosphorylation of β-catenin by GSK3. In loss-of-function mutants β-catenin is not inactivated, and is therefore constitutively active. This leads to the inability to shut off Eph B2 and B3, and to the formation of **polyps**, which are projections into the lumen of differentiated but abnormally organized intestinal tissue. Human patients suffering from adenomatous polyposis coli have many such polyps and a high risk of a polyp developing to cancer (see below). The disease is hereditary and due to loss of one copy of the *APC* gene. When the other, good, copy is lost from an individual cell due to occasional somatic mutations, then that cell will acquire constitutively active β-catenin and develop into a polyp.

In the intestine, as is generally the case for renewal tissues, the stem cells are responsible for producing several types of differentiated cell. It now seems that this is achieved using the Delta-Notch **lateral inhibition** mechanism (see also Chapters 4, 14, and 16). There is a "master switch" at the level of the decision whether to become an ordinary absorptive cell or one of the three specialized cell types (goblet, enteroendocrine, or Paneth), and this is controlled by a bHLH type transcription factor called Math1, which also promotes the formation of Delta (Fig. 13.16). All the cells express Notch and initially have a similar level of Math1. Cells which by chance have a slightly higher level of Math1 produce a little more Delta and signal to surrounding cells. Notch is stimulated in these surrounding cells leading to inhibition of expression of Math1, and hence reduction of Delta. The process will run on until there are a few high Math1-high Delta cells surrounded by a larger number of low Math1-low Delta cells. The cells with low Math1 become absorptive cells, while those with high Math1 become either goblet or enteroendocrine or Paneth, the subsequent decisions depending on further unknown mechanisms. The main evidence for this process is that the knockout of *math1* has an intestinal epithelium which is normal in overall structure, and which contains normal absorptive cells, but totally lacks all the types of specialized cell. Another knockout, of the transcription factor gene *hes1*, shows an opposite phenotype with an elevation of Math1-expressing cells and of the proportion of specialized cell types in the epithelium. Hes1 is on the Notch signaling pathway and its loss will reduce the inhibition of Math1 expression by Notch signaling.

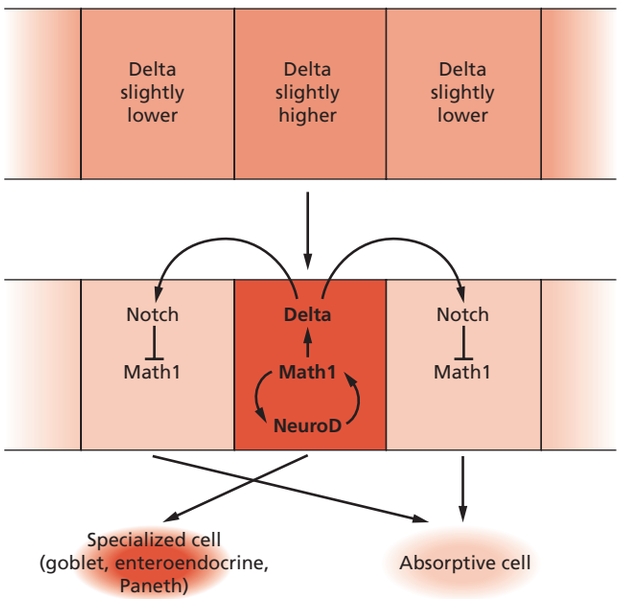


Fig. 13.16 Control of cell differentiation in the intestinal epithelium by lateral inhibition.

Hematopoietic system

In the adult mammal, the **hematopoietic** system resides in the bone marrow within the larger bones of the skeleton. In the embryo it is found at various other sites. Initially it is extra-embryonic, in the yolk sac, then in the **AGM** (**aorta-gonad-mesonephros**) **region** of the mesoderm, then in the liver, then in the spleen and lymph nodes, and finally in the bone marrow. The first two of these sites of production arise *in situ* while the later ones are colonized by cell migration from the earlier sites. This process of cell migration has been established by the grafting of marked cells in amphibian and avian embryos and localizing the progeny of the grafted cells at a later stage.

Like the skin and gut epithelium, the hematopoietic system is a state of continuous cell production and renewal throughout life. There exists a **hematopoietic stem cell** (**HSC**) that can both renew itself and also differentiate into a wide variety of cell types. These include all cells of the blood and immune system together with histiocytes, osteoclasts, and Langerhans cells of the skin.

The cellular components of the blood are as follows:

1 **Erythrocytes** (red cells).
2 **Granulocytes**, comprising neutrophils (phagocytes), eosinophils, and basophils (similar to mast cells).
3 **Monocytes** (similar to **macrophages**/histiocytes).
4 **Megakaryocytes**, giant cells that break up to become platelets.

The above four cell types are collectively known as **myeloid cells**.

5 **Lymphocytes** (T and B cells).

Much of the evidence for the existence of HSCs comes from reconstitution experiments. The bone marrow is the most sensitive tissue in the body to irradiation, and so there is a dose range that will kill by bone marrow failure while other tissues are still potentially able to recover. If a mouse, lethally irradiated with such a dose, is given a graft of marrow cells by injection into the bloodstream, then the graft will colonize the marrow of the host, proliferate extensively, and enable survival of the host. After a few weeks the counts of the various cell types mentioned above have returned to normal and the observation of genetic markers shows that they are all derived from the graft. The ability permanently to rescue lethally irradiated mice is often taken to be the defining feature of the HSC. The use of reconstitution assays to examine the hematopoietic populations in the mouse embryo shows that the earliest HSCs are found in the AGM region at 10–11 days of gestation and in the yolk sac and liver from about 12 days of gestation. This suggests that the hematopoietic population seen in the yolk sac at earlier stages does not contain HSCs capable of long term repopulation.

It is now possible to isolate HSCs directly from bone marrow using the technique of fluorescence-activated cell sorting (**FACS**, see Chapter 5). Mouse HSCs are characterized by high levels of Sca1, low but finite levels of Thy1, and the absence of all other differentiation markers ($Sca1^{+}$, $Thy1^{lo}$, $Lin1^{-}$). Sca1 (stem cell antigen 1) and Thy1 (thymus 1) are both cell surface glycoproteins, Thy1 being abundant on mature T cells. The study of mice transgenic for GFP driven by the *Sca1* promoter shows that the very first HSCs arise in the endothelium of the dorsal aorta. Mouse HSCs can also be isolated because they preferentially exclude certain fluorescent dyes such as Hoechst 33324 or Rhodamine 123, so after exposure to these dyes give a lower fluorescence signal than all other cells in the marrow. The self-renewing properties of the HSC seem to depend on the proto-oncogene *bmi-1*, which encodes a *polycomb* type transcriptional repressor. Knockouts for *bmi-1* develop HSCs but the numbers are greatly reduced postnatally and they have very little reconstitution activity. HSCs are difficult to grow in culture but the numbers, measured by the mouse reconstitution assay, can be increased by introduction of certain genes using retroviruses. These include stabilized (constitutive) β-catenin, suggesting that Wnt signaling may be necessary for HSC self-renewal, as it is for the stem cells of the skin and the intestine.

In addition to the HSC, the marrow contains other cells that can be isolated by different combinations of cell surface markers. In the reconstitution assay they give rise to only a subset of the complete HSC repertoire. This shown the existence of various multipotent progenitors including the common lymphoid progenitor, the common myeloid progenitor, the granulocyte-macrophage progenitor, and the megakaryocyte-erythrocyte progenitor. There are also pluripotent stem cells that have only a temporary repopulating ability, which are believed to represent the next step of maturation after the permanent HSC. A current consensus model for the cell lineage of the hematopoietic system is shown in Fig. 13.17.

A second line of evidence for this model comes from *in vitro* colony assays. It is possible to obtain clones of hematopoietic cells *in vitro* by plating marrow cells in soft agar or methyl cellulose in the presence of the appropriate growth factors. Since most single colonies will be clones derived from a single cell, the production of multiple cell types by a single colony indicates the existence of a multipotent progenitor, and the same potency classes are recovered in these assays as in the whole mouse reconstitution assay. Although the model is generally accepted, it has not yet been confirmed by prospective labeling, which would require the insertion of a permanent genetic marker into HSCs *in vivo*, followed by identification of each type of progeny cell as it is formed.

The *in vitro* colony formation assay has been used to isolate a number of colony-stimulating factors (CSFs), otherwise known as hematopoietic growth factors. Interleukin 3 and stem cell factor (steel factor) can stimulate proliferation of the HSCs, while granulocyte–macrophage CSF (GM-CSF), granulocyte CSF (G-CSF), macrophage CSF (M-CSF), and erythropoietin work in combinations to stimulate the division of the various transit amplifying lineages. Most of the feedback control over the production of the various cell types is exerted by varying the growth rate at the transit amplifying cell level. This is because there are large numbers of such cells and a rapid response can be obtained to changing demand. By contrast, it would take several weeks to alter the rate of production by regulation at the level of the HSC. Several of the hematopoietic growth factors have been prepared

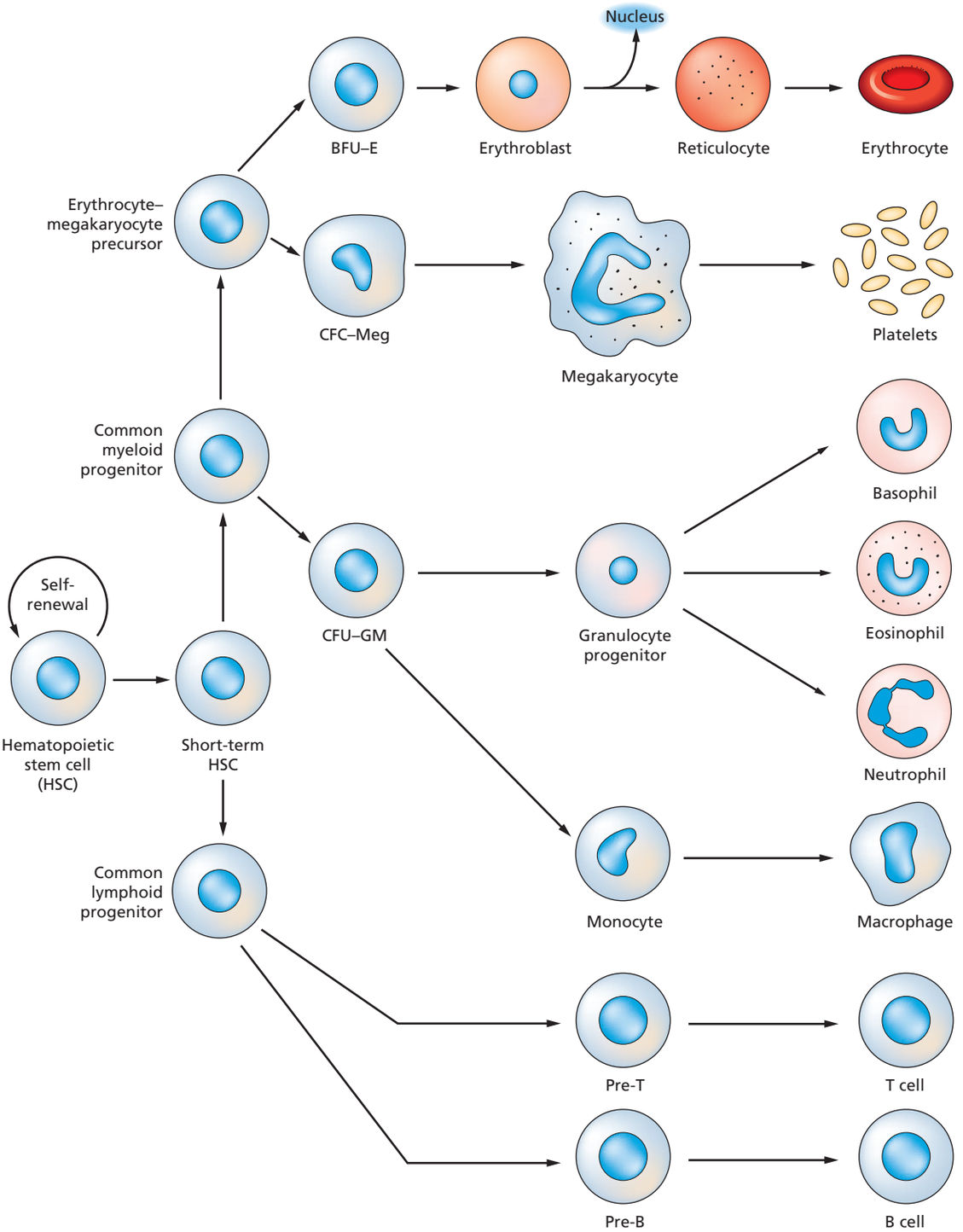


Fig. 13.17 A consensus model for the cell lineage for the cells of the blood and immune system.

in therapeutic quantities by recombinant DNA methods and are now very useful in clinical practice, particularly for the treatment of various types of anemia and for enabling people to rebuild their marrow after cancer therapy.

The transplantable properties of the HSC have also made marrow grafting one of the earliest types of cell therapy to be adopted in human medicine. Indeed the first human bone marrow grafts between identical twins were performed in the 1950s.

Grafting of the patient's own marrow (**autologous graft**) is now routinely used in cancer therapy. Some of the patient's bone marrow is extracted before treatment, the patient is then given a lethal dose of radio- or chemotherapy to kill the cancer, and then the marrow is re-infused to rescue the patient from the radiation. One of the limitations of this method is that there are often cancer cells in the graft which escape the treatment and are reintroduced into the patient. In principle the effectiveness of such grafts could be increased by using pure HSCs instead of whole marrow. It is also possible to graft bone marrow between individuals (**allograft**). However, because the bone marrow is also a factory for the production of immunoactive lymphocytes it is necessary to have a match of major histocompatibility (HLA) antigens in donor and host to avoid both rejection of the graft by the host, and also graft-versus-host disease caused by reaction of lymphocytes in the graft against the host. Even matched grafts are likely to need immunosuppressive treatment to limit the reaction to minor histocompatibility antigens.

Various types of **gene therapy** have been proposed which depend on the possibility of inserting a missing gene into HSCs and then introducing them into the patient. This is likely to be appropriate in cases where the missing gene has a metabolic function, and so would be effective regardless of the tissue in which it is expressed. These methods are still experimental because of the difficult of obtaining enough HSCs, of efficiently introducing genes, and the safety problems associated with random gene insertion events which can sometimes produce cancer-causing mutations.

The reason that the reconstitution assay for HSCs works is that there are a number of stem cell niches for the HSC within the marrow. These are now known to be formed by the **osteocytes** lining the trabecular bone surfaces in the marrow cavity, to which the HSCs attach via N-cadherin (Fig. 13.18). Various treatments that increase the number of trabecular osteoblasts, including downregulation of BMP receptor 1A or injection of parathyroid hormone, also increase the number of HSCs.

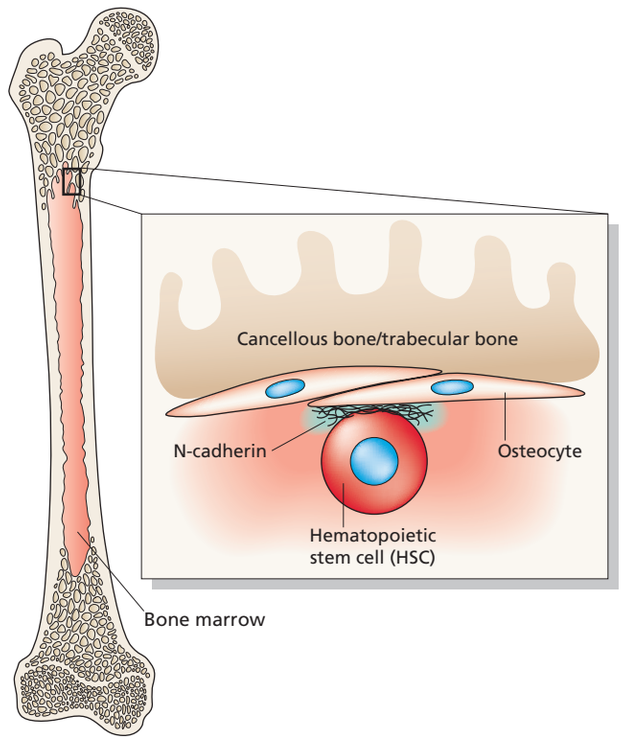


Fig. 13.18 The niche for the hematopoietic stem cell in the bone marrow.

Mesenchymal stem cells and "transdifferentiation"

In addition to the HSCs, the bone marrow contains another type of stem cell. These are called mesenchymal stem cells, or marrow stromal cells, both conveniently abbreviating to **MSC**. It is not yet possible to purify them by cell sorting but they adhere to plastic and long-term cultures can be grown in which the other cell types of the marrow are selected out and disappear. MSCs will form adipocytes, chondrocytes, or osteocytes *in vitro* when cultured in appropriate media. The normal function of MSCs is to produce the various nonhematopoietic cell types found in the bone marrow.

A number of recent studies have suggested that bone marrow cells are capable of colonizing a wide variety of other tissue types when transplanted into irradiated hosts. Some of these are performed with unfractionated marrow, some with enriched or purified HSCs or MSCs. The tissues colonized can include virtually everything including epithelia, muscle, and neurons. This work has generated considerable controversy because it suggests a very different model of development from the conventional one. Instead of cell populations undergoing a series of decisions during embryonic development, in each of which their competence is restricted, the idea is that the whole body is continuously being renewed by highly pluripotent cells from the bone marrow. The phenomenon is known as "transdifferentiation" although this is unfortunate as the term was previously used in a more restricted sense to refer to the rare but well-established cases of direct transformation between differentiated cell types. It now appears that some of the results were due to cell fusion, whereby the genetic markers from donor cells became incorporated into host cells by direct fusion. Other results appear to indicate genuine reprogramming of marrow-derived cells to various other tissue types, but only at very low frequency. Because the hosts are nearly always irradiated, and therefore have considerable tissue damage and tissue regeneration all over the body, it is thought that this situation allows favorable circumstances for the occasional reprogramming event. It is not, however, likely that reprogramming can occur on a large scale, or that the bone marrow is a repository for cells that can regenerate the rest of the body.

Key Points to Remember

- The organs of the body are mostly composed of several tissue layers. Each tissue layer contains multiple cell types.
- Tissues may be classified as postmitotic, quiescent but capable of growth, and renewal. In renewal tissues there is a balance of cell production and cell death.
- Stem cells are cells that can both renew themselves and produce offspring destined to differentiate. They are normally found in "niches" defined by signals from surrounding cells.
- The epidermis is a stratified squamous epithelium. It has proliferative cells only in the basal layer, and the successive layers represent degrees of postmitotic cell maturation. The stem cells are found in integrin-rich clusters.
- Hair follicles contain stem cells in the bulge of the outer root sheath that can populate the entire epidermis and its specializations.
- The small intestine has its proliferative zone in the lower part of the crypts. The stem cells are found just above the Paneth cells at the crypt base. Four types of differentiated cells are produced which move continuously to the upper crypt and villi.
- The bone marrow contains hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). The HSCs continuously renew the cells of the blood and immune system. Their niche is in association with osteocytes on the trabecular bone of the marrow cavity.

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