CHAPTER 5

# The Development of Epithelial Stem Cell Concepts

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## 5.1 INTRODUCTION

Till and McCulloch's groundbreaking work identified and studied the cells that were capable of repopulating hematopoietic tissues, depleting the replacing tissue of cells by exposure to a cytotoxic agent – radiation. Once the endogenous hematopoietic precursors were eliminated by irradiation, the mice were injected with bone-marrow-derived precursors obtained from another animal. The exogenous cells were subject to a variety of treatments prior to transplant. It was discovered that the hematopoietic precursors first circulated in the host, then engrafted into various hematopoietic tissues, including the spleen. Those cells that engrafted into the spleen and possessed extensive regenerative and differentiative potential grew by a process of clonal expansion to form macroscopically visible nodules of hematopoietic tissue 10 to 14 days after transplant. Genetic or chromosome tracking (marking) demonstrated that these nodules were derived from single cells (i.e., they were clones) and that further clonogenic cells were produced within the clones. The colonies were referred to as spleen colonies, and the cells that form the colonies were called colony-forming units (CFUs).

These experiments provided the theoretical basis for subsequent human bone marrow transplant studies. Through a variety of pre-irradiation manipulations and pre- and post-transplantation variables, this technique led to our current understanding of the bone marrow hierarchies or cell lineages and their stem cells. These studies showed that the bone marrow contained undifferentiated self-maintaining precursor cells that generated a variety of cell types representing a range of differentiated lineages. Subsequent studies have suggested that these CFUs are not the ultimate hematopoietic stem cells, but are part of a stem cell hierarchy in the bone marrow.



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Clonal regeneration approaches have subsequently been developed for a variety of other tissues, notably for the epidermis, intestine, kidney, and testis. These studies implicated hierarchical organizations within the proliferative compartments of many tissues. The stringency of the criteria used for defining a clone varied enormously, depending on the number of cell divisions required to produce detectable clones. For epidermis and intestine, the stringency was high since the clones could be large and macroscopic, appearing similar to spleen colony nodules.

One difficulty with interpreting and applying the results of clonal regeneration studies of bone marrow and other tissues was the need to disturb the host tissue to detect regenerating clones, usually by exposing the host tissue to irradiation. Such a disturbance may alter the cellular hierarchies that one wishes to study, and will almost certainly alter the nature (e.g., cell cycle status, responsiveness to signals, susceptibility to subsequent treatment) of the stem cell compartment. This has been referred to as the biological equivalent of the Heisenberg uncertainty principle defined in quantum physics. However, clonal regeneration assays still provide valuable and sometimes unique opportunities to study some aspects of stem cell biology *in vivo*, such as stem cell survival and functional competence under a variety of conditions.

## 5.2 A DEFINITION OF STEM CELLS

Relatively few attempts have been made to standardize the definition of the term stem cells, which has resulted in some confusion in the literature. A variety of terms are seen and the relationship between them is often obscure. Terms include precursors, progenitors, founder cells, and so on. The concept of what constitutes a stem cell is further complicated by the addition of modifiers (e.g., committed precursors or progenitors) and the sometimes confusing use of the term differentiation. One inherent difficulty in standardizing the definition of a stem cell is that it is often dependent on the perspective of the viewer, so different criteria are brought into the use of a given term by embryologists, hematologists, dermatologists, gastroenterologists, and other specialists.

In a 1990 paper published in *Development*, we attempted to define a stem cell. This definition was, admittedly, formulated within the context of the gastrointestinal epithelium, but we felt it had a broader application. The definition still largely holds and can be summarized as follows. Within adult replacing tissues of the body, the stem cells can be defined as a small subpopulation of the proliferating compartment, consisting of relatively undifferentiated proliferative cells that maintain their population size when

they divide, while at the same time producing progeny that enter a dividing transit population within which further rounds of cell division occur, together with differentiation events, resulting in the production of the various differentiated functional cells required of the tissue. The stem cells persist throughout the animal's lifetime in the tissue, dividing a large number of times; as a probable consequence of this large division potential, these cells are the most efficient repopulators of the tissue following injury. If this repopulation requires a re-establishment of the full stem cell compartment, the self-maintenance probability of the stem cells at division will be raised from the steady state value of 0.5 to a value between 0.5 and 1, which enables the stem cell population to be re-established, while at the same time maintaining the production of differentiated cells to ensure the functional integrity of the tissue.

The consequences of this definition are obvious, namely, that stem cells are:

- Rare cells in the tissue, vastly outnumbered by the dividing transit population, and are the cells upon which the entire lineage and ultimately the tissue are dependent;
- The only permanent long-term residents of the tissue;
- Cells at the origin of any cell lineages or migratory pathways that can be identified in the tissue.

The concept of differentiation enters into the definition of stem cells, and this, too, often leads to confusion. In our view, differentiation is a qualitative and relative phenomenon. Cells tend to be differentiated relative to other cells, and hence adult tissue stem cells may, or may not, be differentiated relative to embryonic stem cells (a point of current debate, bearing in mind the controversy in the literature concerning bone marrow stem cell plasticity). Stem cells produce progeny that may differentiate down a variety of pathways, leading to the concept of totipotency and pluripotency of stem cells in terms of their differentiation potential. Potency is actually a strange concept to apply to a stem cell, since it is the progeny that differentiate and not the stem cell itself. The fact that the progeny can differentiate down more than one lineage, as is very obviously the case in the bone marrow, results in bone marrow stem cells being referred to as pluripotent, and the initial dividing transit cells that initiate a lineage ultimately leading to specific differentiated cells are referred to as committed precursors for that lineage.

Some of the instructive signals for differentiation in the hematopoietic cell lineage are now well understood, but such signals for other tissues organized on a cell lineage basis have yet to be determined. There is much debate in the literature concerning the extent to which stem cells may be instructed to produce progeny of specific differentiated types, and whether this is limited

or unlimited. This topic is referred to as the degree of plasticity for stem cells. There are two very distinct issues here:

- The first is whether a stem cell, such as a bone marrow stem cell, is ever instructed by its environment in nature, or in laboratory or clinical situations, to make an apparently unrelated tissue cell type such as a liver, intestinal, or skin cell, and whether it can regenerate these tissues if they are injured. A subsidiary question is not whether this ever happens normally in nature, but whether we, as experimentalists or clinicians, can provide the necessary instructions or environment for this to happen in a controlled situation.
- The second issue relates not only to the stem cells, but also to the early progeny of stem cells from, for example, the bone marrow, and whether these cells, which circulate around the body, might end up in a distant tissue and ultimately express differentiation markers unrelated to the bone marrow cell lineages, but specific to the tissue in which the cell ultimately resides.

The former issue is one of the plasticity of the bone marrow stem cells, and the latter may be more an issue of the plasticity of the bone-marrow-derived cell lineages. If a bone marrow stem cell could ever be instructed to be a gastrointestinal stem cell, it should be capable of undertaking all the functional duties of a gastrointestinal stem cell, including the regeneration of the gastrointestinal epithelium if it is subsequently injured. The cloning of animals by nuclear transfer technology into egg cytoplasm clearly demonstrates that all nuclei of the body contain a full complement of DNA, and that under the right environmental conditions the way that the DNA is expressed can be reprogrammed (or unmasked) by environmental signals to make all the tissues of the body. It should be remembered, however, that such cloning outcomes, such as the one that resulted in Dolly the sheep, are rare and inefficiently produced events. They do, however, clearly indicate the enormous potential that can be achieved if the necessary instructive reprogramming signals are provided. These rare events fuel the belief that, in the future, reproducible instruction can be administered to any adult tissue stem cell and yield any tissue of the body. If and when this becomes the case, the distinction between embryonic stem cells and adult tissue stem cells may disappear.

## 5.3 HIERARCHICALLY ORGANIZED STEM CELL POPULATIONS

The issue here is what determines the difference between a dividing transit cell and a stem cell, and whether that transition is an abrupt or a gradual one. One can think of this transition as being a differentiation event that

distinguishes a dividing transit cell from a stem cell. This is an old argument. Do differentiation signals act on pre-existing stem cells, removing on average half the cells produced by previous symmetric divisions, or do the stem cells divide asymmetrically, to produce a differentiated progeny at division and a stem cell? One possibility is that this distinction is made at the time that a stem cell divides. Indeed, do they need to divide to differentiate? In this case, such divisions must be regarded as asymmetric, with the dividing stem cell producing one stem cell (i.e., for self-maintenance) and one dividing transit cell. This type of asymmetric division may occur in some tissues, such as the epidermis. If this is the case, however, the stem cell must also retain the potential to alter its self-maintenance probability, which for an asymmetric division is 0.5 in steady state, and adopt a value somewhat higher than this if stem cells are killed and require to be repopulated.

The current view regarding the bone marrow stem cells is that the transition between a stem cell and a dividing transit cell is a gradual one that occurs over a series of divisions within a cell lineage, which inevitably implies that one has a population of stem cells with a varying degree of stemness or, conversely, a varying degree of differentiation. For the bone marrow, one issue is whether experimentalists have ever identified the presence of the truly ancestral ultimate bone marrow stem cell. The difficulty here may be one of identifying and extracting such cells, the location of which is probably in the bone where they will be present in increasingly diminishing numbers, as one looks for the increasingly primitive cells.

We have attempted to accommodate as much experimental data as possible into a working model for gastrointestinal cellular organization. Our current working model is that the commitment to differentiation, which produces the dividing transit cells, does not occur at the level of the ultimate stem cell in the lineage, but at a position two or three generations along the cell lineage. If such a concept is drawn as a cell lineage diagram, the proliferative units in the intestine, the crypts, each contain four to six cell lineages and, hence, four to six lineage ancestor stem cells, but up to 30 second- and third-tier stem cells, which under steady state circumstances are inevitably displaced and moved toward the dividing transit compartment. But, if damage occurs in one or more of the ultimate stem cells, they can assume the mantle of the ultimate stem cell and repopulate the lineage. This gives rise to the concept of actual and potential stem cells (see Figure 5.1), which is discussed later in this chapter.

An analogy can be drawn here with the hierarchical personnel structure within an organization such as the army, a concept that was discussed at the time we were formulating the text for the 1990 paper in which we defined stem cells. In a military battlefield environment, the hierarchically organized

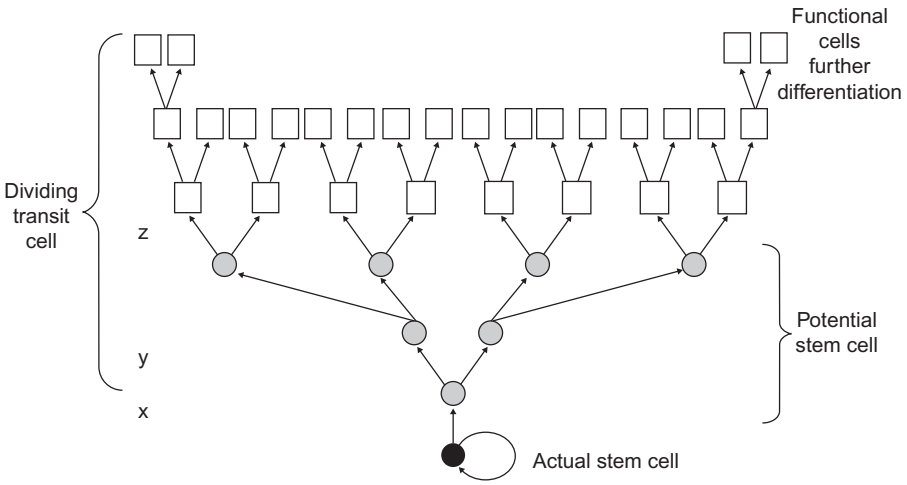


**FIGURE 5.1** **A typical stem-cell-derived cell lineage that may be applicable to most epithelial tissues of the body.**

The lineage is characterized by a self-maintaining lineage ancestor actual stem cell (black) which divides and produces a progeny that enters a dividing transit population. The number of cell generations in the dividing transit population varies from tissue to tissue. The commitment to differentiation that separates the stem cell from the dividing transit population can occur at the point of the actual stem cell division (*x*), in which case the stem cells are dividing asymmetrically on average. This commitment may be delayed to point (*y*) or (*z*), generating a population of potential stem cells that can replace the actual stem cell if it is killed. Under normal steady state circumstances, the potential stem cells form part of the dividing transit population and are gradually displaced down the lineage, undergoing further differentiation events if required to produce the functional mature cells of the tissue.

army is under the control and ultimately dependent upon the highly trained (or so one hopes) general. In the event that the general is killed on the battlefield, there may be a reasonably well-trained captain who can take over command and assume the insignia and uniform, as well as the function of the general. In the event that the captain, too, should be killed, there may be less well-trained officers who will attempt to assume the mantle of command. Ultimately, the vast majority of the troops, the privates, would be insufficiently trained or experienced to be able to adopt the functional role of the commander. However, the Dolly the sheep scenario suggests that occasionally a private, given a crash course in military strategy, might function as the officer-in-command. The analogy could be taken even further to relate to the apoptosis sensitivity that is seen in the gastrointestinal ultimate stem cells. These cells appear to adopt a strategy with such complete intolerance to any genetic damage and a reluctance to undertake repair, since this may be associated with inherent genetic risk, that they commit an altruistic suicide – the

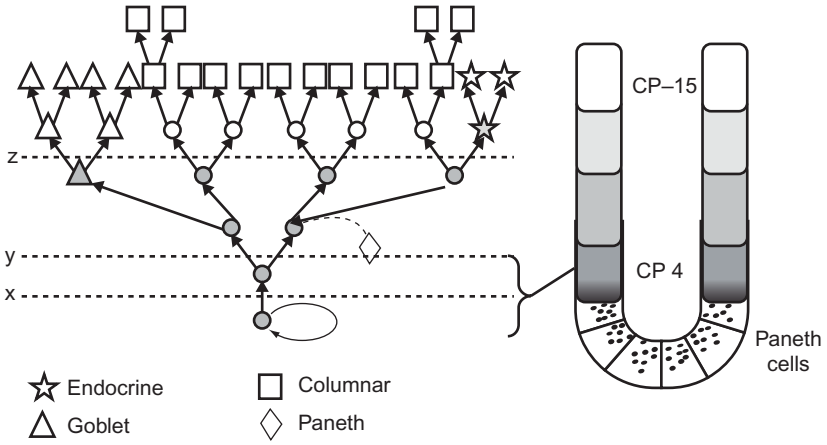


**FIGURE 5.2** **The cell lineage for the small-intestinal crypts.**
It is postulated that each crypt contains four to six such lineages and, hence, four to six lineage ancestor actual stem cells, and there are about six cell generations in each lineage with at least four distinct differentiated cell types being produced. The attractive feature of this cell biological model system is that the position of a cell in a lineage can be related to its topographical position in a longitudinal section through the crypt, as shown on the right.

general who undergoes a nervous breakdown or serious injury and has to be removed from command.

In the small-intestinal crypts, no useful markers yet exist that permit the stem cells to be identified and, hence, studied; such markers are only now being identified. However, even in the absence of markers, the small intestine has proven to be an invaluable biological model system to study stem cells, because the cells of the intestinal cell lineage are arranged spatially along the long axis of the crypt. This spatial localization can be confirmed by cell migration tracking and mutational marker studies. As a consequence, the stem cells are known to be located at very specific positions in the tissue (crypts): at the fourth or fifth cell position from the crypt base in the small intestine and at the very base of the crypt in the mid-colon of the large intestine (see Figure 5.2).

## 5.4 SKIN STEM CELLS

The first suggestion that the proliferative compartment of the epidermis, the basal layer, was heterogeneous and contained only a small subpopulation of stem cells came with the development of the skin macrocolony clonal regeneration assay developed by Withers. This was soon combined with other cell kinetic and tissue organization data to formulate the concept of the

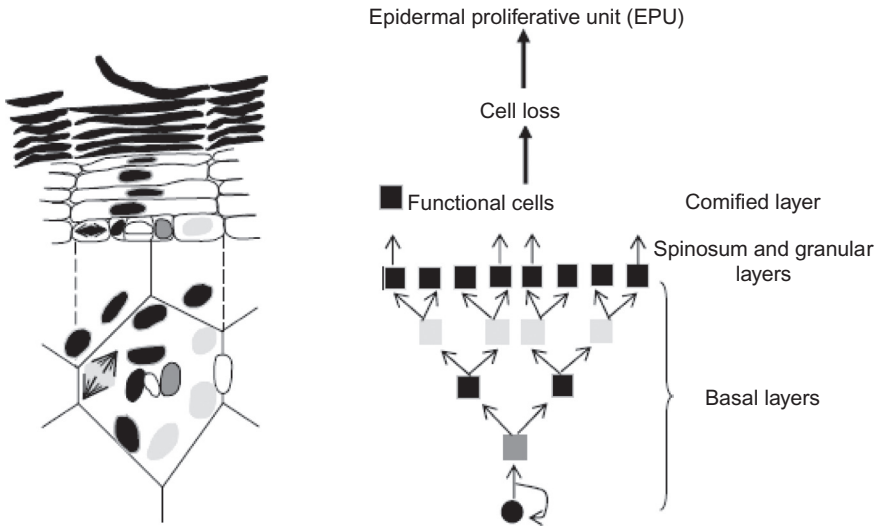


**FIGURE 5.3**

Diagrammatic representation of the cell lineage seen in the interfollicular epidermis and the relationship between the cell lineage and the spatial organization characterized as the epidermal proliferative unit (EPU), as seen in section view (upper portion of the figure on the left) and in surface view in epidermal sheets (lower portion of the figure on the left).

epidermal proliferative unit (EPU) (see Figure 5.3). This suggested that the basal layer consisted of a series of small, functionally and cell lineage-related cells, with a spatial organization that related directly to the superficial functional cells of the epidermis, the stratum corneum. The concept viewed the epidermis as being a series of functional proliferative units. Each EPU had a centrally placed self-maintaining stem cell and a short stem-cell-derived cell lineage (with three generations). The differentiated cells produced at the end of the lineage migrated out of the basal layer into the suprabasal layers in an ordered fashion, where further maturation events occurred, eventually producing the thin, flattened, cornified cells at the skin surface that were stacked into columns (like a pile of plates), with cell loss occurring at a constant rate from the surface of the column (Figure 5.3).

Such an organization is clearly evident in the body skin epidermis of the mouse, its ears, and a modified version of the EPU can be clearly identified in the dorsal surface of the tongue. Debate as to whether this concept applies to human epidermis still occurs. The human body contains many sites where a similar columnar organization can be seen in the superficial corneal layers of the epidermis. What is more difficult in humans is to correlate this superficial structure with a spatial organization in the basal layer. However, the spatial

organization seen in the superficial layers must have an organizing system at a level lower in the epidermis, and it is reasonable to assume that this system is in the basal layer, as is the case for mouse epidermis.

Two techniques used to study the basal organization of epidermis observe clonal regeneration at a macroscopic and a microscopic level; the resulting nodules are very similar in appearance to spleen colonies. The microscopic assay requires less time between irradiation and tissue sampling, but both techniques are fairly labor intensive and have not been used extensively. Together, studies using these two clonal regeneration assays yielded data indicating that only about 10% (or less) of the basal cells have a regenerative capacity (i.e., are stem cells).

The EPU stem cells must have an asymmetric division mode under steady state cell kinetics, because there is only one such cell per EPU. The microscopic epidermal clonal regeneration assay suggests that following injury, such as irradiation, surviving EPU stem cells can change their division mode from asymmetric to symmetric for a period of time to repopulate the epidermis (i.e., change their self-maintenance probability from 0.5 to a value higher than 0.5). These studies also indicated that a significant contribution to re-epithelialization could come from the upper regions of the hair follicles. Studies on the epidermal structure following injury yielded clear data that the epidermis undergoes a reorganization involving hyperplasia to re-establish the spatial distribution of stem cells, in which stem cells are redistributed and eventually re-established into EPU spatial configurations.

The skin contains another important stem cell population associated with hair follicles. Hair is produced over a protracted period of time by rapid divisions in the germinal region of the hair follicle (termed an 'anagen follicle'). Hair growth can continue for fairly sustained periods of time – three weeks in a mouse, months to years in humans, and more indefinite periods for some animal species such as Angora rabbits and Moreno sheep. The germinal matrix of a hair follicle, has considerable spatial polarity much like the intestinal crypt; therefore, it is presumed to have a fixed stem cell population residing in the lowest regions of the germinal matrix that maintains cell division during active hair growth. Very little is known about these stem cells. One complication with hair follicles is that, in mouse and human, hair follicles eventually produce a mature hair, and cell proliferation ceases. The follicle shrinks and becomes quiescent (a telogen follicle). The simplest explanation here is that the telogen follicle, which consists of far fewer total cells than an anagen follicle, contains a few quiescent hair follicle stem cells that can be triggered back into proliferation at the onset of a new hair growth cycle. However, as discussed below, there is some controversy concerning this concept.

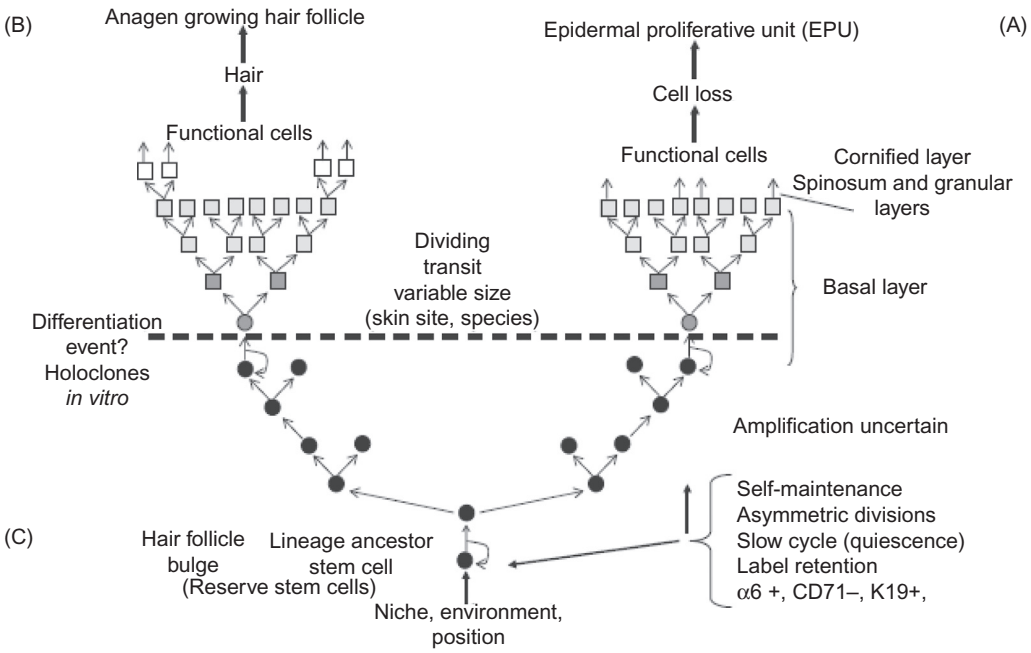


**FIGURE 5.4** **The complexity of the stem cell populations in mammalian skin as characterized in the mouse.**

A distinct cell lineage is proposed (A) for the interfollicular epidermis (EPU); (B) another for the matrix region of the growing hair follicle (anagen follicle); and (C) a potent reserve regenerative stem cell compartment which resides in the upper/outer root sheath or bulge region of the hair follicle. The stem cells in the bulge region can regenerate the epidermis, the hair follicle, and probably other structures such as the sebaceous glands.

It is now very clear that the skin contains a third stem cell compartment located in the upper outer sheath of the hair follicle below the sebaceous glands. This compartment is sometimes visible as a small bulge in the outer root sheath, and so these cells are often called 'bulge cells.' A whole series of extremely elegant, but complicated, experiments have shown that these bulge cells possess the ability, under specialized conditions, to reform the hair follicle if it is damaged and also to contribute to the re-epithelialization of the epidermis. Cells from this region of the follicle were probably responsible for the epidermal re-epithelialization from follicles seen by Al-Barwari. Cells from the bulge can make follicles during development of the skin and also re-establish follicles if they are injured.

The controversy concerns the issue of whether bulge stem cells, which are predominantly quiescent cells, ever contribute to the re-establishment of an anagen follicle under normal undamaged situations. The simplest interpretation is that bulge cells are not normally required to re-establish anagen

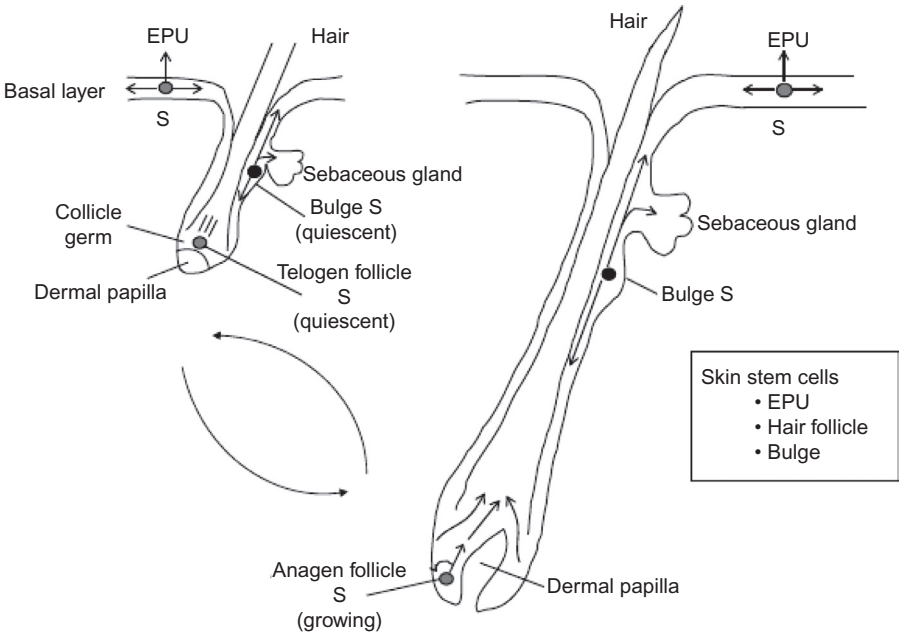


**FIGURE 5.5**
**Diagrammatic representation of a growing anagen hair follicle and a resting or quiescent telogen follicle.** The diagram shows the spatial distribution for the stem cell compartments shown in Figure 5.4.

follicles because such an event would involve activating some very complex cell division and cell migratory pathways, which goes somewhat against the concept of stem cells being fixed or anchored, and also against the concept of keratinizing epithelia being a tightly bound, strong and impervious barrier. What seems likely for the skin is that the EPU stem cell and the hair follicle stem cell have a common origin during the development of the skin from the bulge stem cells, which then become quiescent and are present as a versatile reserve stem cell population that can be called into action if the skin is injured and requires re-epithelialization (see Figures 5.4 and 5.5).

## 5.5 THE INTESTINAL STEM CELL SYSTEM

The intestinal epithelium, like all epithelia, is highly polarized and divided into discrete units of proliferation and differentiation. In the small intestine, the differentiated units are the finger-like villi protruding into the lumen of the intestine. These structures are covered by a simple columnar epithelium consisting of several thousand cells, which perform their specific function, become worn out, and are shed predominantly from the tip of the villus. There is no proliferation anywhere on the villus. The cell loss from the villus tip is precisely balanced in steady state by cell proliferation from the base of the villi – the crypts.

Each villus is served by about six crypts, and each crypt can produce cells that migrate onto more than one villus. The crypts in the mouse contain about 250 cells in total, 150 of which are proliferating rapidly and have an average cell cycle time of 12 hours. The cells move from the mouth of the crypt at a velocity of about one cell diameter per hour, and all this movement can be traced in the small intestine, back to a cell position about four cell diameters from the base of the crypt. The very base of the crypt, in mice and humans, is occupied by a small population of functional differentiated cells, called Paneth cells. Cell migration tracking and innumerable cell kinetic experiments suggest that the stem cells from which all this cell movement originates are located at the fourth position from the base of the crypt in the small intestine, and right at the base of the crypt in some regions of the large bowel.

The crypt is a flask-shaped structure with about 16 cells in the circumferential dimensions. Mathematical modeling suggests that each crypt contains about five cell lineages and, hence, five cell lineage ancestor stem cells. Under steady state kinetics, these cells are responsible for all the cell production, producing daughters that enter a dividing transit lineage of between six and eight generations in the small and large bowel, respectively (see Figures 5.1 and 5.2). The stem cells in the small intestine divide with a cycle time of approximately 24 hours and, hence, in the lifetime of a laboratory mouse divide about 1,000 times. It is assumed that these cells are anchored or fixed in a micro-environmental niche that helps determine their function and behavior. The uniquely attractive feature of this model system, from a cell biological point of view, is that, in the absence of stem cell specific markers, the behavior and characteristics and response to treatment of these crucial lineage ancestor cells can be studied by studying the behavior of cells at the fourth position from the bottom of the crypt in the small intestine. When this is done, one of the features that seem to characterize a small population of cells at this position (about five cells) is that they express an exquisite sensitivity to genotoxic damage, such as is delivered by small doses of radiation. They appear to tolerate no DNA damage and activate a p53-dependent altruistic suicide (apoptosis). It is believed that this is part of the genome protection mechanisms that operate in the small intestine, and accounts for the very low incidence of cancer in this large mass of rapidly proliferating tissue.

Macroscopic clonal regeneration techniques have been used extensively to study the intestinal crypts and suggest the presence of a second compartment of clonogenic or potential stem cells (about 30 per crypt) that possess a higher resistance to radiation and a good ability to repair DNA damage. These observations, together with others, suggest a stem cell hierarchy of the

sort illustrated in Figures 5.1 and 5.2, with the commitment to differentiation that distinguishes dividing transit cells from stem cells occurring about three generations along the lineage. Virtually identical lineage structures can be inferred for the colonic crypts.

There has been an absence of stem cell specific markers in the past, but some may now be available. Antibodies to Musashi-1, an RNA binding protein identified as playing a role in asymmetric division control in neural stem cells, appears to be expressed in very early lineage cells in the small intestine (see Figure 5.6).

Studies have indicated that the ultimate stem cells in the crypt possess the ability to selectively segregate old and new strands of DNA at division and retain the old template strands in the daughter cell destined to remain a stem cell. The newly synthesized strands which may contain replication-induced errors are passed to the daughter cell destined to enter the dividing transit population and to be shed from the tip of the villus five to seven days after birth from division. Cairns developed the concept of selective DNA segregation in 1975. Selective DNA segregation provides a second level of genome protection for the stem cells in the small intestine, and providing further support for the low cancer incidence in this tissue (see Box 5.1). When template strands are labeled with DNA synthesis markers at times of stem cell expansion (i.e., during late tissue development and during tissue regeneration after injury), the label persists and provides a truly specific marker for lineage ancestor cells (see Figure 5.6). Figure 5.6 also illustrates some other ways in which intestinal stem cells may be distinguished from their rapidly dividing progeny.

## 5.6 STEM CELL ORGANIZATION ON THE TONGUE

Oral mucosae are keratinizing, stratified epithelia, similar to skin epidermis in their structural organization. The dorsal surface of the tongue is composed of many small, filiform papillae that have a very uniform shape and size. Detailed histological investigations, together with cell kinetic studies performed by Hume, showed that each papilla is composed of four columns of cells: two dominant and two buttressing columns. The dominant anterior and posterior columns represent modified versions of the EPU and are called tongue proliferative units. Cell migratory pathways in the tongue were mapped using techniques that were applied to intestinal crypts and identified the position from which all migration originated, (i.e., the presumed location of the stem cell compartment). The lineage characterizing tongue epithelium is similar to that of the dorsal epidermis of the mouse – that is, self-replacing, asymmetrically dividing stem cells, occurring at a specific position in the

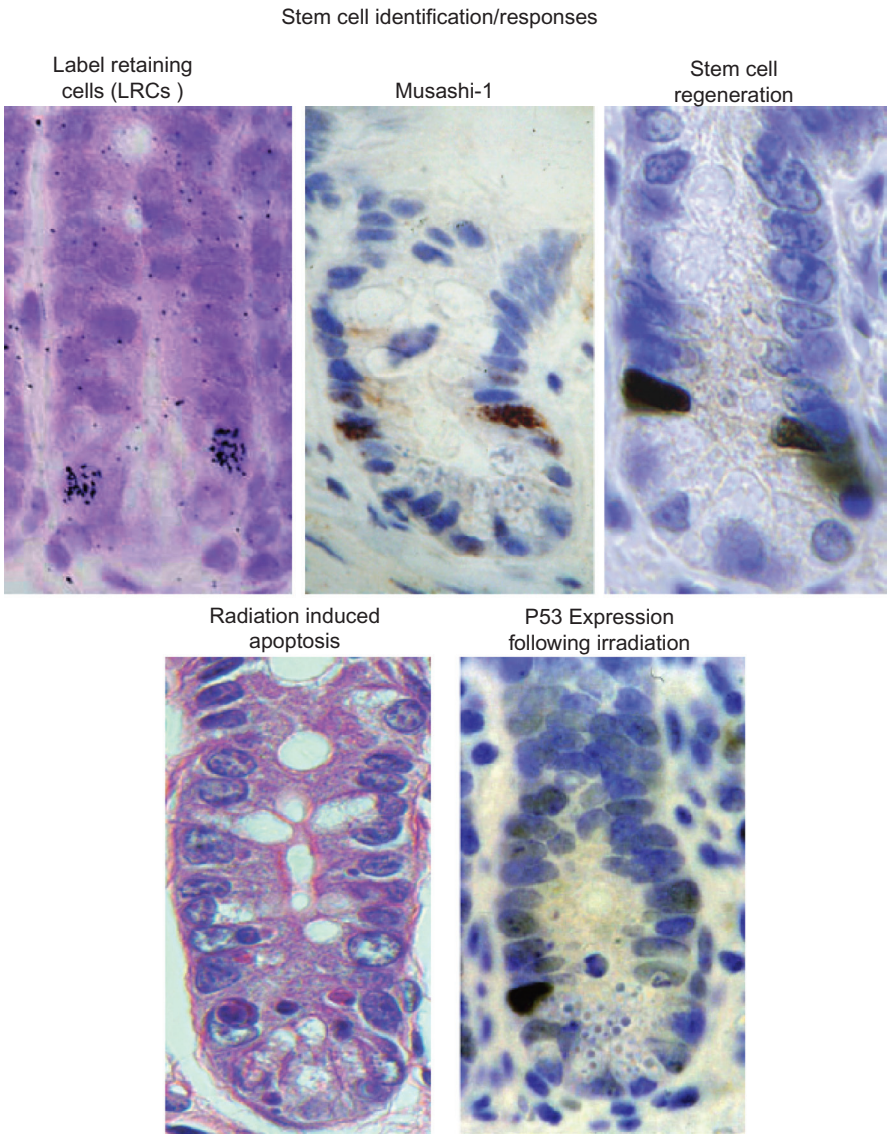


**FIGURE 5.6 Photomicrographs of longitudinal sections of the small-intestinal crypts from the mouse illustrating a range of possible ways of identifying the stem cell compartment.** Making use of the selective strand, segregation hypothesis template strands of DNA can be labeled, generating label-retaining cells at the fourth position from the bottom of crypts. Musashi-1, an RNA binding protein, is expressed in early lineage cells and under some labeling conditions can show specificity for individual cells at around cell position 4. Part of the regenerative or potential stem cell compartment can be seen by S-phase labeling (bromodeoyuridine labeling) at critical phases following cytotoxic injury when these cells are called into regenerative mode. The example shown here is a labeling pattern at 24 hours after two doses of 5-fluorouracil when the only cells in S-phase are a few cells scattered around the

## BOX 5.1 WHY DON'T SMALL-INTESTINAL STEM CELLS DEVELOP MORE CANCERS?

When one considers that, relative to the large intestine, the small intestine has:

- 3–4 times greater mass (length)
- 1.5 times more rapid proliferation
- 2–3 times more total stem cells
- 3–4 times more stem cell divisions in a lifetime

it is quite remarkable that cancers of the small intestine occur 70 times less frequently.

tissue and producing a cell lineage that has approximately three generations (Figure 5.7). The stem cells in tongue have a particularly pronounced circadian rhythm.

## 5.7 GENERALIZED SCHEME

For the major replacing tissues of the body, hierarchical or cell lineage schemes appear to explain the cell replacement processes. These schemes may involve isolated, single stem cells that under steady state circumstances must be presumed to divide asymmetrically, producing a dividing transit population. The size of the dividing transit population differs dramatically from tissue to tissue, the number of generations defining the degree of amplification that the transit population provides for each stem cell division. This amplification is inversely proportional to the frequency that stem cells will be found within the proliferating compartment (see Figure 5.8).

For some systems, such as the bone marrow and the intestine, the commitment to differentiation that separates the dividing transit compartment from the stem cell compartment appears to be delayed until a few generations along the lineage. This generates a stem cell hierarchy with cells of changing (decreasing) stemness or, conversely, increasing commitment, leading to the concept of committed precursor cells. In the small intestine, this delay in the

◀ fourth position from the base of the crypt. As part of the genome protective mechanism, it is postulated that the ultimate lineage ancestor stem cells have an exquisite sensitivity to radiation and the induction of genome damage. When this happens, the cells commit suicide via apoptosis, which can be easily recognized and occurs at about the fourth position from the base of the crypt. These cells do not express p53 protein, at least at the times studied and as detectable by immunohistochemistry. However, some cells do express p53 protein at high levels following radiation exposure, and it is postulated that these are the surviving potential stem cells in cell cycle arrest to allow for repair prior to entering rapid regenerative cell cycles. Under appropriate immunohistochemical preparative procedures, individual wild-type p53 protein expressing cells can be seen at around cell position 4.

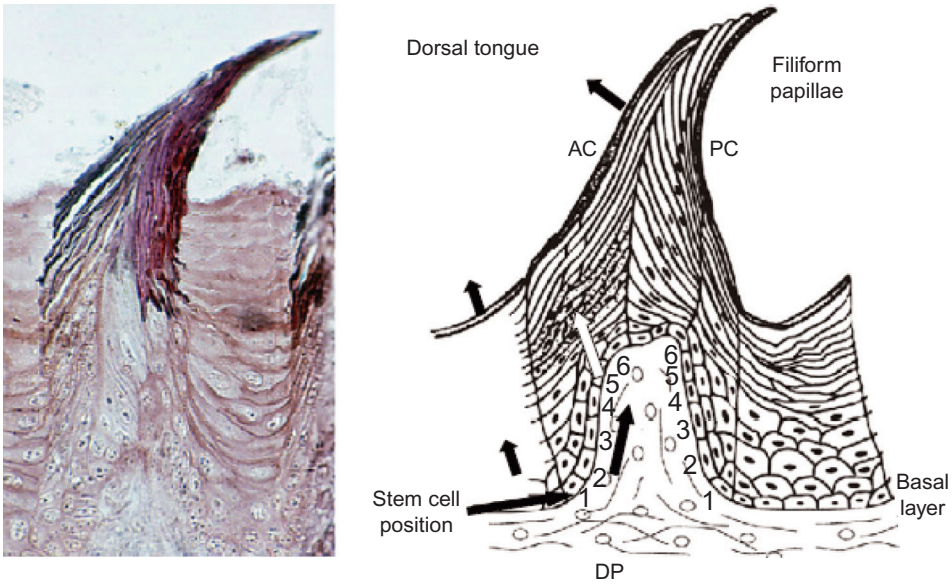


**FIGURE 5.7**

A histological section through the dorsal surface of the tongue (left panel) and a diagrammatic representation of this tissue showing the tongue proliferative units (the dominant anterior column AC, and posterior column PC). Cell migratory pathways have been identified based on cell positional analyses and cell marking and the location of the stem cells identified in the basal layer. The stem cells in this tissue express one of the strongest circadian rhythms in proliferation seen anywhere in the body.

commitment to differentiation to a dividing transit population provides the tissue with a reserve population of potential stem cells that can repopulate the tissue if the lineage ancestor cells are destroyed; providing an added level of tissue protection in this extremely well-protected tissue.

With regard to the bone marrow, committed precursors or even earlier cells appear to circulate in the blood and may lodge in various tissues. Given appropriate microenvironments and local signals, some of these lodged cells may be instructed to differentiate down unusual pathways. This has prompted research into using such cells to repopulate the liver of patients with specific gene defects that result in life-threatening, hepatic metabolic deficiencies.

Although the transdifferentiation theory is attractive, research indicates that the apparent plasticity of stem cells may be less clear-cut. Transplantation experiments in mice with specific gene disorders suggest that transplanted bone marrow cells may 'fuse' with liver cells, and hence complement any gene deficiency in the hepatocytes. These hybrid cells will be viable and undergo clonal expansion. Experimental findings do, indeed, show that

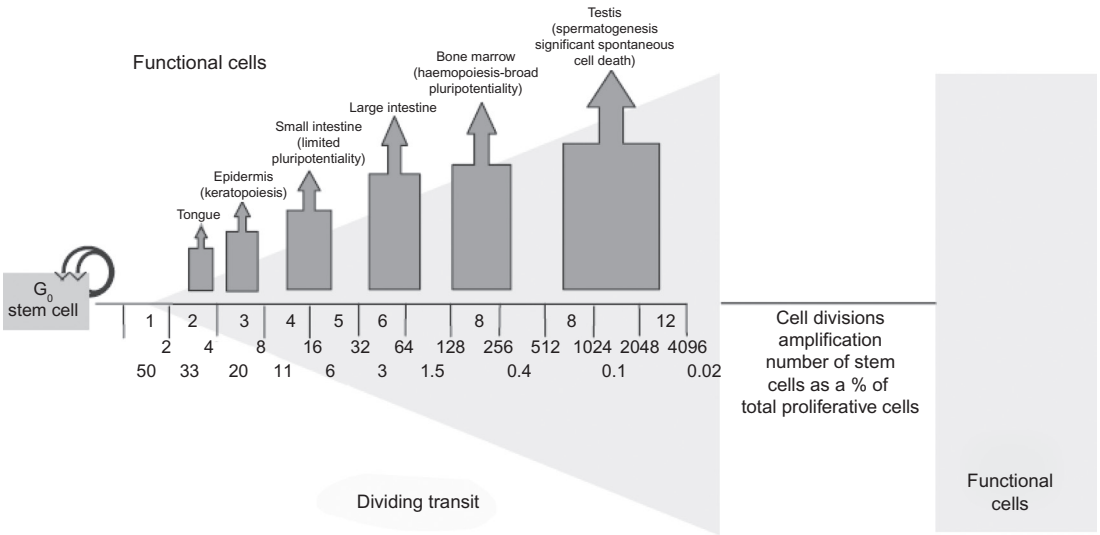


**FIGURE 5.8** **A diagrammatic representation of a stem-cell-derived cell lineage showing the approximate positions for the number of cell generations in the dividing transit population for a range of murine tissues.**
Stratified keratinizing epithelia such as the tongue and epidermis tend to have the shortest lineages, and the bone marrow and the testis tend to have the longest lineages. Also shown is the degree of theoretical amplification that the dividing transit lineage provides for each stem cell division and the inverse relationship between the degree of amplification and the proportion of the proliferative compartment that the stem cells occupy.

cells forming functional liver tissue in the gene-deficient animals have specific genetic markers for both the donor and the host animal. Our concepts of stem cells clearly require further development and refinement.

## 5.8 SUMMARY

Stem cell concepts have evolved dramatically over the last few years, culminating in a rapid expansion of interest in both embryonic and adult tissue stem cells. This chapter explores the evolution of stem cell concepts as applied to adult epithelial tissues. These tissues are characterized by a high degree of polarization and very distinct cell maturation and migration pathways, which permit the identification of specific locations in the tissues which represent the origins of all this cell movement. Cells at the origin of the migratory pathways must represent the cells on which the tissue is ultimately dependent, and the cells that have a long-term (permanent) residence in the tissue, i.e., the stem cells. A variety of cell kinetic studies, together with lineage tracking experiments, have indicated that, in the intestine, the dorsal surface of the tongue, and the interfollicular epidermis, the proliferative compartment of the tissue is divided into discrete units of proliferation, each with

its own stem cell compartment. In the skin, the evolving stem cell studies suggest at least three distinct stem cell populations providing a source of cells for the epidermis, for the growing hair follicle, and a reserve regenerative, highly potent population in the upper follicle region. In the small intestine there are indications that the stem cell compartment itself is hierarchical, with a commitment to differentiation occurring two to three generations down the lineage, resulting in a population of actual stem cells that perform their function in steady state, and a population of potential stem cells that can be called into action if the actual stem cells are killed. Whereas previously, there were no reliable markers for adult intestinal stem cells, new findings have yielded potential markers for identifying these cells. Cancer is rare in the small-intestinal epithelium, which is surprising since this tissue represents a large mass with many stem cells dividing many times. This suggests that effective genome protective mechanisms have evolved, and some aspects of these mechanisms have now been identified.

## FOR FURTHER STUDY

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