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What is a Stem Cell?

In the popular media and even in some medical circles, stem cells are presented as miracle cells that can do anything. When administered to a patient with some serious disease they will rebuild the damaged tissues and make the patient young again. Alas, in reality there are no such cells. However, there are cells that exhibit stem cell behavior and the future of regenerative medicine will undoubtedly be built on a good scientific understanding of their properties. In this chapter these properties are briefly outlined, and in the remainder of the book each of them will be underpinned by an explanation of the relevant areas of science and technology.

A list of characteristics of stem cell behavior that is generally agreed upon is the following:

- Stem cells reproduce themselves.
- Stem cells generate progeny destined to differentiate into functional cell types.
- Stem cells persist for a long time.
- Stem cell behavior is regulated by the immediate environment (the niche).

This is shown diagrammatically in Figure 1.1. The first two items on the list indicate the key abilities of self-renewal and of generation of differentiated progeny. As will be explained below, these abilities may be shown at a cell population level rather than by every single stem cell at every one of its divisions. Also, the second item indicates "destined to differentiate" meaning that cell division may continue for a while before differentiation, but not indefinitely. Cells derived from stem cells that proliferate for a limited number of cycles are called progenitor cells or transit amplifying cells. The third item on the list means that if the stem cell population is one of those that exists in tissue culture then it should be capable of indefinite growth, while if it is part of an organism it should be very long lasting, normally persisting for the whole life of the organism. The fourth characteristic indicates that all stem cells exist in a specific microenvironment that controls their program of division and differentiation. This may seem at first sight only to apply to stem cells within the body and not to those grown in vitro, but in order to get them to grow, the cells in vitro are always provided with specialized medium ingredients that, in effect, mimic the components normally provided in the niche.

This fourfold definition involves not just intrinsic properties of stem cells, but also properties that depend on aspects of their environment such as the lifespan of the animal, the nature of the niche, or the composition of the culture medium. This emphasizes the fact that the goal of stem cell biology is understanding the *behavior* and not just the intrinsic nature of stem cells. To achieve this, the characteristics of the stem cell environment are just as important as the properties of the stem cells themselves. Moreover, understanding stem cell behavior means understanding various aspects of cell and developmental biology which are not always familiar to workers in stem cell laboratories.

The above definition is of value in indicating the special characteristics of stem cell

The Science of Stem Cells, First Edition. Jonathan M. W. Slack.

Companion website: www.wiley.com/go/slack/thescienceofstemcells

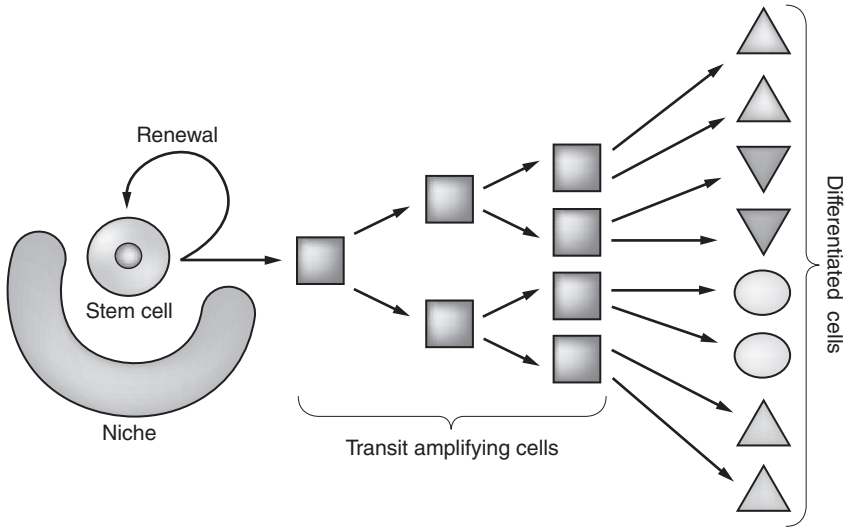

Figure 1.1 A consensus diagram showing stem cell behavior. (Modified from Slack, J.M.W. (2013). Essential Developmental Biology, 3rd edn. Reproduced with the permission of John Wiley and Sons.)

behavior, but is also helpful in indicating what is not stem cell behavior. For example, most of the cells in the body that are dividing are not stem cells. In particular cells in the embryo that differentiate after a certain period of time, such as the earliest cells formed by division of the fertilized egg, are not stem cells. Nor are differentiated cells that divide during postnatal life to generate more of themselves, such as hepatocytes or tissue-resident macrophages. A common term found in the literature is "stem/progenitor cell". This is a singularly unhelpful designation as it conflates two entirely different cell behaviors. Progenitor cells are precisely those that differentiate into functional cell types after a finite period of multiplication. They include the transit amplifying cells that arise from stem cells (Figure 1.1) and also cells of the embryo and of the growing individual that are destined to differentiate after a certain time.

Real stem cells comprise two fundamentally different types: pluripotent stem cells that exist only in vitro, and tissue-specific stem cells that exist in vivo in the postnatal organism. Pluripotent stem cells comprise embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC). There are various subdivisions that will be considered later, but the essential features of these cells are first that they can be propagated without limit in vitro, and second that, under appropriate culture conditions, they are able to give rise to a wide variety of cell types, perhaps all the cell types in the normal organism except for the trophectoderm of the placenta. By contrast, tissue-specific stem cells exist within the body and generate progeny to repopulate the tissue in question. Well-studied tissue-specific stem cells include those of the hematopoietic (blood-forming) system, the epidermis, the intestinal epithelium and the spermatogonia of the testis. Under normal circumstances, tissue-specific stem cells do not produce cells characteristic of other tissue types. There are also some well-characterized stem cells that do not undergo continuous division, but seem to be kept in reserve to deal with tissue regeneration when required. A good example is the muscle satellite cells, which are normally quiescent but are able to be mobilized to divide and fuse to form new myofibers following injury. This type of stem cell behavior is sometimes called facultative.

Many criteria for identifying stem cells have been proposed and used. These are briefly listed here and the concepts and technologies will be developed in later chapters of the book.

Stem Cell Markers

Very often a cell is said to be a stem cell because it expresses one or more gene products associated with stem cells. However, there is no molecular marker that identifies all stem cells and excludes all non-stem cells. Those components required for general cell metabolism and cell division are certainly found in all stem cells, but they are also found in many other cell populations as well.

Pluripotent stem cells (ESC and iPSC) express an important network of transcription factors which are necessary for maintenance of the pluripotent state (see Chapter 6). Transcription factors are the class of proteins that control the expression of specific genes. A key member of the pluripotency group is the POU-domain transcription factor OCT4 (also known as OCT3 and POU5F1). The presence of OCT4 is certainly necessary for the properties of pluripotent stem cells. However it is not expressed in any type of tissue-specific stem cells except at a low level in spermatogonia.

A component that might be expected to be found in all stem cells is the telomerase complex. At the end of each chromosome is a structure called the telomere, made up in vertebrate animals of many repeats of the simple sequence TTAGGG. Because of the nature of DNA replication, the double helix cannot be copied right up to the end, so a part of the telomere is lost in each cell cycle. After enough cycles, the erosion of chromosome ends activates the system which senses DNA double-stranded breaks and causes death of the cell. This process is an important reason for the limited survival time of most primary tissue culture cell lines, which undergo senescence after a certain number of population doublings in vitro. Obviously there must be a mechanism for repairing telomeres in vivo, and this is provided by the telomerase complex, of which the most important components are an RNA-dependent DNA polymerase called TERT, and an RNA called TERC which contains the template CCCTAA for the telomere sequence. High levels of telomerase are found in germ cells, ensuring the survival of full length chromosomes for the next generation. Telomerase is also upregulated in permanent ("transformed") tissue culture cell lines and in most cancers. However most types of somatic cell have little or no telomerase. Tissue-specific stem cells do contain some telomerase; generally enough to maintain cell division for a normal lifetime, but not enough to fully reverse the erosion of the telomeres. In situations such as repeated transplantation of hematopoietic stem cells from one mouse to another, there is an upper limit to the number of possible transplants and this is determined at least partly by telomere erosion. The presence of telomerase can be considered to be a stem cell marker, although it is also found in permanent tissue culture lines, early embryos and most cancers.

In human or animal tissues, various markers have been advanced as characteristic of all stem cells. For example the cell surface glycoprotein CD34 is found on human hematopoietic stem cells (HSCs) and can be used to enrich them from bone marrow by fluorescence-activated cell sorting (FACS). However it is also found on other cell types, such as capillary endothelial cells, and it is unclear whether it is actually necessary for the stem cell behavior of the HSC. In fact, since it is not found on mouse HSC, which are generally similar in behavior to human HSC, it is probable that it is not necessary. CD34 is not found on human embryonic stem cells or on most epithelial stem cell types, indicating that it is not a generic stem cell marker. A molecular marker which is known to be required for stem cell function is LGR5. This is an accessory receptor for the Wnt family of signaling molecules (see Chapter 7) and is found on stem cells in the intestine, hair

follicle, mammary gland and stomach. These types of stem cell all depend on Wnt signaling from their environment for continued cell division, so the presence of the LGR5 is really necessary. However it is not found on other types of stem cell, so is also not a universal marker.

An interesting type of marker is that offered by dye exclusion, in particular exclusion of the Hoechst 33342 dye. This is a bisbenzimide dye, excited by UV light to emit a blue fluorescence. It is widely used as a DNA-binding reagent, but it is also actively pumped out of some cell types. If a subgroup of cells has lost more dye than the rest of the population, then it appears in flow cytometry as a cluster of cells showing less blue fluorescence than average. This is called a side population. The side population is enriched for stem cells in some situations, especially in murine bone marrow where it provides a similar degree of enrichment of hematopoietic stem cells to FACS using a panel of cell surface markers (Figure 1.2). The dye exclusion property is due to the activity of cell membrane transporter molecules including the P-glycoprotein (MDR1) and transporters of the ABC class. Dye exclusion is indicative of an increased capacity for export of all hydrophobic small molecules, many of which are toxic to cells. Although useful to the investigator, it is unlikely that this capacity is really important for stem cell behavior. For example, mouse embryonic stem cells show dye exclusion while human embryonic stem cells do not.

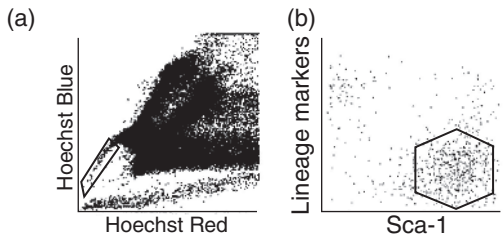

Figure 1.2 Flow cytometry plots showing a side population of cells active in Hoechst dye exclusion. (a) Whole mouse bone marrow, the boxed region is the side population. (b) Side population cells refractionated with regard to differentiated lineage markers, absent from stem cells, and Sca-1, a cell surface marker present on stem cells. (From: Goodell, M.A., Brose, K., Paradis, G., Conner, A.S. and Mulligan, R.C. (1996) Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. Journal of Experimental Medicine 183, 1797–1806. Reproduced with the permission of The Rockefeller University.)

In summary, there is no single gene product which is found in all stem cells and not in any non-stem cell. Many so-called stem cell markers are probably not necessary for stem cell behavior. Of those gene products which are necessary for stem cell behavior, some, such as the cell division machinery and telomerase, are found in stem cells and in some non-stem cells. Others, such as OCT4 or LGR5, are found in some, but not all, types of stem cell.

Label-Retention

When a cell population is exposed to a DNA precursor, such as the nucleoside bromodeoxyuridine (BrdU), which is metabolized by cells in the same way as thymidine, all cells undergoing DNA synthesis will incorporate it into their DNA and so become labeled. The BrdU in the cell nuclei can be detected by immunostaining. After the BrdU supply is withdrawn, so long as cell division is continuing, then the level of BrdU in the DNA will halve with every subsequent S phase and become undetectable to immunostaining after about six divisions. If a cell divides slower than average, it will retain detectable BrdU for longer. This label-retaining behavior is often considered to be a characteristic of stem cells. In Figure 1.C.1 is shown an image of a hematopoietic stem cell (HSC) visualized with an antibody to the cell surface marker CD150. It retains a DNA precursor (EdU) label from a pulse given 30 days previously. Likewise, muscle satellite cells, that enable muscle regeneration following damage, are usually in a quiescent state. This relatively quiescent behavior is considered necessary to maintain regenerative function of some types of stem cell over a lifetime. If the mechanisms

of quiescence are disturbed in mice by knocking out key components, then hematopoietic stem cells or muscle satellite cell populations have been shown to become exhausted during the lifetime of the animal, because they are dividing too much. Relative quiescence also serves to protect the stem cells against the oxidative damage which results from continuous growth with its associated oxidative metabolism.

Slow division is the cause of label retention in stem cells, but it must be remembered that not all label retention is due to stem cell behavior. In particular differentiation to a completely non-dividing (post-mitotic) cell type leads to permanent label retention. This property has been used especially to establish the differentiation time of neurons in embryonic development, and the final mitosis is often referred to as the cell birthday. Moreover, label retention is by no means universal among stem cells. For example, it is not shown by intestinal or epidermal stem cells. It is also, of course, not shown by the pluripotent stem cells (ES or iPS cells) which undergo rapid division in culture.

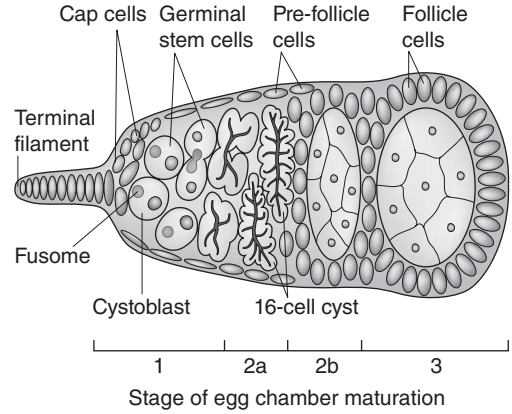

Figure 1.3 The stem cell niche in the Drosophila ovary. Female germ cell stem cells require continued contact with cap cells to remain stem cells. Once they lose contact with cap cells they differentiate into a cyst of one oocyte and 15 nurse cells. (Slack, J.M.W. (2009) Essential Developmental Biology, 2nd edn. Reproduced with the permission of John Wiley and Sons.)

The Niche

The concept of a stem cell niche arose in the 1970s to explain the fact that the spleen colony-forming cells from the bone marrow had a lesser differentiation potency than hematopoietic stem cells in vivo (see Chapter 10). The idea is that stem cells require continuous exposure to signals from surrounding cells in order to maintain their stem cell behavior. This was first proved experimentally using the fruit fly *Drosophila*. In the *Drosophila* ovary there are female germ cells which lie in contact with somatic cells called cap cells. These secrete a TGFβ-like molecule called Decapentaplegic (Dpp). Dpp maintains the stem cells in mitosis. But as they divide, some of the stem cell progeny become displaced from contact with the cap cells, and are then exposed to less of the Dpp. This fall in Dpp lifts a repression on the oocyte maturation process and enables the daughter cell to differentiate to a cystoblast. This then undergoes a fixed differentiation program, dividing four times to generate a post-mitotic complex of one oocyte and 15 supporting nurse cells. This situation illustrates the behavior of a niche very nicely. The stem cells continue to divide so long as they are in contact with the niche, and they differentiate when they are no longer in contact. If a stem cell is removed experimentally, its position may be taken by a progeny cell which would normally have differentiated, but because of its renewed occupancy of the niche it remains a dividing stem cell.

Probably all the stem cells types in the mammalian body exist within specific niches like this which control their behavior. For example the intestinal stem cells lie adjacent to Paneth cells which supply WNT, and spermatogonial stem cells lie adjacent to Sertoli cells that supply them with glial derived neurotrophic factor (GDNF). In both cases the signaling molecules are needed to maintain the stem cells in mitosis, and removal from the niche brings an end to cell division unless the factors are provided experimentally. In the bone

marrow, there has been controversy about the exact nature of the niche, but hematopoietic stem cells are often found adjacent to blood vessels, as shown in Figure 1.C.1.

Asymmetric Division and Differentiated Progeny

It is often thought that all stem cells must undergo asymmetric divisions, with one daughter being a stem cell and the other destined to differentiate. This does sometimes occur, but it is also possible for stem cells to have a less rigid program of cell division with some divisions producing two stem cells, some two progenitor cells, and some producing one of each. Statistically a steady state requires that the stem cell number remains constant, although there may be occasions where it needs expanding, such as during normal growth of the organism or following injury. In the intestine for example it has been shown by cell labeling and by direct visualization that symmetric divisions predominate (see Chapter 10).

By definition, stem cells must produce differentiated progeny, but how many differentiated cell types do they actually produce? The answer is very variable and depends on the tissue concerned. In the intestine, stem cells produce absorptive, goblet, tuft and Paneth cells, together with several types of enteroendocrine cells. In the bone marrow, the hematopoietic stem cells produce all the cell types of the blood and immune system. At the other end of the scale, the spermatogonia of the testis produce only sperm. Epidermal stem cells are often said to produce only keratinocytes, but they can also form a type of neuroendocrine cell called the Merkel cell, responsible for touch sensitivity. The examples of both the intestine and the epidermis indicate that neuroendocrine cells can arise from epithelial stem cells quite distinct from the central or peripheral nervous systems, but they are not indicative of a wider potency enabling other tissue types to be formed.

Around the year 2000 there was a rash of papers indicating that hematopoietic stem cells (HSCs) were able to repopulate many, or perhaps all, other tissue types in the body following transplantation. This phenomenon was known as "transdifferentiation" (a term more usefully reserved for changes of differentiation type between fully differentiated cells). However further investigation showed that the phenomenon could mostly be explained by donor cells lodging within other tissues but not actually differentiating into them, or by cell fusion with cells of other tissues. Unfortunately the idea of very wide plasticity of tissue-specific stem cells became established in many people's minds at this time and has helped promote the present worldwide industry of "stem cell therapy" much of which has no scientific rationale or real clinical effectiveness.

Clonogenicity and Transplantation

It is often supposed that stem cells are those which grow rapidly and can form large clones in vitro. This perception came from early studies on epidermal stem cells, where the proportion of such cells (holoclones) does correlate well with the estimated proportion of stem cells in the basal layer (Figure 1.4). Sometimes, "spheres", such as neurospheres or mammospheres, which contain both stem cells and their differentiated progeny, can be grown from tissue samples in suspension. However, stem cell behavior depends both on the cells and their environment, and it is well-known that cell behavior can be greatly changed by the environment of in vitro culture. For example, neurospheres can be grown from parts of the central nervous system in which there are no stem cells in vivo. As another well-known example, cells of the mammalian embryo epiblast, which rapidly develop into other cell types in vivo, can give rise to pluripotent embryonic stem cells in vitro, which continue to divide indefinitely in an appropriate medium.

Transplantation behavior also looms large in thinking about stem cells. The ability to

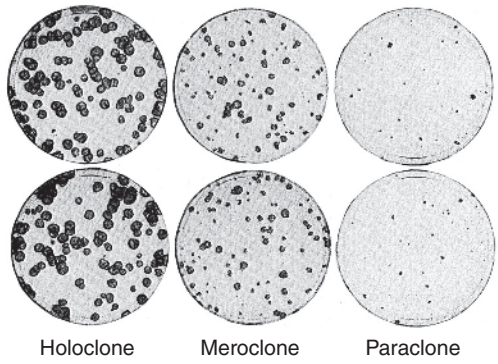

Figure 1.4 Clones of epidermal cells growing in culture. In this study the clones were classified as holoclones (large), meroclones (medium) and paraclones (small). The holoclones were considered to arise from stem cells. (From Barrandon, Y. and Green, H. (1987) Three clonal types of keratinocyte with different capacities for multiplication. Proceedings of the National Academy of Sciences of the United States of America 84, 2302–2306. Reproduced with the permission of Proceedings of the National Academy of Sciences of the United States of America.)

rescue irradiated animals with bone marrow transplants was the original discovery that, decades later, led to the identification of hematopoietic stem cells. It is generally felt that a hematopoietic stem cell is defined by the ability to repopulate the entire blood and immune system of an irradiated host. This is certainly an important property, although a single-minded focus upon it has tended to obscure the important distinction between cell behavior in an extreme regeneration situation and that in normal homeostasis. Some authors even suggest that a cell is not a bona fide stem cell unless it, as a single cell, can repopulate an entire tissue following transplantation. While this has been done a few times and may be a theoretical possibility for all stem cell systems, there are always practical limits to transplantation. All adult vertebrate animals have highly sophisticated immune systems that, as a by-product to their role in defending against infection, cause the rejection of cell and tissue grafts from other individuals. This is a very complex subject, but in general grafting between adults is only possible between genetically identical individuals (e.g. identical twins or inbred mouse strains), or following immunosuppression with drugs, or by using highly immunodeficient strains of animal as hosts.

A type of stem cell defined almost entirely by transplantation is the so-called cancer stem cell. These are subsets of cells from tumors, isolated using various stem cell markers, which will generate tumors in immunodeficient hosts following grafting, under conditions where the majority of cells from the same tumor do not. Cancer stem cells are discussed in Chapter 11.

In Vivo Lineage Labeling

This is the most reliable method for establishing the existence of stem cell behavior in vivo because it can provide direct visualization. So far it has only been widely used in mice, but the wide availability of CRISPR-Cas9 technology will soon make it available for other organisms as well. The principle is to use a DNA recombinase enzyme (Cre) to impart a permanent genetic label to a cell in vivo that expresses a particular gene, or, more precisely, has a particular promoter highly active. The label is subsequently heritable on cell division and is unaffected by any differentiation events occurring in the progeny cells. A modification of the Cre recombinase to make it activatable by estrogen-like hormones (CreER) has been widely used in mice and enables the labeling to be initiated at a specific time. This method is described in Chapter 3. Once it has been labeled a stem cell will produce a sector of labeled tissue in which all its dividing and differentiated progeny carry the label. The labeled sector will grow initially as cells divide and mature and will eventually reach a steady state in which addition of new labeled cells is balanced by the removal of dead ones. This pattern should then remain unchanged in the long term. An example is shown in Figure 1.5, showing intestinal stem cells labeled using the *Lgr5* promoter. These cells reside in the

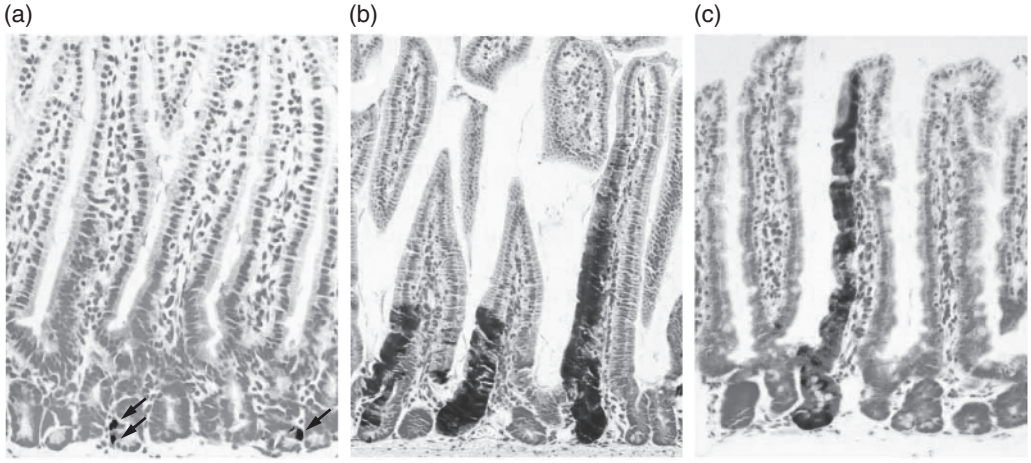

Figure 1.5 Descendants of stem cells in the mouse intestine visualized by the CreER method. The stem cells express a protein, LGR5 whose promoter is used for labeling. (a) The mice were labeled 1 day previously, (b) 5 days previously and in (c) 60 days previously. The initial label is in the LGR5 positive cells themselves (arrows); subsequently, ribbons of descendant cells up the crypts and villi become labeled. (Originally from: Barker, N. et al. (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449, 1003–1007. Reproduced with the permission of Nature Publishing Group.)

intestinal crypts and generate a file of cells up the crypt and onto the neighboring villus. Near the villus tip the cells die and are then lost into the intestinal lumen. Some other examples of stem cell labeling are shown in Figures 10.C.1–10.C.6.

Because it can provide visualization of individual stem cell domains, cell lineage labeling has provided the data for an influential model of stem cell behavior which may be called stochastic. Here the idea is that the stem cells have a certain chance of dividing to form two stem cells, two transit amplifying cells or one of each (Figure 1.6). If the outcome is 50% of new stem cells and 50% of transit amplifying cells, then this gives quantitatively the same outcome as a situation of obligatory asymmetric division in which every division yields one stem cell daughter and one transit amplifying daughter. However it gives different predictions about the behavior of labeled stem cell clones. In the situation of the obligatory asymmetric division, labeled stem cell clones will each comprise one stem cell plus all their descendants. When the steady state has been reached, the labeled clones should persist for life and stay the same size. However in the stochastic model, clones may be lost if their stem cell divides to form two transit amplifying cells. They may also increase in size if the stem cell divides to form two stem cells. This situation has been modeled mathematically and it predicts that the number of labeled clones should steadily decline with time while the size of clones should become progressively more disparate, with the average size increasing. This means that the proportion of the tissue occupied by the labeled clones remains constant, but the number of labeled clones becomes progressively fewer and their size more varied. In fact this behavior is precisely what is observed when lineage labeling data are analyzed quantitatively, at least for the epidermis, spermatogonia and intestinal epithelium. In particular there is a property called "scaling behavior" which means that the frequency distribution of labeled clone sizes, divided by the average clone size, stays the same over time. Under such circumstances, which may turn out to be the norm for mammalian stem cell systems, the key stem cell properties of self-renewal, persistence and differentiation are still maintained, but they exist at a cell population level rather than as the properties of a single cell.

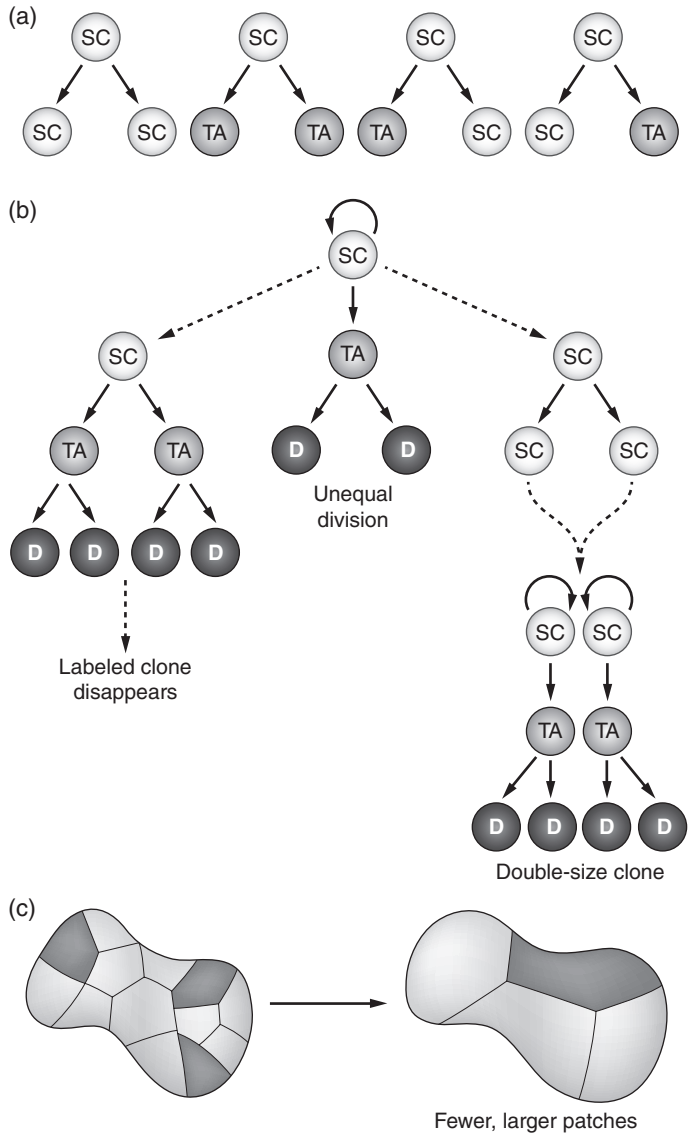

Figure 1.6 Stochastic stem cell model. (a) The four types of stem cell division. (b) Disappearance of labeled clone, and doubling of size of labeled clone. (c) Tendency of labeled clones to become fewer but larger with time. SC: stem cell, TA: transit-amplifying cell, D: differentiated cell.

Conclusions

The above brief discussion indicates that stem cells carry no universal molecular marker, that they are not all quiescent, that they are not necessarily the same as transplantable cells, and even that they may not be definable at the individual cell level at all but only at a population level.

Of course, what is considered to be a stem cell or not to be a stem cell all depends on the definition employed. The definition given at the start of this chapter comprises four properties:

- Stem cells reproduce themselves.
- Stem cells generate progeny destined to differentiate into functional cell types.

- Stem cells persist for a long time.
- Stem cell behavior is regulated by the immediate environment (the niche).

Together, these properties do make up a consensus view of stem cells acceptable to most scientists today. However, some will disagree with one or another element of the set. It is always possible to change the definition and thereby change what counts as a stem cell and what does not. For example, there are some cells at the periphery of the developing mammalian kidney that are sometimes called stem cells because they reproduce themselves and generate new kidney tubules. But these cells only persist for a few cell cycles in late gestation so do not satisfy the third criterion given here. They could be counted as stem cells only if this property were abandoned. As another example, some will insist that a stem cell must be able to give rise to more than one type of differentiated progeny, in which case the spermatogonia have to be removed from the list of tissue-specific stem cells since they produce only one type of differentiated cell: the sperm.

Perhaps because of the high profile of stem cell research generally, some attention has been given to the stem cell concept by philosophers of science. In a recent book, Laplane considers the various proposed attributes of stem cells and classifies these as categorical, dispositional, relational and system-based. A categorical property is essential and intrinsic, for example the presence of OCT4 in pluripotent stem cells. A dispositional property is a property revealed under particular conditions. For example intestinal stem cells need a Wnt signal from their niche in order to proliferate, so this property depends not just on the nature of the stem cell, but also on something else. A relational property is said to exist if it depends entirely on something else: for instance a hypothetical niche which would make any cell whatever that occupied it into a stem cell. Finally a systemic property belongs, in this context, not to individual cells but to the whole system, for example a tumor in which cells might acquire or lose stem cell behavior in specific circumstances. Laplane concludes that stem cells do comprise a "natural kind" (i.e. a real thing, out there, not just a figment of our imagination), but that they require a complex definition with a general part "stem cells are the cells from which tissues are developed and maintained", and a set of specific parts which in effect list the various properties discussed in this chapter. Apparently philosophers will accept that a natural kind may properly be defined by a set of attributes which are both categorical and dispositional, and of which not all need apply in every instance.

Debates such as these may cause momentary panic: how can such an important part of modern life as the stem cell become so intangible when examined critically? The contribution of philosophers may be considered negative if it just makes familiar entities disappear. However the philosophical view is worthwhile if it makes us examine the concepts critically. In the case of stem cell biology what emerges from a critical evaluation is that we should think not about stem cells as such but about *stem-type behaviors* that may be shown by various cell populations in specific circumstances. Defining stem cells is slippery and difficult, but defining stem cell behavior is relatively easy and stem cell behavior is real and important. In order to manipulate it in practical situations we need to understand the complete context, and for this an approach based on the underpinning sciences, such as cell and developmental biology, is really necessary.

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