1 Stem cells and cellular pedigrees – a conceptual introduction

Markus Loeffler and Christopher S. Potten*

*Institute of Medical Informatics, Statistics and Epidemiology, Leipzig, Germany: *CRC Department of Epithelial Cell Biology, Paterson Institute for Cancer Research, Manchester, UK*

SUMMARY

In this chapter, we consider some of the problems involved in current discussions on stem cells in adult mammalian tissues. The present concepts involve a number of pitfalls, logical, semantic and classification problems. This indicates the necessity for new and well defined concepts that are amenable to experimental analysis.

One of the major difficulties in considering stem cells is that they are defined in terms of their functional capabilities which can only be assessed by testing the abilities of the cells, which itself may alter their characteristics during the assay procedure; a situation similar to the uncertainty principle in physics. Hence, a proper description requires the measurement i.e. manipulation process itself to be taken into account.

If such context-dependent interactions exist between the manipulation and measurement process and the challenged stem cells, the question of the number of stem cells in a tissue has to be posed in a new way. Rather than obtaining a single number, one might end up with different numbers under different circumstances, all being complementary. This might suggest that stemness is not a property but a spectrum of capabilities from which to choose. This concept might facilitate a reconciliation between the different and sometimes opposing experimental results. Given certain experimental evidence, we have attempted to provide a novel concept to describe structured cell populations in tissues involving stem cells, transit cells and mature cells. It is based on the primary assumption that the proliferation and differentiation/maturation processes are in principle independent in the sense that each may proceed without necessarily affecting the other.

Stem cells may divide without maturation, while cells approaching functional competence may mature but do not divide. In contrast, transit cells divide and mature showing intermediate properties between stem cells and mature functional cells. The need to describe this transition process and the variable coupling between proliferation and maturation leads us to formulate a *screw model of cell and tissue organization.*

This concept is illustrated for the intestinal epithelium. Reference is made also to other tissues including the basal epidermal cell layer and the haematopoietic system.

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INTRODUCTION

At present there is no experimental way to decide if a given cell in a functional mammalian tissue is a stem cell or not. There are also no morphological criteria to identify such cells. This is partly due to lack of appropriate experimental techniques, and partly due to some conceptual problems. At the present stage it is helpful to discuss stemness as a latent variable which cannot directly be observed and which can only be deduced retrospectively on the basis of some indirect evidence based on measurable observable parameters in specific experimental settings. If one accepts stemness as a hidden property, it becomes obvious that one has to talk about stem cells and stem cell properties within the framework of concepts and models. This clearly implies that there cannot be a canonic unique definition but that a wide variety of models and concepts can be imagined, and have in fact to be proposed to describe various features of stem cell systems. Furthermore, it is evident that such models will differ in their attitude, their methodology and the set of phenomena on which they focus. At present there is no generally accepted standard stem cell model.

This introductory chapter is designed to serve as a framework for the stem cell models discussed in subsequent chapters. Starting with some general definitions, we try to set up criteria that should be fulfilled for stem cells. We point out the conceptual distinction between stem cells and transit cells before we discuss the problem of obtaining measurements on stem cells. We further discuss the basic elements of models used to describe cellular hierarchies and stem cells. In undertaking this exercise we will highlight our present reflections on this topic but also try to relate these to concepts suggested by other authors. In this respect we will extend ideas discussed in a previous paper (Potten and Loeffler, 1990).

DEFINITIONS

General definitions and concepts

In order to understand the full meaning and implications of the definition of stem cells, we need to consider some subsidiary definitions. The most important ones are associated with differentiation and maturation.

Differentiation

Differentiation can be defined as a qualitative change in the cellular phenotype that is the consequence of the onset of synthesis of new gene products, i.e. the non-cyclic (new) changes in gene expression that lead ultimately to functional competence (see Lajtha, 1979c). It may be recognized by a change in the morphology of the cell or by the appearance of changes in enzyme activity or protein composition. Since it is a qualitative change, a cell can be said to be differentiated only relative to another cell, and during its life a cell may be capable of undergoing several differentiation events. Differentiation is commonly identified by the detection of a novel protein. The ability to define a cell

as differentiated thus clearly depends on the sensitivity of the detection procedures. A few molecules of a novel protein may be detectable, as may the changes in the messenger RNA responsible for these molecules, but ultimately the differentiation event involves a change in the repression/activation of the genome, i.e. in transcription, and this may approximate to a quantal phenomenon. According to this definition cells developing from a primitive stage to functional competence may undergo many, even a series of, differentiation events each linked to a novel change in the gene activation pattern. In many circumstances, it may be practically helpful to consider only some primary key (marker) genes as relevant indicators of differentiation, particularly if secondary genes are activated subsequently.

Maturation

Maturation in contrast can be regarded as a quantitative change in the cellular phenotype or the cellular constituent proteins leading to functional competence (see Lajtha, 1979c). Thus the degree of maturation, in principle, could be measured on a quantitative scale, e.g. of the amount of a specific protein per cell. A differentiated cell matures with the passage of time to form a functionally competent cell for that particular tissue. Its passage through time and space could in principle be mapped, as new differentiation events occur changing the path of the cell. This relationship is illustrated in Figure 1.

The terms differentiation and maturation are often used in a loose fashion, interchangeably, with a consequent potential for confusion. It is also common to see the term *terminal differentiation* used without an adequate definition of its meaning, which is presumably an implicit indication of either the activation of the last differentiation event in the cell's life history (e.g. involucrin synthesis for epidermal keratinocytes) or more likely an indication of terminal maturation, i.e. the accumulation of differentiated

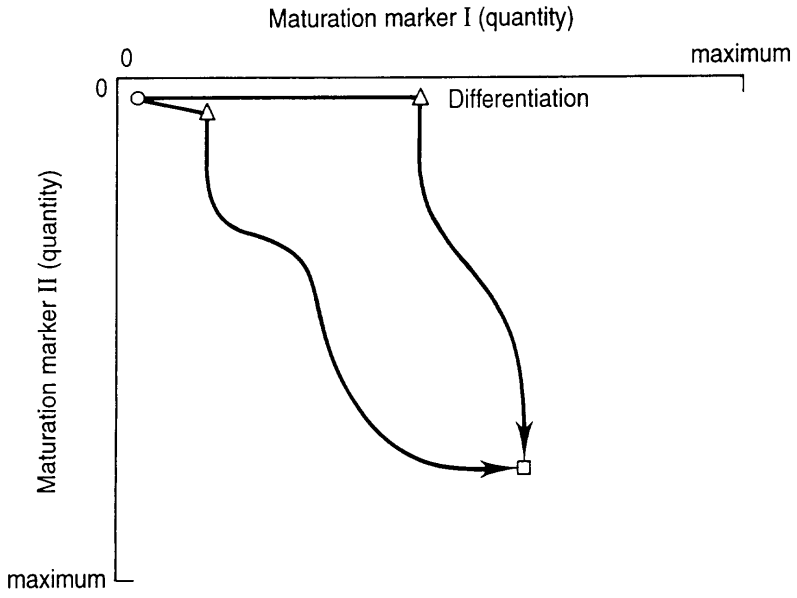

Figure 1 The course of an individual cell can be described in a differentiation–maturation diagram. Acquisition of a qualitatively new marker is defined as differentiation (△), while the trajectory for a given marker (from ○ to △) or, for a set of markers (from △ to □) is defined as maturation. Different maturation/differentiation paths may lead to the same state (□).

product(s) consistent with the final functional role of the cell (e.g. terminal keratinization, or cornification for epidermal keratinocytes).

Proliferation

Proliferation is a process involving a sequential pattern of (cyclic, repeating) changes in gene expression leading ultimately to the physical division of the cells. This is in contrast with cell growth, which involves an increase in cell size or mass. In order to identify a proliferating cell these changes have to be detected, and sensitivity problems similar to those associated with differentiation are encountered. The changes may be represented by discrete step-wise changes in the cellular concentration of, or by sharp peak alterations in, proliferation gene products. Many of these changes can be, and indeed have been, mapped on a time scale represented by the interval in time between two subsequent cell divisions, i.e. mapped in relation to the *cell cycle*. A large number of the gene products of these proliferation-associated genes (which include many cellular oncogenes S-phase and mitotic enzymes, cyclins etc.) have been mapped as transition points in the cell cycle. Traditionally the four major transition points, the onset and termination of DNA synthesis and mitosis have been used to identify proliferative cells, but many other transition points may be equally valid.

There are certain difficulties in distinguishing cells on the basis of our definitions of differentiation and proliferation. The first thing to note about these two processes is that they are not necessarily mutually exclusive. Indeed many cells in the adult body may exhibit differentiation markers, and hence be differentiated relative to cells earlier in tissue development, and yet they also proliferate. Certainly many cells in bone marrow exhibit both properties. Haematopoietic stem cells in the bone marrow are differentiated relative to embryonic stem cells. The stem cells in surface epithelia may be differentiated relative to the bone marrow stem cells and *vice versa*. The characterizations of the state of proliferation and differentiation are dependent upon the ability to identify changing patterns in gene expression and gene products. If these changes are of a cyclical nature they may be associated with proliferation. However, the cells under consideration may divide only once or we may have no knowledge of their previous history, in which case we are unable to tell if a particular gene product has been produced cyclically. Hence, it is more useful to define proliferation on the basis of the appearance of gene products associated with DNA replication, or the cell division process, which are in fact produced in a cyclic fashion. This implies a knowledge of many or all the metabolic processes associated with, and leading to cell division.

The distinction between differentiation, maturation and proliferation appears important as the development from stem cells to functionally competent cells can be viewed as a transition from one extreme (prolif: yes; diff/mat: no) to the opposite extreme (prolif: no; diff/mat: yes). The transition takes place through states of coexistence with some flexibility to accelerate or slow down one or both processes. It is this flexibility that permits cells to be stimulated to differentiate and stop proliferation and vice versa. Below we will introduce the assumption that proliferation, differentiation and maturation are not strictly coupled and in many circumstances should be considered independent of each other.

A special consideration here is to what extent a differentiated cell can dedifferentiate, whether this involves a switching off of the already activated differentiation genes (this

process in itself could be regarded as a differentiation step in certain circumstances) or an inhibition of further maturation. Under certain natural circumstances it appears that some limited categories of cells in the bone marrow and in the intestine can dedifferentiate and assume stem cell potential. To what extent this process can be experimentally manipulated by, for example, providing the correct set of signals/growth factors, remains to be seen.

Definition of stem cells

Criteria for actual and potential stem cells

Stem cells are defined by virtue of their functional attributes. This immediately imposes difficulties since in order to identify whether or not a cell is a stem cell its function has to be tested. This inevitably demands that the cell must be manipulated experimentally, which may actually alter its properties. We will return to this circular problem later. The second problem faced in defining the stem cell population is that the definition can only be relative compared with other cell types. We would define the stem cells of a particular tissue as (a) undifferentiated cells (i.e. lacking certain tissue specific differentiation markers), (b) capable of proliferation, (c) able to self-maintain the population, (d) able to produce a large number of differentiated, functional progeny, (e) able to regenerate the tissue after injury, and (f) flexible use of these options (see Lajtha, 1967, 1979a, 1979b, 1979c; Steel, 1977; Potten and Lajtha, 1982; Wright and Alison, 1984; Potten and Morris, 1987; Hall and Watt, 1989; Potten and Loeffler, 1990). Table 1 gives a summary of these criteria. Ideally, in order to categorize a population of cells as containing stem cells, all of these criteria should be satisfied; in practice, there are experimental limitations. This is further complicated by the fact that not all of these functions have the same weighting. For example, it would not be sufficient to characterize a stem cell by virtue of its ability to proliferate alone. Cells or populations of cells actually fulfilling all these criteria at a given instance will be called *actual stem cells*, while those not actually expressing these capabilities at a particular moment in time, though they possess these capabilities, will be termed *potential stem cells*. It may be possible for a stem cell to cease proliferation, i.e. become *quiescent*, in which case it is not an actual stem cell, but since it can re-enter the cycle and it has the potential to be a stem cell. These cells should

Table 1 Stem cell criteria.

Criteria	Stem cells	Transit cells	Maturing cells
(a) Differentiation marker	no	onset	yes
(b) Capable of proliferation	yes	yes	no
(c) Capable of self-maintenance	yes ($p_{sm} \geq 0.5$ possible)	no (but $0.5 > p_{sm} \geq 0$ possible)	no ($p_{sm} = 0$)
(d) Capable of many progeny cells	yes	limited	no
(e) Capable of regenerating tissues after injury	yes (long term)	temporarily	no
(f) Flexibility in options	(b)–(e)	(b), (d)	no

p_{sm}, self-maintenance probability.

perhaps be termed quiescent actual stem cells to distinguish them from other potential stem cells. Likewise a transit cell (see below) may not normally self-maintain, but may do so under special circumstances, thereby representing a potential stem cell. Without going too much into detail here, it is sufficient to point out that we may have two classes of stem cell; those that actually satisfy the requirements of the definition, i.e. actual stem cells, and those that may have the potential to do so under special conditions, i.e. potential stem cells. We choose the word actual in preference to the term functional, which has been used previously (Steel, 1977; Cairnie *et al.*, 1965; Wright and Alison, 1984).

Some terms outlined in our stem cell definition have stronger weight than others and hence some of them could be accepted on their own as a means of identifying stem cells: (1) self-maintenance and the ability to vary self-maintenance; (2) the ability to produce a large family of differentiated functional cells; (3) the ability to regenerate the tissue or elements of it by producing a large family of differentiated functional progeny following injury. However, it is evident that taken alone any of these criteria will select quite different cell populations. Subsequently we discuss the precise meaning of some of the definitions just used.

Self-maintenance and self-renewal

Self-maintenance, self-renewal, self-reproduction, self-replication and self-regeneration are terms that have been used in connection with stem cells, often interchangeably and without definition, to the detriment of clarity. However, these terms have subtle differences in meaning and should be used with care, as discussed in our earlier review (Potten and Loeffler, 1990).

Maintenance means 'keeping at an existing state or level', and when considered in terms of numbers is a meaningful expression to apply to a stem cell population (see Lajtha, 1979a; Potten and Lajtha, 1982). The ability to maintain its own numbers without input from other cell stages, i.e. self-maintenance, is exclusively a property of stem cells. The term *renewal* can be defined as 'to make like new' which implies an element of rejuvenation. We would like to restrict the term renewal to a specific process to be discussed below in which transit cells regain stemness properties.

The term *reproduction* means to 'give rise to offspring' and is thus a property of all proliferative cells. The term self-reproduction, however, implies that the offspring are identical in every sense, including genetically, with the parent, and is therefore a term best restricted to budding or cell cloning processes. It is clear that self-reproduction has a stronger implication than self-maintenance.

Replication implies duplication or repetition and has connotations somewhat similar to reproduction. Self-replication implies production of identical twins, while self-maintenance implies maintenance of a functional ability (e.g. number) irrespective of the identity.

Regeneration implies 'to make again' something that was already pre-existing. It could apply to a tissue or a population of cells and would be more appropriately used in connection with other processes, to be discussed later. So stem cells may be defined as cells capable of self-maintenance and regeneration under certain conditions.

There are basically two ways to describe stem cell self-maintenance conceptually (Figure 2). The first concept relies on a deterministic description of the entire cell

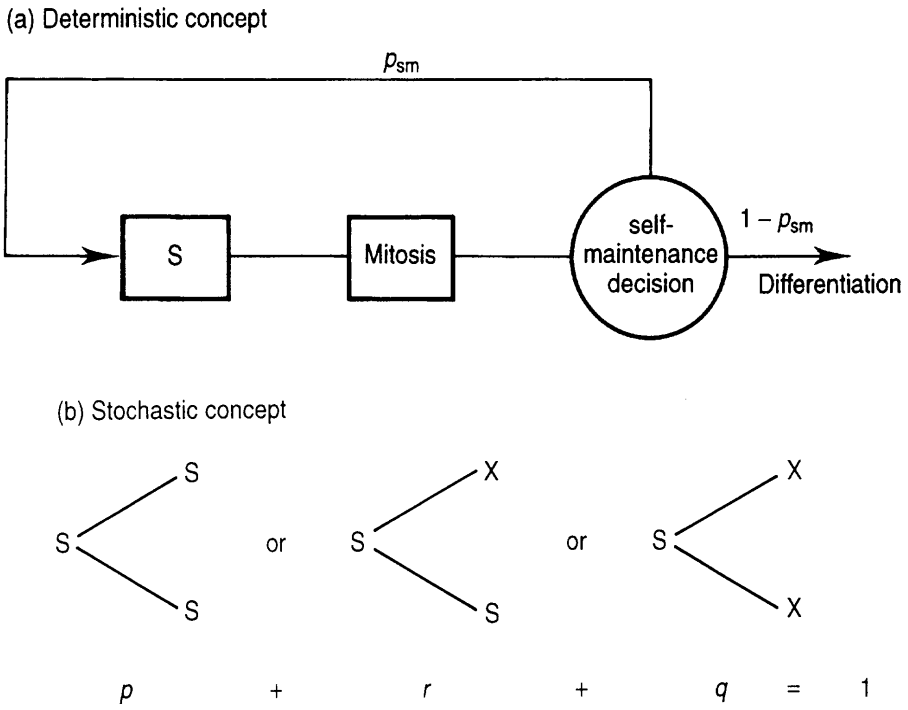

Figure 2 Stem cell concepts. (a) Deterministic concept of population growth; (b) Stochastic concept of single cell growth. (p_{sm}, fraction of postmitotic stem cells remaining in the stem cell pool; steady state condition $p_{sm}=0.5$; p, probability of symmetric cell division generating two stem cells; r, probability of symmetric cell division generating one stem cell; q, probability of stem cell loss by symmetric cell division or extinction; strict steady state $r=1$; note that $p=q\neq 0$ implies long term random extinction for finite populations.)

population (Figure 2a). In this case we consider a compartment, i.e. a pool of many stem cells which are actively cycling and undergo mitosis at a certain rate. The population concept implies that at any given instance in time a fraction p_{sm} of postmitotic stem cells remains in the stem cell pool. The cell population is maintained at a steady state if $p_{sm}=0.5$, indicating that on average half of the daughter cells maintain stem cell properties while the other half leaves the compartment. There is an increase in the stem cell population if p_{sm} becomes larger than 0.5, and there is a decrease if it becomes smaller than 0.5. It should be noted that this quantity has often been termed by us and others 'self-maintenance probability', which is not literally correct, as no random process is involved. The concept describes an average population and does not take into account variations and fluctuations in the population size due to random events. Hence the description is phenomenological as it does not relate the processes of growth of the population to the microscopic events occurring at the single cell level.

In contrast the second frequently used concept relates the population dynamics to the microscopic behaviour of single cells as indicated in Figure 2b. Each stem cell undergoing a cell division can either generate two, one or no daughter stem cells. In order to take our general ignorance about the specific decision in a specific cell into account we describe the processes with probabilities. We distinguish three such probabilities which add up to 1 (i.e. $p+r+q=1$). A strict steady state is maintained if only asymmetric divisions are occurring ($r=1$). On the population scale a stationary state is also possible if r is smaller than 1 provided that $p=q$. However, it should be noted that such a situation

leads to long term random extinction of a finite cell population if p and q are constant with time. This is a particular feature of a stochastic process not present in deterministic concepts. Hence, stochastic models will require special mechanisms to assure long term stability.

Consideration of the particular type of division shown in Figure 2b illustrates a particular conceptual problem. If one looks at individual cells, a cell that produces two daughters that are not stem cells cannot itself be considered a stem cell since it does not satisfy the stem cell criteria of self-maintenance. However, the definition is still applicable to a pool of many such cells contributing as a whole to the self-maintenance process. Thus the two concepts of deterministic population growth and stochastic single cell behaviour do not coincide under all circumstances. This problem of compartment size relating to the stem cell definition reappears on numerous occasions in the consideration of stem cells. Only in the case of a large stem cell population can one equate $p_{sm} = p + 1/2(r+1)$. In the case of small stem cell populations the experimental procedures may lead to a serious estimation bias of p_{sm}. This is illustrated in Figure 3. Here, we have two cell lineages, both characterized by predominantly asymmetric microscopic divisions. In the case of lineage (a), whatever compartment size one considers even down to a single cell division, one could conclude that stem cells are involved and a population determination of p_{sm} yields 0.5. However, in lineage (b) the overall p_{sm} equals 0.5 (steady state), but there is a series of inappropriately small subcompartments for which p_{sm}, if valued, would be very different (box E, 0.0; box C, 0.33; box D, 1.0). Hence the determination of self-maintenance properties of a population on a small sample may be very misleading. Furthermore it is necessary to have constant growth conditions over an appropriate period of time. Thus compartment size is important in considering

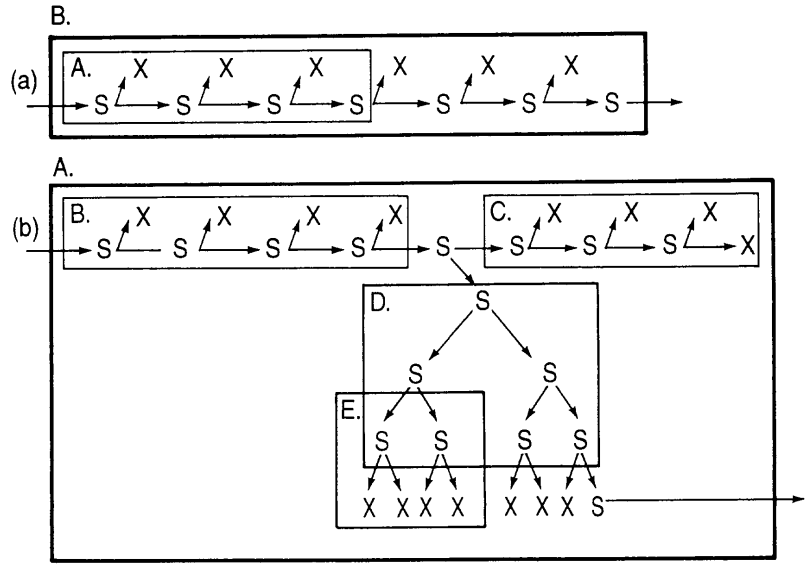

Figure 3 Compartment size considerations. (a) Permanent asymmetric stem cell lineage. Whatever compartment size is considered, self-maintenance is satisfied. (b) A second stem cell lineage. If all the divisions are considered self-maintenance is satisfied (box A). Similarly for a selective smaller box (B) it is satisfied although a similarly sized different compartment (box C) does not satisfy these self-maintenance criteria. Other compartments may show no self-maintenance (box E) or maximum values (box D). The values for p_{sm} in these boxes are: $p_{sm} = 0.5$ for A, $p_{sm} = 0.5$ for B, $p_{sm} = 0.33$ for C, $p_{sm} = 1.0$ for D, $p_{sm} = 0.0$ for E.

self-maintenance but Figure 3 could also be thought of in terms of time frames. Short time frames are as inappropriate as small compartment sizes.

Relativity

One criterion of stem cells is that they are undifferentiated compared with the functional end cells of the particular tissue to which they give rise. This definition is essentially a relative one as it relates the stem cells to the functional end cells, or relates them to cells at earlier stages of the development, or stem cells in other times. Apparently, this definition is compatible with the existence of various stem cells of different tissues as well as of a hierarchy of stem cells for one particular tissue. It is possible that there are specific differentiation markers which would enable a distinction of stem cells in relation to one another and in relation to the functional cells they are eventually producing.

The relativity of stemness is an essential feature to keep in mind and one has to be specific with respect to the particular experimental circumstances. For example, in the haematopoietic system one is presently inclined to distinguish several stem cell classes out of a continuum of stem cells ranging from long term repopulating cells to colony forming units in spleen (CFU-S) (see Chapter 13). A similar continuum has recently been suggested for intestinal stem cells (Potten and Hendry, 1995).

Pluripotency

In the stem cell definition given above, pluripotency was not requested as a prerequisite of stemness. However, it is clear that most tissues contain a range of different specialized functional cells. These may all originate from a common compartment of stem cells in the tissue, the range of variable different differentiation options being facilitated by the length of the transit compartment. Those tissues with the greatest differentiation potential, for example bone marrow, tend to have the longest transit compartment, although the differentiation of individual lineages may well occur earlier in the transit compartment. The limit to the differentiation potential for individual stem cells is unclear and may well differ from tissue to tissue. The ability to produce progeny that differentiate down various lineages (pluripotency) is not necessarily a property of stem cells *per se*, although it appears that many stem cells possess this capability (see Chapters 10 and 13).

Definition of maturing cells

Maturing cells can be defined as cells with (a) full expression of a differentiation marker, (b) no capability of proliferation, (c) no capability of self-maintenance, (d) no capability to produce any progeny cells after injury and (e) hence no ability to regenerate tissue after injury.

Maturing cells therefore represent cell stages which are close to completing their development and becoming functional end cells. In this context, for example, reticulocytes would be maturing cells, as would segmented neutrophils in the bone marrow, villus cells in the intestine and superficial stratified cells in epidermis.

Definition of transit cells

Transit cells can be defined as a cell stage which is intermediate between stem cells and maturing cells. We define transit cells by the following criteria (see Table 1): (a) they are characterized by the onset of differentiation marker expression during their development which are, however, not mandatory; (b) they are capable of proliferation; (c) they cannot self-maintain. This implies that transit cells may be able to operate as amplifying cell stages generating many maturing cells from the few cells entering the transit cell stage. If one would determine the self-maintenance probability p_{sm} for these amplifying cells, one would obtain a value clearly below 0.5. Therefore transit cells would be capable of producing many progeny cells (criterion d) which are temporarily capable of regenerating a tissue after injury (criterion e). However, no long term regeneration and no functional re-establishment of the tissue would be possible.

The essential feature of a transit cell population is that it irreversibly develops towards maturing cells thereby undergoing several rounds of cell division. This process essentially operates as a cellular amplification machine generating numerous end cells from the few cells entering the system. There are again two ways of describing the transit cell stages (Figure 4). One way would be a population description in which a sequence of

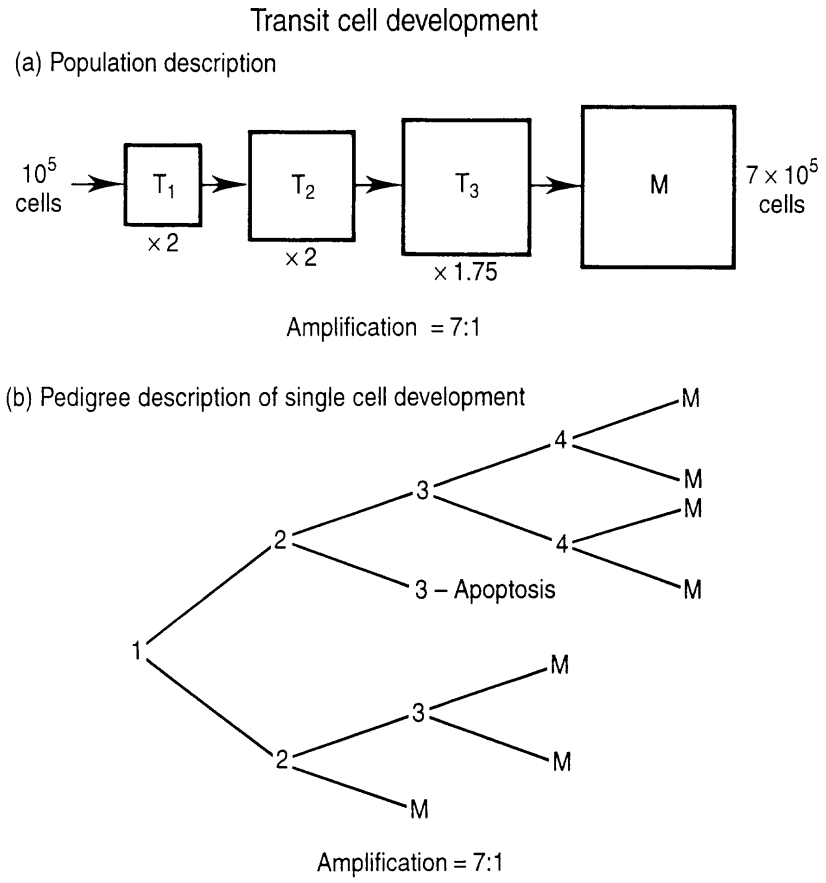

Figure 4 Schemes of transit cell developments. The two diagrams indicate cellular developments which generate numerous mature cells (M) out of less mature and still dividing cells. (a) In a population description the average amplification of many cells can be used as a parameter disregarding single cell development. (b) In a pedigree description the fate of an individual cell is sketched out. Such a pedigree can be quite asymmetric as indicated and may in fact vary between different T_1 cells.

developmental steps (T1, T2, T3, T ...) follow one another. Without looking into the details of the cellular development one could count the cells in each of these stages.

An alternative description relates to the microscopic single cell development and leads to a pedigree description. Figure 4b shows an example of one such pedigree of cells developing out of a primitive transit cell. In this particular pedigree maturing cells originate as early as two cell divisions and as late as four cell divisions after entry into the transit cell stage. This example shows a marked asymmetry in the cellular development. Furthermore, particular processes like apoptosis can be easily introduced into this description. However, at the present stage of cell biological experiments our knowledge is very limited on individual cell development and pedigree descriptions at this level of sophistication are rarely available. Most of our present knowledge is therefore based on the population approach which is an averaging process of the latent pedigree development.

It should be emphasized that a pedigree description or a population description of an age-structured transit cell population as shown in Figure 4 implies a particular distribution of the number of generations cells need to process through. Very little is known at the present stage about this process, its variability and its controls. The question could be rephrased as: 'what determines the number of transit generations?' Is it a clock within the cells counting their divisions or are external signals restricting the division potential? Different systems seem to have selected either the former or the latter, or perhaps some combination of both. It would appear, for example, that in bone marrow the number of transit divisions is under regulation and can be modified, while in some more primitive systems a counting clock may operate. If these processes are under regulatory processes then if the restricting factors are removed transit cells may divide many times (e.g. in culture condition) without exceeding any certain maximum.

A good example of a population description of the cellular developments is given by models of the haematopoietic system discussed by Wichmann and Loeffler (1985) and Loeffler *et al.* (1989). In these examples of the haematopoietic system an entire cell stage (e.g. colony forming unit erythroid (CFU-E) see Chapter 13 for details of cell types) was pooled together into one population compartment. In this compartment a cellular amplification was assumed. Hence, a CFU-E is generating further CFU-E cells which might be misinterpreted as evidence of self-maintenance. In fact there is the mathematical possibility to translate amplification within one compartment into a self-maintenance probability p_{sm}. However, this self-maintenance probability will in no situation exceed the value of 0.5.

PROBLEMS IN MEASURING STEM CELLS

How to check the criteria in practice?

If we consider the stem cell definition presented above, the question arises as to whether it can be used in a practical sense. A stem cell is a proliferative cell but this is the weakest part of the definition. Proliferation can be strictly identified in a population only by determining the future behaviour of the cell in question, i.e. whether it will divide into two cells in the future. In practice it is usually sufficient to identify that the cell, or

population of cells, expresses one or more of the many markers of transition through the cell cycle, the commonest of which is whether it enters DNA synthesis, but the simplest is whether it enters or is in the mitotic phase.

On the other hand it has been suggested that stem cells having the potential for division may in fact be in a resting phase for most of their life and can therefore be identified by appropriate techniques such as long term S-phase label retention. However, as is evident from this feature such cells cannot be participating actively in tissue maintenance.

The second aspect of the definition is whether or not the cell is undifferentiated, which is a qualitative and relative term. It would usually be assessed by observing the morphological status of the cell and whether or not it expresses one or more markers for differentiation.

Another aspect of the stem cell definition relates to the ability of these cells to produce a large progeny of differentiated cells. This again is a question related to the future potential of the cells in question and can only be tested by placing the cell or cells in a situation where they can express this potential, e.g. placing the cells in culture or arranging for a situation *in vivo* where the regulators limiting stem cell growth are removed, as would happen in a situation where some time stem cells were killed, i.e. during a regeneration. We will return to the question of regeneration.

Self-maintenance is the cardinal property of stem cells, but here again it can only be assessed in terms of the future of the cell. Can the cell produce other cells like itself and maintain the population over a period of time?

Self-maintenance is perhaps the most difficult property to determine experimentally. There are at present no easy and reliable assay techniques to measure this feature. Perhaps still the best approaches to the problem are modifications of the original recloning experiments originally used in the haemopoietic system. In a series of classical experiments Till and McCulloch (1961) examined whether a colony produced in the spleen (CFU-S) after transplantation of bone marrow into lethally irradiated mice contained cells which would generate secondary colonies if excised and re-transplanted. Thus the basic idea to investigate self-maintenance is to check by recloning experiments whether one can obtain cells which fulfil the same criteria of stem cells successively. This approach has in fact been used to determine p_{sm} for CFU-S in the early 1960s (see below).

Referring to the boxing phenomena discussed above (Figure 3) it is evident that any determinations of self-maintenance have to be conducted on populations of cells. As a consequence of using populations of cells one loses the information about an individual cell. This necessarily implies that one can only make statistical statements. On the other hand, the problem of investigating a cell population is that one cannot control whether the cell population under investigation receives any input from more primitive cell stages which may contaminate the population. It is therefore always possible, and has in fact been suggested for the CFU-S assay, that some very primitive stem cells have been present in the spleen colonies which gave rise to the CFU-S cells which themselves could be transit cell stages. Thus, the examination of cell populations to determine self-maintenance is often open to criticism, and there are very few biological systems like the intestinal crypt where one can conclude from the anatomical construction of the system that stem cells cannot immigrate and must be constitutive to the cell population.

The final aspect of the stem cell definition, which is associated with the property of

a large proliferative potential, is whether regeneration can be achieved. Besides being a property associated with the future this is specifically a property associated with disturbance of the system. In practice, clonal growth assays are a frequently used way of assessing stem cell function. For adult tissue stem cells, a variety of clonal regeneration assays have been developed *in vivo* and a number *in vitro* (summarized in Potten and Hendry, 1985a, 1985b). The most effective of these is the spleen colony assay for haemopoietic stem cells (Till and McCulloch, 1961) and the micro and macro colony assays for intestinal epithelium (Withers and Elkind, 1969, 1970; Potten and Hendry, 1985b), and epidermis (Withers, 1967). In these cases, the colonies assessed are large and contain very many cells and, if the necessary conditions are satisfied (high doses of radiation), represent *clones* derived from a single surviving stem cell. The fact that these clones contain many cells demonstrates the large division potential of the originator cell (*clonogenic cell*). The fact that the clones can often contain several differentiated cell lineages indicates that the original stem cell was pluripotent. The self-maintenance element can be assessed by either a second clonal regeneration assay starting with the first clone, or simply from the longevity of the clone in terms of maintenance of its cellularity and differentiation and the fact that it eventually repopulates an entire area of the tissue.

Previous functional assays of stem cells were often clonogenic assays. A *clonogenic cell* is thus a cell that is capable of producing from one cell a large number of progeny, i.e. a clone (see Potten and Hendry 1985a). Clonogenic competence is usually measured by looking at surviving clones and as a consequence clonogenic cells might better be described as those cells in a tissue which if all killed will cause the tissue to degenerate. This is really only a technical aspect of stem cell measurement in some specialized circumstances. If the clone can be demonstrated (usually by secondary clonogenic assays) to contain further clonogenic cells, then self-maintenance has been satisfied. Clonogenic cells thus may satisfy some of the criteria for stemness, e.g. clone formation and self-maintenance. However, it is often not clear whether clonogenic cells are able to regenerate the entire tissue in the long run because they are assayed fairly soon after the damage. This raises the question of whether clonogenic cells are a mixture of stem and early transit cells. It is most likely that clonogenic assays measure all, or a part, of the potential stem cells, which may be a considerable overestimation of the number of actual stem cells.

Ontology, uncertainty and probability

A question frequently posed by biologists is: 'is this particular cell a stem cell?' We refer to this as the ontology question. It implies the idea that one can decide about the capabilities of a given cell without relating it to other cells and without testing the capabilities functionally. We believe that this is a very dogmatic and unrealistic point of view.

As we have seen above, the main attributes of stem cells relate to their potential in the future. These can only be studied effectively by placing the cell, or cells, in a situation where they have the opportunity to express their potential. Here, we find ourselves in a circular situation. In order to answer the question whether a cell is a stem cell we have to alter its circumstances, and in doing so inevitably lose the original cell, and in addition we may only see a limited spectrum of responses. This situation has a marked analogy

with *Heisenberg's uncertainty principle* in quantum physics. In simple terms, this states that the very act of measuring the properties of a certain body inevitably alters the characteristics of that body, hence giving rise to a degree of uncertainty in the evaluation of its properties. The analogy holds true for the functional stem cell assay procedures, all of which study the response after a perturbation to the system thereby challenging the different capabilities of the cells in different though complementary ways. Therefore it might be an impossible task to determine the status of a single stem cell without changing it. We hereby postulate a fundamental uncertainty problem for stem cells. This implies that one will not be able to make a definitive statement about whether or not a given cell is a stem cell. It implies that all statements that we can make will be necessarily probabilistic statements about the future behaviour of the cell under consideration. Essentially this has two particular aspects. The first is that we can only make statements about cell populations in the statistical sense of expected values under a given statistical model. This implies that measurements will necessarily be conducted on populations of cells. However, the second essential aspect is that we cannot disregard the experimental procedure by which the stem cell under consideration was challenged. Each particular measurement or perturbation process may induce a different response in one or several of the characteristics of the stem cell. This is constitutive to the stem cell property, as it is thought to be reactive and responsive to various types of perturbations. Hence, all statements about stem cells and their reactions have to be given in the context of the perturbation of the measurement process under which they were obtained.

This type of uncertainty concept makes it obvious that the ontology question can be rather misleading. We therefore advocate focusing research not so much on the question of the causes of effects but much more on the effects of causes. This relates to comparing the behaviour of stem cell populations exposed to different types of manipulation.

Another aspect to consider here is to what extent a stem cell is intrinsically different from a transit cell and to what extent its differences are merely imposed upon it by its environment. It is extremely difficult to make dogmatic statements here but the indications are that at least to some extent some intrinsic properties exist.

MODELS OF CELLULAR HIERARCHIES

In the previous section we have already introduced some theoretical concepts which can be used to describe cellular development. Here we analyse and extend this topic by giving a brief review of model concepts actually used in various descriptions of biological systems.

The essential aspect of the following three types of model is that they all are designed to describe some features of the cellular development from the stem cells to the maturing cells via transit cells.

Compartment models

A frequently used class of models is based on the compartment concept. It is applicable if one considers a large number of cells in which only the average behaviour is the focus

of interest and where variability and fluctuations do not play a role. Usually such models imply the assumption that all cells in a compartment behave alike and that the behaviour of the compartment can be described by some deterministic laws.

A simple population compartment in connection with stem cells would be one which was purely expansionary in growth (see Figure 2). Such compartments may exist when a large number of stem cells is placed in culture, during early embryogenesis, and possibly under some conditions of wound or tissue repair and tumour growth. It is possible to control the expansionary growth by removal of some stem cells which could for example be achieved by applying a simple spatial cut-off. As the cells reach a particular point in the tissue, for example the top of the intestinal crypt, they are instructed by some signal(s) to become mature functional cells. Such a cut-off could operate via some chemical signal from outside the crypt or by a chemical gradient of intracellular factors.

To our knowledge there are few (if any) mammalian tissues which have only stem cells and mature cells. It is unclear why this is so. Perhaps the switch from proliferation to differentiation and maturation is not a simple change in genetic programs but requires time and even cell divisions (Holtzer, 1985). In a situation where there are only stem cells (A) and mature cells (M), there is a large population of stem cells at risk from genetic error (see Cairns, 1975). One solution to these problems might have been to use the time that it takes for maturation for some continued cell proliferation. Such a dividing and maturing cell population allows much of the workload in terms of cell production to be removed from the stem cell compartment, which as a consequence becomes much smaller and hence offers a smaller target for genetic and carcinogenic damage. This maturation time also allows for the generation of diversity of function, i.e. additional differentiation events, whether this requires rounds of cell division (quantal cell cycles, Holtzer, 1978, 1979) or some other mechanism remains uncertain (Lajtha, 1979b).

This reasoning introduces a new class of proliferative cells, the *dividing transit cell compartment* (*T*) (see Lajtha, 1979a, 1979b; Gilbert and Lajtha, 1965). The concept implies that, wherever a high cell production rate is required, a T population might be expected and the higher the cell production rate the more cell divisions could be expected in the T population (see Cairns, 1975; Lajtha, 1979b; Potten and Lajtha, 1982). The converse might also be expected. If turnover is extremely slow the tissue could in principle operate effectively with just stem cells and maturing differentiated cells. Convincing examples of this type of organization are lacking. It is clear from the scheme represented in Figure 5 that the cell production rate, i.e. the number of M cells produced per unit time, is determined by the number of stem cells considered, their cell cycle time and the number of cell divisions (amplification) in the T compartment. The advantage of the transit population is that it enables some genetic protection to be afforded to the stem cells. It effectively amplifies each stem cell division thus minimizing the number of stem cell divisions required, and hence conserves the stem cell genetic load. It also allows for diversity of specialization at a low cost in terms of proliferation and genetic risk for the stem cells. The disadvantage of such a hierarchy is that the length of time spent in the T population can result in an instability in cell output following damage, i.e. overshoots and fluctuations. This can be overcome by introducing feedback loops and dampening phenomena, such as a high variability of cell cycle or transit times (Wichmann *et al.*, 1988). The total cell output can be controlled by either the number of cell generations in the T compartment, which might be controlled, for example, by a feedback loop from the M compartment, or by the output from the stem cells, i.e. their cycle time. If there

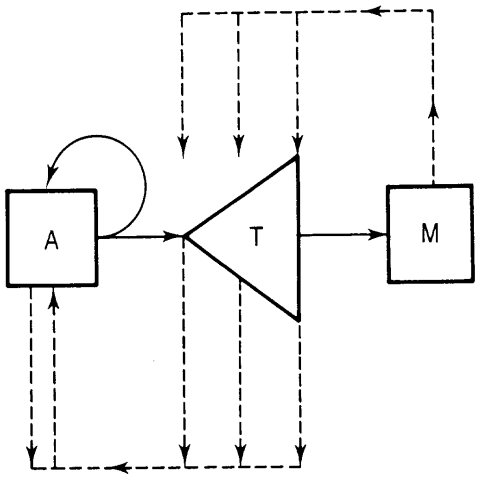

Figure 5 General scheme of an ATM tissue organization with actual stem cells (A), transit cells (T) and mature cells (M). Full arrows indicate cellular development. Dashed arrows indicate possible regulatory feedback. This structure has been proposed for the haematopoietic system (Wichmann and Loeffler, 1985).

are many T generations, there is a logistic problem in terms of the spatial distance in a tissue over which a feedback loop would have to operate from the M population to the stem population. This may be overcome by growth factors that operate over long distances, i.e. hormones, or a breakup of the system into several feedback loops. Such feedback loops are illustrated in Figure 5.

Compartment models are particularly useful to describe cell fluxes and cell production in a dynamic setting to highlight the behaviour with time. A particular advantage is that they are very useful in investigating the assumptions on regulatory control. Such models have in fact been used successfully by many authors who describe the haematopoietic system (e.g. Wichmann and Loeffler, 1985; Mackey, 1978), the intestinal crypt system (Britton *et al.*, 1982; Paulus *et al.*, 1992), the epidermal system and various tumour systems (see also other chapters in this volume). The remarkable technical advantages are that these models can be put into operation using differential equations making them easily manageable by computer simulations.

There are, however, some disadvantages with such models. First of all they are not helpful in taking spatial arrangement of cells and cellular heterogeneity into account. Furthermore, descriptions of systems containing only a few cells will be inadequate, as the stochastic fluctuation in such populations cannot be adequately considered. In addition, compartment models of cellular hierarchies are frequently limited as they include the assumption of a fairly strict link between cellular development towards maturation and the remaining potential for proliferation in the transit cell stage. In other terms such models imply an assumption about a fairly rigid age structure in which the differentiation age and proliferation age are fairly strictly coupled. This implies that cells can only become mature if they have undergone a certain number of transit cell populations. This may not in fact be correct for all tissues under consideration.

Single cell models and pedigrees

In Figure 4 we have already illustrated the principle or idea of a cellular pedigree used to describe the development of a single cell and its progeny. Such a pedigree description would, for example, ideally describe the cellular development seen in an *in vitro* cell culture. Clearly the structure of the pedigree may vary depending on the system, on the growth conditions and on the manipulations previously performed. Furthermore, the temporal development of the cells through the pedigree may be subject to random or systematic variations. It is evident that a cellular pedigree is a much more flexible way of describing cellular development than the compartment approach discussed above. There are various ways of operating such a model technically. One particularly interesting way is to relate the individual cell development to the spatial arrangement of cells. One can, for example, assume that cells are arranged on a two- or three-dimensional lattice and that each cell undergoes a development according to the cellular pedigree. If cells divide on such a lattice, migration processes have to be introduced to rearrange the spatial situation and to remove excess cells from the environment. Such model descriptions usually cannot be described by deterministic rules, and a certain degree of randomness, i.e. stochasticity, has to be introduced. Such models have in fact been successfully developed and applied to the intestinal crypt (Loeffler *et al.*, 1986, 1987, 1988; Potten and Loeffler, 1987; Paulus *et al.*, 1993) and the epidermal basal layer (Loeffler *et al.*, 1987). Such models provide a particular insight into the mechanisms of cellular migration and of development of cells belonging to one pedigree. Similar modelling should also be encouraged for other tissues.

The advantage of a cellular pedigree description is that one can describe systems with only a few cells for which detailed information is available. The conceptual advantage lies in the fact that the pedigrees can vary considerably, become symmetric or become highly asymmetric. Hence the pedigrees provide a microscopic basis for the other models. Furthermore, the pedigree concept permits one to decouple cell proliferation and cell differentiation. It is possible in this concept to develop maturing cells after few transit cell divisions as well as after many. Hence such models are useful in situations where a relative decoupling of the two processes is biologically likely.

Pedigree models technically can be simulated by using stochastic cellular automata. In our view they have not yet been sufficiently exploited.

The screw model

A third class of model is illustrated in Figure 6. Because of its shape we call it the screw model. Its main characteristic is that cell proliferation and maturation/differentiation are assumed to be two independent biological processes. While the cell cycle is represented by a cyclic coordinate on which cells may operate, the maturation/differentiation axis (downwards) indicates the irreversible development towards a mature cell stage. In this cylindrical coordinate system cells travel on a helical downwards spirally. While stem cells are represented by the circle at the top (self-maintenance cycling without differentiation) the transit cell population is characterized by proliferation and a continuous maturation and differentiation downwards. This concept was to our

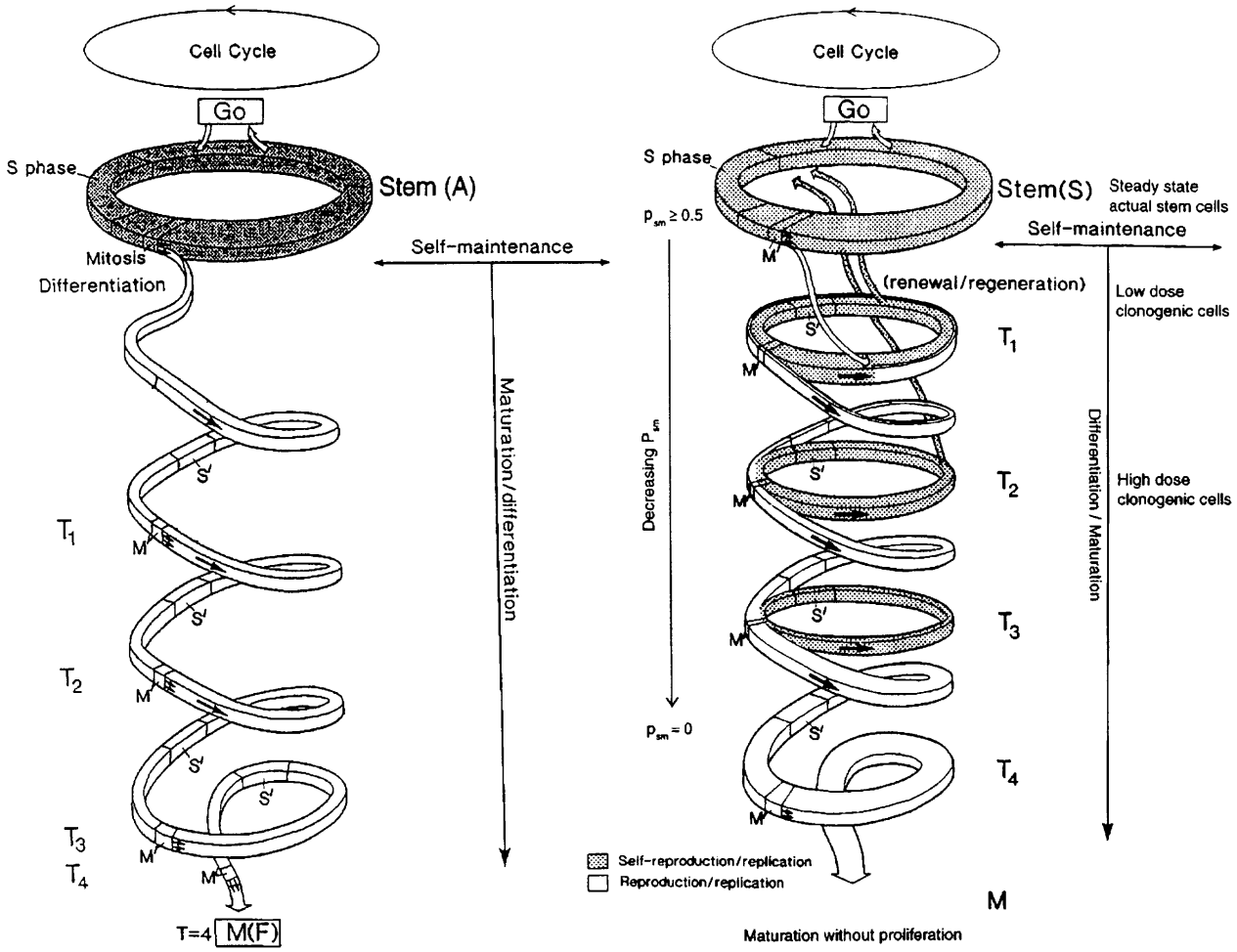

Figure 6 The screw model. Diagrammatic representation of the stem cell (A) and dividing transit (T) and mature (M) cell population. Proliferation is represented by the horizontal cylindrical axis and differentiation/maturation by the vertical axis. The stem cells are characterized by their ability to self-maintain (circle) at the same level of maturation and differentiation with p_{sm} exceeding 0.5 if necessary. The transit cells (T) retain some ability for self-maintenance (horizontal cell cycles as opposed to spiralling cycles). The probability of self-maintenance is always less than that for the stem cells ($p_{sm} < 0.5$) and declines with each transit generation. Thus the probability of a cell progressing down the spiral increases from >0.5 at T_1 generation to 1.0 at the T_4 generation. The ribbons indicate the potential fate of a cohort of cells starting development at the level of A-cells. In a real system the height and width of the ribbons will be much broader and frequently overlapping due to variation in cell cycle times, maturation velocities and self-maintenance processes. Since the T_1 population at least possesses some self-maintenance ability, it can be regarded, when under self-maintenance cell cycle conditions, to be indistinguishable from a stem cell and if a vacant space becomes available in the stem cell environment (niche – see Schofield, 1978) then such a T_1 cell could reoccupy a vacant niche and become an actual stem cell. This represents renewal. Hence renewal is a process that is unique to some transit cells. Having renewed, such a cell could then regenerate the stem compartment, the differentiation spiral(s) and the tissue. Regeneration is thus a property unique to stem cells. The T_1 cells here could be equivalent to the committed stem cells described in some cases. They are also potential stem cells as they have the possibility of renewal. At each level of this diagram, the self-maintenance probability (p_{sm}) changes as does the range of options open to a cell. It is important to realize that since this diagram represents the possible path of a cell in time where there is a bifurcation in the path (cell division in the T_1–T_3 population) the two paths represent the extremes of the options open to individual cells. (a) In the steady state situation self-maintenance at the level of T_1, T_2 and T_3 may not occur and hence the situation would be described by a corkscrew. (b) In regenerative situations self-maintenance and even self-renewal may come into play yielding the picture displayed.

knowledge first proposed by Mackey and Dörmer (1982) and elaborated in a recent review (Potten and Loeffler, 1990).

The situation represented in Figure 6a is one of two alternative possibilities for the T population. It shows a discrete (quantal) change from the stem to the transit population and although the T population in Figure 6a retains the property of division in common with the stem compartment, it cannot self-maintain (stay at the same level of maturation/differentiation). The T population is entirely dependent on an input from the stem compartment. If the stem compartment is removed or destroyed the T population will disappear and has no possibility of maintaining the tissue or itself. In this particular model, only the stem cells have the ability to regenerate the T population and the tissue.

The second and more realistic possibility for the T population is illustrated in Figure 6b, which suggests that the T compartment retains some additional attributes of stemness, i.e. they possess a progressively declining spectrum of stemness and an increasing spectrum of differentiation and maturation. This model suggests that the earlier transit compartments retain a certain ability for self-maintenance, i.e. some do not progress down the screw (i.e. mature) but remain for at least one cycle at the same level. This property may be retained to a lesser extent in the second and third generation (at declining levels). In this case, the feature that distinguishes a transit cell from a stem cell is not so much the question of whether or not it can self-maintain, but its maximum capability of self-maintenance. A transit cell population will by definition always have a p_{sm} value less than 0.5 under steady state conditions. Its ability to vary its p_{sm} may be considerable but restricted, and certainly declines with increasing maturation. The advantage of this model is that some T cells are very similar to the stem cells or indeed indistinguishable from stem cells in some situations but, although these cells may have an ability to behave like stem cells (i.e. $p_{sm} \geqslant 0.5$) in some special circumstances, under normal steady state conditions, they do not. The necessity for considering such a model comes from regeneration experiments *in vivo*, particularly those in the small intestine (see Potten, 1991; Potten *et al.*, 1987; Hendry *et al.*, 1992, and recent developments in techniques for studying bone marrow stem cells (see Chapter 13). It should be noted that the screw model is a much more comprehensive model of the stem–transit–mature (A-T-M) scheme than the compartment concepts in Figure 4 because it inherently allows for a description of a wide variety of different individual cell developments (trajectories). In this respect, it is an extension of the concept of a hierarchical tissue organization (Michalowski, 1981; Gilbert and Lajtha, 1965; Potten, 1974; Loeffler *et al.*, 1987; Clausen and Potten, 1990; Potten, 1991; Potten and Hendry, 1983; Potten and Morris, 1987; Potten *et al.*, 1979, 1982a, 1982b, 1987).

If we consider the scheme in Figure 6b the stem cells within this figure are clearly capable of clone formation and regeneration. A major question is whether any of the T population under conditions of severe cellular depletion could regenerate the epithelium. If this could occur it would involve a sort of rejuvenation process of the T cell, or a renewal of a T cell, i.e. its return to the status of a fully effective stem cell. Thus both stem cells and T_1 cells may be capable of *regeneration* of the tissue but it is only T_1 cells that undergo a process of *renewal*.

Clearly if T_1 cells really represent a spectrum of declining stem cell properties then these questions could in principle be asked concerning the T_2 population and so on.

If we regard the T_1 population as capable of re-entering the stem cell cycle then they constitute a class of *potential stem cells* as distinct from those that are performing stem cell functions which we would term *actual stem cells*. (A second class of potential stem

cells would be those that had actually stopped progression through the cell cycle and therefore were in some *quiescent or* G_0 phase that could at any moment be recalled into proliferation.) We would define the *renewal* of a transit cell population as the fraction of T cells that rejuvenates such that it can perpetually self-maintain and retain the capability of tissue regeneration.

However, the difference between stem cells and T_1 transit cells may be very small and it might be difficult to prove the renewal process on a molecular basis because it would involve the demonstration that an activated T_1 differentiation marker disappears under certain circumstances. On the other hand, there is good experimental evidence in haemopoietic and epithelial systems that fully competent tissue regeneration is still possible even after frequent severe damage, e.g. chronic or fractionated irradiation or drug application.

In the small intestinal crypt such an hierarchical stem cell model has been proposed (Potten and Hendry, 1995). This attempts to take into account the following observations: (a) there are a small number of cells (about six per crypt) that occur at the stem cell location that exhibit an exquisite radiosensitivity and die via apoptosis when small amounts of dosage are induced; (b) after moderate doses of radiation, which would kill all of the above mentioned cells, there are about six other cells that can regenerate the crypt, i.e. are clonogenic; (c) after even higher doses where the above clonogenic cells are sterilized, there are a further 24 resistant clonogenic cells (Hendry *et al.*, 1992; Potten and Hendry, 1995). Altogether they constitute a total of about 36 potential 'stem' cells. The crypt then contains about 124 other proliferating transit cells with no stem cell attributes. The crypt stem cell hierarchy might then be structured as follows:

(i) Up to six actual steady state stem cells – responsible for day to day cell replacement but very intolerant of any DNA damage.
(ii) Up to six potential stem cells which are called into regeneration activity (clonogenic cells) after low levels of injury but when all six actual stem cells are killed.
(iii) Up to 24 other potential stem cells which are the most radio-resistant (good repair system) clonogenic cells called into action after high levels of damage.

STEM CELL MODELS

In the previous section we have discussed descriptions of the cellular hierarchies and in particular the development of cells through the transit cell stages towards the maturing cell stage. In this section we will focus on the various aspects of modelling the stem cell population. This section is not intended to be a review of stem cell models published so far but rather presents a selection of topics that have to be considered.

Clonal succession concept – pseudo stem cell models

There have recently been arguments that several cellular systems may be organized without self-maintaining stem cells. This was advocated in particular for the haemato-

poietic system (Kay, 1965; Abkowitz *et al.*, 1993; Guttorp *et al.*, 1990) and to some extent for the intestinal crypt (Winton *et al.*, 1988; Winton and Ponder, 1990) and possibly for the keratinocyte stem cells found in the hair follice (see Chapter 11). The somewhat simplified basic idea is that a cellular system is maintained by a reservoir of dormant stem cells which are available throughout life time and which can be challenged one after another to enter a transit-cell-like amplification and differentiation/maturation process. Once being triggered such a 'stem cell' would give rise to a large clone of maturing cells. Any of those clones would have a limited life span, as no further input is maintaining the cell flux. After a certain time, which may be long, such a clone would disappear and another 'stem cell' would have to be triggered and recruited to form another clone. This concept is generally considered as the clonal succession theory. The major arguments for these theories stem from observations of somatic mutation experiments in which the occurrence of clones of cells with a specific marker are observed in time. If few such stem cells exhibit the marker it is possible under specific circumstances to observe wide fluctuations in macroscopic clone compositions.

It is evident from these arguments that the clonal succession theory is a concept which is in marked contrast to the stem cell concept of self-maintaining stem cells discussed above. A 'stem cell' in the clonal succession concept is not self-maintaining and can only be viewed as a cell of origin. Once triggered, these cells propagate down the screw of the pedigree development. Hence the clonal succession theory implies that there is a naturally determined age limit for tissues (and organisms in general) which is determined by the exhaustion of the reservoir. It must be noted that in our mind there is so far no clear proof for this concept. As has been analysed for the haematopoietic system and for the intestinal crypt system the majority of findings used to support the clonal succession theory can equally well be integrated into a self-maintaining stem cell concept (e.g. Loeffler *et al.*, 1993). Thus at least for the intestinal system the question on the nature of stem cells is undecided. The basic question of whether or not a small population of self-maintaining stem cells really exists and can regenerate functionally competent stem cells remains to be fully elucidated.

At this point we would like to emphasize that the existence of an age structured stem cell population with a sequence of cells is not by itself an argument for either theory.

The crucial question is whether or not there are cells with self-maintenance capacity in the functional sense. The term 'functional' in this context is important, as it refers to the typical time scale and functional requirements in an organism under stress. For example, it would be sufficient to test whether a stem cell is self-maintaining in the sense that it can reconstitute a tissue for many months, e.g. haematopoiesis, or whether it can sustain this tissue for many years in sequential transplant experiments, which would be a very artificial situation.

Because of the lack of the self-maintaining property in the clonal succession theory we call these concepts pseudo stem concepts. Subsequently we will focus our interests on models which imply functional self-maintenance.

Deterministic models with self-maintenance and self-renewal

As already mentioned above, deterministic models are closely connected to the compartment concept. They represent average cell productions and cell numbers in

single- or multi-compartment systems with or without feedback regulation. Perhaps the most simple stem cell model of this type was already described in Figure 2 where we assumed a homogeneous stem cell pool. A certain fraction 'a' is actively propagating in the cell cycle which has an average cycle time of T. Hence per time unit aS/T cells enter mitosis. Of these cells the fraction p_{sm} self-maintains. This in summary leads to a frequently used differential equation to describe the stem cell dynamics:

$$dS/dt = 2p_{sm}aS/T - aS/T = (2p_{sm} - 1)aS/T.$$

This equation has the important property that p_{sm}, a and T are considered to be independent of each other and therefore can be subject to different regulatory mechanisms. In an extremely simplified case (e.g. tumour growth) all three of these parameters can be constant with time, for example in a small homogeneous tumour. In this circumstance the solution of the differential equation will be an exponential growth characteristic. If we assume that either the fraction of cells in the cycle diminishes with time or the cell cycle time becomes longer with time or the self-maintenance probability reduces with time (e.g. increasing cell loss) then this exponential growth characteristic will slow down, and potentially flatten or even decline. Thus this type of equation can be used for a wide variety of growth circumstances, and several special cases of this type of model are used throughout this book.

We have ourselves used this type of model frequently to describe regulatory processes in stem cells in regenerative situations. It was for example utilized in great detail for the haemopoietic CFU-S population (Wichmann and Loeffler, 1985) and for the intestinal crypt (Paulus *et al.*, 1992).

However, it must be re-emphasized that this model can only be applied to large populations of stem cells where single cell fluctuations do not play a role. Furthermore, more sophisticated and detailed models of the stem cell proliferative process can be envisaged and have in fact been suggested.

In an attempt to understand the recovery behaviour of the small intestinal crypt a deterministic model was used in which self-maintenance properties were attributed to actual stem cells as well as to early transit cell stages under a circumstance of severe demand (Paulus *et al.*, 1992). This was necessary in order to reconcile findings on a small number of actual stem cells in the normal steady state crypts with findings of a much larger number of clonogenic cells becoming active after severe radiation damage. Thus we came to the conclusion that one way of explaining this was to assume a gradual loss of self-maintenance capacity as cells develop through the transit cell stage, e.g. Figure 6b. In the normal situation this capacity may not be utilized and hence these cells appear functionally as transit cells while under other circumstances the same cells can operate in a self-maintaining fashion. Whether this can be regarded as a real self-renewal process with transit cells rejuvenating to become actual stem cells or whether this is simple self-maintenance could not be deduced from these data.

Stochastic models

Stochastic models are legitimate if one considers fluctuations in cell populations due to small cell numbers. The classic model was proposed in the 1960s by Vogel *et al.*, (1968, 1969, reviewed by Wichmann and Loeffler (1983)). It has been used to describe

the CFU-S assay of haemopoietic stem cells. In its basic variant it was described already in Figure 2. We refer to it as the *p, r, q* model, which indicates that three division processes, generating two, one or no daughter stem cells, are possible. In this model one assumes that at each division all three possibilities exist for a given stem cell, that this decision can be described as a random decision and that previous decisions do not play a role (Markovian property). In the model considered by Vogel *et al.* the probabilities *p, r, q* were always assumed to be constant with time. These models were able to explain the large variation of CFU-S numbers found in reseeding and recloning experiments of isolated CFU-S colonies. In fact Vogel *et al.* analysed such data with a restricted version of this model, assuming that only symmetric cell divisions would take place ($r=0$). He found the probability of producing two stem cells was in the range of 0.6–0.7 under most circumstances. As a consequence one obtains a clonal expansion of the stem cell population.

Recently we have applied the *p, r, q* model to the small intestinal crypt (Loeffler *et al.*, 1991, 1993) based on a microscopic growth process of few actual stem cells in the intestinal crypt and were able to explain a wide variety of macroscopic observations of the crypt's behaviour. In particular it was possible to explain a stem cell somatic mutation experiment in which the conversion of a crypt to a new monoclonality of the mutated marker was observed (Winton *et al.*, 1988, 1989; Winton and Ponder, 1990). After about 100 days a crypt with 300 cells converted from one marker status to another starting with an assumed mutation in one single actual stem cell. We deduced from model simulations that if the probability of asymmetric cell division *r* is in the order of 0.95–0.98 with about six actual stem cells being present the data could also be explained (Loeffler *et al.*, 1993). Thus under most circumstances the stem cell division would be asymmetric but in the remaining circumstances symmetric division would cause stochastic fluctuations. In the published version of these models we assumed that the values for *p, q* and *r* were constant with time. However, a number of recent experimental observations hint to the fact that this may not be the case. We have at present good arguments to believe that the probabilities *p, q* and *r* may in fact be variable depending on the actual status a crypt is in. Thus in a situation of severe stem cell depletion there may be a shift towards increasing the probability *p* of generating new stem cells at the expense of the other two processes.

Miscellaneous problems of stem cell biology

There are a number of problems which have so far not been elucidated sufficiently in stem cell models.

The first area of problems relates to the mechanisms of symmetric and asymmetric division. It is somewhat doubtful whether an asymmetric division really exists. We rather believe that quite a number of different processes can contribute to this asymmetry. It is possible that in particular micro-environmental and spatial effects contribute to this process, rendering two daughter cells unequal. Such reasoning would be implied in the niche concept proposed for the haematopoietic system (Schofield, 1978). There may, however, also be cooperative phenomena involved in which the stem cells or other cells send out signals on a local basis, generating something like a morphogenetic field in which cells depending on their position are subjected to such a decision. If such local

processes play a role, the growth situation in an intact *in vivo* situation may be quite different from the *in vitro* environment.

A second area of problems relates to the regulatory processes acting at the stem cell level. Although many people have suggested an autoregulatory process controlling the stem cell population and activity, very little direct biological proof has been gathered. Perhaps one of the most sophisticated stem cell regulatory processes was postulated by our group (Wichmann and Loeffler, 1985) in which the self-maintenance property and the proliferative property of haemopoietic stem cells was assumed to be regulated independently by inter-related feedback loops. One loop relates to the autoregulation of stem cells while the second relates to the demand regulation for differentiated cells. Although this type of regulatory process has shown great potential in explaining a wide variety of experimental situations, still no proof or disproof of this theory has been collected. It should be emphasized that these regulatory concepts are based on the idea of a homogeneous reaction in the tissue which implies that all cells know about each other. In biological terms this mostly implies systemic control processes usually relating to systemically acting growth factor exposure. However, it may well be possible that all these phenomena can also be explained by a different type of concept which originates from the field of synergetics in which self-organization of multiparticle systems is the focus. It will be an interesting area for future research to investigate whether such an idea of cellular next neighbourhood interactions and resulting self-organization leads to new insights into the dynamics of the cellular regenerative tissue. The potential advantage of such an approach is that it takes the spatial arrangement of the cells in their micro-environment constitutively into account.

A third field of problems is the question of how one can describe a hierarchy of stem cells which may develop out of each other and which may gradually lose stemness properties in order to become transit cells. At present one of the strongest limitations of the model discussed so far is that the transition from the stem cell pool to the transit cell pool is a fairly marked transition which may be incompatible with a gradual change as suggested by experiments. Perhaps one has to consider concepts in which the self-maintenance possibility is gradually lost while cells travel down a differentiation/maturation process. It will, however, be a crucial question to investigate whether this is an irreversible process or whether cells can in fact renew to become fully functional stem cells under particular circumstances. Perhaps the best biological system to look at these questions is the intestinal crypt, as we know with great confidence that the crypts are closed systems and all cell regeneration must originate from within the crypt. These crypts live for many hundreds of days with all cells cycling at a high rate. Thus, we assume that stem cells operating in the intestinal crypt not only have self-maintaining properties but may also have self-renewal properties. However, clear proofs for this hypothesis are not present. Similarly in the haematopoietic system it remains to be seen whether long term repopulating cells once having progressed to somewhat more 'mature' cells can become long term repopulating cells again. The basic question is whether the different cell populations develop in a chain fashion or whether they are just different representations of the same type of cells with markers which develop independently of each other.

One of the final considerations relating to stem cells is how they are controlled. How do they know how many stem cells there are and how many there should be? The questions are interesting to consider in relation to the crypt. There are indications that

the six actual steady state stem cells somehow monitor their members and know if an extra stem cell is produced, perhaps as a consequence of occasional symmetric divisions. Unless one stem cell is removed either by differentiation or death the crypt architecture would be lost since an entire extra transit lineage of 32–64 cells would be produced. It is believed that the occasional spontaneous apoptosis seen at the stem cell position removes such excess stem cells. Conversely if using low dose radiation a simple stem cell is killed (induced into apoptosis) there is good evidence that proliferation in the stem cell zone is up-regulated to compensate. The mechanism by which this monitoring of numbers is achieved is conceptually complex and completely unknown. The six stem cells are believed to be located in a ring of 16 cells which on average is at the fourth cell position from the crypt base. However, this is an average. In some cases the stem cell might be at the second cell position while in others it might be at the seventh. Even if they were all at the fourth position they would not be touching since other cells would be interspersed. Nevertheless, one way or another they seem to know that six is the correct number and can detect if the number changes to five or seven and respond accordingly.

There are two further hypotheses which have to be mentioned in the context of stem cell biology and models. One is a concept of chromosomal DNA segregation postulated by Cairns (1975). This hypothesis concept postulated that stem cells might be characterized by a selective chromosomal segregation process which would have a specific function in genetic hygiene by effectively maintaining the template DNA strands in the stem cell and transferring all newly synthesized DNA strands with potential replication errors to the transit cells where they would not be as harmful. At present, however, there is no proof or disproof for this speculation.

The other hypothesis is one of ageing of cells where it is assumed that stem cells have only a limited number of possible cell divisions. This hypothesis is closely related to the concept of clonal succession, although it is not identical. A recent variant of this concept is known as the telomere shortening process (e.g. Vaziri *et al.*, 1994). It was observed that the terminal end of DNA usually contains many repeats of a six base pair motive and that this shortens with the age of an organism. It was therefore suggested that all cells have only a limited division potential and that their life span may expire. However, presently no proof is available that this limitation has an actual impact on stem cell biology.

CONCLUDING REMARKS

In this chapter we have summarized some of the present concepts and models of stem cell behaviour and cellular hierarchies. Sime of the topics that we find relevant at present have been highlighted. Furthermore, it was designed to be an introduction to the various stem cell concepts discussed throughout this book and to illustrate how these concepts relate and differ. It was therefore not intended to be an encyclopaedic summary of the various models published so far and we apologize for all models and groups not mentioned in this chapter.

On the other hand we wanted to point out that a standard or unified stem cell hierarchical model is lacking. We also point out why this is the case and why it will remain a matter of active research in the foreseeable future. We believe that the stem cell problem is closely connected to the question of describing the tissue organization

from an integrated point of view. It does not appear to be very meaningful to model a stem cell population in an isolated way, but rather its description should be integrated into a comprehensive theory of dynamic regenerative tissues.

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