The small intestine as a model for evaluating adult tissue stem cell drug targets¹

Christopher S. Potten, Catherine Booth and Danielle Hargreaves

EpiStem Limited, Manchester, UK

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Abstract. Adult tissue stem cells are defined and some current controversies are discussed. These crucial cells are responsible for all cell production in renewing tissues, and play a vital role in tissue regeneration. Although reliable stem cell markers are generally unavailable for adult epithelial tissues, the small intestinal crypts are an excellent *in vivo* model system to study stem cells. Within this tissue, the stem cells have a very well-defined cell position, allowing accurate definition of stem cell specific events. Clonal regeneration assays for the small intestine allow stem cell survival and functional competence to be studied. The ultimate lineage ancestor stem cells are extremely efficiently protected from genetic damage, which accounts for the low cancer incidence in this tissue. Some of the regulatory networks governing stem and transit cell behaviour are beginning to be understood and it is postulated that p53 plays a crucial role in these processes.

WHAT ARE STEM CELLS?

The replacing tissues of the body such as bone marrow, gastrointestinal epithelium, skin, oral mucosa, some glandular tissues and spermatogenic epithelium are all ultimately dependent upon a small number of specialised proliferating cells, the stem cells. The removal of these crucial cells, by exposure to specific cytotoxic agents, will result in the collapse of the tissue, as it is totally dependent upon these stem cells. For example, the development of mucositis in the intestinal and oral epithelium after exposure to chemo- and radio-therapy is a direct result of damage to the stem cells and the loss of their dependent cell lineages. There have been many attempts to write definitions of stem cells, which often tend to be context-dependent. Hence, subtly different definitions are obtained by development biologists, hematologists, botanists, etc. In 1990, together with Dr Markus Loeffler, we attempted to formulate a definition that would be applicable to all the replacing tissues of the adult body and which took into account the various data sets relating to these cells (Potten & Loeffler 1990). Within a tissue such as the intestinal epithelium, the epidermis, or the bone marrow, the stem cells could be defined as *a small population of relatively undifferentiated, proliferative cells that maintain their population size when they divide, while at the same time producing progeny that enter a dividing transit*

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Correspondence: EpiStem Limited, Incubator Building, Grafton Street, Manchester M13 9XX, UK. Tel.: 44(0)161 6067258; Fax: 44(0) 161 6067348; E-mail: c.potten@epistem.co.uk

population within which further rounds of division occur together with differentiation events which resulted ultimately in the production of the functional cell types required of the tissue. During the course of the animal's lifetime these cells persist, i.e. are anchored, and divide a large number of times. Probably as a consequence of this large division potential, these cells are the most efficient repopulators of the tissue following injury. During this tissue regeneration process, the probability of self-maintenance can be raised from the steady state value of 0.5 to values between 0.5 and 1, thus ensuring an expansion and repopulation of the stem cell compartment, while still maintaining the differentiated cell lineages. It is clear from the definition that stem cells can also be regarded as the cells at the origin of any cell lineages (see Fig. 1a). In the conditionally renewing systems, such as liver, kidney and some glandular epithelium, the existence of a small class of more quiescent stem cells can be inferred, but not always convincingly proven. In other, even less proliferative, tissues such as the central nervous system and muscle, small populations of largely quiescent stem cells have now also been inferred. Thus, there is a strong likelihood that all the tissues of the body contain small numbers of these crucial cells.

Differentiation is regarded as a qualitative change in a cell relative to others in the tissue. It is thus a relative change in a cell's appearance or behaviour. Adult tissue stem cells are undifferentiated relative to most other cells in a tissue but may be differentiated relative to embryonic stem cells.

Under stable steady-state conditions, these cells can be thought of as dividing in an essentially asymmetric fashion, producing one daughter that retains the stem cell characteristics while the second daughter enters a cell lineage within which further divisions may occur and from which differentiated functional cells are ultimately derived. During the development of the tissue, and also following destruction of some of the adult tissue stem cells, this asymmetric division mode must be transiently changed towards symmetric divisions in some stem cells in order to replace the lost or damaged stem cells, and to allow tissue growth during the latter phases of development. Such symmetric division must also occur in the early embryo and during fetal development. These early embryonic cells clearly possess an enormous division potential as well as the ability to produce all the differentiated cell types required by the organism, a process referred to as differentiation *totipotency*. This division potential and totipotency can be clearly seen when embryonic stem cells are extracted from the inner cell mass of a blastocyst and grown in culture.

However, embryonic stem cells are not the subject of this paper. Here we are concerned with the stem cells that reside in the tissues of the adult body, which may have been through a series of progressive restrictions in the range of differentiated products that can be produced. The issue of whether or not adult tissue stem cells have a restricted differentiation potency is a matter of considerable debate in the current literature and, until these issues are resolved with appropriate reproducible and convincing experiments, the topic is perhaps well avoided. It should, however, be pointed out that the stem cells in the bone marrow, that are responsible primarily for the production of the full range of cell types in the blood, seem to have an extraordinarily broad range of differentiation options and are often referred to as being *pluripotent*. Current studies suggest that bone marrow stem cells may even be able to colonise tissues like the adult liver and produce many functional hepatocytes if the liver is subsequently injured, but may not themselves be capable of regenerating a subsequently damaged liver (Alison *et al.* 2000; Lagasse *et al.* 2000; Theise *et al.* 2000; Wang *et al.* 2002). Hence, these stem cells are often referred to as having a greater *plasticity* in terms of differentiation options than was previously thought. Indeed, it may be that given modern developments in cell biological experimentation, adult tissue stem cells, such as those in the bone marrow, may be found to be capable of differentiating into as many different cell types as embryonic stem cells, in which case the distinction between embryonic stem cells and adult tissue stem cells disappears. However, this is yet to be proven.

(a)

(b)

Figure 1. Typical stem cell derived cell lineages. (a) A self-maintaining stem cell (circle) divides to generate a dividing transit lineage (squares). Differentiation events generate different types of functional cells (various symbols). (b) A lineage within which the differentiation event that distinguishes transit cells from stem cells occurs after the third generation in the lineage, thus establishing a potential stem cell compartment.

ACTUAL AND POTENTIAL STEM CELLS

It is clear that in some tissues the differentiation event that distinguishes a stem cell from a dividing transit cell does not necessarily occur at the level of the stem cell division but may be postponed for one or two generations down the dividing transit lineage (Fig. 1b). This may be associated with a gradual loss of stemness as appears to be the case in the bone marrow. Another

way of considering this is that the stem cell compartment of a tissue is itself hierarchical. The advantage here is that the tissue is protected against the sudden loss of the ultimate lineage ancestor stem cells, removal of which can be compensated for by any cell within this early part of the lineage. This has given rise to the concept of *actual stem cells* that are responsible for the day-to-day maintenance of the tissue and *potential stem cells* that can replace the actual stem cells if they are killed (Fig. 1b) (Potten & Loeffler 1990; Potten *et al.* 1997; Potten 1998; Marshman *et al.* 2002). A consequence of such a scheme is the fact that all the stem cells, both actual and potential, now divide in a symmetric fashion as opposed to the inherently asymmetric division process in Fig. 1(a). Of course, to cure a tumour, all actual and potential tumour stem cells would have to be destroyed, ideally without the concomitantly killing of all the actual and potential stem cells in the normal tissues of the body.

One question that such a scheme raises is what is it that distinguishes the actual stem cell from its progeny, the potential stem cells. The most likely explanation here is position within the tissue, the micro-environment, or niche, in which the actual stem cell is located. On division of an actual stem cell, only one daughter can remain within that niche. This inevitably leads to the question of whether stem cells are intrinsically different from other cells in the lineage or are they simply instructed by the micro-environment to function as stem cells. This is a subject of considerable ongoing debate, but there are some indications in some tissues, for example the intestine, that the actual stem cell has some intrinsically different properties (Potten *et al.* 2002). This does not, however, rule out the possibility that other important instructive signals act on these crucial cells.

STEM CELL LINEAGES

One of the major differences that one sees as one moves from one tissue to another is that the size of the cell lineages derived from the stem cells varies, i.e. the number of cell generations within the lineage. Probably the greatest number of generations can be seen in the spermatogenic lineage, closely followed by some parts of the haematopoietic cell lineages. In the spermatogenic lineage there is considerable cell death that occurs so that the cell output is not strictly 2 to the power of the number of generations. In the bone marrow, there is considerably less cell death and large numbers of cells are produced each time a stem cell divides. In the epithelial tissues, skin, intestine, oral mucosa, the number of generations varies from about 3 up to 7 or 8. As a consequence of this type of organisation it is obvious that the stem cells themselves represent a very rare class of cells when the total proliferative compartment of the tissue is considered (see Fig. 2). Thus, stem cells make up only a fraction of 1% of the proliferating cells in the bone marrow and approximately 1–10% of the proliferating cells in epithelial systems. This makes it very difficult to study these rare and elusive, but crucial, cells, particularly as there are few stem cell specific markers. In fact, one characteristic of these undifferentiated cells is that they appear to lack most markers that have so far been studied.

THE ROLE OF STEM CELLS IN THE DEVELOPMENT OF CANCER

It can be argued that epithelial tumours arise from the adult stem cell, for the following reasons. It is a well-established fact that in order for a normal cell to transform into a cancerous cell, several independent mutations have to occur. These changes may take a long time to accrue and

Figure 2. Schematic showing the possible size of the transit cell lineages in various replacing tissues of the body (mouse). The proportional size of the stem cell compartment decreases as the lineages increase.

occur in a specific sequence. These mutations are chance events and to accumulate all that are necessary for cancer formation takes considerable time, accounting for the fact that the latent period between exposure to a known carcinogen and the development of the cancer is months in a mouse, up to decades in man. In practice, this means that the cancer target cell, within the cell lineage of a proliferative tissue, must be a cell that persists in the tissue for months in a mouse and decades in man. The only cells that satisfy this requirement are the lineage ancestor stem cells. For this reason, it is generally accepted that cancer is a disease associated with the tissue stem cells. Mutations may occur in any cells within the lineage, but those that occur in cells other than the stem cells are rapidly discarded through differentiation and cell loss. The only slight complication to this situation is the fact that any accumulating mutations in the stem cells will also be transmitted to all the progeny of stem cells that enter the dividing transit compartment. Hence, given time, the tissue may contain stem cells and entire stem cell-derived lineages that carry all the genetic mutations required for cancer development, except for the last one. This final mutation could occur in any cell in the lineage, and whether or not a cancer develops will depend on where the cell is located, how far it is down the differentiated lineage, and whether it can stop the cell migration and cell-loss process.

The changes in the controls on proliferation, differentiation and cell death of stem cells that occur in cancer formation are yet to be fully elucidated. However, one crucial process that may be involved is the regulation of the probability of self-maintenance. If this were to switch in a permanent way from being 0.5 in steady state to slightly greater than 0.5 (e.g. 0.51), this would result in a gradual increase in the stem cell numbers. As each stem cell produces a stem cell-dependent lineage, the tissue would gradually grow and become hyperplastic (polyp formation) and with further mutations develop into a cancer. It is also clear that, in most cancers, a cell-lineage scheme is maintained, albeit with some permanent change in the controls outlined above. Thus tumours, whether benign or malignant, are likely to contain cancer stem cells and cancer stem cell-derived lineages (dividing transit cells). Understanding the biology of stem cells will obviously help one understand the role that they play in the development of cancer and in the strategies for treating cancer patients.

STEM CELLS AS DRUG TARGETS

Defining the specific response of stem cells to novel therapies, rather than the overall tissue response, can provide predictive information on the likely clinical response. If a drug has stem cell activity, it will impact on the therapeutic index if there is differential effect on tumour and normal stem cells. Furthermore, factors or drugs that control the behaviour of the adult tissue stem cells have enormous commercial potential. The ability to control the behaviour of stem cells in normal tissues would have significant advantages in improving wound healing by speeding up stem cell responses and cell cycle activity. In anti-mucositis strategies in cancer therapy patients there would be advantages in temporarily switching stem cells out of cycle, making them more resistant to some chemotherapy drugs.

There is also significant value in manipulating stem cells in diseased tissue. For example, one might design drugs that have the ability to manipulate cell cycle activity in stem cells in hyperproliferative disorders, such as psoriasis.

In order to cure cancers, but prevent dose-limiting and potentially lethal side-effects (one of the biggest problems faced by clinicians), strategies that specifically target and kill tumour stem cells, but at the same time spare the normal tissue stem cells, are required.

Stem cell responses in animal models are also a useful screen for potential carcinogens, identifying early responses in this persistent population of cells. There is also enormous potential for stem cell manipulation in the treatment of age-related diseases (as such diseases can essentially be attributed again to the long-lived stem cell population).

STEM CELLS AND WOUND HEALING

As stem cells have to support the cell replacement processes and wound healing function in a given tissue for the entire life of the animal, they must have a large division potential. In the small intestine of the mouse, these crucial lineage ancestor cells appear to divide about once a day. In the lifetime of a laboratory mouse (approximately 3 years) this equates to 1000 divisions. In humans, the best estimate for the division potential of the small intestinal stem cells is between 5000 and 6000 divisions in a lifetime. The stem cells appear to be capable of completing these divisions without dramatic deterioration with age and, in the case of the small intestine, without apparently accumulating the genetic errors that lead to cancer (a very rare event within the small intestine) (see Table 1).

Following injury to the tissue, or the destruction using drugs or radiation of some of the stem cell compartment, surviving stem cells repopulate the tissue through a process of clonal growth, hence, these cells are sometimes referred to as *clonogenic cells*. This approach has led to *in vivo* techniques that have allowed the stem cell populations to be studied (Till & McCulloch 1961; Withers & Elkind 1970; Potten & Hendry 1985).

STEM CELLS AND TISSUE ENGINEERING

There is currently considerable interest in the possibility of growing tissues *ex vivo* for subsequent transplantation. In order for this to be successful, it is first necessary to be able to

Table 1. Some estimates for the number of stem cells, cell output, cell-cycle times and cancer incidence data for the small and large intestine of mouse and man

	Mouse	Man
Small intestine		
Length	0.2 m ($\times 3.3$)	5.5 m ($\times 3.7$)
Stem cell cycle	24 h ($\times 1.5$)	~ 120 h ($\times 1.4$)
Total crypts	7.5×10^5 ($\times 1.7$)	5×10^7 ($\times 2.5$)
Total stem cells	4×10^6 ($\times 1.7$)	2.5×10^8 ($\times 2.5$)
Number of divisions in lifetime for a stem cell	10^3 ($\times 1.4$)	5.5×10^3 ($\times 1.5$)
Total stem cell divisions in lifetime	4×10^9 ($\times 2.5$)	1.4×10^{12} ($\times 3.5$)
Cancer incidence/mortality (200 000, UK 1995)		450 M + F 233
Large intestine		
Length	0.06 m	1.5 m
Stem cell cycle	~ 36 h	~ 168 h
Total crypts	$\sim 4.5 \times 10^5$	$\sim 2 \times 10^7$
Total stem cells	$\sim 2.3 \times 10^6$	$\sim 1 \times 10^8$
Number of divisions in lifetime for a stem cell	~ 730	~ 3600
Total stem cell divisions in lifetime	$\sim 1.6 \times 10^9$	$\sim 4 \times 10^{11}$
Cancer incidence/mortality (200 000, UK 1995)		31 230 M + F ($\times 70$) 17 330 ($\times 74$)

The values for mouse small intestine are reasonably well defined. The mouse colon data are less precise while the data for man are based on very limited studies and approximations. The values in brackets represent the fold increase relative to the large intestine which has 70–74-fold more cancers than the small intestine.

maintain and expand the stem cell population *in vitro*, so that ultimately the entire cell lineage organisation, including all the differentiated functional cells of the tissue, can be generated in a controlled manner in a culture dish and that these are subsequently grown on an appropriate scaffold prior to transplantation. For epithelia this has so far proved to be difficult (even for keratinocytes), largely because the crucial stem cell-regulating factors (the growth factors that instruct stem cells to expand their numbers: divide symmetrically) that would be needed in culture have not been identified. However, this is another important area of current stem cell research.

STEM CELLS AND GENE THERAPY

With the development of modern molecular biology approaches that have enabled transgenic cell lines and animals to be established, the concept of gene therapy was formed, whereby genetic defects might be corrected by inserting normal genes into defective cells. Considerable work is still needed to develop reliable and safe vector systems to get the genes into the DNA of the target cells. Transfection into cells within the dividing transit lineage would only result in a transient benefit. In order for a stable permanent cure, the normal genes would need to be inserted in the lineage ancestor stem cells of the tissue. For this to be achieved, the stem cells have to be targeted either *in situ* or by extraction, culturing and expansion, and then transplantation.

Gene therapy offers great hope for the cure of epithelial proliferative disease, but is dependent on further studies into the cell biology and regulation of the tissue stem cells and the ability to target these cells or extract, purify and expand them prior to targeting. Targeting the cells with a safe and reliable vector system to carry the transgene into the stem cell's DNA is a challenge still to be convincingly demonstrated.

STEM CELLS IN THE SMALL INTESTINAL EPITHELIUM

The intestinal epithelium has proved to be a useful cell biological model system for studying some aspects of stem cell behaviour *in vivo*. In both mouse and man, the small intestinal epithelium is divided into discrete differentiated units called *villi* and proliferative units called *crypts*. In the mouse, the crypts contain about 250 cells in a flask-shaped structure, typically with 16 cells circumferentially round the flask. The uniquely valuable feature of this tissue is that the position of cells along the longitudinal axis of the crypt relates exactly to the cell lineage. The very base of the crypt is occupied by a functional differentiated compartment, populated by Paneth cells, and above them sit the stem cells, located in the 4th or 5th annulus of 16 cells from the base of the crypt. A number of characteristics can be attributed to the cells at the 4th or 5th position from the base. These cells have a cell-cycle time in the mouse of 24 h, i.e. they divide once a day. They very rarely develop cancer and, hence, these cells must have very effective protective mechanisms against the accumulation of genetic damage. Both small and large intestine have the type of lineage displayed in Fig. 1(b) with actual stem cells and potential stem cells. It is believed that each crypt contains between 4 and 6 lineages, and that there are about 6 generations within each lineage in the small intestine. The crypt contains about 30 potential stem cells and 4–6 lineage ancestor actual stem cells (see Fig. 3) (Cai *et al.* 1997; Potten *et al.* 1997; Potten 1998; Marshman *et al.* 2002)

DNA strand segregation

DNA replication, which must occur at each cell cycle, is probably the most genetically hazardous event in the life cycle of a cell. It has recently been shown that the actual lineage ancestor cells in the mouse small intestinal crypt protect themselves against replication-induced error by selectively sorting the old and new DNA strands at mitosis and retaining the old template strands which are error free in the daughter cell destined to be the stem cell. (Cairns 1975; Potten *et al.* 1978; Cairns 2002; Potten *et al.* 2002). The newly synthesized strands, which may contain genetic error, are transferred to the daughter cell that enters the lineage and is destined to be lost from the tip of the villus 5–7 days later. It has also recently been shown using cell culture systems that p53, a crucial gene involved in other genome protective mechanisms, plays a role in this selective strand segregation process (Merok *et al.* 2002) (Fig. 4).

It is further hypothesised that, in order to prevent the accumulation of mutations in the immortal or template strands of DNA, those forms of DNA repair that involve strand exchange or local re-synthesis of damaged strands would have to be inhibited. One consequence of which would be the prediction that the actual stem cells would be extremely sensitive to genotoxic agents such as radiation. This is indeed the case in the small intestinal mucosa. If DNA is regarded as the target, four-six cells at the stem cell positions in the crypts have a radiosensitivity equivalent to the ultimate radiosensitivity (Potten 1977; Hendry *et al.* 1982; Paulus 1992; Potten & Grant 1998), one radiation-induced damaging event anywhere in the DNA molecule appears to trigger an altruistic cell suicide or apoptosis rather than cell-cycle arrest and repair. This

Figure 3. Cell lineage believed to account for cell production in the crypts of the mouse small intestine. The position of cells in the lineage can be related to position of cells on the crypt-villus axis. A similar lineage is believed to explain cell production in the large intestinal crypt. (CP: cell position.)

altruistic apoptosis is totally p53 dependent and represents a second highly effective protective mechanism. The selective DNA strand segregation process is an absolute protection for the cells against replication-induced errors, as all errors are transferred to the daughter cell destined for loss, while the accumulation of genetic errors in the template strand or double-strand breaks for example, are rapidly and effectively removed by the death of the cell and, hence, the removal of the damage. This can be achieved as the lineage contains abundant potential stem cells that replace any cell deleted by this altruistic apoptosis (Fig. 5).

There are major implications that result from the selective strand segregation hypothesis for stem cell biology and genetics, cancer biology and ageing. In the latter case, the retention of the template strand consistently in the stem cell line would imply that the telomere shortening problems that have been associated with ageing do not occur in these cells, which accounts for the fact that they seem to be capable of dividing 1000 times in the mouse, without dramatic deterioration in their functional competence.

The selective strand segregation and retention of template strands in stem cells offers the possibility of exclusively labelling these crucial cells. This approach involves the use of DNA synthesis markers such as tritiated thymidine or bromodeoxyureidine delivered to stem cells at times when new stem cells are being made and, hence, new template strands are being synthesised. If this is achieved, and the timings for this are rather difficult to ascertain, then the DNA synthesis markers can be incorporated into the template strands, which will then be permanently marked, as will the cell carrying these strands. This approach has been used effectively in epidermis (Bickenbach 1981; Morris *et al.* 1985) and now also in the intestine (Potten *et al.* 2002) to generate what have been called label-retaining cells (LRCs) (Fig. 6a). This is clearly a way

Figure 4. Hypothetical role of p53 guarding the genome of stem cells in the small intestine. p53 controls damage-induced apoptosis, asymmetric cell divisions and selective strand segregation in stem cells while directing cell-cycle arrest and repair in transit cells.

Protective mechanisms operating at the stem cell level in small intestinal crypts

Figure 5. Diagram illustrating the protective mechanisms operating on the small intestine stem cells.

Stem Cell Identification/Responses

Figure 6. Sections of mouse small intestinal crypts showing label-retaining cells (left panel) (Potten *et al.* 2002a) at cell positions 4–5 and Musashi-1 antibody staining (Potten *et al.* 2002b) of cells also at the same position (next panel to right). Some cells are also exquisitely sensitive to small doses of radiation and die via apoptosis (Potten 1977; Hendry *et al.* 1982; Potten & Grant 1998) (right-hand panel). The second-to-right panel shows the cells that respond to injury (5-fluorouracil exposure) by entering S phase at 24 h (bromodeoxyuridine labelling).

of marking the lineage ancestor stem cells. Other markers for intestinal stem cells are currently being developed. One of these, Musashi-1, is not entirely specific but marks the early part of the lineage (Potten *et al.* 2003). (Fig. 6b). N-Ras may be another similar marker. Musashi-1 is a gene associated with asymmetric divisions in neural progenitors in *Drosophila* (Nakamura *et al.* 1994; Kaneko *et al.* 2000; Imai *et al.* 2001) and is thought to be involved with blocking the translation of Numb protein which is linked to the Notch/Delta signalling pathway (Fig. 7).

p53 regulation

It is clear that p53 plays a role, not only in determining this altruistic apoptosis in the stem cell compartment, but also in cell-cycle arrest and repair processes that are more likely to occur in the dividing transit population. It may also play a role in the permanent cell-cycle arrest or premature differentiation that occurs following doses of radiation, which is associated with the dividing transit compartment (Paulus *et al.* 1992). Recent work suggests that it also plays a role in some cell culture systems in determining asymmetric division and the selective strand segregation process outlined above (Merok *et al.* 2002), both of which are properties associated with the stem cell compartment (Fig. 4). What the small intestinal system seems to suggest is that p53 function differs in the stem and transit population as indicated in Fig. 4.

β catenin regulation

It has recently been shown that part of the regulation of the lineage organisation and the differentiated components in the intestine, together with the migration of cells, is regulated by the β catenin/TCF complex (Battle *et al.* 2002; Booth *et al.* 2002; van de Wetering *et al.* 2002). This interacts with the APC gene product in regulating various aspects of the intestinal epithelium.

Figure 7. Part of the Delta-Notch signalling pathway that is believed to be involved in determining asymmetric cell divisions in various developmental systems. Musashi-1 blocks the translation of M-Numb and is implicated in asymmetric divisions in early neural stem cells (Nakamura *et al.* 1994; Kaneko *et al.* 2000; Imai *et al.* 2001).

This complex interacts with p21 which regulates cell cycle arrest and cMyc that then regulates the proliferative compartment. Another group of genes (tyrosine kinase receptors and their ligands) the EphB/ephrin family appear to play an important role in regulating the differentiation process (See Fig. 8).

UNRESOLVED PROBLEMS ASSOCIATED WITH STEM CELLS

There are a number of problems that still remain to be elucidated by further stem cell studies:

- Further specific stem cell markers are required that will facilitate the *in vivo* study of these cells and their extraction, isolation, purification, prior to use in cell culture systems.
- Further information is needed concerning the genes and proteins that regulate the behaviour of these crucial cells *in vivo* (stem cell growth factors; regulators of proliferation, self-maintenance, differentiation and cell death or apoptosis). The identification of these factors will greatly facilitate the ability to expand stem cells *ex vivo* and also to manipulate them to clinical advantage *in vivo*. These factors clearly have great potential value as new clinical drug targets.
- There is still considerable uncertainty relating to whether stem cells are intrinsically different from other dividing cells or are merely instructed to behave differently by their environment. This inevitably also leads to the question of whether stem cells routinely divide symmetrically or asymmetrically in steady state conditions? These problems have already been alluded

Crowd Control in the Crypt

Figure 8. Schematic showing the interactions between β -catenin/TCF and ephrins in the possible controls on differentiation and cell migration in the small intestinal crypt (Wetters van de *et al.* 2002; Battle *et al.* 2002; Booth *et al.* 2002).

to above. This inevitably also raises the issue of environmental signalling to the stem cells.

- It is still unclear as to whether stem cells exhibit any deterioration in functional competence or division potential with ageing. Are they mortal or immortal cells? The intestinal stem cells are certainly cells that possess an enormous division potential, but it is unclear whether this is limited or not. Do we age because of deterioration in stem numbers or functional abilities? The answer to this question clearly has enormous commercial potential in terms of anti-ageing drugs or treatments.
- The final uncertainty concerning stem cells, which is subject to considerable scientific debate and discussion at present, is the extent to which adult tissue stem cells have the potential for, or can be induced into, producing cells that differentiate down different lineages from those that are normally expected for that stem cell. It is clear that bone marrow stem cells possess a large pluripotentiality in terms of the differentiation options of their daughter cells. The intestinal stem cells can produce daughters that differentiated down at least four or five different pathways. It has recently been shown that bone marrow stem cells can repopulate parts of the liver producing hepatocytes if the liver is injured (Alison *et al.* 2000; Lagasse *et al.* 2000; Theise *et al.* 2000; Wang *et al.* 2002). This indicates a greater plasticity for bone marrow stem cells than was initially thought possible. There are some that believe that some adult tissue stem cells may have considerable plasticity, providing the correct signals are given to

induce the necessary range of differentiation options. Indeed, the nuclear transfer techniques used to clone animals illustrates that even a differentiated cell nucleus can be reprogrammed to act as a totipotent stem cell. This suggests that manipulation of stem cells to make different tissues should be possible but may not be a process that ever occurs in natural conditions. Whether stem cell plasticity is limited or unlimited determines whether adult tissue stem cells are really different from embryonic stem cells or not and this remains to be resolved. Thus, considerable further work and validation is required in this field.

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