REVIEW

Stem Cells in Epithelial Tissues

J. M. W. Slack

Most, if not all, epithelial tissues contain stem cells. They are responsible for normal tissue renewal or for regeneration following damage. Our present knowledge of their properties is limited and is mainly derived from studies of cell kinetics and from clonal analysis.

About 60% of the differentiated tissue types in a mammalian body are epithelia (*1*). The range of their functions is vast and frequently involves the secretion of bioactive materials and absorption of substances as well as the mechanical integrity of surfaces. How epithelia are formed and maintained is one of the key problems of developmental biology and an area in which many basic questions remain unsolved. Some epithelia, such as the skin or intestine, show rapid cell turnover (*2, 3*), whereas others, such as the liver or pancreas, show a very slow turnover under normal conditions but with special adaptations for regeneration (*4–6*).

So all epithelia will probably prove to contain cells that are capable of repopulating them, either during normal life or at least under circumstances of tissue repair. Various definitions for a "stem cell" have been adopted by different authors, but a consensus definition is likely to include at least two ideas: stem cells are able to reproduce themselves throughout the life-span of the animal, and they are able to give rise to differentiated cells (*7*). To this is often added the idea that stem cells are visibly undifferentiated. However, this would exclude some populations that are often described as stem cells, such as cells of the basal epidermal layer or those of the pancreatic and bile ducts. Stem cells are also often thought to undergo obligatory asymmetric division to yield one stem cell daughter and one daughter destined to differentiate. This may be true in some situations, but it is not a necessary attribute because the population of stem cells can still be self-maintaining when some divisions yield two stem cell daughters and others yield two differentiating daughters.

Commitment of Stem Cells

With certain exceptions that will be discussed below, epithelial stem cells are considered to be developmentally committed such that they can form the differentiated cells of their own particular tissue type but not those of any other. In studies on early development, we are now accustomed to the idea that developmental commitment is encoded as a combination of transcription factors (*8*). The same is presumably true for epithelial stem cells, but because of their relative inaccessibility and the difficulty of isolating them for experimentation, there is currently no type that can be characterized by its transcription factor combination.

Cell division is not, in itself, an indication of stem cell status. Cell kinetic studies have shown that stem cells are usually slowly dividing and that most of the dividing cells in a tissue are "transit amplifying cells" that are committed to differentiate after a finite number of divisions (*2, 9*). The presence of the transit amplifying cells means that the tissue can maintain a high output of differentiated cells from a small number of stem cells.

There is some characterization for epidermal stem cells, which have been shown to carry higher levels of certain cell adhesion molecules on their surfaces and also to contain a higher level of β-catenin (*10–12*). In the hair follicle, cytokeratin 15 has been reported as a stem cell marker (*13*). In the small intestine, knockout mice for *TCF4* fail to form a proliferative compartment (*14*). TCF4 is a high mobility group–box transcription factor that normally associates with β-catenin in response to Wnt signaling, so it may be important that these elements of the Wnt pathway have been found playing a role in two different types of stem cell.

Structural-Proliferative Units

In the traditional renewing cell population, there is a clear relation between the activity of the stem cells and the histological structure of the tissue. The dividing cells are located in one place, and the differentiated cells lie elsewhere. For example, in the intestinal crypt, the stem cells are present near the crypt base, the transit amplifying cells occupy perhaps two-thirds of the height of the crypt, and the postmitotic differentiated cells line the upper part of the crypts and the villi (*2*). The histological structure of most other epithelia is also clearly composed of structural units (for example, the glands of the stomach, the acini of the salivary glands, the lobules of the liver, and the nephrons of the kidney). Although good evidence is largely lacking, it is attractive to regard these structures also as units of cell renewal, in other words, to consider each visible histological unit as a "structural-proliferative unit" composed of one or a few stem cells feeding a differentiated compartment [(*9*) and Fig. 1].

Evidence for this concept comes from studies of the clonal makeup of epithelia, and the best analyzed case is that of the small intestine. There have been two main types of study. The first used aggregation chimeras, which are mice formed by the aggregation of two embryos at the preimplantation stage. The cells from the two embryos become well mixed and cooperate to form one single mouse of normal size and normal proportions. If the two embryos differ in the expression of some genetic marker, then it is possible to visualize the clonal composition of the tissues. Intestinal crypts are polyclonal at the time of formation and become monoclonal 1 to 2 weeks after birth (*15–17*). This does not mean, as initially supposed, that there is just one stem cell per crypt, because the genetic diversity of the stem cells may become progressively reduced both by division of the crypt (*18*) and by the differentiation of both progeny of a stem cell (*19*). The second method is mutagenesis to produce a visible cell label. Early experiments again showed monoclonal mutant crypts (*20–22*) but were hampered by problems of clone visualization. Recent work with a positive label in the mutant clone suggests that there are four to five stem cells per crypt (*23*).

To what extent other epithelia are organized as structural-proliferative units is not

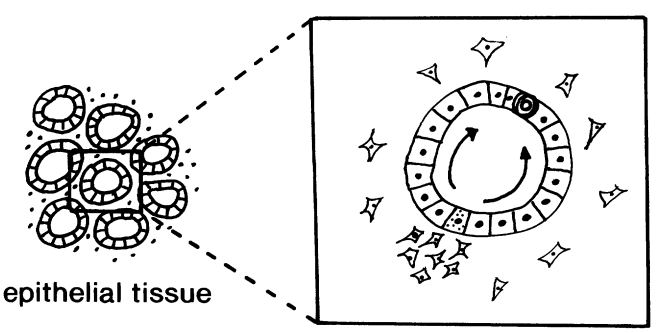

Fig. 1. Structural-proliferative units. In this model of tissue organization, each glandular structure is maintained by slow cellular turnover. There is a "niche" defined by interactions with the stromal tissue, which maintains one or a few cells as stem cells. The progeny of the stem cells will move around the gland such that the oldest cells are removed by apoptosis at the opposite extremity.

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yet clear because the drift to monoclonality will be slow where cell turnover is low (*24*). Gastric glands do follow the rule (*25*); there has been some controversy about the liver (*26, 27*); and in the epidermis, the hair follicles probably are self-contained structural-proliferative units, but the main area of epidermis between the hair follicles is not divided into obvious structures (*28, 29*).

Multi- and Unipotency

Epithelia are usually composed of several distinct cell types, and the ability to form all of them, or "multipotency," is often considered to be an aspect of stem cell behavior. The evidence for multipotency is good although usually derived from situations of severe tissue damage. For example, in the small intestine, there are four classes of mature differentiated cells (absorptive, goblet, Paneth, and enteroendocrine cells). The concept of a multipotent stem cell producing all four types was proposed by Cheng and Leblond (*30*), who followed radiolabeled phagosomes derived from [^{3}H]thymidine labeling from the cells of the crypt base into the differentiated populations. Although it did identify the stem cell region, this work did not prove the existence of multipotent cells. Bipotent (absorptive and goblet) cells have recently been detected by mutagenesis (*23*). Evidence for multipotent cells has been obtained from the use of doses of radiation sufficient to destroy most of the cells, which is followed by regeneration from isolated foci. These were shown to be monoclonal because they consist of just one genotype when examined for X-linked markers in heterozygous females (*31*). Each monoclonal focus can produce at least three of the cell types, although the animals did not survive long enough for the production of Paneth cells. Although this result is unambiguous, the degree of tissue damage produced by the radiation is enormous, so it may not reflect the situation of normal cell turnover.

Multipotent stem cells would presumably resemble the original embryonic rudiment for the tissue in question, which will produce the appropriate mixture of cell types in the course of normal development. For example, the embryonic epidermis forms both stratified epidermis and hair follicles (*32*), the embryonic liver hepatoblasts form both hepatocytes and bile duct cells (*33, 34*), and the embryonic pancreatic epithelium forms both exocrine and endocrine cell types (*35*).

Despite the undoubted existence of some cells that can show multipotent behavior following tissue damage, there is also evidence that, where tissue damage is low or nonexistent, most stem cells are unipotent, producing just one type of differentiated cell. I am here assuming that the definition of "stem cell" can accommodate unipotent as well as multipotent cells. For example, in the liver, regeneration in postnatal life normally proceeds from the hepatocytes (*36*), but if hepatocyte division is inhibited, it can occur from ductular oval cells instead (*4*). In the pancreas, the normal slow cellular turnover in adult life is probably due to intrinsic growth of endocrine and exocrine compartments separately (*6*). But in abnormal circumstances, such as transgenic mice expressing interferon-γ in the pancreas, de novo formation of islets and acini can occur from ducts (*37, 38*). Finally, the recent mutagenesis study of the small intestine suggests that 80 to 90% of long-lived mutant clones are unipotent, forming either absorptive or goblet cells, whereas only 10 to 20% are multipotent (*23*).

All of these examples suggest that steady state cell renewal occurs largely from unipotent stem cells, whereas tissue regeneration following damage may also occur from multipotent stem cells. This suggests that, when regeneration is required, there must be local chemical signals released in tissues, which can activate the dormant multipotent cells. The identification of these signals is potentially of considerable clinical importance, but we know little about them at present. Intriguingly, the overexpression of a stabilized version of β-catenin in the epidermis has been shown to cause the de novo formation of hair follicles (*39*), further evidence for an involvement of the Wnt pathway in the regulation of stem cell behavior.

Metaplasia

Whether multi- or unipotent, most of the time a stem cell will continue to generate the characteristic cell types for its own tissue. Occasionally, and again almost always in association with tissue damage and regeneration, there are errors leading to metaplasia. This is the formation of one differentiated cell type from another in postnatal life, and it happens because one or a few stem cells change their state of developmental commitment. In the embryo, tissues that develop as neighboring rudiments in a common cell sheet will have similar combinations of transcription factors defining their commitment and may differ by the expression of just one transcription factor gene. Assuming that stem cells are indeed the same as the original embryonic progenitors for the tissue, then a change of state of such a gene in later life would cause the stem cells to "flip" from producing one tissue to producing another (Fig. 2).

Metaplasias in epithelia are not uncommon and do in fact often consist of a conversion of a patch of tissue into another type that arose as an adjacent rudiment in the embryo (*40*). For example, patches of ectopic intestinal epithelium are found in the stomach (*41*), colonic type epithelium in the urinary bladder (*42*), endocervical epithelium in the vagina (*43*), or foci of hepatocytes in the regenerating pancreas (*44*).

It is of interest to inquire whether these metaplasias arise from somatic mutation of the genes encoding their commitment or from an epigenetic process that activates or represses the same genes. One approach to this, following the lead of cancer research (*45*), is to inquire whether or not foci of metaplasia are monoclonal. This can be done by examining their composition in mosaic animals that are composed of a mixture of cells of different genotypes. A recent study of intestinal metaplasia showed that foci were polyclonal and must therefore arise from more than one cell (*46*). So, the mechanism in this case is unlikely to be mutation and more likely to be an epigenetic change. Further studies of other types of metaplastic foci will be needed to find whether this is a general rule.

Wider Plasticity of Stem Cells?

The existence of epithelial metaplasias is evidence for some plasticity of stem cells. A more

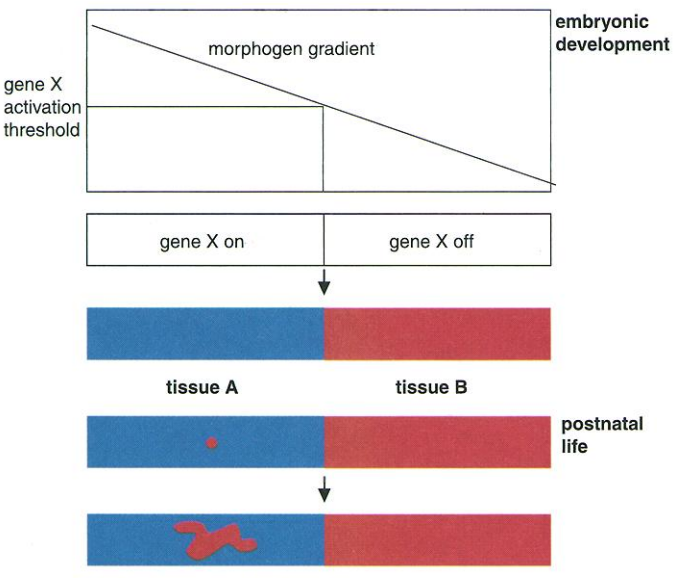

Fig. 2. Metaplasia. In the embryo, two tissue types arise from a common cell sheet because a gene X is activated in one tissue but not in the other. If something later turns this gene off in one or a few stem cells of the tissue, then a metaplasia will result.

dramatic type of reprogramming is suggested by some recent experiments on the grafting of bone marrow cells between individuals. It has recently been shown that genetically marked bone marrow can contribute to the regeneration of skeletal muscle (*47*) and of liver (*48*) in the host animals. In one study, the graft was composed of purified hemopoietic stem cells (*49*). Although the frequency of labeled foci is small and the time for their development is long, this is still remarkable because it implies a much more extreme reprogramming of developmental commitment than that found in endogenous metaplasias. The experiments involve the injection of suspensions of cells, so single graft cells are likely to end up completely surrounded by cells of a foreign tissue. In embryological experiments, isolated single cells often show more developmental lability than extended masses of tissue (*50, 51*), so perhaps this should be expected in the adult animal as well.

The results of such experiments should not confuse us by suggesting that all types of stem cell are the same. The well-characterized hematopoietic stem cell is clearly quite distinct from the equally well studied early embryonic stem cell and probably equally distinct from the epithelial stem cells of the various differentiated tissue types. However, they do show that there is considerable potential scope for reprogramming epithelial stem cells by changes to their environment.

The existence of endogenous processes of tissue repair in many or most epithelia suggests that there is a whole unexplored area of potentially novel therapies based on the stimulation of these regenerative mechanisms. Progress will require better characterization of epithelial stem cells in terms of molecular markers. It will also require the establishment of more in vitro culture systems, like those used for epidermis (*3, 52*), in which the control of stem cell behavior can be investigated in detail. Perhaps the most important advance will be the identification of the mysterious environmental factors that control stem cell behavior, both with regard to self-renewal potential and to the ability to form particular types of differentiated cells.

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REVIEW

Mammalian Neural Stem Cells

Fred H. Gage

Neural stem cells exist not only in the developing mammalian nervous system but also in the adult nervous system of all mammalian organisms, including humans. Neural stem cells can also be derived from more primitive embryonic stem cells. The location of the adult stem cells and the brain regions to which their progeny migrate in order to differentiate remain unresolved, although the number of viable locations is limited in the adult. The mechanisms that regulate endogenous stem cells are poorly understood. Potential uses of stem cells in repair include transplantation to repair missing cells and the activation of endogenous cells to provide "self-repair." Before the full potential of neural stem cells can be realized, we need to learn what controls their proliferation, as well as the various pathways of differentiation available to their daughter cells.

The term "neural stem cell" is used loosely to describe cells that (i) can generate neural tissue or are derived from the nervous system, (ii) have some capacity for self-renewal, and (iii) can give rise to cells other than themselves through asymmetric cell division. Whether stem cells from neural and other tissues are more defined by their tissue of origin or by their multipotentiality is at present unclear. However, neural stem cells can also be derived from more primitive cells that have the capacity to generate neural stem cells and stem cells of other tissues (Fig. 1). Stem cells have varying repertoires. A totipotent stem cell can be implanted in the uterus of a living animal and give rise to a full organism, including the entire central and peripheral nervous systems. A pluripotent

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