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Cell Stem Cell
Review

# Sic Transit Gloria: Farewell to the Epidermal Transit Amplifying Cell?

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**For the past 30 years, the prevailing model of epidermal homeostasis has been that epidermal stem cells give rise to transit amplifying cells, which undergo a limited number of cell divisions before initiating terminal differentiation. Recent studies challenge the existence of a transit amplifying cell compartment and suggest a new paradigm for epidermal homeostasis.**

Mammalian epidermis consists of a multilayered sheet of keratinocytes, interspersed with hair follicles (HF), sebaceous glands (SG), and sweat glands (Fuchs, 2007) (Figure 1). All the compartments of the epidermis are turned over throughout adult life. In the interfollicular epidermis (IFE), proliferation is confined to cells in the basal cell layer that adhere to an underlying basement membrane (Figures 1D and 1F). On commitment to terminal differentiation, basal keratinocytes lose their attachment to the basement membrane and move into the suprabasal cell layers, ultimately reaching the epidermal surface from where they are shed. HF comprise seven to eight distinct types of differentiated cells, including the dead cells of the hair shaft (Niemann and Watt, 2002) (Figures 1A and 1C). HF undergo cyclical growth (termed anagen) followed by regression (catagen), interspersed by periods of quiescence (telogen). Within the apocrine SG, the loss of differentiating sebocytes is compensated by proliferation of cells within the basal layer of the gland (Figures 1A and 1C). Thus there is a continual requirement for proliferation to replace cells lost by differentiation within each epidermal compartment. A key issue in the study of epidermal homeostasis is how these spatially distinct compartments comprising cells of different lineages are maintained.

It has long been argued that stem cells must exist in the epidermis to replace the anucleate terminally differentiated cells that are continually being lost from the HF, SG, and IFE. An epidermal stem cell may be defined as any individual cell that retains a high capacity for self-renewal throughout adult life and is able to produce daughter cells committed to terminal differentiation (Lajtha, 1979).

In this review, we briefly summarize what is known about the different epidermal stem cell compartments and discuss the evidence for proliferative heterogeneity. We then consider a new model of homeostasis in mouse tail epidermis in which tissue maintenance depends on a single population of proliferating cells.

## The Location and Plasticity of Epidermal Stem Cells

The best-characterized stem cells lie in a region of the HF known as the bulge (Claudinot et al., 2005; Cotsarelis et al., 1990; Morris et al., 2004; Tumbar et al., 2004). The existence of stem cells in murine IFE has been inferred from the existence of nonhair bearing epidermis and the observation that the IFE is maintained after ablation of the HF (Ito et al., 2005). Genetic labeling studies also indicate that normal IFE can be maintained without the recruitment of stem cells from the HF bulge (Claudinot et al., 2005; Ghazizadeh and Taichman, 2001; Levy et al., 2005). The evidence for a distinct stem cell pool in the SG comes from retroviral lineage marking experiments, in which labeled SG were observed adjacent to unlabelled HF and IFE (Ghazizadeh and Taichman, 2001). It has recently been reported that the Blimp 1 transcription factor is a marker of candidate SG stem cells (Horsley et al., 2006).

In undamaged epidermis HF, IFE, and SG are each thought to be maintained by their own discrete stem cell population. However, under some circumstances, any of the three stem cell populations is capable of producing any of the differentiated lineages of the epidermis (Owens and Watt, 2003). Bulge stem cells are recruited to regenerate the IFE after wounding, indicating that stem cells or their progeny migrate from the upper HF into the adjacent epidermis (Ghazizadeh and Taichman, 2001; Ito et al., 2005; Levy et al., 2005, 2007; Taylor et al., 2000). When genetically labeled bulge cells are transplanted into the skin, they generate not only HF but also IFE and SG (Oshima et al., 2001; Blanpain et al., 2004; Morris et al., 2004; Tumbar et al., 2004). In response to contact with specialized mesenchyme of the dermal papilla, IFE can form HF and SG (Reynolds et al., 1999). Furthermore, activation of β-catenin in SG and IFE leads to formation of ectopic HF independent of the stem cells in pre-existing follicles (Silva-Vargas et al., 2005; Ito et al., 2007).

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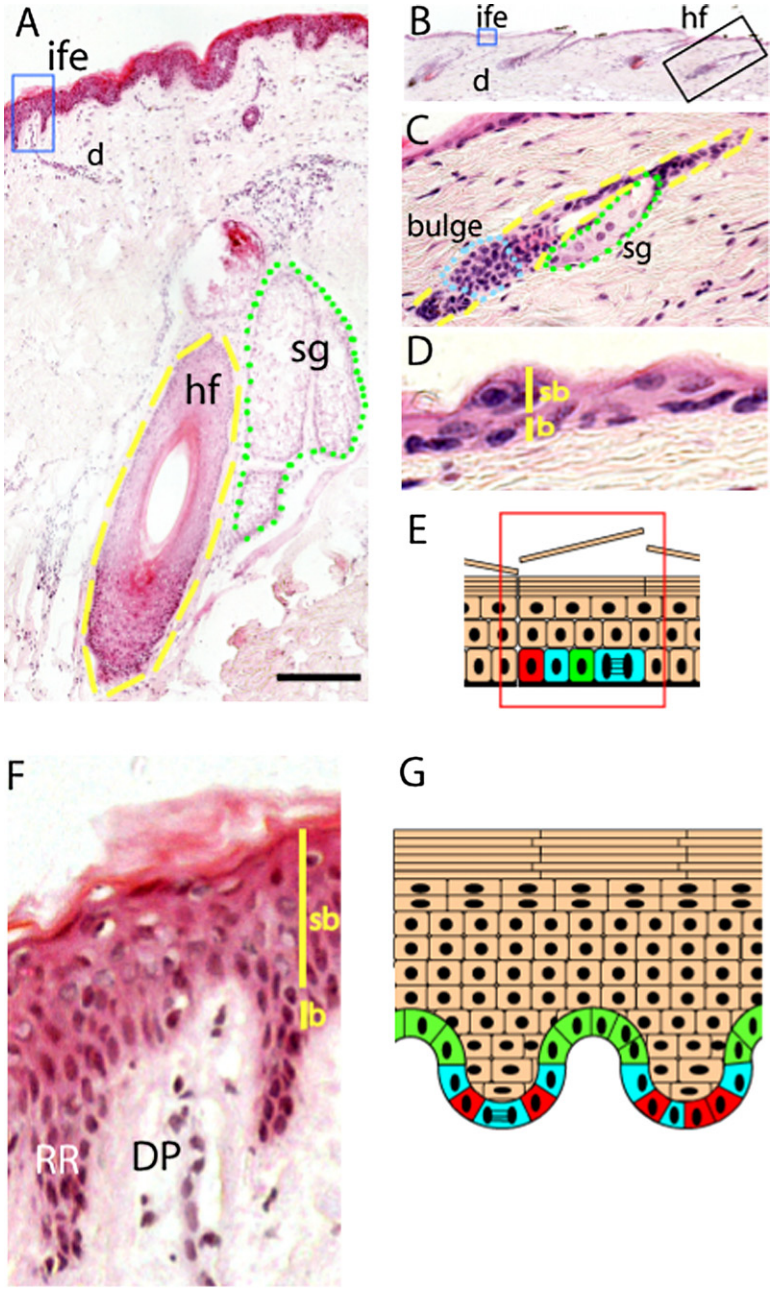


**Figure 1. Structure of Murine and Human Epidermis**

Hematoxylin and eosin-stained sections of human (A and F) and mouse (B–D) skin.

(A) Human skin showing darkly stained interfollicular epidermis (ife), lying above the connective tissue of the dermis (d), with a hair follicle, (hf, dashed yellow line) and its associated sebaceous gland (sg, green dotted line). Scale bar, 500 μm.

(B) Mouse skin taken at the same magnification and labeled as (A).

(C) Mouse hair follicle (shows enlarged view of black box in [B]). Labeling as (A), blue dotted line indicates the hair follicle bulge.

(D) Mouse interfollicular epidermis (shows enlarged view of blue box in B), b indicates basal cell layer, sb suprabasal cells.

(E) Proliferative organization of mouse ife according to Potten (1981). Epidermis is organized into discrete epidermal proliferative units (EPU, indicated by red box). Following the stem/transit amplifying (TA) cell model, each EPU is hypothesized to be maintained by a central stem cell (green), which supports adjacent TA cells (blue) that in turn generate postmitotic basal cells (red), which then exit the basal layer, migrating vertically upward to replace cells shed from the stack of cornified cells overlying the stem cell.

(F) Human interfollicular epidermis (shows enlarged view of blue box in [A]), b indicates basal cell layer; sb, suprabasal cells. The thickness of the epidermis varies; regions where the epidermis projects into the dermis are known as rete ridges (RR), which alternate with regions where the dermis projects into the epidermis known as dermal papillae (DP).

(G) Proliferative arrangement of human epidermis. Clusters of quiescent stem cells (green) overlie the dermal papillae, alternating with regions of proliferating keratinocytes (historically interpreted as TA cells) and postmitotic basal cells. Note that in palmar skin the location of stem cells appears to be reversed; quiescent cells expressing stem cell markers are found in the deep rete ridges (Jones et al., 1995; Lavker and Sun, 1983; Wan et al., 2003).

We thank Adam Giangreco for taking the photomicrographs.

Based on these studies, it seems likely that lineage selection by stem cell progeny is largely determined by local environmental cues. Lineage plasticity, for example, when an IFE stem cell generates progeny that differentiate along the HF lineages, is a response to alterations in environmental signaling after injury, transplantation, or genetic manipulation.

## Transit Amplifying Cells and the Epidermal Proliferative Unit Model

It is widely believed that, in addition to stem cells, the epidermis contains a second population of proliferating cells, known as transit amplifying (TA) cells (Potten, 1981) (Figures 1E and 1G). TA cells are the stem cell progeny that are destined to undergo terminal differentiation; however, prior to cell-cycle withdrawal, they are believed to undergo a limited number of rounds of division. TA cells are further hypothesized to have an internal "memory," which specifies a set number of cell divisions prior to the onset of terminal differentiation (Potten, 1981). Because TA divisions amplify the number of differentiated daughters resulting from each stem cell division, the stem cells need to divide relatively infrequently to maintain epidermal homeostasis (Mackenzie, 1970; Potten, 1974, 1981). Nevertheless, in this stem/TA model, continual slow cycling of stem cells is required to maintain the short-lived TA population.

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By far the most influential model of the role of stem and TA cells in epidermal homeostasis is that of the "epidermal proliferative unit" (EPU). This is based on the observation that in some regions of the mouse IFE cells are arranged in columns, with a single cornified cell at the top (Mackenzie, 1970) (Figure 2). Cornified cells, which have reached the final stage of the terminal differentiation process, are hexagonal in shape, and thus the columns are packed next to one another in a regular array. If one assumes that cells only migrate vertically when they leave the basal layer, it follows that each stack of cornified layer cells will be descended from the cells in the basal layer that lie directly beneath it, forming an EPU.

When the proliferation of the basal layer cells underneath each column of cornified cells was examined, the frequency of mitoses was found to be lower in the cells lying beneath the center of each column compared with the basal cells underneath the periphery. This observation led to the model that a central, slowly cycling stem cell maintains a population of surrounding TA cells that in turn supports the overlying suprabasal cells (Mackenzie, 1970). From the pattern of cell stacks in the cornified cell layer, the size of EPU has been inferred to vary in size from ten basal cells per unit in murine back skin to 25 in tail epidermis (Potten, 1974, 1975). Following the stem/TA model, if the size of an EPU is known, and one assumes each EPU is supported by a single stem cell, it is then possible to infer the behavior of TA cells. In EPUs comprising ten basal cells, there will be three TA divisions prior to the onset of terminal differentiation (Figure 2; Potten, 1975).

## Label Retaining Cells and Actively Cycling Cells

The stem/TA cell model predicts that in the steady state, stem cells will cycle more slowly than TA cells. One way to test this prediction experimentally is to label the DNA of all dividing epidermal cells in neonatal mice (for example, with $^{3}$HTdR or BrdU), the argument being that at this stage the skin is expanding and both stem and TA cells will be dividing (Bickenbach, 1981). As the mice reach adulthood and achieve epidermal homeostasis, TA cells will continue to divide and thereby dilute the label below detectable levels, whereas the relatively quiescent stem cells will be "label retaining cells" (LRC) (Bickenbach, 1981). The HF bulge, now established as the location of stem cells by a variety of different criteria, was originally identified as a reservoir for LRC (Cotsarelis et al., 1990).

The distribution of LRC in the IFE is controversial. In most protocols, scattered LRC are detectable in the IFE, but in no discernible pattern (Braun et al., 2003). This may reflect, at least in part, a failure to label all the IFE cells in neonatal epidermis (Bickenbach, 1981; Braun et al., 2003; Lopez-Rovira et al., 2005). When 95% of IFE basal cells in dorsal epidermis are labeled, using a protocol of $^{3}$HTdR injections every 6 hr for a week, 90% of LRC are found within one nuclear diameter of the center of the EPU (Morris et al., 1985). Although this may indicate the presence of a slow cycling stem cell at the center of an EPU, it is also possible that the central cell is a Langerhans antigen presenting cell (Mackenzie, 1975a; Merad et al., 2002).

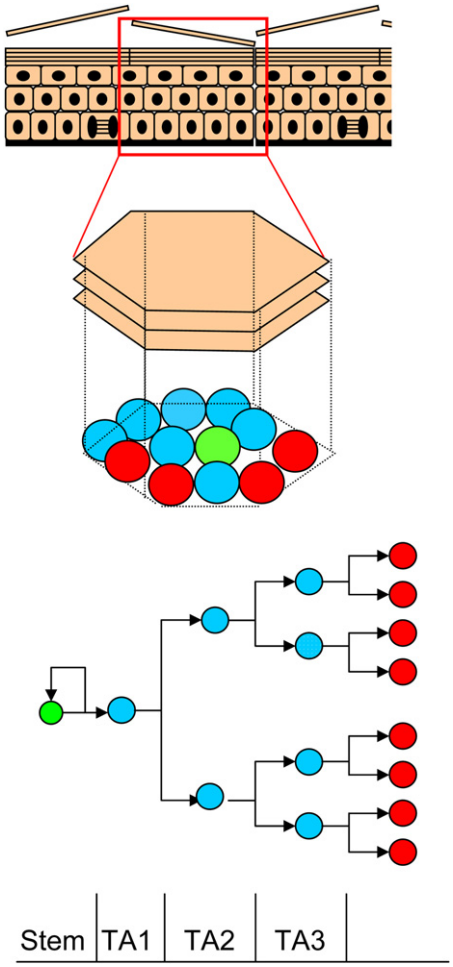


**Figure 2. The EPU Hypothesis**

(A) The EPU as described by Potten (1983). Each stack of cornified cells, see inset, is hypothesized to be maintained by the basal cells underlying it (the intervening suprabasal cells have been omitted for clarity in the inset). In the basal layer, a stem cell (green) lying beneath the center of the stack of cornified cells supports a surrounding population of TA cells (blue), which in turn generate postmitotic basal cells (red) that subsequently exit the basal layer and migrate vertically to the epidermal surface through the suprabasal cell layers.

(B) The rules of cell behavior in the EPU model. The fate of cells derived from a single round of stem cell division is shown. Division of a stem cell (green) generates a stem cell and a first-generation TA cell (TA1). TA1 cells then divide symmetrically to generate TA2 cells, which then produce TA3 cells. TA3 cells then differentiate into postmitotic cells (red), which exit the basal layer. The rates of cell division and differentiation are coordinated so the number of basal cells in each EPU remains constant. Note that the future behavior of a TA cell is fixed by whether it is a first, second, or third generation cell.

The converse of the LRC assay is to look for mitotic cells, although the low frequency of mitoses in the basal layer of normal murine epidermis makes such studies arduous (Frei et al., 1963). Early investigations concluded that there was no pattern in the spatial distribution of mitoses (Frei et al., 1963). However, it was subsequently reported that mitoses were less likely to occur in basal cells lying beneath the center of the EPU, consistent with the LRC results (Mackenzie, 1970). One explanation for the

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discrepancy is that the early studies scored mitoses in an area corresponding to 70 or more EPU, a level of spatial resolution too low for discerning a pattern within individual EPU (Mackenzie, 1975b).

In human epidermis, it is obviously not feasible to generate LRC, although LRC have been identified in human skin grafted onto immunocompromised mice (Lyle et al., 1998). Nevertheless, there is strong evidence that there are some regions of the IFE basal layer in which cells are more likely to be dividing than in others (Jensen et al., 1999; Jones et al., 1995; Rowe and Dixon, 1972; Thuringer, 1928). The putative stem cells of human IFE (identified by their capacity for sustained self-renewal in culture) express a variety of markers that distinguish them from other cells in the basal layer (Frye et al., 2007; Jensen and Watt, 2006; Jones et al., 1995; Jones and Watt, 1993; Legg et al., 2003; Li et al., 1998; Wan et al., 2003). Marker expression suggests that stem cells in human IFE are clustered and tend to have a specific location relative to the topology of the underlying dermis (Figure 1G; Jones et al., 1995). In monkey palmar epidermis, basal cells in regions that extend deepest into the dermis (the rete ridges) are less likely to be in S phase of the cell cycle than more superficial basal cells, consistent with findings in human palm (Jones et al., 1995; Lavker and Sun, 1982).

Using wholemount labeling or conventional histological sections, it is possible to show that in human IFE the actively cycling cells lie outside the stem cell clusters (Jones et al., 1995; Jensen et al., 1999). In addition, the cells that initiate terminal differentiation are not randomly distributed in the basal layer but lie in the regions where the actively dividing cells are concentrated (Jensen et al., 1999). Keratinocytes can initiate terminal differentiation from any phase of the cell cycle, and examination of a skin biopsy from a human volunteer who received a single injection of $^{3}$HTdR revealed S phase cells that were expressing the terminal differentiation marker involucrin (Dover and Watt, 1987). The conclusions from studies of human IFE are therefore that stem cells are much less likely to be dividing than other populations of basal cells and that active cell division is linked to the initiation of terminal differentiation. This, in turn, can lead to the hypothesis that proliferation may increase the likelihood that a keratinocyte will initiate terminal differentiation (Jensen and Watt, 2006).

## Clonal Growth Assays

The conditions for culturing cells from human epidermis have been optimized to allow the cultures to be used as autografts for burns patients, providing strong practical evidence for the persistence of stem cells in culture (Gallico et al., 1984). This has prompted attempts to identify epidermal stem cells on the basis of their in vitro growth characteristics. When primary human keratinocytes are cultured at clonal density and then subcloned to determine their proliferative potential, three types of clone are identified (Barrandon and Green, 1987). Large circular colonies, termed "holoclones," contain thousands of keratinocytes and have a very high proliferative potential, generating in excess of $10^{16}$ cells when subcloned. The cells that generate holoclones have both the ability to self-renew and a very high proliferative potential, consistent with their being stem cells. In addition to holoclones, small, irregularly shaped clones, containing 32–128 cells are also seen. These "paraclones" have either no or only very limited ability to generate new colonies when subcloned, properties expected of TA cells. A third type of colony, the meroclone, has an appearance and proliferative potential intermediate between paraclones and holoclones (Barrandon and Green, 1987). These results demonstrate that in human epidermis not all basal cells have the same proliferative potential in culture.

Other assays of clonal growth have been performed, including one in which the proportion of "abortive colonies" is scored. These colonies contain 2–32 cells, virtually all of which have initiated terminal differentiation within 14 days in culture (Jones and Watt, 1993). The abortive colonies have the predicted characteristics of TA cell founders, whereas the clones that actively self-renew have the expected characteristics of being founded by stem cells (Jones et al., 1995; Jones and Watt, 1993; Zhu et al., 1999; Zhu and Watt, 1999). An interesting feature of these studies is that when the proportion of putative stem cells is expanded, for example by activation of β-catenin or Erk MAPK, the proportion of cells that differentiate when placed in suspension culture is unaltered (Zhu et al., 1999; Zhu and Watt, 1999).

Several techniques have been developed to optimize the in vitro growth of keratinocytes from adult mouse epidermis, including methods that demonstrate that LRC are capable of proliferation in culture (Bickenbach and Chism, 1998; Morris and Potten, 1994; Wu and Morris, 2005). However, using the culture conditions optimized for human epidermis, the clone forming ability of cells from adult mouse epidermis is too low to be informative as a stem cell assay (Romero et al., 1999). This problem has recently been solved by using calcium-free basal medium supplemented with regular fetal calf serum (Blanpain et al., 2004; Silva-Vargas et al., 2005). Although colony size is clearly variable in cultures of adult mouse epidermis, TA cells have not been defined on the basis of colony type. Nevertheless, CD34-positive bulge cells form larger colonies than CD34-negative epidermal cells (Nijhof et al., 2006; Trempus et al., 2003). Activation of β-catenin in vivo, which increases the proportion of cells expressing bulge markers, results in increased colony formation and an increase in colony size in primary cultures (Silva-Vargas et al., 2005).

We can conclude from these studies that cells isolated from mouse or human epidermis are heterogeneous when assayed for clonal growth in culture. The majority of studies of human keratinocytes make use of cells from nonhair bearing skin. However, cultures of mouse keratinocytes are usually derived from hairy skin. There are markers that distinguish bulge from nonbulge keratinocytes in the HF (Nijhof et al., 2006). However, it has not been possible to separate marker-negative HF cells from IFE cells (Nijhof et al., 2006; Silva-Vargas et al., 2005), and so the site of origin of the colony-forming cells has yet to be established.

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## Problems with the Stem/TA Model

From the studies we have outlined, there is no doubt that the dividing cells of the epidermis appear heterogeneous. This is shown by the existence of label retaining cells, the association of actively cycling cells with the onset of terminal differentiation, and heterogeneity of clonal growth in culture. However, there are some observations that do not fit well with the stem/TA cell model.

The first difficulty is that clonal analysis of human epidermal cultures is more consistent with a continuum of proliferative potential than with the existence of two discrete populations of dividing cells. Although culture can never faithfully recreate the in vivo stem cell environment, clonal analysis does have the advantage of analyzing the proliferative potential of individual cells under identical conditions. In the holoclone-type assay, in which growing colonies are subcloned to define their proliferative potential, there are three clonal types, holoclones, TA-like paraclones, and meroclones, which have intermediate properties between the stem cell-like holoclones and TA-like paraclones (Barrandon and Green, 1987). With age, the ability to form holoclone-type colonies is lost, suggesting it may be possible to maintain the epidermis independently of the cells with highest proliferative potential (Barrandon and Green, 1987). In the direct clonal assay, where clones are cultured without subcloning, some cells form abortive, differentiated colonies, whereas others form larger colonies that vary in size (Jones et al., 1995; Jones and Watt, 1993). This spectrum of growth potential suggests that there is heterogeneity among proliferating human keratinocytes but conflicts with the stem/TA model.

A second issue is a lack of markers that define a discrete TA cell population. Several markers have been identified as enriching for human epidermal cells of high clonal growth potential, i.e., the putative stem cells. Some markers, such as MCSP, appear to be expressed in an "on" or "off" fashion (Legg et al., 2003), whereas others, exemplified by the β1 integrins, show graded expression (Jones et al., 1995; Jones and Watt, 1993). Analysis of transcription of individual cultured human keratinocytes reveals cells expressing three different combinations of the putative stem cell markers Delta1 and MSCP: double positives are putative stem cells, double negatives are putative TA cells, and many cells are Delta1 positive, MCSP negative. It remains to be determined whether these differences in gene expression reflect differences in proliferative potential (Jensen and Watt, 2006).

A final issue is that early predictions of the behavior of TA cells were based on the EPU model. However, the proliferative organization of the IFE does not always fit with the classic concept of the EPU. Early studies of chimeric mice generated by aggregating inner cell mass cells of mice expressing different genetic markers revealed large stripes of marker expression in the epidermis (Schmidt et al., 1987). The EPU model predicts that the boundaries of the stripes must run along the borders of EPU, because each EPU must contain cells of a single genotype. In fact, the boundaries of marker expression cut across the EPUs (Figure 3). Later retroviral and transgenic labeling studies

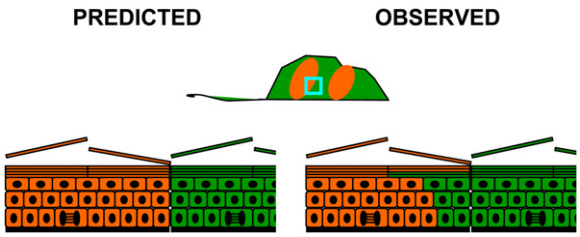


**Figure 3. Predictions of the EPU Hypothesis Compared with Experimental Results: Chimeric Mice**

In mice chimeric for different alleles of the H-2 locus (green/orange), the boundaries of the epidermal regions expressing each allele are predicted to conform to EPU (left panel), as each stem cell must be of either one genotype or the other. What is observed is that the boundaries of mosaic patches cut across EPU (right panel), suggesting the cell stacks in the cornified layer do not define clonal boundaries in the epidermis (Schmidt et al., 1987).

demonstrated the existence of columns of labeled cells that persist for many months in murine IFE, but they are larger and more irregular in shape than predicted for the classic EPU of ten basal cells and again fail to conform to predicted EPU boundaries (Figure 4), (Ghazizadeh and Taichman, 2001; Kameda et al., 2003; Ro and Rannala, 2004, 2005; Taylor et al., 2000; Ito et al., 2005). These studies also reveal that as cells in the suprabasal layers differentiate they can migrate laterally as well as vertically, challenging the assumption that underlies the EPU hypothesis that cornified layer cells are derived from the basal cells immediately beneath them (Ito et al., 2005; Ro and Rannala, 2004; Ro and Rannala, 2005).

The ordered columns of cornified cells seen in the epidermis at some body sites are less apparent in human than in mouse epidermis (Mackenzie et al., 1981). A clonal analysis of human xenografted skin, genetically labeled by lentiviral infection and then maintained in immunocompromised mice for 6 months, revealed the presence of irregularly shaped groups of labeled cells, comprising from one to ten basal layer cells (Ghazizadeh and Taichman, 2005). As in mouse epidermis, the labeled keratinocytes migrated laterally as well as vertically as they traversed the suprabasal cell layers, and the labeled columns exhibited a wide range of shapes and sizes. These marked clones were assumed to derive from stem cells because of their persistence in the tissue for over 6 months.

## The Importance of Lineage Tracing for Analysis of Stem Cell Fate

There are two almost insurmountable challenges in studying stem cells. The first is that no matter how many surface markers are used to enrich for a particular stem cell population it is virtually impossible to obtain 100% purity. The second is that any in vitro assay to quantitate stem cell number will always be imperfect, because it can never completely recapitulate the in vivo environment. For this reason, in vivo lineage tracing, which reveals the fate of the progeny of an individual stem cell, is an essential tool for studying stem cells, as illustrated by elegant studies in *Drosophila* (Fuller and Spradling, 2007). Such

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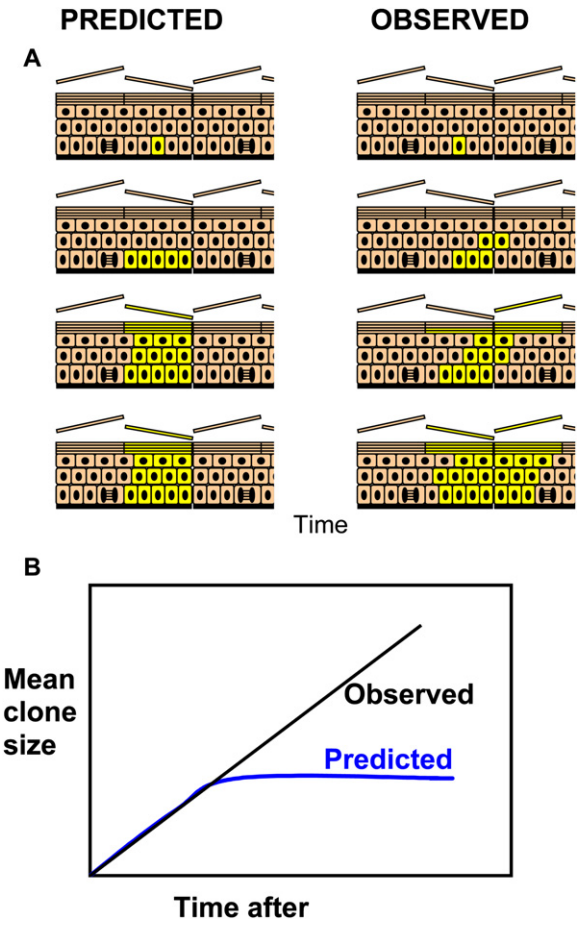


**Figure 4. Predictions of the EPU Hypothesis Compared with Experimental Results: Clonal Genetic Labeling**

(A) In clonal genetic labeling experiments, the EPU hypothesis predicts that labeled TA cells will be rapidly lost from the tissue, while a labeled stem cell (yellow) will populate the TA compartment and then the entire EPU with labeled cells (left panel). Once the EPU is completely labeled, the size and shape of the labeled clones will remain constant. In contrast, the clones observed after induction of a conditional genetic label are irregular in shape, transgress the predicted boundaries of EPU, and continue to expand in size as the time following labeling increases (Clayton et al., 2007; Kameda et al., 2003; Ro and Rannala, 2005).

(B) The variation of the mean number of basal cells in a clone, comparing the predictions of the EPU model (blue) with data from a clonal labeling experiment (black) (Clayton et al., 2007; Klein et al., 2007).

studies offer a window into cellular function in a normal tissue context, avoiding the gross disruption of the cellular environment that occurs when cells are cultured and/or transplanted.

As outlined already, retroviral vectors encoding a β-galactosidase reporter have provided important information about lineage relationships in adult mouse epidermis. However, retroviruses have the disadvantage that they only integrate into dividing cells and therefore label very few cells when injected into normal epidermis (Kameda et al., 2003; Mackenzie, 1997). Attempts to boost transduction, for example by wounding to recruit cells into cycle, inevitably disturb normal tissue homeostasis (Ghazizadeh and Taichman, 2001; Levy et al., 2005).

Alternative strategies to retroviral lineage tracing make use of transgenic mice (Petit et al., 2005). One approach is to engineer a stop codon that prevents expression of a functional reporter protein unless it undergoes an appropriate mutation. This method ensures there is no "leaky" expression of the reporter. However, spontaneous mutations are very rare, resulting in very few labeled cell clones, and use of a mutagen such as ethyl nitrosourea to boost the mutation rate may perturb tissue homeostasis. When this approach has been applied to mouse epidermis, the appearance of the clones detected, though few in number, challenges the EPU model (Ro and Rannala, 2004, 2005).

A different lineage tracing technique, which overcomes the problems of low efficiency and perturbed tissue homeostasis is to express an inducible form of Cre recombinase, such as a fusion of Cre with a mutant the estrogen receptor (CreER) or progesterone receptor under the control of an appropriate keratin promoter in transgenic mice. The mice are then crossed with mice expressing a reporter whose transcription is blocked by a cassette flanked by *loxP* sites (Branda and Dymecki, 2004): reporter gene expression is induced by application of Tamoxifen (Vasioukhin et al., 1999). Unfortunately these inducible Cre mice suffer from leaky recombinase activity in the absence of the inducing drug, which limits the amount of lineage information that can be obtained as the time at which reporter gene expression is induced is unknown (Ito et al., 2005; Vasioukhin et al., 1999). Nevertheless, transgenic strains expressing CreER have been used to map cell fate in the lower HF and also to establish that β-catenin-induced follicles in the IFE are derived from IFE and not bulge stem cells (Legue and Nicolas, 2005; Silva-Vargas et al., 2005).

Recently, the problems of leakiness and low tagging efficiency have been solved by generating a transgenic mouse line, AhcreER, in which the transcription of CreER is under the control of the drug-activated Ah promoter (Kemp et al., 2004). The regulation of Cre at two levels eliminates the problem of background recombination in the epidermis, while Cre activity is highly dependent on the doses of both inducing drugs, allowing clonal frequency labeling of epidermal cells (Clayton et al., 2007; Kemp et al., 2004).

## Lineage Tracing Identifies a Single Population of Dividing Cells that Maintains Mouse IFE

By exploiting the AhcreER mouse for epidermal lineage tracing, the fate of a large, representative sample of cells in interfollicular mouse tail epidermis has recently been tracked at single-cell resolution over a 1 year time course in vivo (Clayton et al., 2007). Clones containing at least one basal layer cell were analyzed: the data could then be used to determine the rules of cell behavior followed by proliferating basal layer keratinocytes. Following the low-frequency labeling of basal cells at clonal induction, the number of clones retaining one or more basal cells fell progressively with time. The remaining clones remained cohesive but varied markedly in size and shape, consistent with previous genetic labeling studies (Kameda et al., 2003; Ro and Rannala, 2004, 2005).

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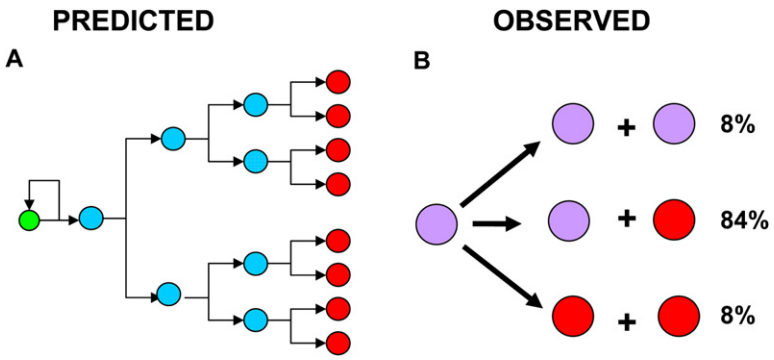


**Figure 5. A New Model of Epidermal Homeostasis**

(A) Rules of cell fate in the EPU model as in Figure 2B.

(B) An empirical, quantitative model of epidermal homeostasis based on clonal labeling data (Clayton et al., 2007; Klein et al., 2007). Cycling basal keratinocytes (purple) may generate daughters with three possible fates: both may remain proliferative, both may terminally differentiate to become postmitotic cells (red), or one may remain proliferative and one postmitotic. The probabilities of cells entering each cell-fate pathway are shown as percentages; note these are the same irrespective of the past history of the cycling cell, so that unlike TA cells, the behavior of all cycling basal cells is stochastic and not determined by the number of cell divisions completed by their parental cell. No stem cells are shown, because quiescent stem cells make no measurable contribution to normal epidermal homeostasis.

It might be thought that the short-lived clones in this experiment derive from labeled TA cells, whereas the clones that persist for many months are maintained by a labeled stem cell. However, the clone fate data are impossible to reconcile with the stem/TA hypothesis. The average number of basal layer cells per clone increases linearly and inexorably with time after induction (Figure 4) (Clayton et al., 2007). Moreover, the observed clone fate data show a remarkable long-time scaling behavior: the probability of finding a clone with between, say, n/2 and n basal layer cells at a time t after labeling is equal to the probability of finding a clone with between n and 2n cells at time 2t postlabeling. Such behavior is incompatible with the existence of a long-lived stem cell population and a short-lived TA cell population (Clayton et al., 2007; Klein et al., 2007), as once the entire TA cell population supported by a labeled stem cell has been labeled, no further expansion of the clone can occur (Figure 4). From the scaling behavior one can infer that clonal evolution is governed by a single rate limiting process. This rules out any model with two cell populations cycling at different rates or explanations of clone growth due to lateral cell migration following stem cell senescence or wounding (Potten, 1981; Ro and Rannala, 2005).

A second set of observations from this study is key to understanding epidermal homeostasis. Conventional histology, wholemount confocal imaging, and lineage tracing all confirm that the overwhelming majority of cell divisions in adult epidermis are in the plane of the basal layer, so that a dividing basal cell generates two basal cell daughters (Clayton et al., 2007; Smart, 1970). Surprisingly, however, clones consisting of two basal cells are found to adopt three different fates: both cells may remain proliferative, both differentiate and exit the cell cycle, or one cell differentiates while the other remains proliferative (Figure 5) (Clayton et al., 2007). Consistent with the latter type of cell division, asymmetric partitioning of numb protein, a marker of asymmetric cell division, is observed between mitotic basal cells (Conboy and Rando, 2002; Zhong et al., 1996).

The entire clone fate data set and the observation that cycling basal cells may generate progeny with three different fates are consistent with a remarkably simple model of epidermal homeostasis in mouse tail involving a single population of proliferating epidermal cells. Clone fate depends simply upon the average cell division rate and the proportion of divisions that result in asymmetric fate (Figure 6). To maintain the steady-state population of proliferating cells, the proportion of cells that generate daughters with each type of symmetric fate must be equal. Moreover, to maintain the population of postmitotic cells in the basal layer at a constant level, their rate of transfer to the suprabasal layer is constrained by the rate of production of basal cells. These constraints allow the rate of cell division (of approximately every 6–7 days) and the probabilities of the three possible fates of a dividing cell to be derived from the clone fate data (Figure 6; Clayton et al., 2007). The ability of the proliferating epidermal cell population to undergo an unlimited number of cell divisions before terminal differentiation allows it to maintain the normal epidermis without recruiting quiescent stem cells into cycle. At each division, proliferating basal cells choose between one of three possible fates at random. The probabilities of each cell fate are set so as to ensure homeostasis over the population of cycling basal cells.

The new model has only been shown to describe mouse tail skin, a specialized scale forming epidermis, raising the issue of whether it is applicable more widely. Technical limitations have prevented similar quantitative analysis of clones in back skin; however, the progressive enlargement of labeled clones observed at this site is qualitatively consistent with the new model (Clayton et al., 2007). The applicability of the model to epidermis at sites that are suitable for quantitative clonal analysis is as yet untested. A further question is whether the homeostasis of human IFE is maintained by a single type of proliferating cells. If proliferating human keratinocytes follow similar rules to cycling cells in mouse tail epidermis, it would explain the observation that almost all the putative stem cells in human IFE are quiescent, suggesting that they are not

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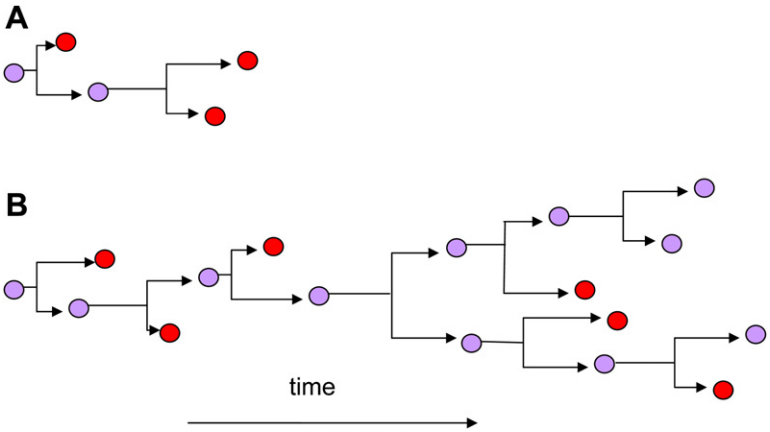


**Figure 6. Generation of Short- and Long-Lived Clones from a Single Population of Cycling Basal Cells**

By following the rules in Figure 5B, identical proliferating basal cells may generate small or large clones in a clonal labeling experiment, as illustrated in the "family trees" of a short-lived clone (A) or a long-lived clone (B); cells are color coded as in Figure 5. Both founding cells are identical, but by chance, the progeny in the smaller clone terminally differentiate so the clone is lost from the tissue, while the larger clone persists and expands. Note that cell division intervals are also stochastic (Klein et al., 2007).

required for normal epidermal homeostasis (Jensen et al., 1999).

## The Characteristics of Proliferating Basal Cells

In the stem/TA model, TA cells are lost from the tissue while stem cells persist. How can a single cell population generate both short- and long-lived clones in a genetic labeling experiment? When a sample of proliferating basal cells is labeled, by chance some cells will generate clones in which all the cells undergo terminal differentiation while identical cells will generate an excess of cycling cells and produce larger clones (Figure 6). As the size of the clone increases, the probability of it being lost from the tissue through differentiation falls, so a few clones attain sufficient size to persist for a long period (Figure 6B). It is important to stress that the cells that found small and large clones are identical: unlike the hypothesized TA cell, proliferating basal keratinocytes have no "memory"; the probability of the progeny of a cycling cell adopting a given fate remains the same irrespective of its past history.

Historically, one of the defining features of stem cells has been thought to be asymmetric cell division (Hall and Watt, 1989). Epidermal homeostasis requires that the number of cycling and differentiated postmitotic basal cells remains constant. The stem/TA cell model predicts that each stem cell division must, on average, produce one stem cell and one cell that undergoes terminal differentiation. This is known as asymmetry of cell fate and it can, potentially, be achieved in two different ways (Hall and Watt, 1988; Watt and Hogan, 2000). One mechanism involves asymmetric divisions, whereby each time a stem cell divides it produces one stem daughter and one differentiated daughter. In a stratified epithelium, this can be achieved by orienting the plane of the mitotic spindle at right angles to the basement membrane, so that one cell remains in the basal (proliferative) compartment and one in the suprabasal, differentiating compartment. This is observed in adult human esophageal epithelium and in developing mouse epidermis, but not in adult mouse epidermis (Clayton et al., 2007; Koster and Roop, 2005; Lechler and Fuchs, 2005; Seery and Watt, 2000; Smart, 1970). Although perpendicular cell divisions do not occur in adult IFE, planar asymmetric divisions, as evidenced by asymmetric distribution of Numb, are seen. Such divisions have previously been reported in *Drosophila* and Zebrafish retinal precursors (Clayton et al., 2007; Das et al., 2003; Gho and Schweisguth, 1998).

The second proposed mechanism for maintaining a constant number of stem cells while generating differentiated cells is known as populational asymmetry (Hall and Watt, 1989; Watt and Hogan, 2000). In this situation, some divisions can yield two stem cells, some two differentiated cells, and some one of each, provided only that the rates of each type of symmetric cell division are the same. The new lineage tracing data reveal that 20% of cycling basal cells undergo both symmetric divisions, the proportion that generate two proliferating cells (10%) being equal to the proportion that produce two postmitotic, differentiating cells (Figure 5). This leads to the surprising conclusion that epidermal homeostasis is achieved by a combination of asymmetric and symmetric divisions (Clayton et al., 2007).

Are the proliferating cells of murine IFE stem cells? As a population, they are able to self-renew to maintain the proportion of cycling cells in the basal layer of the epidermis at a constant level, and apparently by chance, a few of them persist throughout the life of the animal, all characteristics of stem cells. However, while the population of cycling basal cells maintains itself, individual cells and their clonal progeny are transient, the majority being lost from the epidermis within 3 months of labeling. Finally, the existence of a discrete population of quiescent IFE stem cells, as suggested by the detection of interfollicular LRC (Bickenbach and Chism, 1998, Morris et al., 1985), cannot be ruled out, as this study only analyzed the fate of proliferating cells (Clayton et al., 2007). However, if such a stem cell population exists, it does not make a measurable contribution to normal epidermal homeostasis.

## Challenging the Status Quo

Although normal epidermal homeostasis can be maintained by a single population of dividing cells, what happens when there is a requirement for an increase in the proportion of proliferating cells, for example following wounding (Potten et al., 2000)? There are three potential

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mechanisms by which the balance between proliferation and differentiation could be tipped in favor of proliferation. One way is by mobilizing stem cells that are normally quiescent. This is consistent with the observation that keratinocytes derived from the HF bulge migrate into IFE following wounding (Ito et al., 2005; Claudinot et al., 2005; Ghazizadeh and Taichman, 2001; Levy et al., 2005). It is also known that LRC in the bulge, SG, and IFE are all recruited into cycle in response to treatment with the phorbol ester TPA (Braun et al., 2003). A second mechanism to generate additional cells is to increase the proportion of cell divisions, resulting in two proliferating daughters (Figure 5); this hypothesis is yet to be tested by clonal analysis. A further possibility is that cells that are committed to undergoing terminal differentiation revert to stem cells, as occurs in the *Drosophila* germline (Fuller and Spradling, 2007). Such reversal of differentiation has been observed in cultured human keratinocytes after expression of the E1A oncogene (Barrandon et al., 1989). It is also implicit in the observation that ectopic follicles induced by β-catenin in adult mouse IFE are not obligatorily clonal in origin, if indeed stem cells are distributed singly (Figure 1E) rather than clustered (Silva-Vargas et al., 2005).

A final challenge in explaining stem cell function in the epidermis is the ability of cells in the IFE, SG, and HF to give rise to different epidermal lineages in response to appropriate stimuli. It has been noted that bulge LRC neither migrate nor undergo rounds of division in response to activation of Myc or β-catenin or overexpression of N-terminally truncated Lef1, all of which cause radical changes in epidermal lineage selection (Braun et al., 2003; Silva-Vargas et al., 2005). It remains to be determined whether the proliferating cells that maintain mouse IFE are a uniform population in terms of their capacity to undergo lineage reprogramming, such as the induction of HF from adult IFE(Estrach et al., 2006; Ito et al., 2007; Silva-Vargas et al., 2005).

## Beyond the Epidermis

The concepts of stem cell quiescence, TA cells, and asymmetric divisions are by no means unique to the epidermis (Fuchs et al., 2004). In particular, it is widely held that the maintenance of the intestinal epithelium and the blood is depended on populations of slow-cycling stem cells. Many of the experiments used to characterize stem cells in these tissues rely on reconstitution after injury. It will be interesting to discover whether the application of detailed quantitative lineage tracing to undamaged tissue will also challenge prevailing models of homeostasis.

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