

More than one way to skin . . .

Elaine Fuchs¹ and Valerie Horsley

Howard Hughes Medical Institute, Laboratory of Mammalian Cell Biology and Development, The Rockefeller University, New York, New York 10065, USA

Epithelial stem cells in the skin are specified during development and are governed by epithelial–mesenchymal interactions to differentially adopt the cell fates that enable them to form the epidermis, hair follicle, and sebaceous gland. In the adult, each of three epithelial lineages maintains their own stem cell population for self-renewal and normal tissue homeostasis. However, in response to injury, at least some of these stem cell niches can be mobilized to repair an epithelial tissue whose resident stem cells have been damaged. How do these stem cell populations respond to multiple signaling networks, activate migration, and proliferation, and differentiate along a specific lineage? Recent clues add new pieces to this multidimensional puzzle. Understanding how these stem cells maintain normal homeostasis and wound repair in the skin is particularly important, as these mechanisms, when defective, lead to skin tissue diseases including cancers.

Our bodies are encased by the skin epidermis, which serves as a protective barrier against external environmental insults and loss of internal bodily fluids (Fuchs 2007; Koster and Roop 2007). These functions exist through a single layer of proliferative cells that gives rise to terminally differentiating stratified layers whose cells are sloughed from the skin surface and continually replaced by inner cells moving outward. The skin epithelium is separated from the dermis by a basement membrane (BM) that is rich in extracellular matrix and tyrosine kinase growth factors, which provide proliferative stimuli to the innermost basal layer of the epidermis.

Epidermis is remarkable in its ability to generate appendages. In haired body regions, the primary appendages formed by epidermis are hair follicles (HFs) and their associated sebaceous glands (SGs), which lubricate the skin surface with oils that exit through the canal of the “pilosebaceous” unit. In nonhaired regions, the major appendage is the sweat gland, which brings fluids to the body surface for cooling. These markedly different epithelial structures arise during development, as external cues stimulate a single layer of epidermal stem cells

(SCs) to stratify to form the architecture of the mature epidermis and its appendages.

Signals from specialized mesenchymal cells within the dermis orchestrate the decision to form HFs or sweat glands. Once HFs begin to mature, they initiate differentiation of the SGs, which are the last of the appendages to form. In most mammals, a dense coat of HFs provides the bulk of protection to the body surface and, concomitantly with coat formation, the epidermis becomes less proliferative and thins. In the adult, the epithelial lineages of the skin undergo continual turnover and rejuvenation.

The three best-studied lineages of the skin—epidermis, HFs, and SGs—all have distinct SCs that are capable of self-renewing, generating their resident tissue in its entirety and maintaining homeostasis once the tissue is formed. In this review, we focus on the current mechanisms known to regulate SC character in epithelial niches of these three lineages and how defects in these mechanisms contribute to tumorigenesis.

Regulation of the epidermis

The epidermis begins as a single layer of ectodermal cells that are specified to the epidermal lineage. Basal cells of the interfollicular epidermis (IFE) maintain a population of progenitors that retain their proliferative potential basally but can also stratify and differentiate progressively upward to generate multiple suprabasal layers. The differentiation program of the epidermis exists as three morphologically and biochemically distinct phases (Fig. 1). Cells of the spinous and granular layers remain transcriptionally active. Spinous cells synthesize an extensive network of keratin filaments interconnected to desmosomes to generate an integrated mechanical infrastructure in the differentiating layers, while granular cells produce lipid-rich lamellar granules. Granular cells also make lysine- and glutamine-rich proteins that become irreversibly cross-linked by transglutaminase to form the cornified envelope. As granular cells transit to the stratum corneum, all metabolic activity ceases, cytoplasmic organelles are lost, and the cornified envelope serves as a scaffold for lipid bilayers that are extruded to make the epidermal barrier at the skin surface (Fuchs 2007; Koster and Roop 2007).

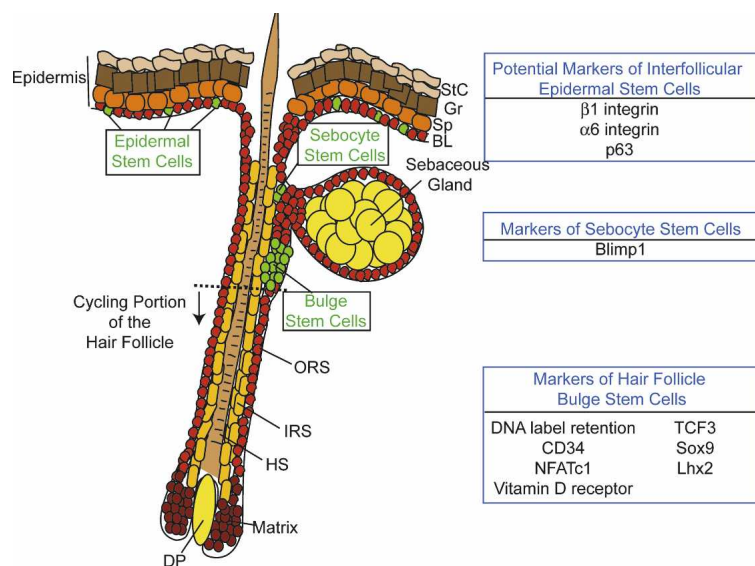
Genetic lineage tracing has revealed that the IFE contains a population of SCs that are distinct from those in the HF and that are responsible for maintaining normal

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¹Corresponding author.

E-MAIL fuchslb@rockefeller.edu; FAX (212) 327-7954.

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NFATc1. Bulge cells generate cells of the ORS, which are thought to fuel the highly proliferative matrix cells that are adjacent to the mesenchymal DP. After spurts of rapid proliferation, matrix cells differentiate to form the hair channel, the IRS, and the HS.

homeostasis (Ito et al. 2005; Levy et al. 2005). Two different mechanisms have been described to account for how a single layer of proliferative basal cells generates a multilayered differentiated epidermis. In the first model, a small population of slow-cycling basal SCs gives rise to a large number of more rapidly proliferating but transiently amplifying (TA) cells that, after a few divisions, undergo a decline in expression of surface integrins, leading to detachment from the BM and suprabasal differentiation. Although this model has been widely accepted, recent studies show that basal epidermal cells can also polarize and localize key regulatory proteins to distinct cortical domains. This process could lead to divisions that asymmetrically partition proteins that specify SC versus differentiating cell fates to the two daughter cells (Lechler and Fuchs 2005; Clayton et al. 2007). Moreover, in the absence of $\beta 1$ integrin or α -catenin, asymmetric divisions do not occur properly, underscoring the requirement of the BM and cell–cell junctions in this process (Lechler and Fuchs 2005). Whether the committed basal cell delaminates in the act of or after the division is still unclear. It could be that the differences observed in this regard reflect differences in the relative rates of basal cell proliferation versus stratification and/or differentiation, which vary considerably between embryonic and adult skin.

Where and how many SCs reside within the basal layer has long been debated, and the identification of asymmetric divisions has only added fuel to this fire. Classically, epidermal SCs were defined by their slow-cycling characteristics and their ability to form proliferative units (EPU) composed of hexagonally packed cells that can be outlined either histologically or by lineage tracing experiments (Potten 1974; Mackenzie 1975; Ghazizadeh and Taichman 2001). Cell kinetic analysis in vitro have supported the notion that infrequently dividing epider-

Figure 1. Epithelial SC compartments in the skin. The epidermis contains a population of epidermal SCs (green) that reside in the basal layer (BL). In models where a small number of SCs and a large number of TA cells (red) reside within the basal layer, SCs are proposed to express elevated levels of $\beta 1$ and $\alpha 6$ integrins and differentiate by delamination and upward movement to form the spinous layer (Sp), the granular layer (Gr), and the stratum corneum (StC). Recent findings suggest that by virtue of their ability to undergo asymmetric divisions, many if not all basal cells may have the capacity for self-renewal and epidermal stratification and differentiation. The SG contains a small number of progenitors that express the transcriptional repressor Blimp1 and reside near or at the base of the SG. SG progenitors produce proliferative progeny that differentiate into the lipid-filled sebocytes that signify the gland. The HF SCs reside in the bulge compartment below the SG. HF SCs are slow-cycling and express the cell surface molecules CD34 and VdR, as well as the transcription factors, TCF3, Sox9, Lhx2, and

mal SCs produce TA daughters that differentiate after a limited number of divisions and then terminally differentiate (Potten 1974; Jones et al. 1995). In vivo pulse-chase experiments with BrdU show that only 5%–10% of basal epidermal cells are label-retaining cells (LRCs) (Potten 1974). However, it has been difficult to address whether basal epidermal LRCs represent SCs, since the use of BrdU precludes the subsequent analyses of the physiological properties of LRCs. Moreover, when an inducible YFP reporter was used for lineage tracing in mouse tail skin epidermis, the number of labeled basal cells increased with time, a feature inconsistent with an EPU model in which a fixed number of basal layer SCs regionally maintain a defined number of surrounding TA cells (Clayton et al. 2007). While these recent data on asymmetric divisions can be explained mathematically without invoking the existence of TA cells, the studies do not rule out an SC-TA hypothesis and, in addition, regional and age-related differences could yield considerable variation in the percentage of cells within the basal layer that possess high proliferative and tissue regenerative capabilities; i.e., the physiological properties expected of SCs.

If there is a discrete subset of SCs within the basal layer of the epidermis, their identification will be predicated on defining distinguishing markers for them and on conducting lineage tracing experiments to document their importance. In addition to basal cell heterogeneity in label retention, expression of $\beta 1$ integrin is also graded, at least in human epidermis, and in vitro, elevated $\beta 1$ integrin can enrich for human epidermal cells with greater proliferative potential (Figs. 1, 2; Jones et al. 1995). That said, $\beta 1$ is not a specific epidermal SC marker (Ghazizadeh and Taichman 2001), and although $\beta 1$ integrin-null basal cells fail to maintain proliferative capacity in vivo, this has been attributed at least in part

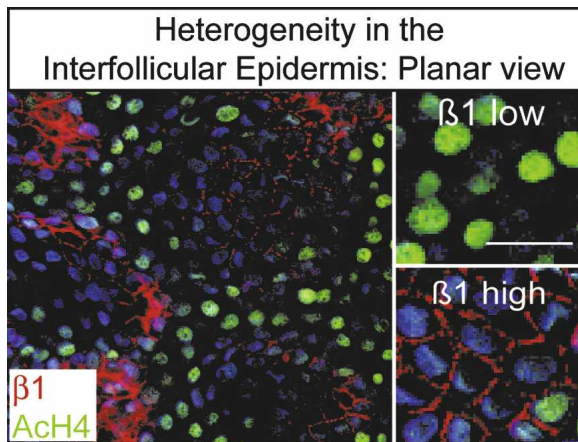


Figure 2. Heterogeneity in the basal epidermal layer. Expression of $\beta 1$ integrin and acetylated histone H4 (AcH4) are differentially expressed in human epidermis. In $\beta 1$ integrin-high cells, acetylated histone H4 levels are reduced, suggesting that chromatin remodeling might play a role in the regulation of SCs within the epidermis. (Images courtesy of F. Watt) (Frye et al. 2007).

to associated defects in BM deposition and organization (Brakebusch and Fassler 2005).

Adding another interesting twist to $\beta 1$ integrin regulation within the basal layer is $\beta 1$'s role in asymmetric divisions (Lechler and Fuchs 2005). This link suggests the intriguing possibility that through the act of asymmetric cell divisions, SCs may be able to generate a $\beta 1$ -high cell that maintains its stemness and a $\beta 1$ -low daughter that commits to terminally differentiate. Integrin expression and spinous layer differentiation are also inversely linked to Notch signaling. When active Notch signaling results in the cleavage of the intracellular domain of Notch, NICD is freed to form a bipartite transcription factor with the RBPj DNA-binding protein, which in turn results in transcriptional changes required for basal cell detachment and for spinous differentiation (Rangarajan et al. 2001; Blanpain et al. 2006). These findings make it difficult to distinguish the specific effects of $\beta 1$ integrin from those of other cellular changes. Thus, although the importance of the BM in maintaining epidermal SC proliferation and maintenance is clear, it has not been documented in vivo that $\beta 1$ integrin levels determine the number of proliferative rounds a basal cell has prior to embarking on a terminal differentiation program.

Signaling through the transmembrane receptor tyrosine kinases (RTKs), insulin growth factor receptor (IGFR), and epidermal growth factor receptor (EGFR) also regulate proliferative behavior in the epidermis (Fig. 3). Both EGFR and IGFR ligands act as potent mitogens for keratinocytes in vitro (Rheinwald and Green 1977; Barrandon and Green 1987). Overexpression or injection of EGF can promote epidermal thickening (Atit et al. 2003 and references therein), while deletion of Mig6, an attenuator of EGFR signaling, leads to hyperproliferation of epidermal keratinocytes and increased susceptibility

to tumorigenesis (Ferby et al. 2006). Consistent with these studies, Lrig1, a negative modulator of EGFR responsiveness, appears to be expressed differentially within the basal layer and can repress keratinocyte proliferation in vitro (Jensen and Watt 2006).

Adding complexity to the control of epidermal homeostasis is the transforming growth factor β (TGF β) signaling pathway, which can cause epidermal keratinocytes to transiently withdraw from the cell cycle by inducing G₁ cyclin-dependent kinase (cdk) inhibitors (Massague and Gomis 2006). Conversely, loss of TGF β s, expression of a dominant-negative TGF β type II receptor (TGF β RII), and conditional deletion of the *TGF β RII* gene in skin lead to epidermal hyperproliferation (Glick et al. 1994; Guasch et al. 2007). However, TGF β s can also promote apoptosis in keratinocytes, and in *TGF β RII*-null skin, hyperproliferation is counterbalanced by enhanced apoptosis,

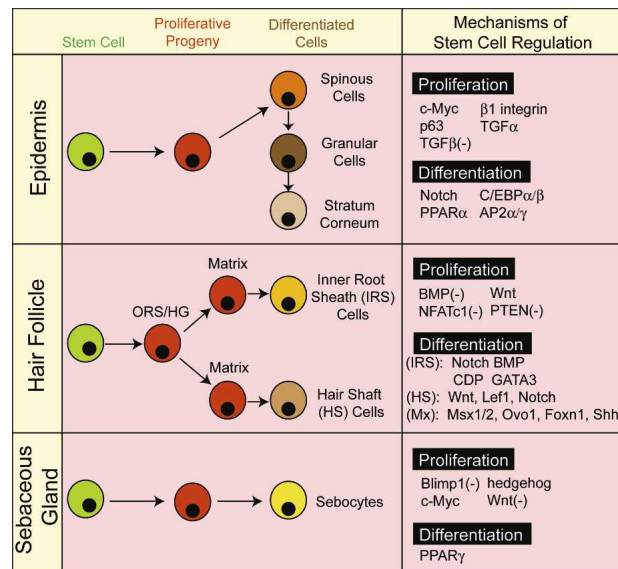


Figure 3. The molecular mechanisms that control epithelial SC proliferation and differentiation in the skin. Epidermal SCs produce three differentiated cell types: the spinous cells, the granular cells, and the stratum corneum. The proliferation of epidermal SCs is regulated positively by $\beta 1$ integrin and TGF α , and negatively (-) by TGF β signaling. In addition, the transcription factors c-Myc and p63 control epidermal proliferation. Notch signaling and the transcription factors PPAR α , AP2 α / γ , and C/EBP α / β control the differentiation of epidermal cells. The HF SCs in the bulge region produce multiple progenitor cells in the ORS/HG and the matrix, which ultimately produce two differentiated lineages: the HS and the IRS. The proliferation of the bulge cells is controlled negatively (-) by BMP signaling and the transcription factors NFATc1 and P-TEN, and positively (+) by Wnt signaling. Differentiation of the IRS is controlled by Notch and BMP signaling and the transcription factors CDP and GATA-3. HS differentiation is controlled by Wnt signaling and its downstream transcription factor Lef1. Matrix (Mx) cells are controlled by Msx1/2, Ovo1, Foxn1, and Shh. The unipotent SG SCs are regulated negatively by the transcription factor Blimp1 and Wnt signaling, and positively by c-Myc and hedgehog signaling. The differentiation of sebocytes is thought to be directed by PPAR γ expression.

enabling the epidermis to maintain homeostasis (Guasch et al. 2007). The homeostasis achieved by *TGF β RII*-null epidermis is precarious, and when challenged with an additional genetic alteration—e.g., oncogenic Ha-Ras—squamous cell carcinoma ensues (Guasch et al. 2007).

Consistent with the role of TGF β signaling in cancer formation, conditional epidermal deletion of *SMAD4* results in hyperproliferation of epidermal keratinocytes and squamous cell carcinoma development with age (Yang et al. 2005). SMAD4 is the cofactor for both TGF β and bone morphogenetic protein (BMP) signaling-activated SMAD members to mediate their transcription. While SMAD4's effects impact on both pathways, conditional deletion of the *BMP receptor 1A* (*BMPRIA*) has not revealed defects in epidermal proliferation, although it has marked consequences for HFs. Thus, unless functional redundancy through the additional BMP receptor 1B has obscured a key relevance of BMP receptor signaling in the *BMPRIA*-null epidermis, SMAD4's effects are likely mediated by TGF β signaling in this tissue.

Given that RTKs promote and TGF β s inhibit epidermal proliferation, it is not surprising to find one of their target transcription factors, c-Myc, at the crossroads of skin homeostasis. Functional studies confirm that overexpression of c-Myc in the epidermis results in hyperproliferation, endogenous c-Myc is dispensable for normal skin homeostasis, but c-Myc-deficient epidermis is resistant to Ras-mediated chemically induced tumorigenesis (Oskarsson et al. 2006 and references therein). Recently, it was found that human basal epidermal keratinocytes express the repressive chromatin mark trimethylated Lys 9 on histone H3, and this epigenetic mark is lost when c-Myc is overexpressed (Fig. 2; Frye et al. 2007). In the future, it will be interesting to explore how epigenetic modifications are established naturally and how they are altered as epidermal SCs commit to terminally differentiate.

In addition to RTK and TGF β signaling circuitries, functional studies suggest a role for the p63 isoform Δ Np63 in the maintenance of the proliferative potential of epidermal cells (Senoo et al. 2007 and references therein). p63 is a relative of the established tumor suppressor p53, which typically restricts proliferation by inducing cell cycle arrest or apoptosis. One mechanism by which p63 might act to control proliferation is in concert with p53. This has been most convincingly demonstrated in human epidermal organotypic cultures, where simultaneous knockdown of both p53 and p63 with siRNAs was found to rescue the cell proliferation defects that occurred with knockdown of p63 alone (Truong et al. 2006). p63's importance in epidermal biology across the vertebrate kingdom has become increasingly apparent (Truong et al. 2006; Senoo et al. 2007), and its governance is controlled both transcriptionally and through microRNAs (Rangarajan et al. 2001; Yi et al. 2008).

Regulation of the HF

In mice, HF morphogenesis occurs in waves from embryonic day 14.5 to 18.5 (E14.5 to E18.5) to give rise to

the distinctive hairs (guard, awl, zigzag, and auchenne) that constitute the adult hair coat (for review, see Schmidt-Ullrich and Paus 2005). HF morphogenesis begins with the downward invagination of the epidermis into the underlying dermis to form a hair placode. This process is dependent on cues received from neighboring epidermal cells as well as underlying dermal cells that assemble into a condensate to form what will become the dermal papilla (DP), which drives further HF formation. As the HF extends into the dermis, it is encapsulated by the highly proliferative (matrix) cells at the leading front.

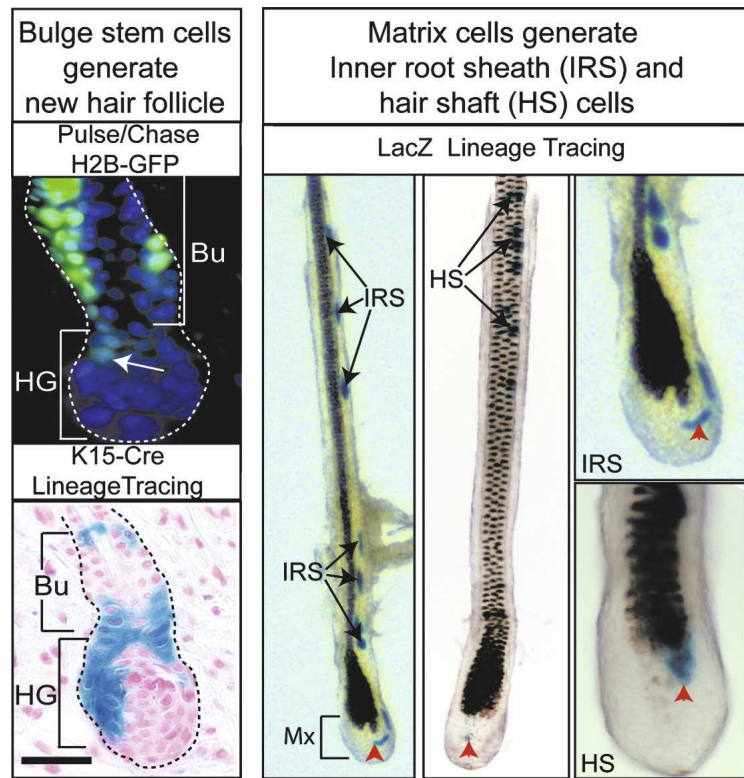
Once HF extension into the dermis is complete, matrix cells at the base (bulb) of the HF continue to proliferate as they generate the upwardly terminally differentiating cells that form the inner root sheath (IRS) and hair shaft (HS) (Figs. 1, 4; Legue and Nicolas 2005). The IRS acts as a channel that guides the HS to the skin surface. IRS specification is regulated by BMP signaling, Notch signaling, and transcription factors CDP and GATA-3 (Fig. 3; for review, see Blanpain and Fuchs 2006). HS specification is regulated by Wnt signaling and transcription factor LEF1 (Fig. 3; Gat et al. 1998; Ito et al. 2007). The outer layer of cells, called the outer root sheath (ORS), is contiguous with the epidermis and contains a region known as the bulge (Fig. 1).

Following this initial period of hair growth, which is completed by approximately day 14 in the mouse, HFs enter a degenerative phase (catagen) involving the apoptosis and loss of the lower two-thirds of the HF. During this process, an epithelial strand brings the DP upward to rest beneath the bulge. Upon full regression, the HF enters a resting stage (telogen) in which the bulge anchors the old hair (club) but waits before initiating a new cycle of hair regeneration (anagen). As a new round of growth begins, the emerging hair germ (HG) begins to proliferate and grow downward, in a process resembling HF morphogenesis (Fig. 4). The cyclic nature of degeneration and regeneration supports the notion that SCs within the HF exist and maintain this miniorgan. With each new hair cycle, the resting phase expands, suggesting that whatever the cues required for stimulating entry into anagen, the threshold becomes increasingly difficult to achieve as the animal ages.

Located within the ORS and just below the SG, the bulge compartment of the HF was identified initially on its histological appearance and is the most well-defined SC niche in the skin (Blanpain and Fuchs 2006). Operating on the premise that SCs are used sparingly and hence cycle infrequently, researchers have used nucleotide labels in pulse-chase experiments to identify the bulge as the residence of the slow-cycling LRCs within the skin (Cotsarelis et al. 1990).

The LRCs of the HF cycle less frequently than those of the IFE. Moreover, multiple studies have confirmed that these slow-cycling cells are SCs that regenerate the HF. When activated to form a new hair during anagen, some LRCs exit the niche and become proliferative, as initially evidenced by their incorporation of a second DNA nucleotide tracer administered at this time (Taylor et al. 2000). Similarly, when bulge LRCs are labeled by pulse and

Figure 4. Lineage relationships in the HF. (*Left panels*) The DNA LRCs of the bulge can be marked by a 4-wk chase of histone H2B-GFP (green), regulated specifically in K5-positive keratinocytes in a tetracycline-controllable fashion (Tumbar et al. 2004). Once hair growth occurs, the bulge (Bu) remains GFP-high, and cells with diminishing GFP can be detected in the growing HG. Similar findings were obtained through genetic lineage tracing of bulge cells marked by expression of a *Rosa26 lox-stop-lox lacZ* transgene rendered active through *K15-Cre recombinase*, active in the bulge cells and their progeny. (Reprinted by permission from MacMillan Publishers Ltd: *Nat. Biotechnol.*) (Morris et al. 2004). (*Right panels*) The matrix (Mx) cells of the HF can also be lineage-traced using regulatable *lacZ* expression. (Courtesy of E. Legue and J.F. Nicolas; reproduced with permission of the Company of Biologists) (Legue and Nicolas 2005). The isolated HFs shown here exhibit clonal contribution of Mx progeny to both the IRS and the HS.



4-wk chase of a GFP-labeled histone (H2B-GFP) that is regulated by tetracycline, the H2B-GFP LRCs that exit the niche divide rapidly, diluting the fluorescence label (Fig. 4; Tumbar et al. 2004). Although a trail of a few H2B-GFP-positive LRCs can be detected in the ORS during anagen, the majority of bulge LRCs remain H2B-GFP-bright, indicating that only a subset of LRCs are used with each hair cycle and that the bulge as a whole remains largely slow-cycling (Tumbar et al. 2004).

Lineage tracing experiments *in vivo* have been used to document the relation between bulge cells and the new, regenerating HF. By crossing *Rosa26 lox-stop-lox lacZ* mice to those expressing a regulatable Cre recombinase controlled by the keratin 15 (K15) promoter, Morris et al. (2004) genetically marked telogen-phase bulge cells and followed their progeny during anagen induction. Expression of *lacZ* was detected in the growing HG as well as in the ORS and matrix (Fig. 4).

To demonstrate the multipotent behavior and enormous regenerative potential of bulge cells by clonal analyses, researchers have exploited the capacity to culture bulge SCs *in vitro* from either microdissected rat whisker HFs (Claudinot et al. 2005 and references therein) or FACS-purified mouse pelage HFs (Blanpain et al. 2004). In both cases, even after prolonged passaging *in vitro*, cells derived from a single bulge cell could still generate epidermis, SGs, and HFs when engrafted onto the backs of *Nude* mice (Blanpain et al. 2004; Claudinot et al. 2005). Intriguingly, the grafted cells formed HFs that cycle and displayed a new bulge compartment, suggesting that the SC niche can be regenerated. In striking

parallel to that observed from lineage tracing analyses (Ito et al. 2005), the contribution of bulge cells propagated for >100 generations outside their native niche showed long-term contribution to the pilosebaceous unit, but only transient contribution to the epidermis during the wound-related period when the graft was still healing (Claudinot et al. 2005). Human epidermal SCs also possess this remarkable intrinsic ability to maintain their features of stemness outside their native niche, a feature that has been exploited for treatment of badly burned patients (Blanpain and Fuchs 2006).

How the bulge niche regulates SC activity is just beginning to be revealed (Fig. 3). Transcriptional profiling reveals signs of elevated BMP signaling in the bulge (Blanpain et al. 2004; Morris et al. 2004; Tumbar et al. 2004) and, *in vitro*, BMPs cause bulge cells to withdraw transiently from the cell cycle, suggesting a role for BMPs in controlling SC quiescence (Blanpain et al. 2004). Additionally, BMPs and BMP inhibitors are expressed by the DP cells, which transmit external regulatory cues to the bulge (Kulesa et al. 2000; Rendl et al. 2005; Kobiela et al. 2007) in cyclic fashion (Plikus et al. 2008).

When conditionally targeted for ablation in telogen-phase bulge cells, loss of *BMP receptor 1A (BMPR1A)* results in global activation of the SCs, leading to expansion of the niche and inactivation of the dual specificity phosphatase (PTEN) (Kobiela et al. 2007). Interestingly, conditional deletion of *SMAD4* also results in enhanced proliferation in the ORS progenitors of the bulge cells, which leads to defects in hair cycling and alopecia. The phenotype of the *SMAD4*-null skin is augmented by loss

of PTEN (Ito et al. 2005), and since conditional loss of the *TGFBR1* does not induce HF cycling or cause hyperproliferation in the bulge niche (Guasch et al. 2007), it seems most likely that the effects of SMAD4 in the HF are mediated by BMP signaling to control PTEN activation and coordinate quiescence.

Recently, we discovered that BMP signaling may also act to regulate transcription of *NFATc1*, encoding a transcription factor specifically expressed in cells of bulge HFs (Tumbar et al. 2004; Horsley et al. 2008). Loss-of-function studies suggest that NFATc1 activity is required for maintaining the slow-cycling nature of bulge SCs, where it acts at least in part through transcriptional repression of the cell cycle regulatory gene encoding CDK4. In order for NFATc1 to function in nuclear transcription, it must be modified through a calcium/calci-neurin mechanism. Although the underlying mechanisms remain to be elucidated, we have found that the immunosuppressive drug cyclosporine A (CSA), which inhibits calcineurin, acts on the bulge to promote proliferation. This finding is particularly intriguing in light of the well-known side effects of CSA on hair growth, and the established, often negative, effects of calcium on cell cycle control (Schmidt-Ullrich and Paus 2005). From these data, a model emerges whereby BMP and calcium act coordinately to regulate NFATc1 and PTEN, which together function by holding bulge cells in a quiescent, slow-cycling state.

In addition to BMP signaling, the bulge niche appears to be in a repressed state of Wnt signaling. Quiescent bulge SCs express soluble Wnt inhibitors such as FRP1 and Wif (Morris et al. 2004; Tumbar et al. 2004). In addition, they fail to display signs of bulge-specific Wnt reporter gene activity, which requires the presence of members of the LEF1/TCF family of DNA-binding proteins and their bipartite transcriptional cofactor nuclear β -catenin (Blanpain and Fuchs 2006). The rate-limiting step for activation of Wnt signaling in the bulge appears to be the presence of sufficient levels of nuclear β -catenin, since bulge SCs express the cognate Wnt receptors (Frizzleds and LRP6) (Morris et al. 2004; Tumbar et al. 2004) as well as TCF3 and TCF4 (Nguyen et al. 2006).

β -Catenin is stable at adherens junctions, where it participates in cell–cell adhesion. However, excess cytoplasmic β -catenin is typically phosphorylated by the GSK3 β kinase and targeted for ubiquitin-mediated turnover. To accumulate threshold levels of β -catenin for gene expression, the GSK3 β kinase must be phosphorylated and inactivated. This can be accomplished through several different mechanisms, including Wnt signaling and phosphatidylinositol 3-kinase (PI3K)-Akt. Since PI3K is inhibited by PTEN, BMP and Wnt signaling pathways are likely to converge to negatively and positively regulate bulge SC functions that are dependent on β -catenin stabilization. Activation of the small GTPase, Cdc42, can also contribute to inactivation of GSK3 β to control bulge SC activity (Wu et al. 2006). Whether this is due to PTEN/PI3K-mediated effects on Cdc42 is not yet clear.

What functions are dependent on β -catenin stabilization? The earliest indications arose from transgenic studies

in which a constitutively stabilized form of β -catenin, lacking the GSK3 phosphorylation sites in the N terminus (Δ N β cat), resulted in expansion of the cell fate choices afforded to adult IFE and excessive HF formation (Gat et al. 1998). Conversely, *TCF4* knockout mice failed to maintain progenitors in the intestinal crypt (Reya and Clevers 2005), which prompted the hypothesis that LEF1/TCF factors and their partners play an important and perhaps universal role in the activation of proliferation of SC reservoirs followed by a discrete differentiation program (Gat et al. 1998). This has held true for nearly all of the SC reservoirs characterized to date (Reya and Clevers 2005), and Wnt signaling in SCs appears to be critical not only for regulating tissue homeostasis but also for wound repair (Ito et al. 2007; Stoick-Cooper et al. 2007).

In the skin, conditional loss of β -catenin supports a role for β -catenin in follicle SC maintenance and self-renewal (Huelsenken et al. 2001), while elevated TCF3 expression suppresses all three epithelial lineages (Nguyen et al. 2006). When Δ N β cat is induced in resting (telogen-phase) HFs, HF regeneration is activated precociously (Van Mater et al. 2003). Among the Wnt target genes that are activated during this transition are *cyclin D2*, *Sox 4*, and *biglycan* (Lowry et al. 2005). Interestingly, although the threshold for SC proliferation and activation appears to be reduced by increasing the pool of stabilized β -catenin, the microenvironment of the SC niche appears to override this signal as, once activated, the bulge niche returns to a quiescent state (Lowry et al. 2005). This contrasts with loss of BMP receptor and/or NFATc1 signaling, which compromises quiescence (Kobielak et al. 2007; Horsley et al. 2008).

One model consistent with these collective results is that when the levels of nuclear β -catenin are low, TCF3/4 can act to maintain bulge SCs in their undifferentiated state, but as the levels rise, SCs exit the niche, activate Wnt target genes, proliferate, and embark on their journey along the HF lineage. An intriguing possibility is that the activation by LEF1/TCF/ β -catenin genes may vary depending on the specific LEF1/TCF family member and/or the overall concentration of β -catenin. If so, additional fine-tuning of gene expression may be achieved through reductions in BMP signaling and elevations in Wnt signaling.

A number of players in bulge SC maintenance have been identified, but as yet their placement within the framework of our knowledge of SC behavior still remains elusive (Fig. 3). Repression of *c-Myc* also appears to be critical for controlling bulge SC behavior, as *c-Myc* overexpression can lead to excessive proliferation and differentiation into epidermal and sebaceous fates (Braun et al. 2003 and references therein), while early loss of *c-Myc* in the skin represses bulge SC proliferative capacity and results in alopecia (Zanet et al. 2005). The Rho GTPase Rac-1 may also be involved in bulge SC maintenance, as evidenced by conditional loss-of-function mutations in *Rac-1* (for review, see DiPersio 2007). Finally, three transcription factors that are elevated within the bulge include the LIM homeobox transcription factor *Lhx2*, *Sox9*, and the vitamin D nuclear receptor (VdR)

(Blanpain et al. 2004; Morris et al. 2004; Tumber et al. 2004; Rhee et al. 2006). Loss of *Lhx2* results in increased proliferation of cells within the follicle SC niche as well as the loss of follicle SC markers such as CD34 and tenascin C (Rhee et al. 2006), while loss of either *Sox9* or *Vdr* results in alopecia (Vidal et al. 2005; Cianferotti et al. 2007). It has been suggested that *Vdr* interferes with Wnt signaling (Cianferotti et al. 2007 and references therein). It also heterodimerizes with RXR α and complexes with the transcriptional repressor Hairless, two other proteins that, when mutated, result in a loss of follicle SCs and alopecia (Xie et al. 2006).

Once activated and committed to a HF program of differentiation, SCs go through several proliferative steps prior to making the so-called TA matrix cells that fuel the formation of the HF. Bulge ORS cells and some early progeny express *Sox9*, *Tcf3*, *Lhx2*, and E-cadherin, while another population of progeny express *Sox4*, *Lhx2*, and sonic hedgehog (Rhee et al. 2006; Kobiela et al. 2007).

A number of questions remain: How does the HF SC niche maintain a constant SC pool size despite its expenditure of SCs with each successive round of regeneration? How is the balance between SC self-renewal, quiescence, proliferation, and commitment fine-tuned? How do bulge SCs exit and migrate the niche and what governs this process? The answers to these questions should unfold in the next few years.

Regulation of the SG

A third lineage afforded to epithelial cells in the skin is the SG, which develops late in embryogenesis in the upper portion of the HF. In mature skin, the SG resides above the bulge. During differentiation of the gland, mature sebocytes are produced that synthesize specialized lipids, or sebum. As sebocytes differentiate, they lyse, releasing sebum into a canal and onto the skin's surface. The constant turnover of mature sebocytes requires a continuous source of cells to maintain the gland, suggesting that SCs might be involved in SG homeostasis.

HF bulge SCs have the capacity to differentiate and produce SGs, as evidenced from grafting studies with FACS-purified bulge cells and their cultured progeny (Blanpain et al. 2004; Morris et al. 2004). However, several lines of evidence suggest that within the SG there also exists a resident pool of progenitor cells. In addition, retroviral lineage tracing experiments reveal that the SG can be marked specifically over multiple hair cycles, supporting the notion that a population of long-lived cells can maintain the SG independent of the HF bulge (Fig. 5; Ghazizadeh and Taichman 2001). Pulse-chase experiments further suggest the existence of slow-cycling cells in the gland (Braun et al. 2003).

Recently, it was shown that a small cluster of cells at the base of the SGs is marked by expression of a transcriptional repressor, *Blimp1* (Fig. 1; Horsley et al. 2006). Genetic lineage tracing experiments show that the *Blimp1*-expressing cells are progenitors that give rise to all of the cells within the SG (Fig. 5). Moreover, conditional ablation of *Blimp1* results in increased sebocyte proliferation

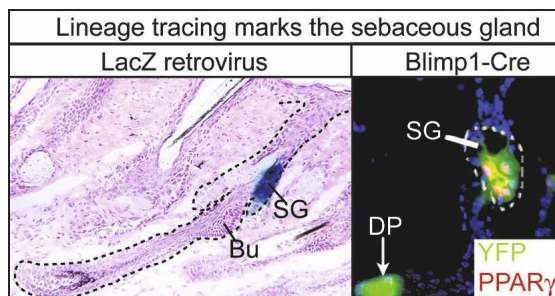


Figure 5. Evidence for a SG progenitor population. (*Left panel*) Skin sections are shown from mice in which retroviruses expressing lacZ were injected into the skin following wounding. The skin was then analyzed 70 d after injections. X-gal staining (blue) marks the SG and not the HF bulge (Bu) or the epidermis. (Reprinted by permission from MacMillan Publishers Ltd: *EMBO J.*) (Ghazizadeh and Taichman 2001). (*Right panel*) Skin sections of *Rosa26-flox-stop-flox-YFP* mice that also express Cre recombinase under the *Blimp1* promoter. YFP becomes activated in all *Blimp1*-positive cells and their progeny, which here include the differentiated sebocytes that are PPAR γ (+). (Reprinted from *Cell*, 126, with permission from Elsevier) (Horsley et al. 2006).

and expansion of the glands, indicating that *Blimp1* acts as a gatekeeper in the SG progenitor cells. The link between *Blimp1* and proliferation is especially interesting in that *Blimp1* regulates *c-Myc* gene expression in B lymphocytes and elevated *c-Myc* expression in skin results in enlarged SGs (Braun et al. 2003), much like the loss-of-function mutations in *Blimp1* (Horsley et al. 2006). Together, these findings have shed insight into the nature of this unipotent SC pool.

The best-characterized signaling pathway involved in sebocyte proliferation is hedgehog signaling (Fig. 3). Expression of a mutant smoothen receptor that constitutively activates hedgehog signaling results in ectopic sebocyte development (Allen et al. 2003). Similarly, treatment of sebocytes in vitro with Indian hedgehog (IHH) drives sebocyte proliferation (Niemann et al. 2003). In contrast, inhibition of hedgehog signaling by overexpression of a dominant-negative mutant of *Gli2*, a downstream transcriptional hedgehog effector, can suppress sebocyte development (Allen et al. 2003).

Several lines of evidence also suggest that inhibition of Wnt signaling may be required for SG lineage specification. The first indications came from expressing a dominant-negative mutant form of *LEF1*, Δ NLEF1, which resulted in SG differentiation at the expense of HFs. Additionally, dominant-negative mutations in humans and in mice result in sebocyte tumorigenesis (Takeda et al. 2006). The negative role of Wnt signaling appears to extend to β -catenin, as overexpression of *Smad7*, which promotes β -catenin degradation through a novel mechanism involving *Smurf2*, also results in SG hyperplasia (Han et al. 2006).

Despite these compelling studies, there are a few paradoxes in the Wnt connection to SG development. One arises from the fact that *Smad7* also functions as an antagonist of TGF β , BMP, and activin signaling pathways

(Massague and Gomis 2006), which under some circumstances can also lead to enhanced β -catenin stabilization (Kobiela et al. 2007). Another is that TCF3 overexpression in skin results in suppression of sebocyte transcriptional regulators and the lack of SG formation (Nguyen et al. 2006). While further studies will be needed to fully understand the basis for these differences, the data provide increasing support for the notion that SC fate in the skin is intricately linked to changes in Wnt signaling.

The Wnt pathway may be linked to hedgehog signaling in sebocytes, as overexpression of Δ NLef1 can lead to up-regulation of IHH in sebocytes (Niemann et al. 2003). Furthermore, sebaceous tumors display up-regulation of patched, the hedgehog receptor (Niemann et al. 2003), supporting a role for hedgehog signaling in sebocyte proliferation and suggesting that the timing and balance between hedgehog and Wnt signaling is crucial for proper sebaceous development.

The little that is known about the SG differentiation program suggests that it resembles that of adipocytes. Like adipocytes, sebocytes appear to govern transcription of lipid metabolism genes through transcription heterodimers of peroxisome proliferator-activated receptor γ (PPAR γ) and retinoid X receptors. Although PPAR γ -null mice are not viable, PPAR γ -null embryonic stem (ES) cells have been found to contribute poorly to the SGs of chimeric mice, lending some supporting functional evidence to its role in vivo (Rosen et al. 1999). Conversely, treatment of PPAR γ ligands can induce sebocyte differentiation in vitro and increase sebum production in humans (Trivedi et al. 2006). Notch signaling is also required for SG development (Pan et al. 2004).

Concluding remarks

The three lineages of the skin—the epidermis, HF, and SG—display a robust regenerative capacity that maintains each lineage through our lifetime and following injury. The mechanisms that regulate SCs within each lineage are only beginning to unfold. Common signaling pathways impinge on the regulation of SCs within each lineage and when these signals go awry, tumorigenesis can occur. How and when these signaling pathways act appears to be critical in controlling SC behavior. Thus, Wnt signaling is key for regulation of SCs in the HF and for HS specification and differentiation, but has inhibitory roles on SG maintenance and differentiation. BMP signaling differs in that it plays a positive role in governing HF SC quiescence, a negative role in HF SC activation, and then a positive role again in specifying late-stage differentiation to make a functional hair. While BMP signaling has no reported effect on the epidermis or SG lineage, TGF β signaling appears to be important in controlling epidermal homeostasis, while Notch signaling appears to be a universal regulator of all three different SC lineages in the skin epithelium.

Does each SC population possess distinct intrinsic features that define their ability to respond differently to these external cues, or is there a single type of keratino-

cyte SC that receives its identity from the microenvironment of its niche? While the answer to this question remains unclear, it is interesting that all three niches are in close proximity to a BM rich in extracellular matrix ligands and growth factors that provide it with a plethora of external stimuli. In addition, however, these niches are also distinguished by variations in the surrounding cellular milieu. The bulge BM, for instance, is encased by a dermal sheath and a rich array of blood vessels and nerve endings, while beneath the epidermal BM is a dense array of dermal fibroblasts. Whether intrinsic or extrinsic, SCs within distinct SC niches of the skin ensure that proper differentiation programs are executed in controlling a remarkable diversity in tissue structure and function and an amazingly rapid flux of transient epithelial cells through these tissues. The additional ability of skin epithelial tissues to respond and repair frequent wounds and injury to our body surface make it all the more fascinating that the balance between SC quiescence, activation, proliferation, and differentiation only occasionally goes awry in the course of the lifetime of these tissues.

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