The multifaceted adult epidermal stem cell

Laure Gambardella and Yann Barrandon*

Adult epidermal stem cells renew the epithelial compartment of the skin throughout life and are the most accessible of all adult stem cells. Most importantly, epidermal stem cells can be efficiently cultivated and transplanted, a significant advantage for cell and gene therapy. Recent work has pointed to the hair follicle as the main repository of multipotent stem cells in skin. Hair follicles, which are often affected in the mouse by spontaneous or man-made mutations, have become superb model systems to study the cellular and molecular factors that regulate the proliferation, migration and fate of adult stem cells.

Addresses
Laboratory of Stem Cell Dynamics: School of Life Sciences, Swiss Federal Institute of Technology, Lausanne, Switzerland; and Department of Experimental Surgery, Lausanne University Hospital, 1015 Lausanne, Switzerland
*e-mail: yann.barrandon@epfl.ch

Current Opinion in Cell Biology 2003, **15**:771–777

This review comes from a themed issue on
Cell differentiation
Edited by Fiona Watt and Elaine Fuchs

0955-0674/$ – see front matter

DOI 10.1016/j.ceb.2003.10.011

Abbreviations
BMP bone morphogenetic protein
DEB dystrophic epidermolysis bullosa
EPU epidermal proliferative unit

Introduction

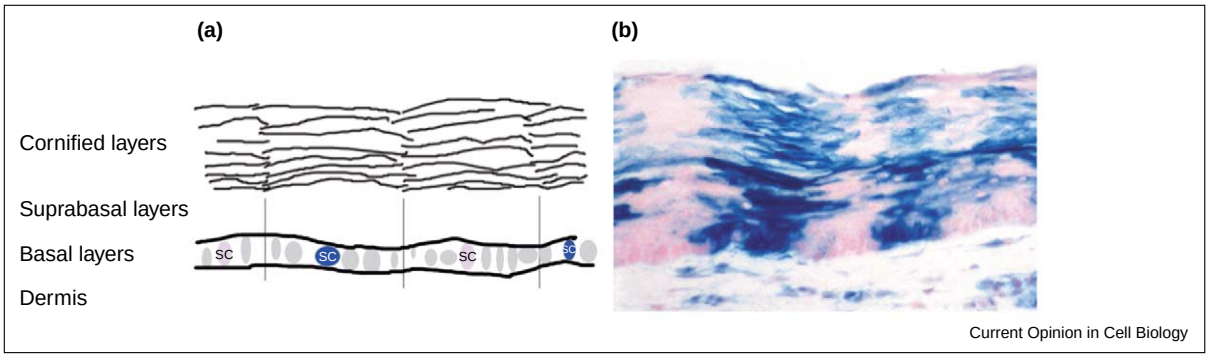
The epidermis is the outermost layer of the body and is in direct contact with the external environment. It is a stratified keratinised epithelium mainly composed of keratinocytes, the specialised epithelial cells responsible for epidermal renewal, cohesion and barrier function. Other inhabitants of the epidermis are the antigen-presenting Langerhans cells and the epidermal T lymphocytes, which are both derived from bone-marrow, the pigment-forming melanocytes, which are of neural crest origin, and the neuroepithelial Merkel cells, whose origin and function remain unknown. The epidermis is in continuity with the epidermal appendages — the hair follicles and the sebaceous and sweat glands, which form through complex epithelio-mesenchymal interactions during embryonic life and are extended deep in the dermis. Epidermal appendages have long been known to contribute to epidermal repair and regeneration [1,2]. The epidermis is constantly renewed and hair follicles are regularly remodelled (the hair cycle), maintaining a fine equilibrium between proliferation and differentiation. Unbalanced renewal and remodelling can lead to a variety of diseases, including improper scarring and cancers [3,4]. Several features make the epidermis and its appendages a superb model system to study stem cells and tissue renewal. Epithelial stem cells are the most accessible of all adult stem cells as they are easily isolated from a plucked hair [5]. Most importantly, they can be very efficiently expanded in culture [6,7] and transplanted [8]. Furthermore, the large numbers of spontaneous or man-made mutations in the mouse that result in epidermal or pelage abnormalities [9,10] provide unique tools to better comprehend skin homeostasis. The recent advances in understanding the molecular events controlling stem-cell fate exquisitely demonstrate this. These advances [11•,12] include the demonstration of the importance of the transcription factor tcf3 in maintaining the phenotype of bulge stem cells and of the role of the β-catenin/lef-1 pathway in the commitment of the stem cells to hair lineages [13–16] in relation to E-cadherin expression and Wnt signalling [17••,18••]. Furthermore, significant progress has been made in dissecting the roles of several other signalling proteins implicated in the control of stem cell fate, including Notch [19•,20], Ectodysplasin and its receptor [21–23], c-Myc [24,25], sonic hedgehog (Shh) [26] and bone morphogenetic proteins (Bmps) [27]. The putative role of the transcription factor CDP (CCAAT displacement protein) in determining the inner root sheath lineage has also been revealed [28]. However, there are still a lot of unanswered questions and controversial issues regarding the dynamics of epidermal renewal. Here we will review some of the recent work addressing them.

Location of the epidermal stem cells

Epidermal stem cells, like other adult stem cells, are best defined by their capacity to self-renew and to generate large amounts of tissue for an extended period of time or even a lifetime [29]. This functional definition implies that a cell with these properties qualifies as a stem cell: all other properties, including the capacity to divide infrequently [30], clonogenicity [31] and the presence of a specific repertoire of cell-surface molecules [32,33], are then circumstantial [34]. Most importantly, this means that at some point during skin development and renewal there may exist stem cells that are not slow-cycling, that are not clonogenic or that express different repertoires of surface molecules, but that maintain their full stem-cell identity. Future research may lead to the identification and characterisation of these cells in the skin.

It is well established that the glabrous epidermis, the interfollicular epidermis and the hair follicle contain epithelial cells that are slow cycling (label-retaining cells

Figure 1



Columnar organisation of the epidermis. **(a)** The epidermis of the mouse is organised into functionally distinct EPUs comprising a stem cell and its transient amplifying cell progeny, which give rise to a column of differentiated keratinocytes. SC, stem cell. **(b)** Columnar organisation of the epidermis illustrated by the expression of the transcription factor egr1 in the epidermis of the footpad of a *egr1*$^{+/-}$ mouse ([78]; L Gambardella and Y Barrandon, unpublished data). The *egr1* knock-in allele is expressed in some columns (blue) and not in others (pink).

or LRCs) [30], and cells that are clonogenic with extensive growth potential [7,31–33,35]. Most importantly, it has been demonstrated that adult hair follicles contain multipotent stem cells that can reconstitute a wounded epidermis [36] and can respond to skin morphogenetic signals by forming epidermis, hair follicles and sebaceous glands [37]. However, the contribution of these multipotent stem cells to epidermal renewal under normal conditions is highly debated. Indeed, the epidermis of the mouse is organised into functionally distinct epidermal proliferative units (EPUs) (Figure 1) comprising a central stem cell and its transient amplifying cell progeny (around 10 basal cells), which give rise to a column of differentiated keratinocytes located directly above the multiplying cells [38,39]. Ghazizadeh and Taichman have nicely demonstrated by infecting multiplying epidermal interfollicular keratinocyte with a defective retrovirus bearing a marker gene that EPU can self-renew for months independently of any contribution from the hair follicles, hence demonstrating that hair-follicle stem cells contribute little if anything to epidermal renewal under physiological conditions in the adult mouse [40]. There is then compelling evidence that stem cells with differing epithelial lineage potentials are present at several locations in the skin: for example, the basal cell layer of the epidermis contains unipotent epidermal stem cells whereas the upper region (the bulge) of the hair follicle contains multipotent stem cells. The hair follicles and possibly the sweat glands are then reservoirs of stem cells that can migrate out of their niches to adopt an epidermal fate in response to a skin defect. Nevertheless, there are still difficulties in establishing a definite lineage hierarchy even if there is no doubt that the different epithelial stem cells residing in the skin are related to each other [41].

Identification of the epidermal stem cells

Hematopoietic stem cells and progenitor cells are efficiently identified by their repertoire of cell-surface molecules [42]. By analogy, many groups have searched for a universal epidermal stem cell marker, the epidermal stem cells usually being identified on the basis of clonogenicity, growth potential and ability to reconstitute an epidermis. This has led to the demonstration that epidermal stem cells express high levels of β1 integrin [32] and α6 integrin and low levels of the transferring receptor (CD71), whereas transient amplifying cells express high levels of α6 integrin and of CD71 [33]. However, neither β1 integrin nor α6 integrin can be considered to be specific stem-cell markers [43]. Interestingly, epidermal stem cells can be efficiently sorted by Hoechst 33342 dye exclusion from the skin of the new-born mouse [44,45••]. This technique in combination with selection by size yielded a virtually homogenous population of epidermal stem cells that did not express hematopoeitic markers such as CD34 or Sca-1.

It has recently been reported that expression of keratin 15 (K15) is restricted to cells located in the bulge region of human hair follicles, and consequently K15 has been proposed to be a stem-cell marker [46]. However, further work demonstrated that K15 is present in the entire outer root sheath of the human hair follicle [47], in agreement with previous results obtained by *in situ* hybridisation experiments [48]. Differences in the affinity of the antibodies used in these experiments probably explain these apparently conflicting results. This said, further work is necessary to definitively evaluate the relation between stem cells and K15 expression, as a significant number of stem cells are distributed over a large portion of the outer root sheath in human hairs [7].

It has recently been reported that CD34 is specifically expressed on stem cells isolated from the bulge region of mouse hair follicles [49]. However, cells that are CD34-negative also show stem-cell properties [45••,50]. Again further work is needed to clarify these conflicting results. AC133-2, an isoform of CD133 recently identified as a

hematopoeitic stem cell marker, is expressed in basal cells but its usefulness as a stem-cell marker awaits further investigation [51]. Most interestingly, mice in which p63, a transcription factor homologue of the p53 tumour suppressor gene, had been inactivated lack all stratified epithelia [52,53]. Whether p63 acts on stem-cell maintenance or on stratification is still debated [54]. Pellegrini and others [35] showed that p63 expression nicely correlates with the proliferative potential of cultured keratinocytes, with the cells with the highest potential (holoclones, i.e. stem cells) expressing high levels of p63 and the cells with the lowest proliferative potential (paraclones, i.e. transient amplifying cells) expressing no p63. However, p63 expression is not sufficient to unambiguously identify epidermal stem cells *in vivo* as it is expressed in all basal cells as well as in a significant number of suprabasal cells of the epidermis and of the outer root sheath of the hair follicle. Moreover, p63 is also highly expressed in the hair matrix, which is thought to contain only cells with limited growth capacity ([55]; L Gambardella and Y Barrandon, unpublished data). In conclusion, we cannot yet unambiguously identify a stem cell on a skin section.

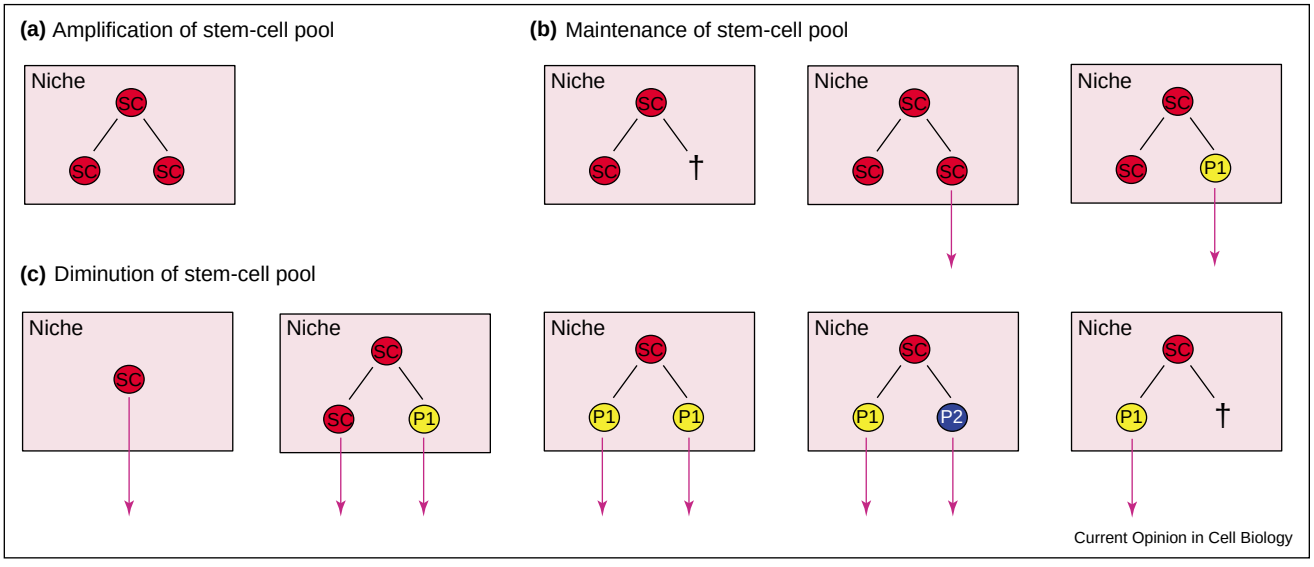
Do stem cells undergo asymmetrical divisions in adult skin?

The pool of adult stem cells that are present in self-renewing tissues such as the blood, intestine or skin must be maintained throughout life. Moreover, the mechanisms that regulate stem-cell proliferation and differentiation must be tightly controlled to avoid depletion or amplification of the pool, a situation that can possibly lead to diseases. It is commonly accepted that an efficient way to control the number of stem cells while maintaining tissue homeostasis is through asymmetrical cell divisions [56]. A stem cell that divides asymmetrically generates two daughter cells, one of which is identical to the dividing stem cell while the other is different (Figure 2). Asymmetrical cell division results either from the unequal segregation of cell-fate determinants (proteins or mRNA) in each of the daughter cells or from the selective response of each daughter cell to a gradient of signalling molecules present in the immediate environment. Numb appears to be one of the main cell-fate determinant proteins segregated during asymmetrical divisions in *Drosophila* [57] as well as during the development of the central nervous system of vertebrates [58,59]. Interestingly, Numb acts by inhibiting notch signalling, which seems to play a pre-eminent role in the hair bulb and the epidermis. The cell that leaves the stem-cell compartment can either proliferate, usually for a limited number of divisions, or differentiate. The protein $p21^{WAF1/CIP1}$ seems to be one of the players that control stem-cell proliferation in the skin of the mouse [60]. Indeed, $p21^{WAF1/CIP1}$ knock-out mice present an excess of stem cells, suggesting that the mechanism controlling the asymmetrical divisions of stem cells is inefficient. Further research will certainly reveal with greater precision the role of asymmetrical divisions in controlling stem-cell fate and skin renewal.

Do stem cells migrate out of their niches?

The bulge-containing region of the hair follicles seems to be the main reservoir of multipotent stem cells [36]. Most

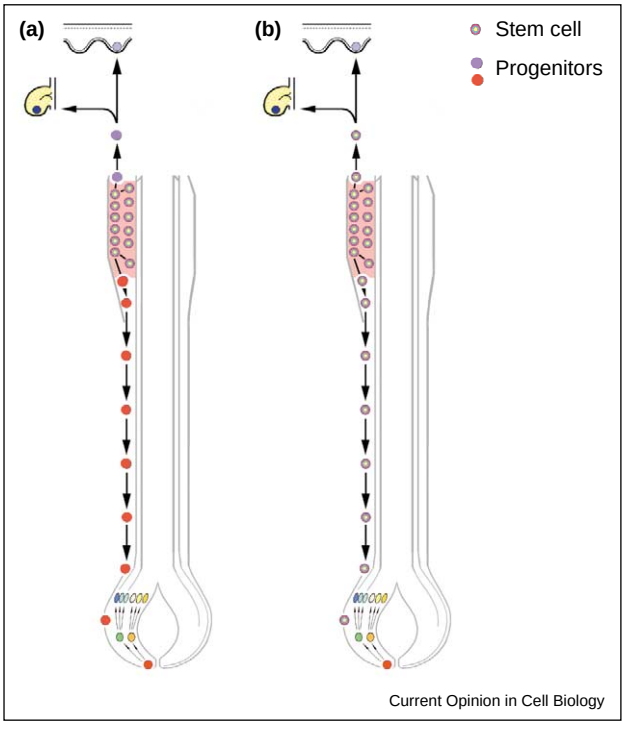
Figure 2



Schematic fate of a dividing stem cell in an epidermal niche. The stem cell (SC) can divide symmetrically or asymmetrically. The resulting daughter cells can be stem cells or committed progenitor cells (P). Daughter cells can stay in the niche, leave it or die. The combination of these events can result in **(a)** the amplification of the stem-cell population, **(b)** the strict maintenance of the stem-cell population, or **(c)** the diminution of the stem-cell population. Arrows indicate that the cells move out of the niche, dagger symbols that the cell dies.

importantly, transplantation experiments that replaced part of the bulge of a wild-type mouse-whisker follicle with a bulge obtained from a Rosa 26 adult mouse that constitutively expressed a *LacZ* gene demonstrated that a hair follicle is a very dynamic structure [37]. Indeed, bulge cells can migrate down the follicle to form the different lineages indispensable to the formation of the outer root sheath, the inner root sheath and the hair or can migrate out of the follicle to generate sebaceous glands and epidermis. Are the cells leaving the bulge committed progenitors or stem cells? How is the pool of stem cells kept constant? These are important questions to address. A possibility is that stem cells divide asymmetrically in the bulge to generate committed progenitor cells that then leave the bulge depending on their fate (Figure 3a). Stem cells then need to divide fairly frequently to generate the numerous progenitors needed to sustain hair growth, but cell kinetics experiments demonstrate that the bulge is not a mitotically active region [37]. Furthermore, it is widely accepted that stem cells generate committed progenitors in response to specific mesenchymal cues originating from the follicular papilla or from the fibroblasts underneath the epidermis or in the vicinity of the sebaceous glands. Therefore it is unlikely that stem cells divide asymmetrically in the bulge, at least in the whisker follicle. An alternative proposal is that stem cells divide symmetrically in the bulge, and that half of the new generation of stem cells remains in place to maintain the pool constant while the other half leaves (Figure 3b). According to this hypothesis, the stem cells excluded from the bulge are fully multipotent and can divide symmetrically or asymmetrically to generate committed progenitors depending upon the signals that they receive on their way. The demonstration that the lower region of the whisker follicle contains cells that respond to skin morphogenetic signals by forming epidermis, hair follicles and sebaceous glands strongly supports this hypothesis [37]. Experiments investigating the fate of cells in pelage follicles labelled by means of defective retroviruses bearing a *LacZ* marker gene [40] and the fate of labelled cells in chimeric pelage follicles [61•] also support the hypothesis that the lower region of the hair follicle contains multipotent progenitors or stem cells [62]. However, only experiments exploring the fate of individual cells isolated from the upper and lower region of the follicle will permit definite conclusions.

Figure 3



Schematic fate of multipotent stem cells in the hair follicle. **(a)** Stem cells can divide asymmetrically in the follicle upper region (bulge; shown in pink) and generate committed progenitors that are specifically sorted out of the bulge depending on their fate and migrate to their new niches. **(b)** Stem cells can divide symmetrically in the follicle upper region (bulge). One of the daughter stem cells remains in the bulge to maintain the stem cell pool, while the other one leaves to generate specific committed progenitors when it reaches its new niche. The structures shown above the bulge are the sebaceous gland (yellow) and the epidermis (waved). The grey and dark blue cells are epidermal and sebaceous progenitors respectively.

Cell therapy and plasticity

The capacity to cultivate stem cells is a *sine qua non* condition for many applications in cell and gene therapy. Adult human epidermal stem cells form colonies that can be serially cultivated under appropriate conditions, leading to a large expansion of their population *in vitro* [6,7]. This fantastic growth capacity has been successfully used to produce cultured epithelia that are transplanted to treat extensive autologous deep burn wounds [8]. Surprisingly, little is known about the behaviour of the transplanted stem cells, although the fact that the regenerated epidermis self-renews for years indicates that cells with significant growth potential — holoclones and possibly some meroclones — are permanently engrafted ([63]; Rochat and Barrandon, unpublished data). A significant development in skin-cell therapy is the use of fibrin matrices to ease the transplantation of cultured epithelia [63,64]. Significant progress has also been made towards the use of genetically modified stem cells to treat crippling skin diseases. Different strategies have been explored to deliver a gene of interest to epidermal stem cells, including the use of naked DNA, adenoviruses and lentiviruses [65,66]. Chen *et al.* transduced a lentivirus vector containing a *COL7A1* cDNA into dystrophic epidermolysis bullosa (DEB) keratinocytes and fibroblasts and reconstituted anchoring fibrils when the gene-corrected cells were transplanted onto immune-deficient mice [67•]. Also, Ortiz-Urda *et al.* succeeded in correcting keratinocytes obtained from patients with junctional epidermolysis with a gene encoding laminin 5 using a

non-viral system permitting the genomic integration of an expression plasmid with the ϕC31 integrase [68•]. The future of gene therapy looks promising, but the immunological response to non-native proteins in patients carrying null mutations is a concern, as it could lead to immunorejection of the restored protein [69]. This said, gene therapy using genetically modified autologous epidermal stem cells remains the only hope to improve the life of patients with disabling epidermal genodermatoses.

Reports on the plasticity of adult stem cells isolated from bone marrow or the brain have generated tremendous interest [70] even if the concept of stem-cell plasticity is highly debated [71,72]. For instance, Wang *et al.* have recently reported that bone-marrow-derived hepatocytes arise from cell fusion of bone-marrow cells with resident hepatocytes and not from a true differentiation of haematopoietic stem cells in hepatocytes [73••]. Nevertheless, many groups in the skin field are exploring the putative plasticity of epidermal stem cells because of their accessibility and the efficacy of their cultivation in comparison to other adult stem cells. Liang and Bickenbach have nicely demonstrated that mouse epidermal stem cells injected into a blastocyst contribute to different tissues originating from the three germ layers [45••]. This indicates that it might be possible to reprogram adult epidermal stem cells in the future. Interestingly, cells derived from transplanted bone marrow stem cells can target the epidermis of a recipient mouse and adopt an epithelial phenotype [72]. The presence of bone-marrow-derived epithelial cells has also been reported in the buccal epithelia [74] as well as in the epidermis of humans transplanted with bone-marrow stem cells [75]. However, in one study [75] the keratinocyte-like cells could not be cultivated and were unlikely to be stem cells. Several questions then need to be addressed before an adult stem cell can be diverted from its original fate for cell therapy. For instance, it will be of great importance to identify the genes determining the stem phenotype.

Conclusions

In this review, we have focused on the cellular aspects of the renewal of the epidermis and its appendages in adult mammalian skin. However, it is important to emphasise the similarities of the mechanisms involved in development and skin renewal. Comprehending the cellular and molecular mechanisms that control stem-cell fate in embryonic skin is therefore of great importance to our understanding of the behaviour of adult stem cells (e.g. the role of the niche and the regulation of proliferation, migration and lineage commitment) especially from the perspective of cell and gene therapy [11•]. For instance, it will be of great interest to determine when and how stem cells switch from an embryonic to an adult phenotype [76]. For this purpose, embryonic stem-cell lines may be extremely valuable. It is also of prime importance to improve our understanding of the relationship between the epidermal stem cells and other stem cells inhabiting the skin, as illustrated by the close relation between the melanocyte stem cells and the keratinocyte stem cells in the hair follicle [77••].

Update

The laboratory of E Fuchs has recently reported that in the absence of the transcription factor GATA-3, the inner root sheath of the hair follicle fails to develop properly as a result of defects in cell lineage determination [79••].

Acknowledgements

We thank Ariane Rochat for critical reading of the manuscript. This work was supported by funds from the Swiss Federal Institute Lausanne (EPFL) and from the Lausanne University Hospital (CHUV).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Al-Barwari SE, Potten CS: **A cell kinetic model to explain the time of appearance of skin reaction after X-rays or ultraviolet light irradiation**. *Cell Tissue Kinet* 1979, **12**:281-289.

2. Argyris TS: **Kinetics of epidermal production during epidermal regeneration following abrasion in mice**. *Am J Pathol* 1976, **83**:329-340.

3. Owens DM, Watt FM: **Contribution of stem cells and differentiated cells to epidermal tumours**. *Nat Rev Cancer* 2003, **3**:444-451.

4. Perez-Losada J, Balmain A: **Stem-cell hierarchy in skin cancer**. *Nat Rev Cancer* 2003, **3**:434-443.

5. Limat A, Noser FK: **Serial cultivation of single keratinocytes from the outer root sheath of human scalp hair follicles**. *J Invest Dermatol* 1986, **87**:485-488.

6. Rheinwald JG, Green H: **Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells**. *Cell* 1975, **6**:331-344.

7. Rochat A, Kobayashi K, Barrandon Y: **Location of stem cells of human hair follicles by clonal analysis**. *Cell* 1994, **76**:1063-1073.

8. Gallico GGI, O'Connor NE, Compton CC, Kehinde O, Green H: **Permanent coverage of large burn wounds with autologous cultured human epithelium**. *N Engl J Med* 1984, **311**:448-451.

9. Tong X, Coulombe PA: **Mouse models of alopecia: identifying structural genes that are baldly needed**. *Trends Mol Med* 2003, **9**:79-84.

10. Nakamura M, Sundberg JP, Paus R: **Mutant laboratory mice with abnormalities in hair follicle morphogenesis, cycling, and/or structure: annotated tables**. *Exp Dermatol* 2001, **10**:369-390.

11. • Alonso L, Fuchs E: **Stem cells in the skin: waste not, Wnt not**. *Genes Dev* 2003, **17**:1189-1200.
An excellent review that nicely covers the molecular aspects of stem-cell fate in the skin.

12. Watt FM: **Stem cell fate and patterning in the mammalian epidermis**. *Curr Opin Genet Dev* 2001, **11**:410-417.

13. Merrill BJ, Gat U, DasGupta R, Fuchs E: **Tcf3 and Lef1 regulate lineage differentiation of multipotent stem cells in skin**. *Genes Dev* 2001, **15**:1688-1705.

14. DasGupta R, Rhee H, Fuchs E: **A developmental conundrum: a stabilized form of β-catenin lacking the transcriptional activation domain triggers features of hair cell fate in epidermal**

cells and epidermal cell fate in hair follicle cells. *J Cell Biol* 2002, **158**:331-344.

15. Huelsken J, Vogel R, Erdmann B, Cotsarelis G, Birchmeier W: **β-Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin**. *Cell* 2001, **105**:533-545.

16. Niemann C, Owens DM, Hulsken J, Birchmeier W, Watt FM: **Expression of δNLef1 in mouse epidermis results in differentiation of hair follicles into squamous epidermal cysts and formation of skin tumours**. *Development* 2002, **129**:95-109.

17. •• Jamora C, DasGupta R, Kocieniewski P, Fuchs E: **Links between signal transduction, transcription and adhesion in epithelial bud development**. *Nature* 2003, **422**:317-322.
This report reveals the importance of E-cadherin down-regulation in relation to Wnt and Bmp signalling in hair bud formation.

18. •• Andl T, Reddy ST, Gaddapara T, Millar SE: **WNT signals are required for the initiation of hair follicle development**. *Dev Cell* 2002, **2**:643-653.
Ectopic expression of Dickkopf 1, a potent diffusible inhibitor of Wnt action, in the skin results in a complete failure of placode formation. Activation of Wnt signalling is thus required for the initiation of hair follicle placode formation.

19. • Nicolas M, Wolfer A, Raj K, Kummer JA, Mill P, van Noort M, Hui CC, Clevers H, Dotto GP, Radtke F: **Notch1 functions as a tumor suppressor in mouse skin**. *Nat Genet* 2003, **33**:416-421.
Inactivation of Notch1 in the skin of an adult mouse results in an epidermal phenotype in the central cornea and the development of basal-cell carcinoma-like tumours in the epidermis.

20. Yamamoto N, Tanigaki K, Han H, Hiai H, Honjo T: **Notch/RBP-J signaling regulates epidermis/hair fate determination of hair follicular stem cells**. *Curr Biol* 2003, **13**:333-338.

21. Headon DJ, Emmal SA, Ferguson BM, Tucker AS, Justice MJ, Sharpe PT, Zonana J, Overbeek PA: **Gene defect in ectodermal dysplasia implicates a death domain adapter in development**. *Nature* 2001, **414**:913-916.

22. Laurikkala J, Pispa J, Jung HS, Nieminen P, Mikkola M, Wang X, Saarialho-Kere U, Galceran J, Grosschedl R, Thesleff I: **Regulation of hair follicle development by the TNF signal ectodysplasin and its receptor Edar**. *Development* 2002, **129**:2541-2553.

23. Gaide O, Schneider P: **Permanent correction of an inherited ectodermal dysplasia with recombinant EDA**. *Nat Med* 2003, **9**:614-618.

24. Waikel RL, Kawachi Y, Waikel PA, Wang XJ, Roop DR: **Deregulated expression of c-Myc depletes epidermal stem cells**. *Nat Genet* 2001, **28**:165-168.

25. Arnold I, Watt FM: **c-Myc activation in transgenic mouse epidermis results in mobilization of stem cells and differentiation of their progeny**. *Curr Biol* 2001, **11**:558-568.

26. Oro AE, Higgins K: **Hair cycle regulation of hedgehog signal reception**. *Dev Biol* 2003, **255**:238-248.

27. Botchkarev VA, Botchkareva NV, Sharov AA, Funa K, Huber O, Gilchrest BA: **Modulation of BMP signaling by noggin is required for induction of the secondary (nontylotrich) hair follicles**. *J Invest Dermatol* 2002, **118**:3-10.

28. Ellis T, Gambardella L, Horcher M, Tschanz S, Capol J, Bertram P, Jochum W, Barrandon Y, Busslinger M: **The transcriptional repressor CDP (Cutl1) is essential for epithelial cell differentiation of the lung and the hair follicle**. *Genes Dev* 2001, **15**:2307-2319.

29. Lajtha LG: **Stem cell concepts**. *Differentiation* 1979, **14**:23-34.

30. Cotsarelis G, Sun TT, Lavker RM: **Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis**. *Cell* 1990, **61**:1329-1337.

31. Barrandon Y, Green H: **Three clonal types of keratinocyte with different capacities for multiplication**. *Proc Natl Acad Sci USA* 1987, **84**:2302-2306.

32. Jones PH, Watt FM: **Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression**. *Cell* 1993, **73**:713-724.

33. Tani H, Morris RJ, Kaur P: **Enrichment for murine keratinocyte stem cells based on cell surface phenotype**. *Proc Natl Acad Sci USA* 2000, **97**:10960-10965.

34. Blau HM, Brazelton TR, Weimann JM: **The evolving concept of a stem cell: entity or function?** *Cell* 2001, **105**:829-841.

35. Pellegrini G, Dellambra E, Golisano O, Martinelli E, Fantozzi I, Bondanza S, Ponzin D, McKeon F, De Luca M: **p63 identifies keratinocyte stem cells**. *Proc Natl Acad Sci USA* 2001, **98**:3156-3161.

36. Taylor G, Lehrer MS, Jensen PJ, Sun TT, Lavker RM: **Involvement of follicular stem cells in forming not only the follicle but also the epidermis**. *Cell* 2000, **102**:451-461.

37. Oshima H, Rochat A, Kedzia C, Kobayashi K, Barrandon Y: **Morphogenesis and renewal of hair follicles from adult multipotent stem cells**. *Cell* 2001, **104**:233-245.

38. Mackenzie IC: **Relationship between mitosis and the ordered structure of the stratum corneum in mouse epidermis**. *Nature* 1970, **226**:653-655.

39. Potten CS, Morris RJ: **Epithelial stem cells *in vivo***. *J Cell Sci* 1988, **10**:45-62.

40. Ghazizadeh S, Taichman LB: **Multiple classes of stem cells in cutaneous epithelium: a lineage analysis of adult mouse skin**. *EMBO J* 2001, **20**:1215-1222.

41. Niemann C, Watt FM: **Designer skin: lineage commitment in postnatal epidermis**. *Trends Cell Biol* 2002, **12**:185-192.

42. Weissman IL: **Stem cells: units of development, units of regeneration, and units in evolution**. *Cell* 2000, **100**:157-168.

43. Janes SM, Lowell S, Hutter C: **Epidermal stem cells**. *J Pathol* 2002, **197**:479-491.

44. Dunnwald M, Tomanek-Chalkley A, Alexandrunas D, Fishbaugh J, Bickenbach JR: **Isolating a pure population of epidermal stem cells for use in tissue engineering**. *Exp Dermatol* 2001, **10**:45-54.

45. •• Liang L, Bickenbach JR: **Somatic epidermal stem cells can produce multiple cell lineages during development**. *Stem Cells* 2002, **20**:21-31.
Adult epidermal stem cells of the mouse can contribute, when transplanted into blastocysts, to several tissues originating from the three germ layers. Adult epidermal stem cells like other adult stem cells can be reprogrammed if given the appropriate environment.

46. Lyle S, Christofidou-Solomidou M, Liu Y, Elder DE, Albelda S, Cotsarelis G: **The C8/144B monoclonal antibody recognizes cytokeratin 15 and defines the location of human hair follicle stem cells**. *J Cell Sci* 1998, **111**:3179-3188.

47. Porter RM, Lunny DP, Ogden PH, Morley SM, McLean WH, Evans A, Harrison DL, Rugg EL, Lane EB: **K15 expression implies lateral differentiation within stratified epithelial basal cells**. *Lab Invest* 2000, **80**:1701-1710.

48. Whitbread LA, Powell BC: **Expression of the intermediate filament keratin gene, K15, in the basal cell layers of epithelia and the hair follicle**. *Exp Cell Res* 1998, **244**:448-459.

49. Trempus CS, Morris RJ, Bortner CD, Cotsarelis G, Faircloth RS, Reece JM, Tennant RW: **Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34**. *J Invest Dermatol* 2003, **120**:501-511.

50. Albert MR, Foster RA, Vogel JC: **Murine epidermal label-retaining cells isolated by flow cytometry do not express the stem cell markers CD34, Sca-1, or Flk-1**. *J Invest Dermatol* 2001, **117**:943-948.

51. Yu Y, Flint A, Dvorin EL, Bischoff J: **AC133-2, a novel isoform of human AC133 stem cell antigen**. *J Biol Chem* 2002, **277**:20711-20716.

52. Yang A, Schweitzer R, Sun D, Kaghad M, Walker N, Bronson RT, Tabin C, Sharpe A, Caput D, Crum C *et al.*: **p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development**. *Nature* 1999, **398**:714-718.

53. Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR, Bradley A: **p63 is a p53 homologue required for limb and epidermal morphogenesis**. *Nature* 1999, **398**:708-713.

54. Fuchs E, Raghavan S: **Getting under the skin of epidermal morphogenesis**. *Nat Rev Genet* 2002, **3**:199-209.

55. Tsujita-Kyutoku M, Kiuchi K, Danbara N, Yuri T, Senzaki H, Tsubura A: **p63 expression in normal human epidermis and epidermal appendages and their tumors**. *J Cutan Pathol* 2003, **30**:11-17.

56. Sherley JL: **Asymmetric cell kinetics genes: the key to expansion of adult stem cells in culture**. *Stem Cells* 2002, **20**:561-572.

57. Le Borgne R, Schweisguth F: **Unequal segregation of neuralized biases notch activation during asymmetric cell division**. *Dev Cell* 2003, **5**:139-148.

58. Zhong W: **Diversifying neural cells through order of birth and asymmetry of division**. *Neuron* 2003, **37**:11-14.

59. Cayouette M, Raff M: **Asymmetric segregation of Numb: a mechanism for neural specification from *Drosophila* to mammals**. *Nat Neurosci* 2002, **5**:1265-1269.

60. Topley GI, Okuyama R, Gonzales JG, Conti C, Dotto GP: **p21(WAF1/Cip1) functions as a suppressor of malignant skin tumor formation and a determinant of keratinocyte stem-cell potential**. *Proc Natl Acad Sci USA* 1999, **96**:9089-9094.

61. • Kopan R, Lee J, Lin MH, Syder AJ, Kesterson J, Crutchfield N, Li CR, Wu W, Books J, Gordon JI: **Genetic mosaic analysis indicates that the bulb region of coat hair follicles contains a resident population of several active multipotent epithelial lineage progenitors**. *Dev Biol* 2002, **242**:44-57.
The authors present a lineage analysis of hair follicles of chimeric mice, and suggest that the hair bulb contains symmetrically dividing stem cells.

62. Panteleyev AA, Jahoda CA, Christiano AM: **Hair follicle predetermination**. *J Cell Sci* 2001, **114**:3419-3431.

63. Ronfard V, Rives JM, Neveux Y, Carsin H, Barrandon Y: **Long-term regeneration of human epidermis on third degree burns transplanted with autologous cultured epithelium grown on a fibrin matrix**. *Transplantation* 2000, **70**:1588-1598.

64. Pellegrini G, Ranno R, Stracuzzi G, Bondanza S, Guerra L, Zambruno G, Micali G, De Luca M: **The control of epidermal stem cells (holoclones) in the treatment of massive full-thickness burns with autologous keratinocytes cultured on fibrin**. *Transplantation* 1999, **68**:868-879.

65. Mecklenbeck S, Compton SH, Mejia JE, Cervini R, Hovnanian A, Bruckner-Tuderman L, Barrandon Y: **A microinjected COL7A1-PAC vector restores synthesis of intact procollagen VII in a dystrophic epidermolysis bullosa keratinocyte cell line**. *Hum Gene Ther* 2002, **13**:1655-1662.

66. Kuhn U, Terunuma A, Pfutzner W, Foster RA, Vogel JC: ***In vivo* assessment of gene delivery to keratinocytes by lentiviral vectors**. *J Virol* 2002, **76**:1496-1504.

67. • Chen M, Kasahara N, Keene DR, Chan L, Hoeffler WK, Finlay D, Barcova M, Cannon PM, Mazurek C, Woodley DT: **Restoration of type VII collagen expression and function in dystrophic epidermolysis bullosa**. *Nat Genet* 2002, **32**:670-675.
The authors report the formation of anchoring fibrils by DEB keratinocytes and fibroblasts transduced by a lentivirus vector containing a *COL7A1* cDNA. This work opens the way to gene therapy disabling genodermatoses using transduced skin stem cells.

68. • Ortiz-Urda S, Thyagarajan B, Keene DR, Lin Q, Calos MP, Khavari PA: **PhiC31 integrase-mediated nonviral genetic correction of junctional epidermolysis bullosa**. *Hum Gene Ther* 2003, **14**:923-928.
The authors achieve correction of epidermal stem cells obtained from patients with junctional epidermolysis with a gene encoding laminin 5 by using a non-viral system permitting the genomic integration of an expression plasmid with the ϕC31 integrase. The method is an alternative to viral-based vectors.

69. Ghazizadeh S, Kalish RS, Taichman LB: **Immune-mediated loss of transgene expression in skin: implications for cutaneous gene therapy**. *Mol Ther* 2003, **7**:296-303.

70. Goodell MA: **Stem-cell 'plasticity': befuddled by the muddle**. *Curr Opin Hematol* 2003, **10**:208-213.

71. Wagers AJ, Sherwood RI, Christensen JL, Weissman IL: **Little evidence for developmental plasticity of adult hematopoietic stem cells**. *Science* 2002, **297**:2256-2259.

72. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ: **Multi-organ, multi-lineage engraftment by a single bone-marrow-derived stem cell**. *Cell* 2001, **105**:369-377.

73. •• Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M, Lagasse E, Finegold M, Olson S, Grompe M: **Cell fusion is the principal source of bone-marrow-derived hepatocytes**. *Nature* 2003, **422**:897-901.
Bone-marrow-derived hepatocytes arise from cell fusion of bone marrow cells with resident hepatocytes and not from a true differentiation of haematopoietic stem cells in hepatocytes. This is a major argument against the plasticity of adult stem cells.

74. Tran SD, Pillemer SR, Dutra A, Barrett AJ, Brownstein MJ, Key S, Pak E, Leakan RA, Kingman A, Yamada KM *et al.*: **Differentiation of human bone marrow-derived cells into buccal epithelial cells *in vivo*: a molecular analytical study**. *Lancet* 2003, **361**:1084-1088.

75. Hematti P, Sloand EM, Carvallo CA, Albert MR, Yee CL, Fuehrer MM, Blancato JK, Kearns WG, Barrett JA, Childs RW *et al.*: **Absence of donor-derived keratinocyte stem cells in skin tissues cultured from patients after mobilized peripheral blood hematopoietic stem cell transplantation**. *Exp Hematol* 2002, **30**:943-949.

76. Coraux C, Hilmi C, Rouleau M, Spadafora A, Hinnrasky J, Ortonne JP, Dani C, Aberdam D: **Reconstituted skin from murine embryonic stem cells**. *Curr Biol* 2003, **13**:849-853.

77. •• Nishimura EK, Jordan SA, Oshima H, Yoshida H, Osawa M, Moriyama M, Jackson IJ, Barrandon Y, Miyachi Y, Nishikawa S: **Dominant role of the niche in melanocyte stem-cell fate determination**. *Nature* 2002, **416**:854-860.
The authors have identified the niche of melanocyte stem cells in the vicinity of the epidermal niche in the hair follicle. This raises the question of a putative functional cooperation between stem cells of different origin.

78. Topilko P, Schneider-Maunoury S, Levi G, Trembleau A, Gourdji D, Driancourt MA, Rao CV, Charnay P: **Multiple pituitary and ovarian defects in Krox-24 (NGFI-A, Egr-1)-targeted mice**. *Mol Endocrinol* 1998, **12**:107-122.

79. •• Kaufman CK, Zhou P, Pasolli HA, Rendl M, Bolotin D, Lim KC, Dai X, Alegre ML, Fuchs E: **GATA-3: an unexpected regulator of cell lineage determination in skin**. *Genes Dev* 2003, **17**:2108-2122.
The authors identify the transcription factor GATA-3 as being a key regulator of inner root sheath lineage determination.