Hair Follicular Stem Cells: The Bulge-Activation Hypothesis

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The hair follicle is a complex structure originating from a group of rapidly dividing keratinocytes located in the bulb (or matrix) area that almost completely encloses and intimately interacts with specialized mesenchymal cells (dermal papilla; Fig 1*a*). Cells leaving the bulb area undergo several distinctive pathways of terminal differentiation resulting in the formation of multiple, concentrically arranged "rings" of specialized cell layers including the inner root sheath, cuticle, cortex, and medulla (the central cell column, Fig 1*a*). Because classically it was thought erroneously that stem cells can be identified as ^{3}H-thymidine-incorporating cells, which are predominantly found in the bulb, it has long been assumed that follicular stem cells reside in this area. However, this prevailing dogma failed to answer several important questions. (i) If stem cells indeed reside in the bulb, how can one explain the experimental observation that follicles can regenerate even after the entire bulb is destroyed by x-ray irradiation or removed by surgery? (ii) What is the mechanistic basis of hair cycle control? (iii) Why is the formation of skin tumors in response to topically applied chemical (complete) carcinogens strictly hair-cycle dependent?

To address these questions, it is important to understand the current concept of stem cells. Recent data indicate that stem cells are (i) slow cycling but can be stimulated to proliferate by demand, (ii) relatively undifferentiated, and (iii) located in a well-protected, well-nourished, and innervated location (for a review, see [1]). The division of stem cells is a relatively rare event and is presumably tightly regulated. On average, one of the two daughter cells of a stem-cell division will leave the stem-cell environment (the "niche") and become a "transient amplifying" (TA) cell. These TA cells proliferate rapidly and readily incorporate ^{3}H-thymidine. They have only a limited proliferative potential and will later become terminally differentiated. Although this concept was originally based on studies of blood cells [1], it has been applied, with remarkable success, to several keratinocyte systems suggesting its validity in stratified squamous epithelia. For example, we were able to identify a subpopulation of basal cells in monkey palm epithelium that (i) is relatively slow cycling, (ii) possesses a relatively undifferentiated cytoplasm, (iii) is heavily pigmented and thus well protected from solar damage, and (iv) gives rise to an immediately adjacent and suprabasally located population of rapidly proliferating (transient amplifying?) keratinocytes [2,3]. These results led us to propose that these ("non-serrated") basal cells are the stem cells of palm epithelium [3]. Our observation that over 70% of the ^{3}H-incorporating palm epithelial cells are located in the suprabasal layers, coupled with the concept of "stem cells → transient amplifying cells → terminally differentiated cells," also allowed us to address the possible biologic significance of keratinocyte proliferation in the suprabasal compartment [3]. In another series of experiments, we were able to identify in corneal epithelium some basal cells, located at peripheral cornea in the limbal zone, that (i) lack a major 64-kD keratin and are thus relatively undifferentiated [4], (ii) are slow cycling but could be preferentially stimulated to undergo transient proliferation in response to a central corneal epithelial wound [5], and (iii) provide an excellent tissue source for corneal epithelial transplantation. These recent data provide an explanation for the preponderance of corneal epithelial neoplasms in the limbus, and have important implications on the pathogenesis of certain corneal epithelial diseases [4,5].

Using a similar approach, we have recently identified a subpopulation of hair-follicular keratinocytes that shares a number of important properties with other keratinocyte stem cells. The location of these putative hair-follicular stem cells was unexpected. They were found exclusively in the upper follicle in a region known as the bulge, which consists of a discrete collection of outer root sheath cells located near the insertion site of arrector pili muscle (Fig 1*a* [6]). The putative follicular stem cells in the bulge possess the following properties. (i) They are normally slow cycling because they are rarely labeled by a pulse administration of ^{3}H-thymidine. However, once they incorporate ^{3}H-thymidine by long-term labeling they retain the isotope for a prolonged period of time during subsequent "chasing;" they can therefore be identified as "label-retaining cells." (ii) They apparently can be stimulated to proliferate by tumor promotor TPA. (iii) They are ultrastructurally relatively primitive. (iv) Although the bulge may be inconspicuous in some adult follicles, it represents a prominent structure in embryonic follicles. (v) The bulge marks the lower end of the permanent portion of the hair follicle; keratinocytes below this area degenerate during catagen and telogen and thus appear to be "dispensable."

We have incorporated these data and other known important histologic and kinetic events occurring during different stages of the hair cycle in a schematic model (the "Bulge-Activation Hypothesis;" Fig 1 [6]). This hypothesis consists of four important elements. (i) Bulge cells are normally slow cycling but can be stimulated/activated by dermal papilla to undergo transient proliferation during early anagen (Fig 1*c*,*d*). (ii) During anagen IV dermal papilla cells undergo transient proliferation and de-condensation possibly in response to a matrix signal (Fig 1*e*). (iii) Matrix cells, being derived from the bulge (stem) cells, are transient amplifying cells and thus have only a limited proliferative potential (Fig 1*a*). (iv) The upward movement of DP during late catagen is crucial to allow the subsequent physical interaction or activation of the resting bulge cells by DP cells, and to start a new cycle of hair growth (Fig 1*b* [6]).

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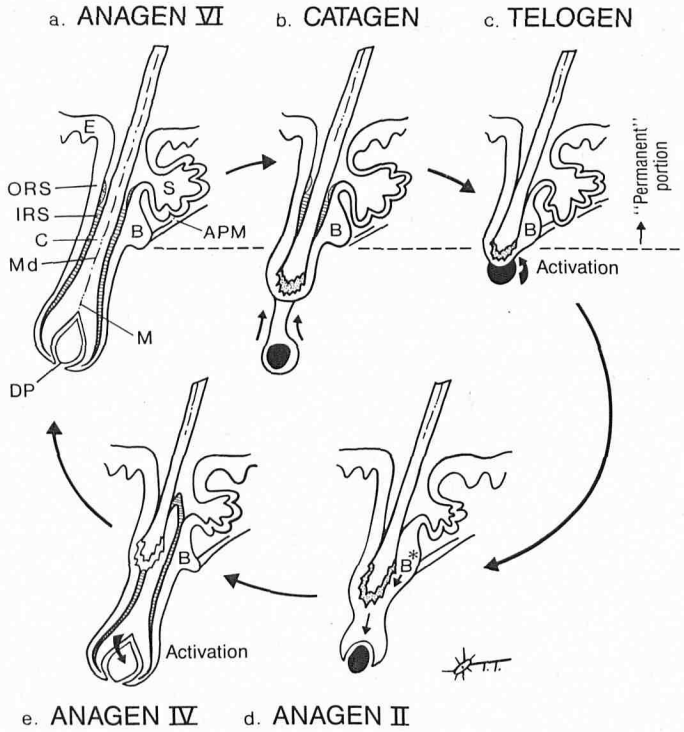

Figure 1. The bulge-activation hypothesis. For explanations see text. Abbreviations: arrector pili muscle (APM), bulge (B), cortex (C), dermal papilla (DP), epidermis (E), inner root sheath (IRS), matrix (M), Medulla (Md), outer root sheath (ORS), and sebaceous gland (S). B and B* denote quiescent and activated bulge cells, respectively. (Reproduced with permission from Cotsarelis et al, Cell 61:1329–1337, 1990.)

Although there are still many unknowns in this scheme, it provides a new conceptual framework allowing us to explain some earlier, paradoxical observations [6]. Firstly, it explains why the hair follicle can regenerate even after the entire bulb area is removed—so long as two conditions are met: (i) the upper follicle including the bulge area remains intact and (ii) dermal papilla can regenerate from the remaining connective tissue sheath. Secondly, it explains the cyclic growth of hair follicle. Thirdly, the greater accessibility of the putative follicular stem cells during telogen (versus anagen) to topically applied chemical carcinogens raises the interesting possibility that most of the experimentally induced skin tumors are follicle derived. This also offers a possible explanation for the greatly increased skin tumor yield during telogen of the hair cycle. Finally, this may explain the difficulties we and others have encountered in the past in culturing the hair-follicular matrix cells (which according to our model [Fig 1] are transient amplifying instead of stem cells). Experiments are in progress to test some of these hypotheses and to refine our understanding of the controlling mechanisms and physiologic regulation of the hair cycle.

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