The Major Pathways of Keratinocyte Differentiation as Defined by Keratin Expression: An Overview*

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The keratinocyte is the major cell type present in the epidermis and other stratified squamous epithelia, including those covering the surfaces of cornea, esophagus, tongue, and exocervix.[1] Although keratinocytes of different epithelia are characterized by distinct morphologic and biochemical features, and there is clear evidence suggesting that many of them have diverged "irreversibly" from one another during development, keratino-

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cytes as a group do share many common properties.[2, 3] Thus, all keratinocytes replicate predominantly in the basal layer; they undergo progressive morphologic and biochemical changes during terminal differentiation; and they can be propagated in vitro under similar cell culture conditions (e.g., in the presence of lethally irradiated 3T3 feeder cells).[4, 5] Moreover, all keratinocytes make large quantities of a group of cytoskeletal proteins called *keratins*, which form a cytoplasmic network of 10-nm intermediate filaments.[5] These proteins constitute about 30% of the total proteins in the lower, viable cell layers and become even more predominant (i.e., >80%) in superficial, cornified cells through the selective degradation of nonkeratin molecules.[6] Although the detailed function or functions of keratin proteins remains unclear, the fact that keratinocytes devote such a large proportion of their biosynthetic machinery and energy toward making these cytoskeletal molecules strongly implies that keratins must play an extremely important role in maintaining the structural integrity or protective function (or both) of stratified epithelia.

Epidermis as a Model System for Studying Keratinocyte Differentiation

Because of its accessibility, the epidermis has been studied extensively as a model of keratinocyte differentiation. Such studies have been particularly fruitful during the past decade due to the recent advances in cell culture techniques for growing keratinocytes, in hybridoma techniques for making monoclonal antibodies to various epidermal antigens, and in nucleic acid cloning techniques for studying the molecular biology of keratins and other epidermal proteins. Armed with these powerful techniques, researchers have accumulated a great deal of data that are beginning to shed light on some fundamental questions regarding keratinocyte differentiation. The question that forms the central theme of this chapter relates to the pathways of keratinocyte differentiation. Specifically, when a keratinocyte becomes terminally differentiated, how many options does it have in its keratin expression program? Does it have only a few options? Or does it have numerous options, as the tremendous morphologic variations in epidermal diseases and cultures may indicate? A related question is: What goes wrong in psoriatic epidermis? If the normal sequence of epidermal differentiation is represented by steps A→B→C→D, does psoriatic epidermis follow the same pathway but stop short (A→B or A→B→C) because it does not have enough time to fully mature?[7, 8] Or in this case do the cells actually follow an alternative pathway of differentiation (A→X)? What about keratinocytes from other tissues such as the cornea or esophagus? What are the relationships among these keratinocytes as far as their major differentiation programs are concerned?

A prerequisite of any meaningful discussion of a differentiation pathway is the availability of well-defined biochemical markers. In this respect, ker-

atins, being so abundant, provide a good marker for studying the major differentiation pathways of keratinocytes. Studies on other less abundant differentiation markers such as filaggrin or involucrin may also be useful, although the relationship between the expression of these markers and that of the keratins is currently not well understood.[9–11]

To establish that specific keratins can, indeed, be regarded as markers for different pathways of keratinocyte differentiation, we will review briefly in this chapter the biology and biochemistry of keratin proteins. To do so, we will address, in turn, keratin expression in the epidermis, corneal epithelium, esophageal epithelium, hair follicles, and nail matrix. During our discussion, we will develop a set of rules that govern the expression of most keratin molecules. Although there are exceptions to these rules, as one might expect when dealing with a biologic system, the realization of such rules has greatly simplified our thinking and has enabled us to gain insights on the pathways of keratinocyte differentiation.

Keratin Subfamilies

Although keratin proteins were classically thought to be present only in the epidermis and its appendages such as hair and nail, the work of Franke et al.,[12] Sun and Green,[13] Sun et al.,[14] and Franke et al.[15] showed about 10 years ago that keratin-related proteins form intermediate filaments in almost all epithelia. The fact that keratins are present in epithelial neoplasms (carcinomas) but not in neoplasms of nonepithelial origin has prompted the clinical use of antibodies to keratins as an immunohistochemical tool for distinguishing carcinomas from sarcomas and other nonepithelial neoplasms.[16–20]

Detailed biochemical analyses of human epithelial keratins by Franke et al.[21] and Moll et al.[22] have established the existence of 25 to 30 keratins (cytokeratins). Since all of these keratins are in a rather narrow molecular weight range of 40 to 70 kilodaltons (kD), they are best resolved by two-dimensional polyacrylamide gel electrophoresis, which separates proteins by both charge and size. Figure 1 is a schematic diagram showing the position of all the known human keratins as resolved by such a gel. In this diagram, each keratin is represented by several interconnected circles indicating charge heterogeneity due to phosphorylation.[6, 21, 23] To facilitate their identification, we have marked all keratins not only by their molecular weights but also by their catalogue numbers (designated by Moll and co-workers), which are becoming an accepted standard in the field.[22]

This complex situation of having to deal with more than 25 keratins was somewhat simplified recently when it was realized that all keratins can be divided into two mutually exclusive subfamilies.[24] Fuchs et al.[25] and Kim et al.[26] were the first to demonstrate by the hybrid selection technique that

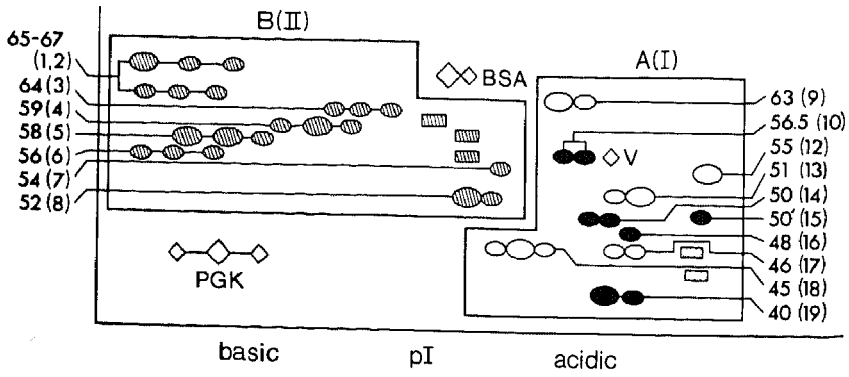

FIG 1.
Schematic diagram of human keratins (also known as cytokeratins) as resolved by two-dimensional gel electrophoresis. Keratins, labeled with their molecular weights and Moll's catalogue numbers (in parentheses), are divided into acidic and basic subfamilies according to their charge and immunoreactivity. The ovals and rectangles represent "soft" and "hard" (hair) keratins, respectively. All basic keratins are AE3 reactive *(hatched ovals* and *rectangles)*, whereas most acidic keratins are AE1 reactive *(solid circles)*. The acidic hair keratins are AE13 reactive *(dotted rectangles)*. *A(I)*, acidic, type I keratins; *B(II)*, basic, type II keratins; *pI*, isoelectric point; *V*, vimentin; *BSA*, bovine serum albumin; *PGK*, 3-phosphoglycerate kinase. (From Lynch MH, O'Guin WM, Hardy C, et al: Human hair follicle differentiation: Coordinate expression of acidic and basic hair keratins in upper cortical and cuticle cells and the relationship between "hard" (hair/nail) and "soft" keratin. *J Cell Biol* 1986; 103:2593–2606. Reproduced by permission.)

the keratins of cultured human epidermal and mesothelial cells belong to two groups that share sequence homology with types I and II wool keratins. Independently, Moll et al.[22] and Schiller et al.[27] showed that many basic keratins, including those expressed by numerous nonepidermal epithelia (of cornea, esophagus, liver, etc.), are closely related since they exhibit similar peptide mapping patterns. Using two monoclonal antibodies (AE1 and AE3) prepared against human epidermal keratins, we demonstrated that keratins from a wide range of epithelia can be divided into two mutually exclusive groups according to their immunoreactivities.[28] Further analyses showed that our AE1 and AE3 antibodies recognize the acidic and basic keratins, respectively.[24, 29, 30] The data obtained independently by three groups using very different techniques are therefore remarkably consistent with one another and support the division of keratins into two subfamilies. Members of the acidic (type I) subfamily are, in general, smaller and relatively acidic, they share a high degree of sequence homology with a type I wool keratin, and many are recognized by our AE1 monoclonal antibody. On the other hand, members of the basic (type II) subfamily are, in general, larger and relatively basic (pI >8), they share

sequence homology with a type II wool keratin, and they all are recognized by our AE3 monoclonal antibody.

Keratin Pairs of the Epidermis

The biological significance of the two keratin subfamilies was made clear by the experimental observations from several groups showing that at least one acidic and one basic keratin are required for the in vitro reconstitution of 10-nm keratin filaments.[31–34] Moreover, when we compared the expression patterns of individual keratins in the two subfamilies, we found that most of the acidic keratins have a preferred partner with which it coexpresses (forming a pair).[24, 30, 35, 36] Although many of the keratin pairs could be recognized based on tissue distribution data alone, the validity of some of these pairs was greatly strengthened by keratin localization data showing coexpression within a particular cell layer of a stratified epithelium. For example, tissue distribution data showed that the acidic 56.5-kD keratin (catalogue number K10) and basic 65- to 67-kD (K1 and K2) keratins are expressed mainly by keratinized tissues such as the epidermis.[22, 28] Immunolocalization and cell fractionation data further indicated that in the epidermis these two keratins are associated primarily with suprabasally located cells (Fig 2[37, 38]; for related data on mouse epidermis, see references 39 to 43). Taken together, these results strongly suggest that the acidic 56.5-kD keratin and the basic 65 to 67-kD keratins represent a coexpressed pair and may be regarded as markers for an advanced stage of keratinization or skin-type differentiation.[28, 37, 44]

In addition to the two suprabasally expressed keratins, the epidermis possesses two other major keratins, namely, an acidic 50-kD keratin and a basic 58-kD keratin, which are synthesized mainly in basal cells.[37–45] These two keratins are not epidermal specific but are present in basal cells of many stratified squamous epithelia.[46, 47] They may therefore be regarded as useful markers for keratinocytes in general.[22, 28]

A third keratin pair of the epidermis consists of an acidic 48-kD keratin and a basic 56-kD keratin. Despite some earlier controversies, it is now generally agreed that these two keratin proteins are absent from normal epidermis but are expressed in a wide variety of epidermal diseases, including psoriasis, actinic keratosis, verrucae, basal cell epithelioma, and squamous cell carcinoma.[48–50] They are also synthesized in large quantities by cultured epidermal keratinocytes that tend to adopt a *nonkeratinized* morphology (with no discernible granular or cornified layers) under most of the standard tissue culture conditions.[4] Thus, cultured epidermal cells make 50-/58-kD plus 48-/56-kD keratins, whereas keratinized, normal epidermis makes 50-/58-kD plus 56.5-/65- to 67-kD keratins (Fig 3).[30] Between these two extremes are a large number of epidermal diseases whose keratin patterns form a continuous spectrum depending on their degree of keratinization, hyperproliferation, or both (see Fig 3 and later discussion).[48]

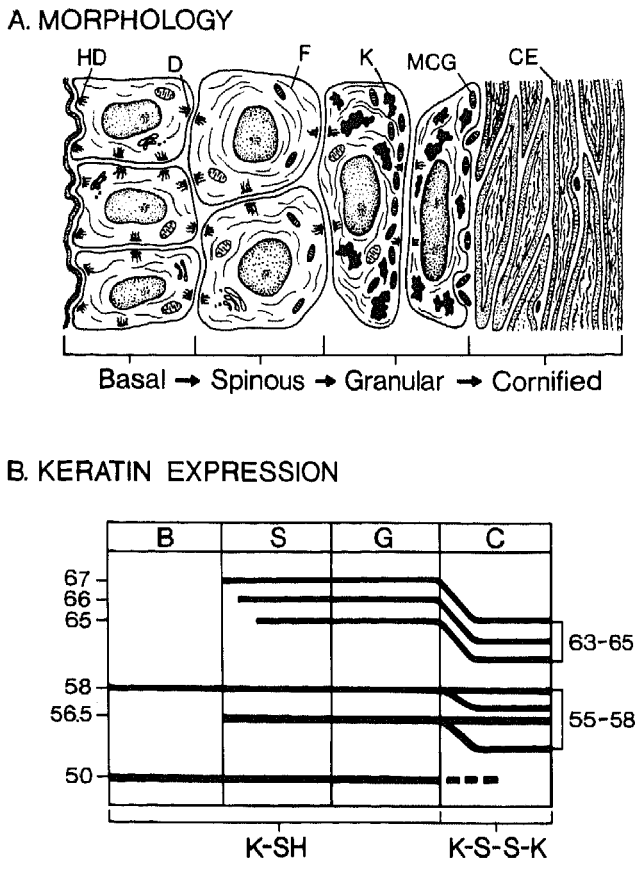

FIG 2.
Diagrammatic representation of the morphologic and biochemical changes accompanying normal epidermal differentiation. Basal layer expresses predominately the acidic 50-kD and basic 58-kD keratins. Production of the acidic 56.5-kD and basic 65- to 67-kD keratins occurs primarily in the suprabasal layers. On reaching the cornified layer, the keratins become partially degraded (resulting in slightly smaller molecular weights of 63 to 65 kD and 55 to 58 kD) and become cross-linked by intermolecular disulfide bonds (K-S-S-K). *HD*, hemidesmosome; *D*, desmosome; T, tonofilaments; *K*, keratohyaline granules; *MCG*, membrane coating granules. (Data from references 35 and 37.)

Keratin Pairs of Corneal Epithelium

Like the epidermis, corneal epithelium can also express a total of three major keratin pairs. Two of them are in common with the epidermis; they are the 50-/58-kD keratins made by basal cells and the 48-/56-kD keratins of the suprabasal cells expressed under hyperproliferative conditions.[46]

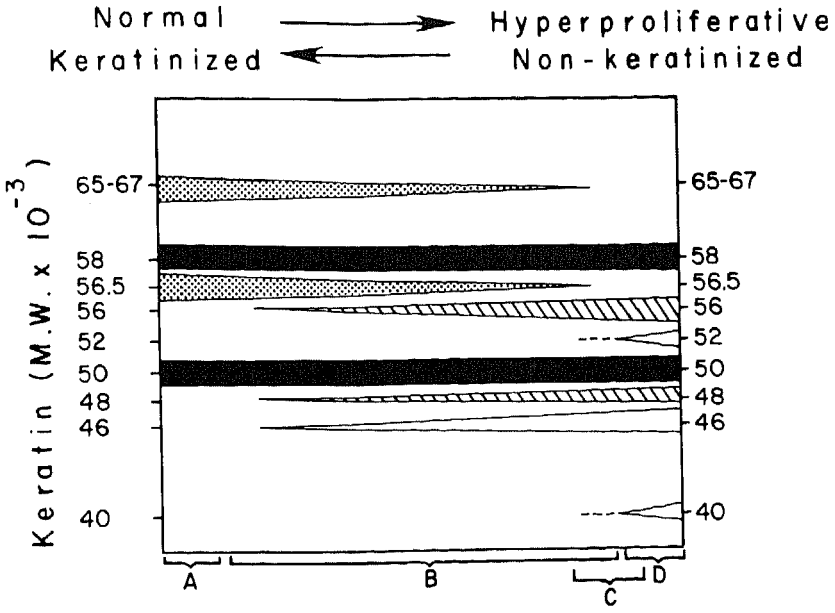

FIG 3.
Spectrum of epidermal keratin expression in normal and diseased states. The stippled bars denote the 56.5-kD and 65- to 67-kD keratin markers of skin-type differentiation (keratinization). The hatched bars denote the 48- and 56-kD keratin markers of hyperproliferation. The solid bars denote the 50- and 58-kD keratins made by basal keratinocytes. The 40-, 46-, and 52-kD keratins *(open bars)* are not keratinocyte specific; they are present in simple epithelium and in cultured keratinocytes and some epidermal diseases. *M.W.*, molecular weight. *A*, the keratins expressed by normal in vivo epidermis and by nonhyperproliferative epidermal disease such as icthyosis vulgaris (50, 58, 56.5, and 65 to 67 kD). *B*, keratin pattern of hyperproliferative epidermal diseases such as psoriasis, actinic keratosis, and squamous cell carcinoma. The keratin pattern of these diseases is intermediate between that of the normal in vivo epidermis and that of cultured keratinocytes. *C*, the keratin pattern of basal cell epithelioma closely approximates that of cultured keratinocytes. *D*, keratins produced by keratinocytes cultured in the presence of 3T3 feeders and various growth-stimulating factors such as epidermal growth factor, vitamin A, and hydrocortisone. (From Weiss RA, Eichner R, Sun T-T: Monoclonal antibody analysis of keratin expression in epidermal diseases: A 48 kd and a 56 kd keratin as molecular markers for hyperproliferative keratinocytes. *J Cell Biol* 1984; 98:1397–1406. Reproduced by permission.)

The only keratin pair that is somewhat unique to corneal epithelium consists of an acidic 55-kD keratin and a basic 64-kD keratin.[22, 24] These two keratins are expressed suprabasally in cultured corneal epithelium and therefore may be regarded as markers for an advanced stage of corneal keratinocyte differentiation.[46] These two proteins are designated as mark-

ers for corneal-type differentiation because they are also found in a few other stratified epithelia that cover moist, exposed surfaces, including the lip and, in bovine, the snout.[51] The keratin expression patterns of (cultured) corneal epithelial cells and epidermis are therefore quite analogous: their basal cells make the 50-/58-kD keratins, whereas their suprabasal cells have the option of choosing between two pathways (one common to all stratified epithelia and one somewhat tissue specific).

Keratin Pairs of Esophageal Epithelium

Esophageal epithelium exhibits the by now familiar pattern of expression: its basal cells make predominantly the 50-/58-kD keratins, whereas its suprabasal cells make either the 51-/59-kD keratins (markers for esophageal-type differentiation) or the 48-/56-kD keratins (markers for hyperproliferation).[47, 52] The 51-/59-kD keratins are expressed as the major differentiation-related markers not only in esophageal epithelium but also in several other internal, nonkeratinized, stratified epithelia, including those of the tongue and exocervix.[22]

Keratin Pairs of Hair and Nail

Recent analyses of the keratins of human hair revealed the existence of a group of two to four closely related acidic (44- to 46-kD) and another group of three to four closely related basic (56- to 59-kD) keratins.[53, 54] These "hard" keratins contain many more sulfhydryl groups than the "soft" keratins that we have discussed so far and are synthesized in upper cells (10 to 15 cell layers away from the basement membrane) of the hair cortex and cuticle.[53] These sulfhydryl-rich keratins may therefore be regarded as markers for an advanced stage of hair-type differentiation.

An identical set of hard keratins are synthesized in the upper cell layers of nail matrix.[55, 56] However, the differentiation of the nail matrix is distinguishable from that of the hair follicle in two important aspects. First, nail matrix synthesizes several soft keratins (the 50-/58-kD keratin markers of basal keratinocytes and the 48-/56-kD keratin markers of hyperproliferation), which account for about 10% to 20% of the total nail plate proteins.[53] Second, nail matrix does not give rise to inner or outer root sheaths, which are major structures in hair follicles.

Keratin Pairs as Markers of Differentiation Pathways

We have therefore identified several keratin pairs that are associated with particular pathways or states of keratinocyte differentiation.[24, 30] These include the 56.5-/65- to 67-kD pair (markers for skin-type differentiation), the 55-/64-kD pair (corneal-type differentiation), the 51-/59-kD pair

(esophageal-type differentiation), the 50-/58-kD pair (keratinocyte), and the 48-/56-kD pair (hyperproliferation). An interesting feature of all of these keratin pairs is that their basic keratins are larger than their acidic partners by approximately 8 kD.[24, 57] This size difference is also observed in another keratin pair consisting of an acidic 45-kD keratin and a basic 52-kD keratin, which are characteristic of simple epithelia such as mesothelium or small intestinal epithelium.[22, 58] Such a constant size difference within most of the known human keratin pairs is illustrated in Table 1 and in a schematic diagram shown in Figure 4.

The mechanism of coexpression of the acidic and basic members of a keratin pair is not well understood. We do know, however, that (1) in at least two keratin pairs (the 56.5-/65- to 67-kD skin markers and the 55-/64-kD corneal markers) the expression of the basic keratin precedes that

TABLE 1.
Classification and Distribution of Human Epithelial Keratins: Keratin Pairs Defined by Coexpression*

Marker For	Catalogue No.	Mol Wt (kD)	Distribution
Skin-type differentiation†	1,2/10	65–67/56.5	Keratinized (cornified) stratified squamous epithelia
Corneal-type differentiation†	3/12	64/55	Corneal, lip, and snout epithelia
Esophageal-type differentiation†	4/13	59/51	Many internal, nonkeratinized, stratified squamous epithelia
Palm/sole-type differentiation†	−/9	−/63	Thick, glabrous epithelia of palm and sole
Hair/nail-type differentiation†	——	H55, H59, H60/H44, H46	Hair cortex and cuticle, and nail plate
Keratinocytes‡	5/14,15	58/50,50'	All keratinocytes
Hyperproliferation§	6/16,17	56/48,46	Embryonic, cultured, and certain diseased keratinocytes (including carcinoma)
Simple epithelia	8/18	52/45	Simple epithelia and their derivatives

*Adapted from Lynch MH, O'Guin WM, Hardy C, et al: Human hair follicle differentiation: Coordinate expression of acidic and basic hair keratins in upper cortical and cuticle cells and the relationships between "hard" (hair/nail) and "soft" keratin. *J Cell Biol* 1986; 103:2593–2606.
†Differentiation-specific keratins that are made primarily in upper cell layers.
‡Common to all stratified epithelia, made mainly in basal layer.
§Expressed suprabasally in all stratified epithelia during hyperproliferation.

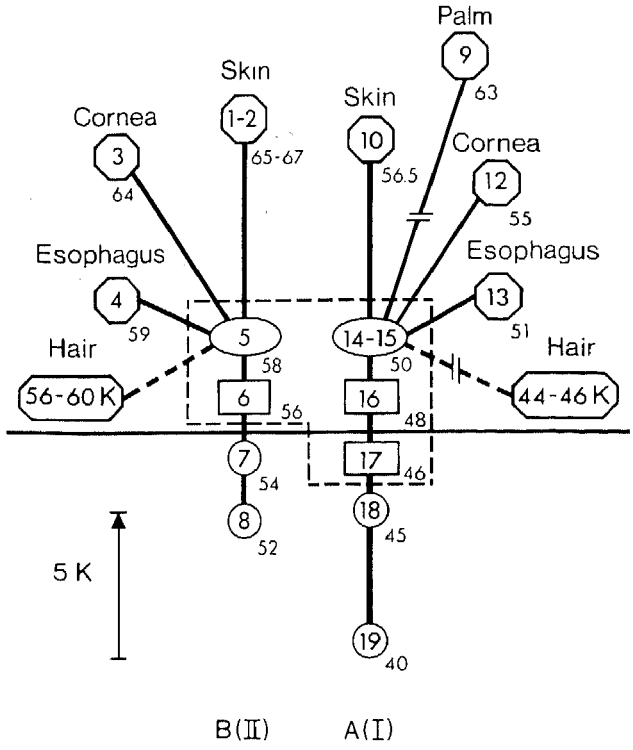

FIG 4.
Schematic diagram summarizing the keratin expression patterns in various simple and stratified epithelia. The acidic (type I) keratins are arranged vertically according to their relative size (see the molecular weight [*M.W.*] scale). The basic keratins are arranged according to a molecular weight scale that is 8 kD higher than that used for the acidic ones. Each keratin is labeled with its molecular weight (40–67 kD) and Moll's catalogue number (1–19). Keratins below the horizontal line are expressed mainly by simple epithelia except for the 46-kD keratin, which is synthesized in a large quantity by hyperproliferative keratinocytes. Keratins above the horizontal line are found mainly in stratified or complex epithelia. The keratin pattern consisting of 46, 48/56, 50/58 kD, as enclosed by the dashed box, is common to all stratified epithelia in neoplasms, hyperproliferative diseases, and culture. (Adapted from Sun T-T, Eichner R, Schermer A, et al: Classification expression, and possible mechanisms of evolution of mammalian epithelial keratins: A unifying model, in Levine A, Topp W, Van de Woude G, et al [eds]: *The Cancer Cell: The Transformed Phenotype.* Cold Spring Harbor, NY, Cold Spring Harbor Laboratories, 1984, vol 1, pp 167–176; Cooper D, Schermer A, Sun T-T: Classification of human epithelial and their neoplasms using monoclonal antibodies to keratin: Strategies, applications and limitations. *Lab Invest* 1985; 52:243–256; Lynch MH, O'Guin WM, Hardy C, et al: Human follicle differentiation: Coordinate expression of acidic and basic hair keratins in upper cortical and cuticle cells and the relationship between "hard" [hair/nail] and "soft" keratin. *J Cell Biol* 1986; 103:2593–2606.)

of the acidic partner[46, 59, 60]; (2) transfection of fibroblasts with a basic keratin gene can induce the expression of an as yet unidentified acidic keratin[61]; and (3) conversely, inhibition of the expression of the acidic 45-kD keratin can lead to the suppression of the basic 52-kD keratin gene.[62] More data are needed to elucidate the detailed mechanism or mechanisms underlying these regulatory events.

The model as depicted in Figure 4 has several implications. First, it points out that detailed keratin analysis can give important clues not only about the epithelium's origin (e.g., the presence of large keratins above the horizontal line in Figure 4 strongly suggests a stratified or complex epithelial nature) but also about its *pathway* or pathways of differentiation (skin, corneal, esophageal, hair, palm types of differentiation or a combination of two or more of the above).[24, 53, 63] In special cases where an epithelium expresses keratin markers for more than one differentiation pathway, immunohistochemical staining with a panel of monoclonal antibodies specific for individual keratins can help to "dissect" the epithelium into several well-defined compartments. In a later section, we will use human dorsal tongue epithelium as an example to illustrate this point.[64]

Another implication of the model is that keratin analysis can yield information about the *state* of differentiation of a single keratinocyte. Thus, in the epidermis the 56.5-/65- to 67-kD keratin markers (of skin-type differentiation) are made predominantly by suprabasal cells.[37, 38, 47] The few basal cells (<5%–10%) expressing these two keratins are considered to be partially differentiated cells about to leave the basal layer.[39, 65] Using a similar approach, we have analyzed the differentiation state of corneal epithelial cells and have obtained some unexpected results suggesting that corneal epithelial stem cells are actually located at the outer edge of the cornea in an area called the *limbus*.[46] This new finding and its clinical implications will be discussed in a later section.

The third implication of the model relates to how many options a suprabasally located keratinocyte may have as far as its major differentiation pathways are concerned. We will use psoriasis as an example to illustrate this point. As an extension of this hypothesis, we will discuss the relationship among skin, corneal, and esophageal keratinocytes with an emphasis on the roles of intrinsic divergence and extrinsic modulation in regulating the differentiation of these three prototype epithelia.

What Is Wrong in Black Hairy Tongue?

The dorsal surface of human tongue is histologically complex since it is covered with numerous primary filiform papillae, each of which has 5 to 15 smaller, secondary filiform papillae projecting mainly from its periphery.[66] Biochemical and immunohistochemical staining data indicate that each secondary filiform papilla consists of a central column of cells express-

ing hair-related keratins (defined immunologically using a monoclonal antibody, AE13, specific for the acidic hair keratins),[53] surrounded by a ring of cells expressing 56.5-/65- to 67-kD keratin markers for skin-type differentiation. The remaining epithelium of the primary papilla, located outside the secondary papillae, expresses 51-/59-kD keratin markers for esophageal-type differentiation (Fig 5).[64] In black hairy tongue, there is a significant retention of the keratinized cells in the "hair" and "skin" compartments of secondary papillae, which thus form highly elongated spines. The formation of these cornified spines in the peripheral region of primary papilla is accompanied by the hyperkeratosis of the esophageal-like epithelium in the center, resulting in the formation of a column of cornified cells with a total diameter approximating that of the primary papilla.[67] These results suggest that the projections in black hairy tongue are indeed hair related and that defective desquamation, hyperplasia, or both may play a role in the disease process.

Where Are Corneal Epithelial Stem Cells?

All self-renewing tissues, including the epidermis and corneal epithelium, by definition must contain stem cells that are ultimately responsible for tissue renewal and regeneration. A subpopulation of epidermal basal cells located at the bottom of deeper rete ridges are believed to represent epidermal stem cells.[68–70] A similar situation exists in dorsal tongue epithelium where a few cells located at the bottom of the skin and hair compartments are presumed to represent the stem cells.[70] However, the situation in corneal epithelium turned out to be quite unique and somewhat unexpected.

Using cultured rabbit corneal epithelial cells as a model system, we have established that the 55-/64-kD keratins are expressed in suprabasal cells and are therefore associated with an advanced stage of corneal epithelial differentiation.[46] Thus, this situation is analogous to the epidermis and esophageal epithelium where the tissue-specific keratin pairs are expressed suprabasally. Studies using the in vivo tissues showed that this 64-kD keratin is also expressed suprabasally at the peripheral corneal epithelium in a region known as the *limbus* (the transitional zone between the cornea and conjunctiva).[46] However, we were surprised to find the strong presence of this keratin in the basal layer of central corneal epithelium. This finding implies that as far as the expression of 64-kD keratin marker is concerned, the central corneal basal cells are in a more advanced state of differentiation than limbal basal cells. Since there are data suggesting that corneal epithelial cells actually migrate centripetally (toward the central cornea),[71] the simplest explanation for these data would be to assume that corneal epithelial stem cells are located in the limbus (Fig 6) instead of distributing uniformly throughout the entire corneal surface, as was previously thought.[46] This model provides an explanation for the preponderance of corneal carcinomas at the limbus[72, 73] and has implications on the

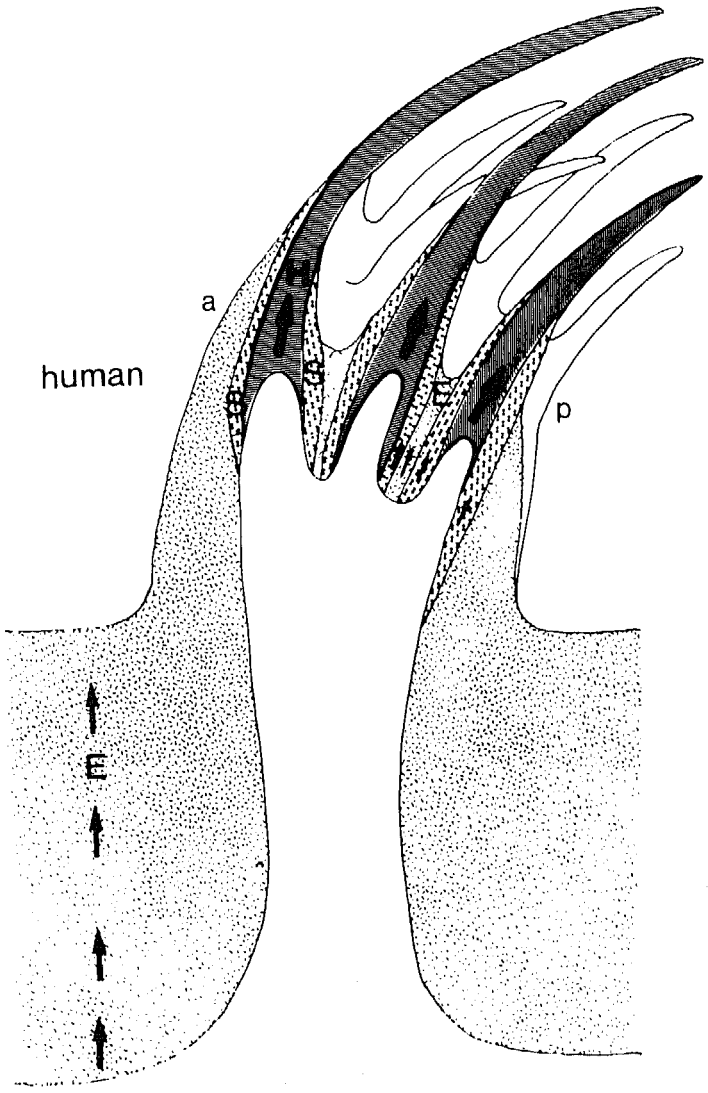

FIG 5.
Schematic representation of the dorsal tongue epithelium demonstrating the compartmentalization of keratin production. Keratinocytes expressing hair, skin, and esophageal types of keratin markers are segregated as illustrated. *H*, hair; *S*, skin; *E*, esophagus; *a*, anterior, *p*, posterior. (From Dhouailly D, Xu C, Manabe M, et al: Three distinct populations of dorsal tongue keratinocyte express keratin markers for hair-, skin- and esophageal-type of differentiation. 1988 [in submission]. Reproduced by permission.)

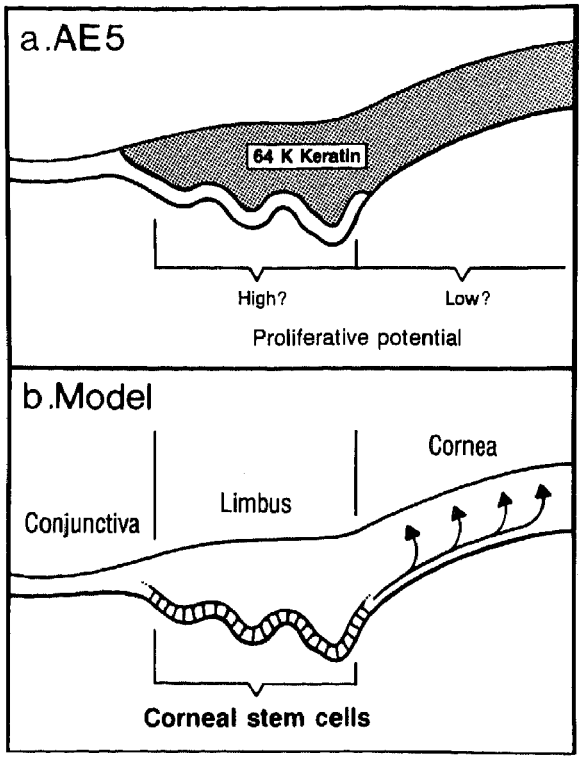

FIG 6.
Model of corneal epithelial differentiation. *a*, expression of 64-kD keratin in corneal and limbal epithelia as detected by AE5 staining. Recent tritiated thymidine labeling data support our earlier suggestion that the proliferative potential of the limbal basal cells is greater than that of the 64-kD positive, corneal basal cells (R. Lavker, personal communication). *b*, schematic diagram demonstrating the limbal location of corneal epithelial stem cells and their centripetal migration pattern. (From Schermer A, Galvin S, Sun T-T: Differentiation-related expression of a major 64K corneal keratin in vivo and in culture suggests limbal location of corneal epithelial stem cells. *J Cell Biol* 1986; 103:49–62. Reproduced by permission.)

mechanism of other corneal epithelial diseases, including persistent corneal epithelial defect.[46]

Mutually Exclusive Expression of Differentiation and Hyperproliferation Markers: A Binary Decision Hypothesis

In the study mentioned earlier in which we analyzed keratins of various epidermal diseases, we found that the level of 56.5-/65- to 67-kD keratins

correlated quite well with the degree of morphologic keratinization.[48] The data also indicated that the 48-/56-kD keratins are expressed in many hyperproliferative diseases at a level inversely proportional to that of the keratinization markers (see Fig 3).[48] We have recently extended this observation to cultured rabbit corneal epithelial cells by ^{35}S-methionine incorporation.[47] Taken together, the data suggest a control mechanism by which the total level of differentiation and hyperproliferation markers in a stratified epithelium is kept roughly constant. This can be easily explained by assuming that a given suprabasal cell at any one time can express either a differentiation marker pair or the 48-/56-kD hyperproliferative pair but not both. According to this hypothesis, keratinocytes of suprabasal layers constantly make such an "either/or" or binary decision with regard to keratin expression.

In making such a decision, keratinocytes most likely respond to various growth factors and extracellular matrix molecules.[11, 74, 75] There is also circumstantial evidence that these decisions may not be irreversible since cells already possessing some differentiation markers appear to be able to respond later to environmental cues by making the 48-/56-kD markers.[46] This hypothesis is illustrated in a cartoon in Figure 7. A similar diagram can be made for corneal and esophageal epithelial cells simply by changing the label (and keratin markers) of the upper pathway to corneal or esophageal type of differentiation. It should be emphasized, however, that this scheme represents a reductionist's current view of a complex and evolving problem. In formulating our hypothesis, we assumed that the hyperproliferation markers are synthesized in the upper layers[46] despite an earlier suggestion that they are made by basal cells of even normal epidermis.[76] The value of our hypothesis lies in the fact that (1) it explains the mutually exclusive expression (on a tissue level) of keratin markers for hyperproliferation and differentiation,[48] (2) it explains the continuous spectrum of keratin expression patterns observed in psoriasis and other epidermal diseases (by having up to 100% of the keratinocytes adopting one pathway vs. another),[48] (3) it supports Mansbridge's recent suggestion that keratinocytes in psoriasis (and, we believe, in many other hyperproliferative epidermal diseases as well) may adopt an alternative pathway of differentiation instead of simply undergoing incomplete keratinization,[77] (4)it presents the 48-/56-kD keratins as markers for an alternative pathway of differentiation (not as markers for immortality), and (5) it suggests a novel mechanism of binary decision-making in keratinocyte differentiation.

Keratinocyte Differentiation: An Integrated View

Over the last 10 years we have used skin, corneal, and esophageal epithelia as prototypes of stratified squamous epithelia to study the relationships among keratinocytes. Although they are morphologically distinct, the three epithelia share many common features as far as keratin expression is con-