Molecular Biology of Keratinocyte Differentiation

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Epidermal keratinocytes (skin cells) are highly specialized epithelial cells designed to perform a very specific function, separation of the organism from its environment. To accomplish this the cells synthesize precursors and assemble them into two distinct structures, the cornified envelope and keratin intermediate filaments. The intermediate filaments are assembled from keratin monomers and the cornified envelope is assembled from a protein called involucrin and several other proteins. Expression of involucrin and the keratins genes are regulated as a function of the stage of keratinocyte differentiation and by various external agents such as calcium and vitamin A. To study the function of these structures and the regulation of precursor production we have cloned cDNA and genomic clones encoding involucrin and four of the keratin polypeptides.

Retinoids profoundly alter the differentiation pattern of human epidermal keratinocytes, but the underlying biochemical basis of this change is not known. In this report we describe retinoid-promoted changes in keratin gene expression that may, in part, be responsible for the alteration in cellular phenotype in the presence of the vitamin. We also describe the novel structure of the human 40 kD keratin, a member of the keratin family that is retinoid responsive and is likely to be important during epidermal development. Finally, we describe the structure of the envelope precursor protein, involucrin, as determined from its DNA sequence and speculate on its role in cornified envelope formation.

Introduction

The skin provides a vitally important protective separation between the internal and external environments. It is a stratified squamous epithelium consisting of several cell types including Langerhans cells, melanocytes, and keratinocytes. By far, the most abundant cell type is the keratinocyte [1], which synthesizes major structural components of the epidermal barrier through a programmed process of differentiation. They arise as basal cells at the innermost layer of the epidermis and following cell division, some progeny remain in the basal layer as stem cells while others migrate into the upper epidermal layers. The migrating cells ultimately terminally differentiate to form the dead surface layer of the skin (stratum corneum) and during the transit from the basal layer to the surface the cells change dramatically both biochemically and morphologically (Fig. 1). During this process keratinocytes initiate synthesis of important differentiation-dependent structural and catalytic proteins including involucrin [2,3], the keratins [4], filaggrin [5], and transglutaminase [6]. In addition, they go through marked morphological and cytostructural changes [1,7]. These changes include the appearance in the spinous and granular layers of various intracellular lamellar bodies (membrane coating granules, keratohyalin granules, Odland bodies, etc.) [1]. In addition, desmosomal links appear in the spinous layer (Fig. 1). In the upper granular layer, the nucleolytic and proteolytic degradation of the bulk of the intracellular organelles and macromolecules [7] and release of lipid-containing lamellar bodies [1] occurs. Simultaneously, the keratins become stabilized by disulfide bonds [7] and involucrin, the precursor of the cornified envelope [2], becomes covalently cross-linked to membranous proteins by a calcium activated enzyme, transglutaminase, thus forming the cornified envelope [6,8,9]. These events are largely completed in the stratum lucidum, a transition zone between living and dead layers of the epidermis. The stratum corneum consists primarily of disulfide-linked keratin filaments surrounded by a cross-linked envelope of protein (Fig. 1). These structures are held together by desmosomal junctions and lipids released from the lamellar bodies. A characteristic feature of this process is that it is strictly regulated in time and space. When this balanced regulation is disturbed, skin disease and diminished barrier function may ensue.

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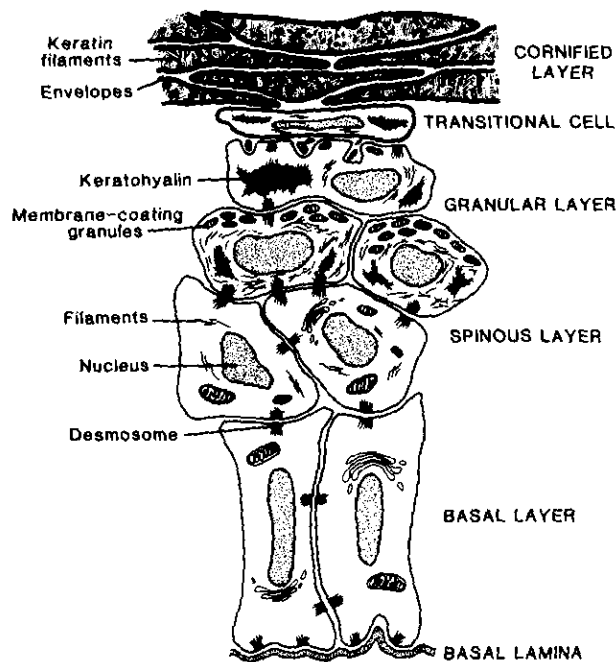
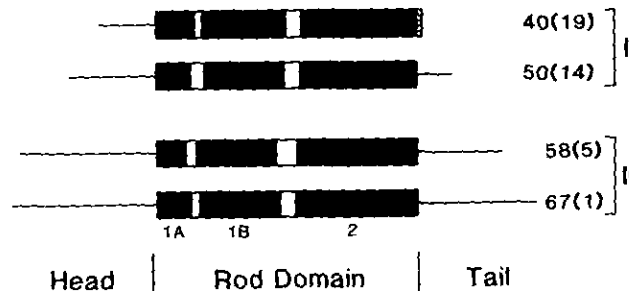FIGURE 1. Keratinocyte differentiation in the epidermis.

FIGURE 2. Structure of 40 kD keratin. The structure of the 40 kD human keratin is compared with the structure of other keratin intermediate filament precursors. Shown are the head and tail segments (single line) and the rod domain. The coiled-coil-forming α-helical segments within the rod domain, coils 1A, 1B, and 2 are shown as darkened blocks, and the nonhelical spacer regions, L1 and L12, are indicated by open regions within each block. The α-helical extension of the 40 kD keratin is indicated by the stippled region. The numbers in parentheses are those assigned to each keratin according to the catalog of Moll et al. (11). Two type I (40 and 50 kD) and two type II keratins (58 and 67 kD) are shown for comparison.

Despite considerable descriptive information about the process, there was until recently very little information concerning its regulation or the structure of the peptides involved in the process. Our laboratory is interested in understanding how keratins form the intermediate filaments in keratinocytes and how the keratin genes are regulated by retinoids and other agents. A discussion of these aspects will form a major portion of this review. We will also discuss the function of involucrin, the envelope precursor, in the context of currently available information.

Keratin Peptide Structure and Filament Formation

Intermediate filaments are structures formed in virtually all cell types and are intermediate in size between the actin and tubulin filaments (10). In epithelial cells, including keratinocytes, these filaments are formed by keratins (7), which consist of a family of at least 20 closely related proteins that are expressed in a cell-type specific manner (11,12). Keratins and all other intermediate filament precursor proteins have a structure consisting of three major segments; a non-α-helical head segment, a non-α-helical tail segment, and an α-helical central rod domain (13) (Fig. 2). The central α-helical domain contains a heptad repeated structure of the type $(abcdefg)_n$ where a and d are nonpolar amino acids. Nonpolar residues placed in this manner form an inclined hydrophobic stripe that wraps around the length of the α-helix. Proteins containing this repeated structure wrap around one another by aligning this hydrophobic stripe to form a coiled-coil structure (i.e., two α-helices wound together to form a larger coil) (13). The coiled coil is further stabilized by favorable

ionic interactions. Keratin filament formation requires the association of type I (acidic) and type II (basic) partners to form the coiled-coil structure. The central core segment of the keratin peptide is essential for this pairing, but segments of the non-α-helical end domain may also be important. These dimers are then assembled into a tetrameric structure that is likely to be the precursor of the extended filament (13).

In keratinocytes, four keratins are expressed in abundance. These have molecular weights of 50, 58, 56.5, and 67 kD (14). The 50 and 58 kD peptides preferentially pair in vitro, as do the 56.5 and 67 kD peptides. However, the nature of the filaments formed by each pair differs. The 56.5/67 kD pair form large macrofibrillar bundles, whereas the 50/58 kD complex forms relatively typical intermediate filament structures, as described above. The macrofibrillar structures formed by the 56.5 and 67 kD pair are likely to be important in lending rigidity to the epidermis. This is the first evidence for a functional difference between keratin filaments assembled from different keratins (14). Additional keratins are expressed in hyperproliferative skin diseases (15), in tumor cells (16), and in simple epithelial cell types (11). A current area of interest is whether all keratin pairs or combinations of pairs have different cellular functions, as has been described for the major epidermal keratins. This might be expected, since keratins display marked tissue specific patterns of expression. Later sections will describe the sequence of the 40 kD keratin, a structurally unique keratin that is expressed in squamous cell carcinomas and in simple epithelial cells. Its structure is unique among all IF proteins and may suggest a special function (17).

Cornified Envelope Formation

The second major structure elaborated by keratinocytes is the cornified envelope (2). During keratinocyte differen-

tiation, the cells become increasingly permeable to calcium and other ions. As noted above, this occurs in the upper granular layer and the stratum lucidum (Fig. 1). The mechanism of this increased permeability is not known; it may be a programmed part of keratinocyte differentiation or it may result from the action of some as yet undefined extracellular trigger. The calcium influx activates transglutaminase, which then catalyzes formation of the cornified envelope. This envelope consists of a minimum of seven proteins that become covalently cross-linked into a stable submembranous structure (2). The mechanism of envelope formation is largely unknown, but recent findings have suggested some important features of the process (8,9,18,19) that will be discussed later.

Retinoids Regulate Keratinocyte Differentiation

Retinoids have been known since the early 20th century to profoundly affect epithelial cell organization (20), and many different cell types are developmentally regulated by retinoids (17,21–28). Retinoid treatment of human keratinocytes in culture drastically changes the morphology of these cells. The stratification process, shown in Figure 1, is dramatically suppressed, resulting in the appearance of far fewer layers (29). As part of this inhibition, the cornified layer is reduced or absent and squame (envelope) formation is reduced 10- to 100-fold (28–30).

Striking biochemical changes are also produced by retinoids. Keratin gene expression is markedly altered by retinoid treatment of cultured keratinocytes (17,22). To study these effects, we have isolated cDNA (21) and genomic clones (22) encoding human epidermal keratins number 5 (58 kD), 6 (56kD), 13 (54 kD), and 19 (40kD) (21). These clones were selected for study because two are upregulated and two are downregulated by retinoids (22). Each clone is specific for one keratin as evidenced by hybrid selection of a single keratin mRNA and hybridization to a single band of mRNA in blot hybridization experiments (21,22). All are expressed in cultured human keratinocytes (21,22).

To begin to understand the process of regulation we have cultured human keratinoctyes using the method of Rheinwald and Green (32). In this method, the keratinocytes are cultured on a feeder layer of cobalt-irradiated 3T3 cells. The 3T3 (feeder) cells are irradiated such that they are mitotically inactive but metabolically active. The feeders provide a supporting matrix for attachment and growth and may also release some unknown stimulators of epithelial cell growth and differentiation.

In our initial experiments, we treated cultured human keratinocytes with retinol, *trans*-retinoic acid (tRA), and a synthetic analog of tRA known as Ro 13-6298 and monitored the dose and time dependence of changes in keratin peptide and mRNA levels (22). Retinol promotes changes in keratin mRNA and protein levels only at higher doses (30). Ro 13-6298, by contrast, produces very dramatic changes (Figs. 3 and 4). tRA produces similar changes, only it is slightly less potent.
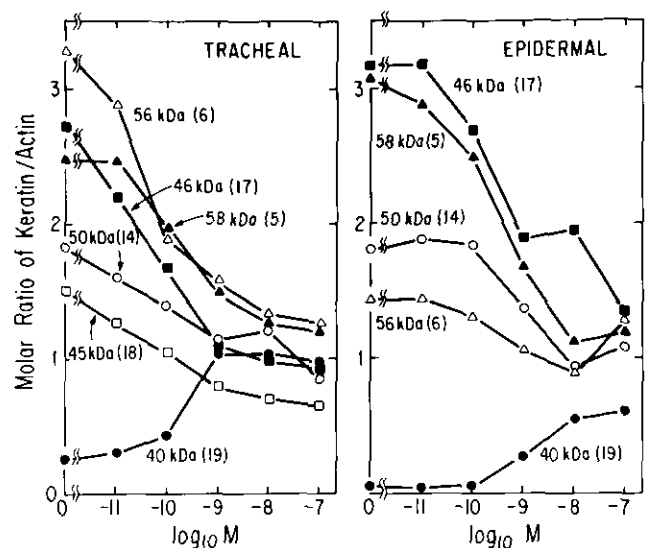
FIGURE 3. Effect of retinoid concentration on the content of individual keratins in tracheal and epidermal keratinocytes. Epidermal or tracheal keratinocytes were treated for 4 days in the presence of the indicated concentration of arotinoid Ro 13-6298. Keratins were electrophoretically separated and then quantitated by Coomassie staining and laser densitometry (22).
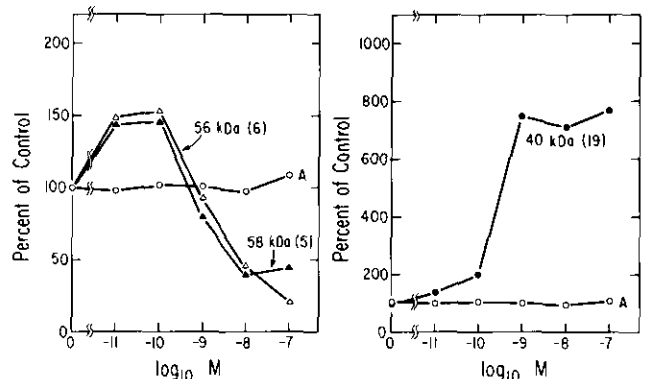
FIGURE 4. Retinoid effects on keratin mRNA levels. Cultured fetal tracheal keratinocytes were grown in vitamin A-free medium supplemented with the indicated concentrations of Ro 13-6298. Poly (A)$^+$ RNA was prepared, electrophoresed, transferred to membranes, and probed with nick-translated probes encoding the 40, 56, and 58 kD keratins. After exposure of the blots on X-ray film, the exposure intensity was quantitated by laser densitometry (22). Results were similar with epidermal keratinocytes.

Several mechanistically important features are evident from these experiments. First, the level of each keratin changes in parallel with the level of the corresponding mRNA, suggesting that keratin level is regulated not by posttranslational mechansims but by control of keratin mRNA synthesis and/or degradation. Second, these experiments demonstrate that keratin levels can be downregulated by retinoids and that changes in mRNA content mediate the reduction. In fact, as shown in Figure 5, the total keratin content is drastically reduced in retinoid-treated cells. This change reduces total keratin content to 1/3 the level observed in untreated cultures (22). Since ker-
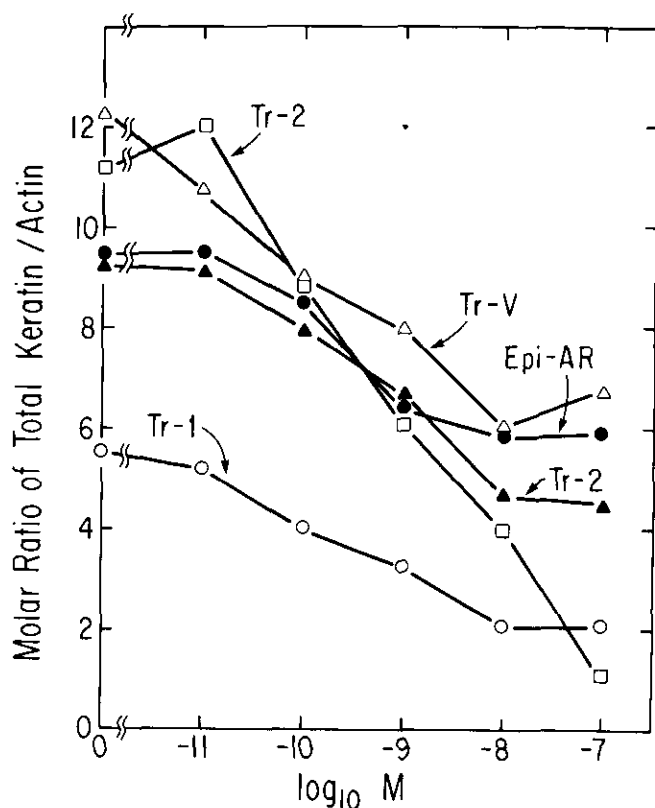
FIGURE 5. Reduction in total keratin content in cultured keratinocytes treated with Ro 13-6298. Cells were treated and proteins quantitated as described in Fig. 3. Total keratin content is plotted as a function of dose of retinoid. The strains are: Tr-1, fetal tracheal keratinocyte; Tr-2, fetal tracheal keratinocyte; Tr-V, adult tracheal keratinocyte; Epi-AR epidermal keratinocyte.

atins comprise 10 to 20% of the total protein content of cultured keratinocytes, this is a reduction that substantially alters the protein composition of the cell. Third, there is a coordinate regulation of keratin mRNA and protein levels in opposite directions, with the 40 and 54 kD keratins increasing and the 56 and 58 kD (and other keratins) decreasing. We are currently investigating whether these changes are due to regulation of RNA synthesis, turnover, or both. The simplest explanation for the coordinate pattern of simultaneous up/downregulation of these genes is that the same factor affects the expression of each gene at some common point in the vitamin A regulatory pathway. One possibility is that simultaneous activation of transcription of one set of genes and suppression of the second set is mediated by a common regulatory protein (trans-acting factor) plus the appropriate ancillary factors. Alternatively, the modulation may involve direct regulation of mRNA turnover. An interesting, but less likely possibility, is that the genes are regulated by two distinct pathways, each of which has very similar sensitivity to retinoid. In this scheme, one mRNA species may be elevated by increased synthesis (pathway one), and another may be elevated by changes in mRNA stability/processing (pathway two). If this is so, it is simplest to assume that vitamin A modulation of the mRNA levels of each group of keratins (stimu-

lated and suppressed) would be mediated solely by one or the other mechanism. Experiments in progress should distinguish among these possibilities.

The kinetics of the responses to retinoid shown in Figure 6 indicate that the cells respond to very low levels of retinoid and that a plateau in stimulation is reached after which there is no further increase. This shows that there is no toxic downregulation at pharmacological concentrations of retinoid and suggests that some step in the retinoid response pathway becomes rate-limiting around 10 nM retinoid. As shown in Figure 4, at low concentrations of Ro 13-6298 (10^{-11} and 10^{-10} M) there is an increase in the level of 56 and 58 kD keratin mRNAs. This increase suggests that the control cells (grown in vitamin A free medium) are actually vitamin A deficient and that low concentrations of retinoid are necessary for maintaining optimal expression of these keratins. This transient increase is not reflected at the protein level (Fig. 3) for reasons that are related to the differentiated status of the cells (22). Time course studies shown in Figure 6 indicate that the genes are not fully activated/suppressed for 2 days following retinoid administration, suggesting that there are intermediate steps between retinoid interaction with the cell and activation/suppression of the keratin genes. A definitive answer to this question will, however, require the use of more sensitive assays to look at earlier times. Keratinocytes are known to contain the cellular retinoic acid binding protein (cRABP) (33) found in many cell types and thought to mediate retinoid responses. This particular molecule is a 20 kD protein that has an affinity for retinoic acid in the nanomolar range (34). Thus, the affinity of this potential mediator matches the dose of retinoid required to initiate changes in keratin gene expression (22), but no evidence currently exists to confirm its direct involvement. If the recently described retinoid receptor (23) is expressed in keratinocytes, it may also be important.
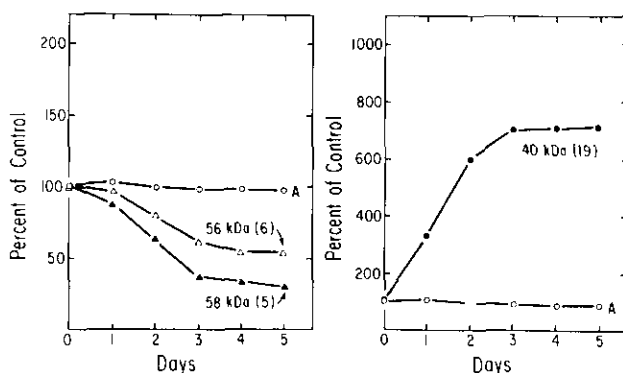

FIGURE 6. Time course of changes in keratin mRNA content in keratinocytes treated with Ro 13-6298. Cultured keratinocytes were treated with 20 nM Ro 13-6298 for 1 to 5 days. Poly (A)$^+$ RNA was prepared, electrophoresed, transferred to membranes, and probed with nick-translated probes encoding the 40, 56, and 58 kD keratins. After exposure of the blots on X-ray film, the exposure intensity was quantitated by laster densitometry (22). Results were similar with epidermal keratinocytes.

40 kD Keratin Has a Novel Structure

Intermediate filament proteins are expressed in virtually all cell types, and keratins are the precursors of these filaments in epithelial cells (11,12,35). These proteins integrate intracellular space and form a network that lends structural strength to the keratinocyte. Thus, the terminally differentiated keratinocyte has a network of disulfide cross-linked keratin intermediate filaments that comprise the bulk of intracellular protein. We have sequenced several of these keratins in an effort to determine whether the individual structure of each is related to its function.

As discussed above, one of the primary effects of retinoids on keratin gene expression is upregulation of the level of the 40 kD keratin and its mRNA (21,22). This keratin is increased 10- to 40-fold within 2 days after adding 20 nM Ro 13-6298 to the cultures (21). In unstimulated cells it is relatively unabundant, representing much less than 0.1% of total cell protein. It is in many ways a unique keratin in that it is the only one that does not have a type II partner, it is the smallest keratin, it is the first keratin to appear during development, and probably the most primitive phylogenetically (12).

The 40 kD keratin is expressed in epidermal keratinocytes in culture following treatment with retinoids (22), in transformed cells (36), and in simple epithelial cells (11). As part of our studies aimed at elucidating the function and regulation of these genes and proteins, we have sequenced the coding and 5′ upstream regions of the 40 kD keratin (17). When compared to other keratins, or for that matter, all other intermediate filaments, the 40 kD keratin (keratin #19) (11) has a unique structure (17) (Fig. 2) in that it lacks a non-α-helical tail domain. In its place is a 13 amino acid α-helical extension that retains the heptad structure in phase with the coiled segments.

As mentioned previously, the tissue-specific pattern of keratin expression suggests that keratins may have unique functions (11,12). The α-helical core appears to be necessary for keratin coiled-coil formation (13). The head and tail regions appear to be less involved in keratin pairing and filament formation and may be more important for inter-

action of the filaments with cellular structures. The observation that in two distantly related species, the human and bovine, the 40 kD keratins are highly identical (nearly 90% identity throughout the entire length) suggests that it has an essential function (17). The unique structure of this keratin may, at least in part, be responsible for the alterations in cell morphology and function associated with its presence in transformed cells (27) and retinoid-treated keratinocytes (22).

The α-helical extension of the 40 kD keratin extends only 13 amino acids and is under structural constraint due to its α-helical nature. This unusual tail segment could alter the properties of the keratin filaments containing the 40 kD keratin or the interaction of such filaments with other cellular structures. The precise role of this keratin in mediating changes in cell structure and function will require further investigation.

Formation of Cornified Envelopes

The cornified envelope in keratinocytes is formed during the terminal stages of keratinocyte differentiation (2,7). As the cells migrate into the upper skin layers they become more permeable to calcium and other ions (7). The resulting calcium influx is responsible for activation of a keratinocyte-specific transglutaminase that catalyzes formation of the envelope (2,6) (Fig. 7). A main precursor of the envelope in human keratinocytes is a 68 kD protein known as involucrin (2). Involucrin is synthesized as a soluble precursor that is positioned, by an unknown mechanism, adjacent to the interior face of the plasma membrane and then cross-linked to various membrane proteins by the keratinocyte-specific transglutaminase (6). The linkage formed is a γ-glutamyl-lysine bond between glutamine residues on involucrin and primary amino groups of selected membranous envelope precursor proteins (8,9) (Fig. 7). The subcellular localization of these precursors plus the amino acid sequence of involucrin makes it possible to construct a rudimentary model of envelope formation (Fig. 8) that involves the participation of at least eight proteins. Seven are located in the membrane, six being
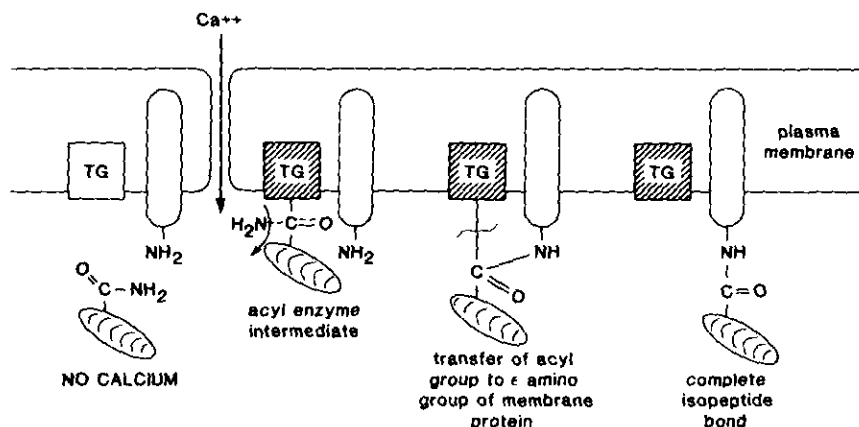

FIGURE 7. Mechanism of transglutaminase-mediated formation of isopeptide bonds. The unshaded transglutaminase is inactive and the shaded form is active.
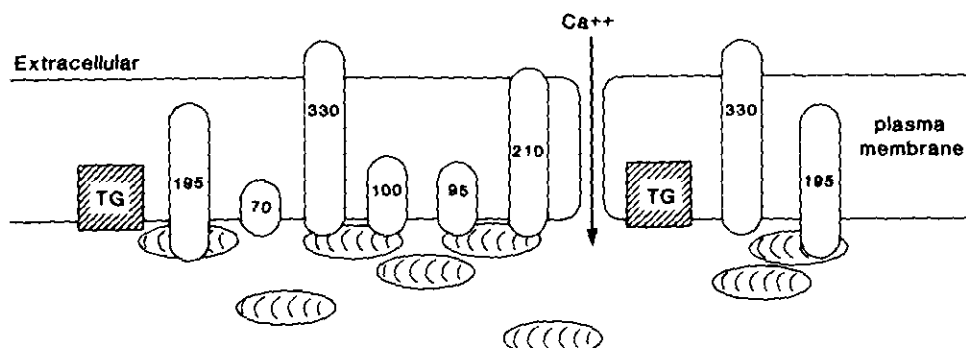
FIGURE 8. A model of cornified envelope formation. The close juxtaposition of involucrin molecules is meant to suggest the possibility of involucrin homopolymer formation during envelope formation.

membranous precursors of the envelope (70, 95, 100, 195, 210 and 330 kD), and the seventh being the enzyme responsible for synthesis of the ε-γ-glutamyl-lysine bonds (Fig. 7), transglutaminase (6). The eighth is involucrin.

As intracellular calcium levels increase, presumably by influx of extracellular calcium and release from intracellular stores, transglutaminase is activated and formation of isopeptide bonds ensues (7). This links the amine acceptor (involucrin) to the membrane-associated (amine donor) proteins. Specific membrane localization of transglutaminase has recently been reported (6). Its localization assures that cross-linking will occur beneath the plasma membrane and not at other intracellular locations. The presence of these proteins in other compartments could result in inappropriate cross-linking of other cellular proteins and organelles.

The structure of involucrin contributes to its being an efficient amine acceptor in the cross-linking reaction (18). Involucrin consists of a series of approximately 40 highly conserved repeats of a 10 amino acid sequence having the consensus sequence (GLU-GLN-GLN-GLY-LEU-LYS-HIS-LEU)$_n$ plus an additional 19 repeats of lesser similarity, 15 at the amino and 4 at the carboxyl terminus (18). Two important features of the sequence that are likely to have functional implications are apparent. First, glutamic (GLU) acid and glutamine (GLN) residues are spaced at equal intervals along the entire length of involucrin. Second, the primary sequence predicts an α-helical structure.

The high content of glutamic acid (20%) regularly spaced along the length of the molecule explains why involucrin and fragments of involucrin migrate as discrete bands on acrylamide gels in the absence of detergent (19); the negative charges apparently act like a coating of sodium dodecyl sulfate. These residues are also largely responsible for the acid pI of involucrin at 4.5 (2). More importantly, these negatively charged groups are likely to have a role in maintaining extension of the involucrin α-helix via intramolecular charge repulsion.

The GLN residues are the reactive (amine acceptor) sites within the involucrin molecule. The repeated distribution and high content (20%) of GLN residues places potential amine acceptor sites at regular intervals along the entire length of the involucrin peptide (18), thus potentiating cross-linking and making these sites accessible to more than one amine donor. The α-helical structure of the mol-

ecule further contributes to the reactivity of involucrin by providing a conformation in which the GLN and GLU residues are pointing out away from the axis of the helix and thus enhancing accessibility for reaction. Because involucrin is a very elongated molecule, it is possible to suppose that it could cross-link two membrane proteins spaced at some distance along the plane of the membrane, creating a very stable envelope structure. It is much less likely that these functions could be carried out by a globular (nonelongated) protein or a protein without a regularly repeating structure.

Many questions remain unanswered concerning envelope production. With the exception of the 195 kD precursor, which may be an internally directed peripheral membrane protein (37), it is not known whether the membranous precursors proteins are integral or peripheral proteins (they are drawn as shown only for convenience). They clearly are exposed to the intracellular space, but may also project into the extracellular environment. A significant intracellular extension could make cross-linking sites available deep in the cytoplasm.

Second, the sequence of assembly is not known. Transglutaminases are activated by a calcium-induced change in conformation and then react to form an acyl enzyme intermediate (45). In the presence of amine donors the enzyme then catalyzes formation of an isopeptide bond between the amine donor (glutamyl acceptor) and amine acceptor (glutamyl donor) before dissociating (Fig. 7). This scheme requires that involucrin initially bind to the enzyme and then be transferred to various amine donors. It seems likely that involucrin is transferred to membranous glutamyl acceptor proteins based on the acceptors concentration and ability to react with transglutaminase. It is known that involucrin acts as a substrate at multiple sites (19), suggesting that it may potentially form multiple cross-links simultaneously or sequentially. It can be presumed that there are steric limitations on the number of linkages made by each involucrin molecule. Transglutaminase may not be capable of accessing potential cross-linking sites after several proteins have been linked to involucrin. This hindrance may decelerate the rate of envelope formation as the process proceeds. A further factor that most likely limits the extent of envelope formation is that the cell is dying (how fast this occurs is not known), and as a result

new precursors and transglutaminase are not being synthesized. In addition, as the membrane is destroyed and the cross-linking proceeds, the available precursors and transglutaminase are likely to become less mobile in the membrane and therefore less able to react. Third, it is not known whether any of the membranous precursors are themselves cross-linked or if involucrin must mediate all cross-linking events. It does appear, however, that involucrin can markedly accelerate and augment the rate and extent of envelope formation (8), suggesting that its primary role may be as a facilitator.

Finally, any model of envelope assembly must explain the progressive thickening of the envelope that occurs during terminal differentiation. The envelope forms in a uniform manner beneath the plasma membrane with very little difference in depth over its entire circumference. This is a mechanical problem that can be stated: How can a progressively thickening and shape-limiting envelope form below the cell plasma membrane if the membranous precursors are only available in the plasma membrane? The simplest explanation is that involcrin homopolymer formation, (involucrin)$_n$, accounts for the bulk of the thickening that occurs. Involucrin homopolymers have been observed *in vitro* (8). However, this assumes that involucrin molecules can get sufficiently close to link together despite the high negative charge repulsion of the glutamic acid residues. Another alternative is recruitment of other cytoplasmic proteins to the envelope. However, available data suggest that involucrin is the main cytosolic component of the envelope (8). A third alternative is that involucrin is first anchored to the membrane precursor proteins at one end and that it then points into the cell at right angles to the membrane, like a dense forest of tree trunks directed into the cytoplasm. This would place many potential cross-linking sites in the cell interior and would also allow for involucrin homopolymer formation between adjacent involucrin molecules. This, however, seems unlikely. Assuming a uniform reactivity of all potential cross-linking sites along its length, the probability of involucrin being initially cross-linked in the central region of the molecule seems as likely as this initial link being formed on either end. *In vivo*, the involucrin rod is likely to be linked in a variety of orientations relative to the plane of the membrane.

Regulation of Envelope Formation

Retinoids are potent suppressors of envelope formation in human keratinocytes (29). A remarkable feature of keratinocyte physiology is the differential effects of retinoids on cornified envelope formation and modulation of keratin gene expression (30). Envelope formation is suppressed half-maximally at 10- to 100-fold lower concentrations of retinoid than the concentration required to modulate keratin gene expression (22). This suggests that these two systems are likely to be controlled via different mechanisms. Vitamin A has been reported to exert its effects by interacting with intracellular binding proteins (38), by membrane effects (38), and by altering glycoprotein synthesis (40). The recently cloned retinoid receptor may also have

a role (23). It is currently not known if any or all of these mechanisms are important in regulating keratinocyte function. It is conceivable that multiple mechanisms are important.

Retinoid effects on envelope formation in transformed keratinocytes has been extensively studied by Rice and coworkers (41,42) and have also been examined in normal keratinocytes (30). The results argue that the differentiation process is modified in two ways in squamous cell carcinoma (SCC). First, retinoids and culture conditions appear to affect the levels of envelope precursors; that is, these agents affect their synthesis and/or stability, and second, the membranes of these cells may not allow calcium influx, since they do not spontaneously form envelopes under any circumstances. In normal cells, in contrast, there is less regulation of envelope precursor levels and the membranes appear to become spontaneously permeable to calcium during the course of differentiation.

These data suggest two potential mechanisms for regulating the extent of envelope formation: a) modulation of the levels of essential precursors, or b) changing membrane permeability to calcium. The evidence suggests that both mechanisms operate *in vivo*: There is very little, if any, involucrin expression in the basal layer in epidermis (2) or in small (basal) cells from keratinocyte cultures (46). This profile is paralleled by the 195 and 210 kD membranous precursors of the envelope (9) and by transglutaminase (6). Although we know of no direct evidence of changes in membrane permeability during differentiation, the physiology of keratinocyte differentiation indicates that large changes in membrane permeability do not occur until the cells reach the upper granular and transitional layers when changes in membrane permeability alter transglutaminase activity and promote organellar destruction. Thus, envelope precursor levels are regulated during early and intermediate stages in differentiation, but major changes in membrane permeability occur only later during terminal differentiation.

Summary

In recent years much progress has been made in our understanding of the molecular events underlying differentiation in epidermis. We now know that keratinocyte differentiation is enchanced by calcium (43), hydrocortisone (41,42), and vitamin A depletion (21,22,44) and that differentiation is generally suppressed by retinoids (22) and low calcium levels (43). Progress has also been made in understanding the regulation of genes expressed in human keratinocytes (21,22) and in elucidating their structure (17,18) and function (14). Other participants involved in keratin filament assembly and cornified envelope formation have also been identified (5,8,9). Future experiments can be expected to describe in ever increasing detail the function of these proteins in the differentiating keratinocyte.

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