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Transcription factor regulation of epidermal keratinocyte gene expression

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The skin – a protective barrier

The epidermis, the outermost skin layer, provides the first line of defense against the external environment. The major cell type in epidermis, and the cell type responsible for constructing the protective barrier, is the epidermal keratinocyte [1, 2, 3]. Keratinocyte differentiation is the process whereby a relatively undifferentiated keratinocyte is converted into a corneocyte. This process is shown schematically in Figure 1 and is outlined below. The basal lamina separates the epidermis from the dermis. The basal layer (stratum basale) consists of a single layer of undifferentiated, proliferative cells that are anchored to the basal lamina [4, 5]. This layer provides a continuous supply of cells that repopulate the epidermis [4, 5]. The spinous layer, the second epidermal cell layer is characterized by dense desmosomes. Early markers of keratinocyte differentiation, including involucrin and transglutaminase type 1 (TG1), are first expressed in this layer [6] (Figure 1). The next layer, the stratum granulosum or granular layer, is rich in granules. These granules contain products of keratinocyte differentiation that are used to assemble various terminal keratinocyte structures, including the corneocyte cornified envelope [7, 8, 9]. Granular layer cells are living, metabolically active cells. The stratum lucidum is called the transition zone and is the layer that separates the dead from living epidermal layers. This is a region of systematic destruction of cellular organelles and nucleic acids that coincides with assembly of the keratin intermediate filament bundles and the cornified envelope. The cornified cells, corneocytes, are the terminal product of keratinocyte differentiation. These dead, flattened polyhedron-shaped structures occupy the stratum corneum and are uniquely adapted to provide a protective surface. They consist of a stabilized array of keratin filaments contained within a covalently cross- linked protein envelope [10]. Keratin intermediate filament bundles course through their interior and connect at various points to the surrounding marginal band (i.e., the cornified envelope). The marginal band is a thick band that forms beneath the inner leaflet of the plasma membrane and is composed of a variety of proteins that are covalently connected by ϵ-(γ-glutamyl)lysine protein-protein crosslinks [6, 11]. These crosslinks are formed by transglutaminase [12, 13, 14]. Adjacent corneocytes are held together by modified desmosomes and by an interlocking system of ridges and grooves [6]. The composite of billions of terminal keratinocytes (cornified envelopes) forms the protective epidermal surface.

The transition from basal cell to corneocyte is a complex process that requires the simultaneous activation and inactivation of a wide variety of genes. For differentiation to succeed, i.e., to produce a normal epidermal surface, these genes must be expressed at the correct time and in the correct location. A major goal of keratinocyte-related research is to identify the mechanisms that regulate this process. In the present review, we describe several transcription factor families and signal transduction pathways that have been identified in keratinocytes. Among these pathways, activation of protein kinase C (PKC), followed by activation of activator protein 1 (AP1), have been studied and appear to be important in the regulation of keratinocyte differentiation. A review of the literature reveals gaps in our knowledge regarding how ligands activate gene expression in keratinocytes. While some information is available regarding events early and late in these signal transduction pathways, little information is available regarding intermediate steps. In addition, for many of the structural genes, the initial stimulus that activates

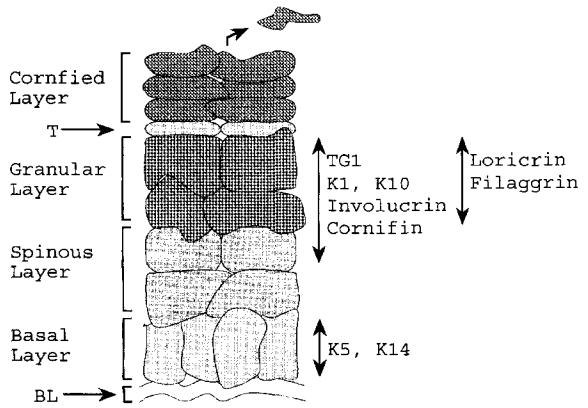

Figure 1. Epidermal keratinocyte differentiation. The major morphological layers of epidermis are shown (i.e., basal, spinous, granular and cornified, layers). The transition zone (T) and the basal lamina (BL) are indicated and are described in the text. The basal lamina is a dense layer of collagen and related proteins that separates the epidermis from the dermis. The transitional cell layer is the region where the living cells of the granular layer are converted into the dead cells of the cornified layer. The location of expression of some of the differentiatin marker proteins discussed in this review are indicated to the right (TG1, K1, K10, involucrin, cornifin, loricrin, filaggrin, K5 and K14).

the signal transduction cascade has not been conclusively identified.

Regulation of keratinocyte gene expression

During the process of differentiation, keratinocyte stem cells are converted to differentiated keratinocytes (Figure 1). An important question is how the expression of the genes that are required to be turned on and/or off during the differentiation process is regulated. Although some progress has been made in identifying factors that regulate gene expression [11, 15, 16, 17, 18], much work needs to be done.

Many of the keratinocyte genes discovered so far are regulated at the transcriptional level. The fact that many of these genes are expressed in a coordinate manner during keratinocyte differentiation might predict a common mechanism of gene regulation. However, initial efforts in this field have not revealed such a unifying mechanism. In fact, most genes that are specifically expressed in keratinocytes are regulated in an independent manner by at least one regulatory agent and the gene regulatory regions do not appear to be conserved.

The cytokeratins, cornified envelope precursor proteins, and transglutaminase are useful markers of gene expression in keratinocytes, both *in vitro* and *in vivo* [11, 15, 17, 18]. The cytokeratins are a family of over thirty proteins that are assembled to form the intermediate filaments in epithelial cells [19]. Based on sequence homology and expression pattern, the keratins can be divided into acidic and neutral-basic families [19, 20, 21]. Formation of keratin filaments involves the assembly of neutral-basic and acidic keratins [22]. This assembly involves formation of a tetramer consisting of two acidic and two neutral-basic keratins [22, 23, 24]. Keratins are expressed in pairs (i.e., as partners) to assure the presence of a neutral-basic and acid partner to permit filament assembly [21]. In epidermis, cytokeratins 5 (K5) and 14 (K14) are expressed in the basal cells and form an intermediate filament network that is suitable for cells that are proliferative [25]. In contrast, cytokeratins 1 (K1) and 10 (K10) are expressed in suprabasal cells [21]. These keratins are involved in formation of the intermediate filament bundles that are present in differentiated keratinocytes [26]. The keratin bundles are stabilized by disulfide bonds and occupy the interior volume of the cytoplasm in terminally differentiated cells [26]. The presence of these bundles provides structural stability to the corneocyte. Profilaggrin, the precursor of filaggrin, is expressed in the suprabasal epidermal layers. It is converted to filaggrin, which packages the keratin filaments into fibrils [8].

Involucrin and loricrin are envelope precursor proteins that are expressed in the epidermal suprabasal layers [8, 11, 14, 27, 28]. These proteins are crosslinked to form the cornified envelope by the enzyme(s) transglutaminase which catalyzes the formation of interprotein ϵ-(γ-glutamyl)lysine bonds. Thus the envelope is a structure comprised of covalently crosslinked proteins that is assembled beneath the deteriorating plasma membrane during the final stages of keratinocyte differentiation.

In the following sections, we discuss our current understanding regarding the mechanisms responsible for regulation of keratin and cornified envelope precursor gene expression.

Regulation of cytokeratin gene expression. Endo A and Endo B are mouse keratins which correspond to human K8 and K18, respectively. They are the earliest keratin genes expressed during embryogenesis. In the adult mouse, these keratins are expressed in simple epithelia. Although Endo A and Endo B are not epidermal keratins, we will briefly discuss the regulation of these

keratins, since they are regulated by AP1 and their methylation state appears to regulate expression.

In mouse fibroblasts, the Endo B gene is hypermethylated and inactive, while the exogenously introduced gene is active [29]. This suggests that the endogenous gene may not be active in fibroblasts because it is not accessible to transcription factors because it is hypermethylated. The human K18 promoter (the human keratin corresponding to mouse Endo B) is not active in F9 embryonal carcinoma cells [30] which express low levels of AP1 transcription factors [31], but activity is increased when F9 cells are cotransfected with constructs encoding c-*jun* or c-*fos*, or when the cells are treated with retinoic acid, which increases c-*jun* and c-*fos* levels [30]. The DNA element that mediates this response is present in the first intron of the human K18 gene and includes an AP1 binding site [30]. The K8 gene (the human keratin corresponding to mouse Endo A), which encodes the neutral-basic keratin partner to K18, also has a potentially active AP1 site [32, 33]. These studies illustrate that AP1 has a role in regulating keratin gene expression during embryogenesis, indicate that regulatory regions in keratin genes can be present in introns and downstream of the gene and show that two keratins which are coexpressed need not be regulated by identical regions that are localized at similar positions in each gene.

Cytokeratins K5 and K14 are expressed in the basal layer of most stratified epithelia, including epidermis. Several transcription factors, including AP1, Sp1 and AP2, may be important in regulating cytokeratin K5 expression [34, 35, 36, 37]. A 6 kb segment of the human K5 promoter upstream regulatory region confers basal cell specific expression in transgenic mice and a truncated promoter containing the 90 bp most proximal to the K5 TATA box directs expression to the suprabasal epidermal layers. Thus, the distal promoter region is required to maintain differentiation-appropriate expression, but the proximal promoter is sufficient to direct tissue specific expression [34]. The bovine keratin K5 promoter displays cell type-specific expression *in vitro* and the activity maps to an AP1 site-containing enhancer located between −762 and −1009 upstream of the transcription start site [38]. However, factors other than AP1 may be important in mediating tissue specific regulation, since the AP1 site produces identical footprints using cell extracts from K5 expressing and non-expressing cells.

Acidic keratin K14 is the expression partner of K5. The sequence 5′-GGCTGCAGGC–3′ is contained in the proximal promoter and point mutations in this element, which binds AP2, reduces gene activity [39, 40]. However, a strong correlation between AP2 levels and K14 promoter activity has not been observed [39], suggesting that although AP2 may be important for keratin gene activation, it is not alone sufficient for cell type-specific expression.

Cytokeratins K1 and K10 are expression partners that are specifically expressed in the suprabasal epidermal layers. A calcium responsive regulatory region has been localized in the 3′ end of the K1 gene [41, 42, 43, 44] and this region includes an AP1 site and two inverted repeats of a hormone response element [42]. These elements appear to mediate the effects of calcium (AP1 element), vitamin D and retinoids (hormone response element).

Thus, a variety of transcription factors, including AP1, Sp1 and AP2, appear to be involved in regulation of keratinocyte keratin gene expression. AP1 appears to be an important regulator for a number of these genes and appears to confer calcium and phorbol ester responsiveness. In addition, these results indicate that for keratin genes, the AP1 response elements can be located upstream or downstream of the coding region or within introns.

Regulation of envelope precursor gene expression. Loricrin, a precursor that is a component of the cornified envelope, is a marker of late keratinocyte differentiation (i.e., expression in the upper granular layer) [27, 28]. *In vitro* studies show that loricrin transcription is increased by calcium and phorbol ester, and decreased by retinoic acid [45, 46]. Transgenic studies show that a 6.5 kb segment of the mouse loricrin promoter confers cell and tissue-specific expression. However, this region does not confer differentiation-specific regulation. A 9 kb segment of the human loricrin gene 5′ flanking region can direct tissue and differentiation-specific expression in mice [47]. Promoter activity requires a conserved AP1 site within the proximal promoter [48].

Involucrin, a marker of early keratinocyte differentiation, is expressed in the upper spinous and granular layers [49]. It is covalently incorporated into the cornified envelope during the terminal stages of differentiation [50, 51]. Involucrin levels are regulated by phorbol ester [52, 53], calcium [53], hydrocortisone [54], retinoids [54] and vitamin D [55]. Human involucrin promoter studies show that the involucrin upstream regulatory region is necessary and sufficient to target involucrin expression to the epidermis in transgenic mice [56, 57]. AP1 is an important regulator of invol-

ucrin gene expression. Cotransfection of an involucrin promoter construct with c-*jun* and c-*fos* increases activity [58]. DNA sequencing experiments indicate five potential AP1 sites located between −2473 and −7 bp upstream [59] of the involucrin transcription start site [60]. Truncation of the upstream region reveals that AP1-5, the most distal AP1 site, and AP1-1, the most proximal AP1 site, are essential for promoter activity. Each site accounts for approximately 40% of promoter activity. Gel supershift analysis shows that *jun*B, *jun*D and *Fra*-1 are the major AP1 transcription factors interacting at both sites [59]. The other three AP1 sites in the involucrin upstream region, AP1-2, AP1-3 and AP1-4, do not appear to be functional in this context [59]. Thus, not all sites for a particular transcription factor need to be used in any given promoter. Phorbol ester produces a 10-fold increase in promoter activity, and mutagenesis of AP1-1 and AP1-5 indicate that these sequences mediate part of the TPA-dependent increase. In addition, several studies show that the involucrin upstream regulatory region displays cell type-specific expression *in vitro* [53, 59, 61].

Transglutaminases are the enzymes responsible for cornified envelope assembly. Expression of one member of this family, transglutaminase type 1 (TG1) is coordinately regulated with involucrin expression. The TG1 gene appears to contain an active AP1 site [62, 63]. A segment of DNA including 0.82 kb of the human TG1 gene mediates a phorbol ester concentration-dependent increase in transcriptional activity in rat keratinocytes and inhibitors of protein kinase C, or the retinoid 9-cis-retinoic acid, reduce the phorbol ester-dependent activity by 50% [63]. Co-transfection with c-*jun* increases TG1 promoter activity, while co-transfection with c-*fos* inhibits activity. The element(s) that mediate the TPA response of the TG1 promoter is not presently known.

These studies provide strong evidence that AP1 is an important regulator of involucrin, transglutaminase and loricrin expression. In addition to these genes, the human papillomavirus (HPV) upstream regulatory region (URR) is a well studied keratinocyte specific enhancer that contains two closely spaced AP1 sites that interact with *jun*B and *jun*D [64]. AP1 sites within the involucrin promoter interact with *jun*B, *jun*D and *Fra*-1 [59]. Thus, two AP1 transcription factors are used in by both promoters. In addition, studies of the involucrin promoter show that not every site that binds a particular transcription factor is utilized (e.g., only two of five AP1 consensus sites appear to be functional in the involucrin promoter) [59]. Table 1 lists several genes that are expressed in keratinocytes and the transcription factors that have been implicated in their regulation.

Role of the protein kinase c signaling cascade in regulating keratinocyte proliferation and differentiation

Protein kinase C (PKC) signal transduction. The above studies indicate that AP1 is an important regulator of gene expression in epidermis. Protein kinase C (PKC) is an upstream component of the pathway that regulates AP1 in many systems. The protein kinase C (PKC) family consists of eleven distinct isozymes [65, 66]. Eight of these isozymes function in a calcium-independent manner while three forms, alpha, beta and gamma, require calcium for activity. The PKC pathway is activated by binding of external signaling agents (hormones, growth factors, etc.) to receptors that are coupled to and activate phospholipase C (PLC). PLC hydrolyzes phosphotidylinositol to produce two short-lived products, inositol triphosphate (IP_3) and diacylglycerol (DAG) [66, 67]. IP_3 is involved in the release of calcium from intracellular stores and DAG is an activator of PKC [66, 67]. TPA (12-*O*-tetradecanoylphorbol-13-acetate) is a pharmacological agent that activates PKC by binding to the same site on PKC as the natural ligand, DAG [68]. PKC phosphorylates target proteins on serine/threonine residues to alter their activity. Downstream gene products targeted for phosphorylation by this pathway include the transcription factors c-*fos* and c-*jun*. For any particular cell type, various PKC agonists can produce different responses. This can result from selective regulation of activity and/or level [69, 70] or post-translational modification [71, 72] of specific PKC isozymes. PKC subcellular distribution is also important, since it influences interaction with cofactors, substrates and lipids [73, 74]. PKC activity is also influenced by other signalling pathways including the retinoic acid receptor system [75, 76].

PKC in mouse keratinocytes. Five PKC isoforms, PKC-alpha, -delta, -epsilon, -zeta and -eta, are present in mouse epidermal keratinocytes. Each PKC isozyme in epidermis appears to have a distinct function [77, 78, 79, 80] and to be selectively expressed during epidermal differentiation. For example, as in other epithelia [81], PKC-eta is detected in the late stages of terminal differentiation in epidermis [82, 83]. *In vitro*,

Table 1. Transcription factor regulation of keratinocyte gene expression

Protein	*In vivo* Expression	Regulation/ stimulus	Response element/ (Transcription factor)[a]	Ref.
K18	simple epithelia (early development)	basal activity	AP1 site, intron 1	[30]
K8	simple epithelia (early development)	basal activity	AP1 site, 3′ end	[33] [32]
K5	basal layer	basal activity	AP1 site, 5′ end	[38]
K14	basal layer	basal activity	AP2 site, 5′ end	[39]
K1	granular layer	calcium response	AP1 site, 3′ end	[42]
		vitamin D response	Steroid hormone response motif, 3′ end	[42]
K17	inflammation	IFNγ response	STAT factor site 5′ end (STAT-91)	[170]
TG1	spinous/granular	TPA response	putative AP1 site, 5′ end	[62] [63]
Loricrin	granular layer	calcium and TPA response	upstream AP1 site is proximal to basal promoter, 5′ end	[48] [46]
Involucrin	spinous/granular	basal activity, phorbol ester response	upstream AP1 sites proximal and distal to basal promoter, 5′ end (*jun*B, *jun*D, *Fra*-1)	[59]
HPV16	suprabasal	basal activity	URR of P_{97} promoter (*jun*B, *jun*D)	[173] [174] [175] [64]

[a]With one exception, this table lists studies in which the indicated transcription factor has been clearly implicated and the binding site identified within the gene. The specific transcription factor(s) involved in the regulation are presented in parenthesis. The location of transcription factor binding site is indicated as located downstream of the gene (3′ end), upstream of the transcription start site (5′ end) or in an intron.

addition of extracellular calcium to murine keratinocytes changes the subcellular distribution [84], phorbol ester-binding properties [85] and level [86] of PKC. It has been suggested that translocation of PKC-alpha to the membrane is directly correlated with induction of cell differentiation [87] (i.e., with downregulation of K1 and K10 and upregulation of transglutaminase type 1, loricrin and profilaggrin [88, 89, 90]). PKC-delta may also be a regulator of differentiation [91].

Phospholipase C (PLC) is the major regulator of PKC activity. PLC activation results in the release of DAG which, in turn, activates PKC. Mouse keratinocytes express PLC-gamma-1 (145 kDa) and PLC-delta 1 (85 kDa), but not PLC-beta 1 [92]. PLC activity is known to be differentially regulated by calcium, phorbol ester and mitogens. Calcium increases the level of phosphatidylinositol-specific PLC-gamma and -delta [92]. These, in turn, influence the level of inositol phosphates [88, 93, 94], intracellular calcium [95], and DAG [88, 96]. Other agents produce different effects on PLC isozymes. For example, the calcium-dependent increase in PLC-gamma and PLC-delta does not appear to involve tyrosine phosphorylation of the PLC enzymes [92]. In contrast, TPA, which also increases differentiation, increases serine phosphorylation of PLC-gamma. TGFα dependent cell proliferation is associated with increased PLC levels and tyrosine phosphorylation of PLC gamma [92]. These results indicate that in keratinocytes the PLC-triggered cascade can be utilized in many different regulatory

contexts and that the pathways responsible for TPA-dependent and calcium-dependent differentiation are distinct. The fact that these pathways differ suggests that some genes may be more responsive to phorbol ester (i.e. PKC activation) and less responsive to calcium regulation. Other genes, in contrast, may display the reverse sensitivity.

PKC in human keratinocytes. Like murine keratinocytes, human keratinocytes express PKC-alpha, -delta, -epsilon, -zeta, and -eta, but not PKC-beta, -gamma, or -theta [97]. Protein kinase C activators, 12-0-tetradecanoylphorbol 13-acetate (TPA) and bryostatin-1, rapidly stimulate translocation of cytosolic PKC-alpha and -epsilon to the membrane; prolonged treatment induces complete downregulation of PKC-alpha and -epsilon levels, but neither the distribution nor level of PKC-delta, -zeta or -eta are changed by agonist treatment [97]. This translocation is correlated with an increase in human keratinocyte differentiation as measured by a reduction in cell proliferation [98], increased cornified envelope formation and involucrin expression [99, 100]. Surprisingly, various PKC inhibitors produce a concentration-dependent inhibition of human keratinocyte proliferation [99, 101]. Staurosporine, a relatively non-specific PKC inhibitor, produces effects identical to TPA [99]. In contrast, Ro31-8220, a relatively specific PKC inhibitor, antagonizes the TPA responses [99]. Other investigators report that calcium-dependent keratinocyte differentiation is not inhibited by PKC inhibitors [98] and that PKC elevation does not always trigger cell differentiation. Ultraviolet A (UVA) elevates PKC levels in human keratinocytes and increases the fraction of PKC bound to membrane [102]. However, in contrast to TPA stimulation, the UVA-dependent PKC increase is not associated with enhanced differentiation [102]. Thus, as with murine keratinocytes, not all agents that induce PKC activity result in increased differentiation of human keratinocytes. This could be related to the rate of turnover of PKC isozymes or the strength with which they are activated.

PKC is also regulated as a function of human keratinocyte differentiation. In a reconstituted epidermal system, PKC activity is higher in differentiated than non-differentiated cultures [103]. In this system PKC-delta and -eta mRNA levels are increased with differentiation. However, in contrast, these mRNAs are downregulated by phorbol ester induced differentiation [103].

PKC activation produces transient increases in intracellular calcium. Studies using thapsigargin, an inhibitor of the endoplasmic reticulum Ca^{++}-ATPase, have shed some light on the role of the PKC-dependent increase in intracellular calcium in regulating differentiation [104]. Thapsigargin produces a transient increase in intracellular calcium in the absence of IP_3 or diacylglycerol (DAG) production, or PKC activation [104]. This treatment does not increase cell differentiation or increase involucrin levels, suggesting that a transient increase in intracellular calcium is not sufficient to promote differentiation [104]. These results also suggest that PKC activation may be necessary for differentiation.

Activator protein 1 (AP1) as a regulator of keratinocyte gene expression

Activator protein 1. Activator protein 1 is a major target for phosphorylation following activation of protein kinase C. Activator protein 1 describes a family of transcription factors belonging to two major families, the *fos*-related and *jun*-related transcription factors [105]. The *fos*-related family includes *Fra*-1, c-*fos*, *fos*B and *Fra*-2 [106, 107, 108, 109], while the *jun* family includes c-*jun*, *jun*D and *jun*B [110, 111, 112]. These proteins interact with specific DNA response elements to regulate gene transcription [113]. c-*fos* is a 380 amino acid nuclear phosphoprotein (55 kDa) which can be modified by phosphorylation [114]. The c-*fos* protein, and all other AP1 family member proteins, contain a region of periodically repeated leucine residues spaced every seven amino acids (leucine zipper) located adjacent to a basic (arginine- and lysine-rich) region [105]. This region is necessary for heterodimerization with the c-*jun* protein to form the active AP1 complex [105]. c-*fos* cannot form homodimers and only interacts with DNA as part of a c-*jun*/c-*fos* heterodimeric complex [105]. In contrast, c-*jun*, a 39 kDa protein can form c-*jun*/c-*jun* homodimers or heterodimers with *fos* family proteins [105]. c-*jun*, unlike c-*fos*, can bind directly to DNA response elements having the consensus sequence 5′-TGA(C/G)TCAG-3′ [105]. AP1 levels are modulated by a wide variety of mitogens, differentiation factors and pharmacologic agents [115, 116, 117, 118]. The protein kinase C (PKC) and adenylate cyclase pathways have been implicated in this regulation [105]. The first AP1 factor family members described, c-*jun* and c-*fos*, are transiently increased in response to agents that stimulate cell pro-

liferation. Thus, c-*fos* and c-*jun* were characterized as immediate early genes (i.e., an early part of the proliferation response) [119]. In terminally differentiating systems, such as epidermis, central nervous system and epiphysis, however, AP1 proteins appear to have a different role, as sustained expression of c-*fos* precedes cell death [120, 121].

Activator protein 1 in keratinocytes. The AP1 literature is complicated and sometimes contradictory. An impressive array of studies suggest that members of the activator protein 1 family play a key role in regulation of epidermal differentiation and gene expression. AP1 factors are present in epidermis and in cultured cells, are expressed in a differentiation-dependent manner [122, 123, 124, 125, 52] and appear to mediate phorbol ester and calcium effects on expression of specific genes [41, 42, 43, 59, 126]. Other agents, including ultraviolet light and tissue trauma, also regulate epidermal AP1 factor expression [127, 128, 129, 130, 131, 132].

However, knocking out c-*fos* expression *in vivo*, does not alter epidermal differentiation [133], suggesting that other AP1 factors may substitute for c-*fos* in the epidermis of these mice, or that c-*fos* is not a required regulator of keratinocyte differentiation. This is supported by Dotto et al. [134] who used primary cultures of mouse keratinocytes and showed that raising extracellular calcium levels induces growth arrest and increases differentiation without altering c-*fos* levels. However, the same study showed that stimulation of differentiation with serum or TPA increases c-*fos* mRNA levels [134].

c-*fos* also interfaces with growth regulatory pathways in keratinocytes. EGF and IGFs are potent stimulators of keratinocyte proliferation [135,136]. In EGF-stimulated BALB-MK cells, the level of c-*fos* expression is rapidly decreased by calcium, but calcium does not affect c-*fos* expression in the absence of EGF [135]. Constitutive overexpression of c-*fos* in BALB/MK cells appears to potentiate the response of the cells to the mitogen insulin-like growth factor-1 (IGF-1)[136].

Taken together, these results indicate that c-*fos* may play a role in keratinocyte proliferation and differentiation, but this role is not well understood. Thus, additional studies are required to clarify the role of all of the AP1 factors expressed in epidermis (*Fra*-1, *jun*B, *jun*D, c-*fos*, *fos*B, c-*jun*, etc. [52]) and to determine the role of each individual transcription factor in regulating keratinocyte proliferation and differentiation.

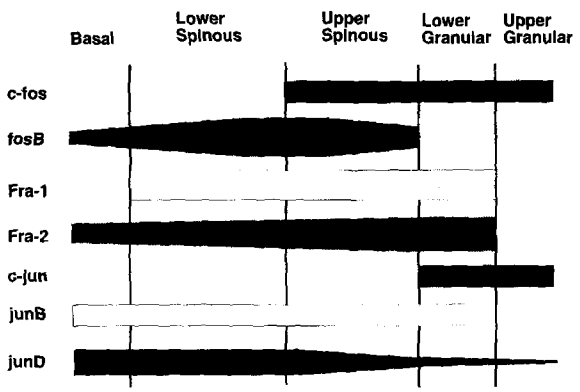

Figure 2. Localization of AP1 proteins in epidermis. The pattern of expression, abundance and cytoplasmic/nuclear distribution of the AP1 family members is shown as a function of the stage in keratinocyte differentiation. The width of each bar indicates the abundance level of each factor as measured by immunofluorescence intensity in sections stained with an antibody specific for each protein [52]. Because different antibodies are used, interprotein comparisons are not possible. Black indicates nuclear localization, white indicates cytoplasmic localization and grey indicates nuclear and cytoplasmic localization. Results are shown for c-*fos*, *fos*B, *Fra*-1, *Fra*-2, c-*jun*, *jun*B and *jun*D. Only *fos*B shows a change in cytoplasmic/nuclear localization during differentiation.

Recent studies suggest that different AP1 family members regulate expression of different genes. For example, *jun*B, *jun*D and *Fra*-1 are required for optimal expression of the human involucrin gene [59], while *Jun*B and *jun*D are important for papillomavirus promoter activation [64]. Additional unpublished results indicate that other subsets of *fos*/*jun* family members are important in the regulation of other epidermal genes. Although all of the known AP1 family members are expressed in epidermis [52], not all of them are likely to participate in the regulation of individual genes. The effects of different AP1 factors on gene expression probably depend upon 1) the exact DNA sequence flanking the AP1 site in the target gene [137], 2) which AP1 factors are expressed in each epidermal layer [52], whether the AP1 complex associates with other transcription factors [138], 4) the presence of cytosolic modulators of AP1 activity [122, 139], 5) the state of phosphorylation of the AP1 factors and 6) the subcellular distribution of the resident AP1 proteins [140, 141]. Phosphorylation is important to consider, since it is known to affect AP1 dimerization and transactivating activity [140, 141].

Because heterodimer composition may influence the activity of the AP1 complex, the localization of transcription factor expression in epidermis may be particularly important. Figure 2 shows the pattern of

AP1 protein expression in adult epidermis [52]. *Fra*-1, for example, is expressed in the immediate suprabasal layers at a time when involucrin expression is activated. Thus, Fra-1 could have a role in regulating involucrin expression [52, 139]. Other proteins, such as loricrin which are expressed later in differentiation, may be regulated by AP1 factors that are expressed later in differentiation.

Pathways that modulate AP1 activity. As outlined above, AP1 transcription factors have been implicated in the regulation of several keratinocyte genes [142, 143, 144, 145]. However, an understanding of the complete pathway that links stimulation and activation of gene expression has not been achieved for any of these.

AP1 transcription factors are activated via several pathways in various cell types. In some systems AP1 factors are activated by phosphorylation via a mitogen activated protein kinase (MAPK)-dependent pathway [142, 143, 144, 145]. Furthermore, MAP kinase-2 (MAPK-2) and other MAP kinases have been shown to form a complex with AP1 [146]. Ras (p21), a G protein-like signal transducing protein that is embedded in the cytoplasmic face of the plasma membrane, also appears to be an upstream signal leading to MAPK activation and subsequent AP1 factor activation in some cell types [147, 148]. In other systems, Raf-1, a cytoplasmic serine/threonine protein kinase, is directly activated by PKC with subsequent phosphorylation of MAPK and induction of c-*jun* [149].

Mitogen activated protein kinases (MAPK) phosphorylate c-*jun* at two N-terminal serine residues (ser_{62}, ser_{72}) that result in activation of c-*jun* transactivating activity [147]. In contrast, phosphorylation of three residues near the basic region of the DNA binding domain (residues 227–252) inhibits DNA binding and, in turn, inhibits transactivation [150]. Phosphorylation of the amino terminal serines and dephosphorylation of the inhibitory residues within residues 227–252 are necessary for full activation of c-*jun*. These events are simultaneously stimulated by TPA via a PKC-dependent mechanism in some systems [147, 150]. On the other hand, phosphorylation of the inhibitory residues, and the resulting decrease in c-*jun* activity, has been linked to the action of glycogen synthase kinase-3 [150]. In addition, phosphorylation appears to produce differing effects on different *jun* proteins. For example, in contrast to the results with c-*jun*, the trans-activation potential of *jun*B is not activated by amino terminal phosphorylation [151]. This suggests that AP1 factor activity is differentially regulated by phosphorylation [151].

Unfortunately, the phosphorylation and dephosphorylation of *fos* and *jun* proteins cannot account for the full complexity of the modulation of AP1 activity in keratinocytes. While it is known, for example, that phorbol esters increase interleukin-1-alpha (IL-1α) mRNA levels in keratinocytes by a mechanism that involves PKC activation upstream of AP1 activation, MAPK is not involved in this pathway [91]. In contrast, IL-1α autoregulation of its own mRNA utilizes a MAPK-dependent pathway upstream of AP1 activation [91].

Other mechanisms that modulate AP1 activity have been described in several systems. Thus, some non-AP1 family member proteins are known to modulate AP1 activity [122, 150]. For example, nuclear receptors have been shown to interact directly with the DNA binding domains of c-*fos* and c-*jun*, and thus to inhibit the formation of the AP1-DNA complex [153, 154]. In other systems, however, nuclear receptors have been shown to associate with *fos*/*jun* heterodimers and affect gene regulation, without influencing the binding of AP1 to DNA [155]. In addition, in several systems, cooperativity with factors binding to adjacent sites has been demonstrated, as has the formation of ternary complexes with other transcription factors [42, 156]. Furthermore, different AP1 heterodimers have been reported to differentially regulate gene expression [157, 158]. The sequences flanking the AP1 binding sites themselves may have an influence on the selectivity of the AP1 binding site for different dimers [137]. However, a further level of complexity is introduced by the fact that different heterodimers can successively occupy the same AP1 binding site, and can thus contribute to multiphasic patterns of gene regulation [159]. This finding is particularly intriguing in the context of epidermal differentiation as we have recently shown differentiation specific patterns of expression of the *jun* and *fos* family members in the epidermis (Figure 2) [52].

These results suggest several general conclusions. First, PKC activation is a signal for terminal differentiation in murine and human keratinocytes. Second, activator protein 1 is a frequent target of PKC activation. Third, AP1 activity appears to directly regulate the transcription of several keratinocyte genes. A challenge in the next few years will be to identify the signalling pathways that link PKC activation and gene response.

Other transcription factors that regulate keratinocyte function

AP2. Several lines of evidence suggest that activator protein-2, a 52 kD protein transcription factor that binds to the consensus sequence 5′-GCCNNNGGC-3′ (N is any nucleotide), has a role in the regulation of keratinocyte differentiation [160]. AP2 motifs are localized in the upstream regulatory regions of a number of keratinocyte genes and AP2 has been implicated in the regulation of keratinocyte genes [39, 40, 161]. Arsenate treatment of cells results in a dramatic suppression of tyrosine phosphorylation which is associated with a reduction in human involucrin promoter activity [161]. It is likely that the reduction in AP2-site mediated human involucrin promoter activity is due to reduced phosphorylation of AP2. Finally, AP2 is expressed in early development following a pattern similar to, but preceding cytokeratin expression [162].

POU homeodomain factors. These proteins are important developmental regulators in a variety of tissues [163]. Several POU factors have been localized in epidermis. These include Skn-1a and Skn-1i that are expressed in the suprabasal epidermal layers during mouse development and in the adult mouse [164]. Skn-1a is thought to be an activator of gene expression, as it activates expression of the human keratin 10 promoter [164], which is normally expressed in the suprabasal layers. Skn-1i a suppressor of gene activity [164]. In contrast, Oct-6, as measured by *in situ* hybridization, is expressed in all epidermal layers and inhibits the activity of the K5 and K17 keratin promoter/enhancers [165]. In addition, a variety of POU domain proteins inhibit expression of the human involucrin promoter [166], which is normally expressed in the epidermal suprabasal layers. Thus, evidence to date, indicates that POU domain proteins can inhibit or activate expression of suprabasal gene markers, and can activate expression of basal marker genes. Given the complexity of the expression pattern of these factors in epidermis, it will be interesting to see how the role of these proteins is eventually defined.

Hox-B6. Homeobox genes are thought to be involved in developmental regulation in a wide variety of organisms [167]. They are also expressed in the epidermis where they may function in regulating cell proliferation and/or differentiation. Hoxb-4, Hoxb-6, Hoxb-7 and Hoxa-10-B6, for example, are expressed in mouse epidermis, and the levels are elevated in hyperproliferative epidermis [168].

STAT proteins in epidermis. Signal transducer and activator of transcription (STAT) transcription factors are phosphorylated by members of the Jak family of kinases and mediate the effects of a variety of ligands [169]. The phosphorylated STAT proteins translocate to the nucleus where they regulate gene expression by binding to specific DNA response elements [169]. Epidermal contact hypersensitivity stimulates translocation of the STAT-91 factor to the keratinocyte nucleus. This translocation is associated with activation of cytokeratin K17 expression via binding to GAS (IFN-γ-activated-sequence) elements in the promoter [170]. Thus, STAT proteins provide a molecular link between immune system cells, which release cytokines that trigger STAT activation, and gene regulation in keratinocytes that may be important in inflammatory diseases.

Basonuclin. Basonuclin is a zinc finger protein that is localized in the nuclei of basal and near-basal layer cells of the epidermis [171, 172]. In the hair follicle, basonuclin is expressed in the outer root sheath. It has been suggested that basonuclin is a regulatory factor whose presence is linked to maintenance of proliferative potential [171, 172]. Although basonuclin has zinc fingers and is localized in the nucleus, no information is currently available that definitively identifies this factor as a regulator of gene expression.

Summary

The epidermis is a tissue that undergoes a very complex and tightly controlled differentiation program. The elaboration of this program is generally flawless, resulting in the production of an effective protective barrier for the organism. Many of the genes expressed during keratinocyte differentiation are expressed in a coordinate manner; this suggests that common regulatory models may emerge. The simplest model envisions a 'common regulatory element' that is possessed by all genes that are regulated together (e.g., involucrin and transglutaminase type 1). Studies to date, however, have not identified any such elements and, if anything, the available studies suggest that appropriate expression of each gene is achieved using sometime subtly and sometime grossly different mechanisms.

Recent studies indicate that a variety of transcription factors (AP1, AP2, POU domain, Sp1, STAT factors) are expressed in the epidermis and, in many

cases, multiple members of several families are present (e.g., AP1 and POU domain factors). The simultaneous expression of multiple members of a single transcription factor family provides numerous opportunities for complex regulation. Some studies suggest that specific members of these families interact with specific keratinocyte genes. The best studied of these families in epidermis is the AP1 family of factors. All of the known AP1 factors are expressed in epidermis [52] and each is expressed in a specific spatial pattern that suggests the potential to regulate multiple genes. It will be important to determine the role of each of these members in regulating keratinocyte gene expression.

Finally, information is beginning to emerge regarding signal transduction in keratinocytes. Some of the early events in signal transduction have been identified (e.g., PLC and PKC activation, etc.) and some of the molecular targets of these pathways (e.g., AP1 transcription factors) are beginning to be identified. Eventually we can expect to elucidation of all of the steps between the interaction of the stimulating agent with its receptor and the activation of target gene expression.

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