Mini-Review

Epidermal Differentiation: The Bare Essentials

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In the past ten years, there have been a number of major scientific discoveries in the field of epidermal differentiation. We owe many of these findings to the development of model tissue culture systems, and to technological advances in molecular biology. In this article, I will review some important aspects of the biology of terminal differentiation in vivo and in vitro, and highlight the recent advances in elucidating the molecular mechanism underlying these processes.

The Program of Terminal Differentiation

The epidermis is a stratified squamous epithelium that forms the protective covering of the skin. Only the innermost, basal layer of epidermal cells has the capacity for DNA synthesis and mitosis. Under an as yet unidentified trigger of terminal differentiation, a basal cell will begin its journey to the skin surface. In transit, it undergoes a series of morphological and biochemical changes that culminate in the production of dead, flattened, enucleated squames, which are sloughed from the surface, and continually replaced by inner cells differentiating outward.

The process of epidermal growth and differentiation has been subdivided into four parts. Basal cells are distinguished by an intracellular cytoskeleton composed of a relatively dispersed, but extensive, network of keratin filaments. These filaments are made of a 1:1 ratio of two distinct keratin proteins: K5 (58 kD) and K14 (50 kD) (Nelson and Sun, 1983). Additional hallmarks of basal cells, also common to spinous cells, are desmosomes, which are calcium-activated membrane junctions that interconnect the cells into a three-dimensional lattice (for review, see Franke et al., 1987). The four to eight layers of suprabasal spinous cells are postmitotic, but metabolically active. These cells devote much of their protein synthesizing machinery to manufacturing two new keratins, K1 (67 kD) and K10 (56.5 kD), forming cytoskeletal filaments that aggregate into thin (tonofilament) bundles (Eichner et al., 1986). In addition, spinous cells make glutamine and lysine-rich envelope proteins, such as involucrin, which are deposited on the inner surface of the plasma membrane of each cell (Rice and Green, 1979). Also made at this time are membrane-coating granules, which will subsequently fuse with the plasma membrane and release lipids into the intercellular spaces of granular and stratum corneum cells (Swartzendruber et al., 1989). As spinous cells reach the granular layer, they stop generating keratin and envelope proteins, and make their final tailoring in protein synthesis, including production of filaggrin, a histidine-rich, basic protein which may be involved in the bundling of tonofilaments into larger, macrofibrillar cables (Fleckman et al., 1985; Rothnagel and Steinert, 1990). This process of increased filament packing is thought to enable the keratin filaments to be among the few survivors of the massive destructive phase which soon ensues. Loricrin, a recently described and major component of the cornified envelope is also synthesized at this late stage (Mehrel et al., 1990). As each differentiating cell becomes permeable, a calcium influx activates epidermal transglutaminase, which then catalyzes the formation of ϵ-(γ-glutamyl) lysine isopeptide bonds, thereby biochemically cross-linking the envelope proteins into a cage (Rice and Green, 1979). As other lytic enzymes are released, all vestiges of metabolic activity terminate, and the resulting flattened squames are merely cellular skeletons, filled with macrofibrils of keratin filaments. The stratum corneum, composed of terminally differentiated keratinocytes sealed together by lipids, is an impermeable, insoluble, and highly protective fortress, which keeps microorganisms out and essential bodily fluids in.

Reproducing Epidermis In Vitro

Among the most significant contributions to epidermal cell growth in culture are the pioneering studies of Rheinwald and Green (for review, see Rheinwald, 1980), who realized that epidermal cells are not only dependent upon EGF, but also some as yet unidentified fibroblast factor. Thus, when seeded on a lawn of x-irradiated or mitomycin C-treated fibroblasts, and in medium containing the appropriate serum lot, cAMP-inducing agent, growth factors, and various additional nutrients, epidermal cells grow optimally for several hundred generations in culture. Despite the desirability of this system for generating rapidly growing keratinocytes, it is not optimal for differentiation, since only a few layers of cells form at confluence, and many of the biochemical changes characteristic of terminal differentiation, such as K1, K10 and filaggrin expression, do not occur (Fuchs and Green, 1980; Asselineau et al., 1986).

A number of laboratories have contributed to methods aimed at optimizing terminal differentiation in vitro, and from these studies, clues to extracellular controls of keratinization have been obtained. One of these factors is calcium, which is a necessary prerequisite for a variety of differentiative processes, including stratification, assembly of desmosomes, and activation of epidermal transglutaminase (Rice and Green, 1979; Hennings et al., 1980; Watt et al., 1984; Duden and Franke, 1988). Another important group of regu-

lators are retinoids, which diminish features of terminal differentiation in stratified squamous epithelia both in vivo (Fell and Mellanby, 1953) and in vitro (Yuspa and Harris, 1974; Fuchs and Green, 1981). When retinol is removed from serum, cultured epidermal cells display many of the morphological and biochemical characteristics of terminal differentiation (Fuchs and Green, 1981). These features include stratification, cell adhesiveness, reduced cell motility, and expression of K1 and K10.

While calcium and retinoids are clearly important, cultures grown on feeder layers in the presence of low concentrations of retinol and high concentrations of calcium produce basal keratinocytes and one to two layers of squames, but generate very few spinous and granular cells. A clue for retaining these earlier stages of epidermal differentiation in culture came from studies by researchers culturing epidermal cells on floating lattices of collagen at the air–liquid interface (see Asselineau et al., 1986 for previous references). In initial attempts, basal cells were often flattened rather than cuboidal, and no visible granular layer was present. Despite these shortcomings, four to five layers of spinous-like cells and four to five layers of cornified cells were consistently obtained with floating cell cultures. From these studies, it was clear that when epidermal cells are exposed to the air and receive their nutrients via diffusion through an artificial dermis, their differentiation is significantly enhanced (Asselineau et al., 1986).

We now know that a combination of optimal serum lots, calcium, retinoids, and other nutrients, coupled with culturing epidermal cells on a floating raft of collagen and fibroblasts at the air–liquid interface, results in a near optimal balance between growth and differentiation (Kopan et al., 1987; Choi and Fuchs, 1990, see Fig. 1 *A*). Tritiated thymidine labeling and examination of mitotic indices reveal a single layer of cuboidal-shaped basal cells, which express K5 and K14 (Kopan and Fuchs, 1989). As cells leave this layer, they synthesize spinous proteins, including K1 and involucrin (Asselineau et al., 1986; Kopan et al., 1987; see Fig. 1, *B* and *C*, respectively). Six to eight layers of K1/K10-synthesizing cells are produced before a filaggrin-expressing granular layer is reached (Fig. 1 *D*). Above the granular layer, stratum corneum-like layers are seen. Unlike epidermis in vivo, where dead squames are sloughed from the skin surface, the raft squames have nowhere to go and accumulate at the top of the tissue.

Molecular Controls of Epidermal Growth and Differentiation: Positive Growth Regulators

The search for an optimal culture system uncovered a number of extracellular regulators controlling the balance between growth and terminal differentiation. Stimulators of keratinocyte growth include EGF (Rheinwald, 1980), transforming growth factor α (TGF-α)[1] (Coffey et al., 1987; Barrandon and Green, 1987), low (10^{-7}–10^{-10} M) concentrations of retinoic acid (Fuchs and Green, 1981; Kopan and Fuchs, 1989), keratinocyte growth factor (Finch et al., 1989), and two cytokines, IL-6 (Grossman et al., 1989) and IL-1α (Ansel et al., 1988; Kupper et al., 1989). Of these, EGF and TGF-α have been the most extensively studied. The targets of both EGF and TGF-α are the tyrosine kinase activatable EGF receptors, located primarily on the surface of basal cells (Green et al., 1987). However, as implied by a number of experiments, these factors do not act simply as mitogens (Barrandon and Green, 1987). In fact, when keratinocyte colonies are small, cell growth is exponential and usually not markedly influenced by additional growth supplements. It is only when colonies become larger that supplemental factors play significant role in colony expansion. This acquired factor dependency appears to arise from the fact that colony growth occurs predominantly at the periphery, and hence larger colonies are dependent upon factors that increase cell migration. TGF-α (Barrandon and Green, 1987) and retinoids (Fuchs and Green, 1981) are especially potent in enhancing migration of keratinocytes, and it may be that the growth stimulatory effects of these factors are at least in part related to this ability.

TGF-α differs from EGF in that it is synthesized by keratinocytes both in vitro and in vivo (Coffey et al., 1987; Gottlieb et al., 1988; Elder et al., 1989). Since epidermal cells autoregulate their own growth via TGF-α production, it is perhaps not surprising to find that such control can go awry, leading to uncontrolled growth. Several reports have implicated an increase in EGF receptors with epidermal tumorigenesis (Yamamoto et al., 1986; Ozanne et al., 1986). In addition, it was recently reported that psoriatic epidermis contained higher levels of TGF-α than normal skin, suggesting that overexpression of TGF-α might contribute to hyperproliferative diseases of the skin (Gottlieb et al., 1988; Elder et al., 1989). This hypothesis is an attractive one, although it remains to be shown whether the increase in TGF-α in psoriasis is causal or consequential. Thus, while there are later steps in the keratinocyte growth cycle that can be altered thereby leading to epidermal hyperproliferation and malignant transformation (for examples, see Roop et al., 1986; Bailleul et al., 1990; Greenhalgh et al., 1990; Wilson et al., 1990), TGF-α seems to be involved in the initial events controlling the proper balance between growth and differentiation.

Negative Growth Regulators

Withdrawal from the cell cycle seems to be a prerequisite for irreversible commitment of a keratinocyte to terminally differentiate. In recent years, several noncytotoxic, autocrine inhibitors of keratinocyte growth have been uncovered. While epidermal chalones (see, for example, Richter et al., 1990) will undoubtedly receive more attention in the future, the most extensively studied negative regulators are the TGF-βs, which act at picomolar concentrations to inhibit DNA synthesis and cell division (Shipley et al., 1986; Kopan et al., 1987; Bascom et al., 1989). The effect of TGF-βs on growth is reversible, at least within a 48-h frame, and is not accompanied by gross changes in protein or RNA synthesis (Bascom et al., 1989). While TGF-βs inhibit growth of basal cells, their natural expression in epidermis seems to be predominantly in suprabasal, differentiating layers. Indeed during mammalian development, expression of TGF-β2 mRNA (Pelton et al., 1989) and a related Vgr-1 mRNA (Lyons et al., 1989) coincides with epidermal stratification and keratinization. An interesting parallel occurs in *Drosophila*, where a TGF-β-like gene called decapentaplegic is expressed dor-

1. *Abbreviations used in this paper*: RA, retinoic acid; RAR, retinoic acid receptor; TGF, transforming growth factor.

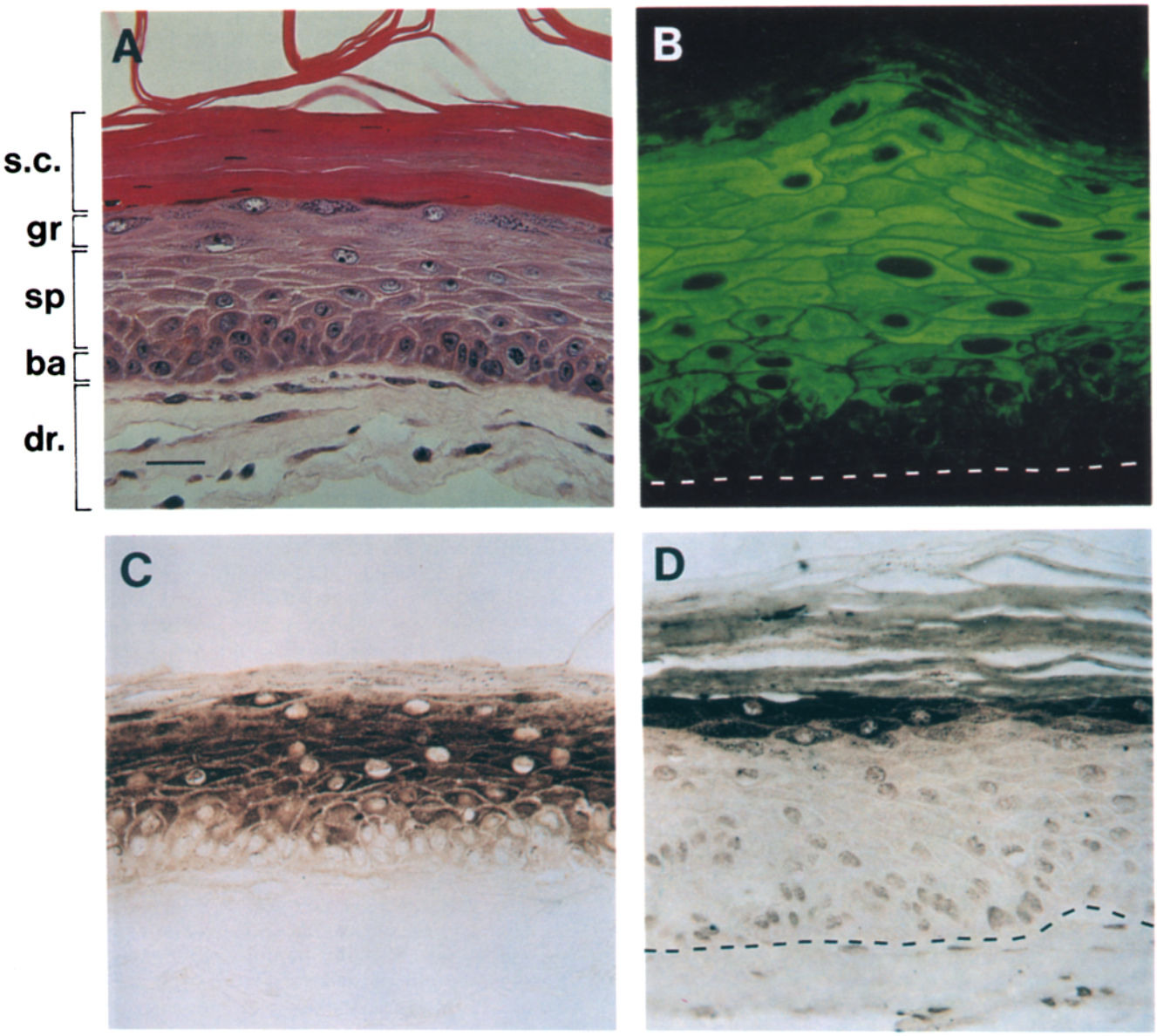

Figure 1. Morphology and expression of biochemical markers of differentiation by cultured human epidermal cells. Human epidermal cells from newborn foreskins were isolated and cultured according to Rheinwald (1980). Cells were transferred to a dish containing a lattice of collagen and fibroblasts, and at confluence, the epidermal cells and the lattice were raised to the air-liquid interface, and cultured for an additional three weeks (Asselineau et al., 1986; Kopan et al., 1987). The "tissue" was then fixed in Carnoy's solution, embedded in paraffin, and sectioned (5 μm). (*A*) Section stained with hematoxylin and eosin (Choi and Fuchs, 1990); (*B*) section treated with anti-human K1 antisera and subjected to indirect immunofluorescence (Kopan et al., 1987); (*C*) section treated with anti-human involucrin and subjected to indirect immunogold enhancement (Choi and Fuchs, 1990); (*D*) section treated with anti-human filaggrin and subjected to indirect immunogold enhancement (Choi and Fuchs, 1990). *s.c.*, stratum corneum; *gr*, granular layer; *sp*, spinous layers; *ba*, basal layer; *dr*, dermis. Bar: (*A*, *C*, and *D*) 27.5 μm; (*B*) 15.5 μm.

sally in early embryos and localized later in the imaginal disks, which will form much of the adult epidermis of the fly (Padgett et al., 1987). In adult mammalian epidermis both in vivo (Thompson et al., 1989) and in vitro (Shipley et al., 1986; Van Obberghen-Schilling et al., 1988; Glick et al., 1989, 1990; Bascom et al., 1989; Pelton et al., 1989), TGF-β1 and TGF-β2 mRNAs are low, but detectable in the differentiating layers. In adult epidermis, TGF-β mRNAs can be upregulated (*a*) in an autoregulatory fashion (Van Obberghen-Schilling et al., 1988; Bascom et al., 1989), and (*b*) when mouse skin is treated with tumor-promoting agent (TPA) (Akhurst et al., 1988) or calcium (Glick et al., 1990), agents known to induce epidermal keratinization. Curiously, TPA also induces TGF-α expression, presumably in basal epidermal cells (Pittlekow et al., 1989), a phenomenon that may explain the seemingly antagonistic effects of TPA, enhancing growth and inducing differentiation in epidermis. Collectively, the timing and location of TGF-β mRNA expression suggests that members of the TGF-β family may be involved in maintaining the cessation of growth in the differentiating cells of epidermis.

Elucidating the functional significance of TGF-β expres-

sion has been hampered by the fact that TGF-βs are produced and secreted by cells in a latent form, which must then be activated before interaction with TGF-β receptors on the cell surface (Lyons et al., 1988; Miyazono et al., 1988). Mere elevation of TGF-β mRNA expression is therefore not automatically an indication that active TGF-βs are being produced. An example of this is the production of latent TGF-β1 by human keratinocytes cultured under serum-free conditions at neutral pH (Bascom et al., 1989). Thus, even though the correlation between suprabasal TGF-β mRNA expression and cessation of growth in keratinizing cells is compelling, a causal relationship has not yet been unequivocally demonstrated, nor is it clear to what extent the expression of TGF-β mRNAs in vivo is an accurate reflection of active TGF-β secretion.

Because the effects of TGF-βs on basal cell growth appear to be largely reversible, it has been assumed that TGF-βs alone are not sufficient to induce terminal differentiation, a process thought to be irreversible. In support of this notion were early in vitro studies, showing that the biochemical indicators of terminal differentiation were not induced upon treatment of keratinocytes cultured on plastic with TGF-βs (Kopan et al., 1987; Bascom et al., 1989). More recent studies with differentiating culture systems have revealed that at greatly elevated levels, TGF-βs can influence biochemical markers of keratinization, but at these high levels, they inhibit rather than promote, K1, K10, and filaggrin expression (Mansbridge and Hanawalt, 1988; Choi and Fuchs, 1990). At high concentration, TGF-βs also enhance expression of K6 (56 kD) and K16 (48 kD), keratins more commonly associated with suprabasal layers of epidermis undergoing (*a*) wound healing (Mansbridge and Knapp, 1987) and (*b*) hyperproliferation (Weiss et al., 1984; Stoler et al., 1988). Studies using floating epidermal cultures have shown that induction of K6 and K16 is accompanied by morphological changes typical of squamous cell carcinomas, including increased stratification, vacuolization, and coilocyte formation (Choi and Fuchs, 1990). Interestingly, TGF-βs seem to elicit these changes in keratinizing epidermal cells at least in part independently of their action on basal cells. Collectively, these studies have led to the notion that the epidermal phenotype associated with wound healing and many hyperproliferative diseases may not relate to hyperproliferation, per se, but rather is a reflection of environmental changes, which, in some circumstances, may include active TGF-βs (Mansbridge and Hanawalt, 1988; Kopan and Fuchs, 1989; Schermer et al., 1989; Choi and Fuchs, 1990).

A priori, the apparent reversibility of TGF-β-mediated growth inhibition may seem ironic in light of more recent findings that (*a*) TGF-β mRNA expression is largely confined to suprabasal, terminally differentiating cells, and (*b*) TGF-βs can upregulate their own expression (Van Obberghen-Schilling et al., 1988; Bascom et al., 1989). However, the effects of TGF-βs at various stages of epidermal differentiation seem to be quite different (Choi and Fuchs, 1990), and hence it may be relevant that the reversibility studies were conducted on dividing cells of the population, while TGF-β mRNA synthesis and autoregulation seem to take place in nondividing cells. In addition, it seems increasingly apparent that while cessation of cell growth by TGF-βs may be necessary, it is not sufficient for commitment to terminal differentiation. In a model where a cascade of biochemical changes is necessary to enter and maintain the differentiation state, TGF-β expression may be among the early steps in the pathway. Interestingly, one step after TGF-βs in this putative cascade may be downregulation of c-*myc* expression, which occurs in response to TGF-βs and is abrogated by viral transforming proteins (Pietenpol et al., 1990). As the receptors of TGF-βs are identified and localized, and as further studies are conducted, the molecular mechanisms underlying the effects of TGF-βs on epidermal growth and differentiation should become more apparent.

Retinoids

While TGF-βs seem to accentuate abnormal and inhibit normal differentiation, retinoids appear to inhibit both forms of epidermal differentiation (Kopan and Fuchs, 1989). Thus, for example, in organ culture of chick epidermis, vitamin A can induce its transition from a keratinizing to a secretory epithelium (mucous metaplasia) (Fell and Mellanby, 1953). Excess retinoids in mammalian epidermal cultures can suppress differentiative features including K1/K10 expression (Fuchs and Green, 1981), K6/K16 expression (Kopan and Fuchs, 1989), cornified envelope production (Yuspa and Harris, 1974), and filaggrin expression (Fleckman et al., 1985). Many of these effects are at least at the level of mRNA expression (Fuchs and Green, 1981; Kopan et al., 1987; Kopan and Fuchs, 1989), and quite likely at the transcriptional level as well (Agarwal et al., 1990; Blumenberg et al., 1990).

Recently, it was shown that 10^{-6} M retinoic acid inhibited proliferation and concomitantly induced active TGF-β2 in mouse keratinocytes (Glick et al., 1989). Conversely, when exposed to 10^{-7}–10^{-6} M retinoic acid, human keratinocytes showed an increase in proliferation and cell migration, with no detectable TGF-β2 induction (Choi and Fuchs, 1990). Whether these differences are a reflection of species-specific variations in dose response to retinoids awaits further investigation. Nevertheless, the discovery of an autocrine regulatory loop between retinoids and TGF-βs in some keratinocytes under some conditions is exciting, and suggests that both factors may be involved in some common regulatory pathways.

Determining the mechanism of action of retinoids on epidermal differentiation has been hampered by the fact that there are a number of intracellular regulators of retinoids. Among the first to be identified were cellular retinol binding protein (CRBP) and cellular retinoic acid binding protein (CRABP), barrel-shaped proteins initially thought to mediate the steroid hormone-like effects of retinoids on epidermal gene expression (Chytil and Ong, 1983). With the recent discovery of retinoic acid receptors (RARs) with sequence homologies to classical steroid receptors (see Krust et al., 1989; Mangelsdorf et al., 1990), it now seems more likely that CRBP and CRABP play a role in storage or transport of retinoids, rather than in mediating the steroid hormone-like effects of retinoids.

RAR-α and RAR-γ are expressed in human epidermis (Rees and Redfern, 1989; Kim, M., and E. Fuchs, unpublished data; Noji et al., 1989), and of these, RAR-γ seems to be unique to skin (Krust et al., 1989). In situ hybridization studies have shown that RAR mRNAs are most abundant in the keratinizing layers of epidermis (Noji et al., 1989). No

analogous retinol receptors have been detected, and it therefore seems likely that the effects of retinol are mediated via intracellular conversion to retinoic acid and subsequent interaction with RARs. That retinoic acid (RA)-induced suprabasal RARs mediate biochemical changes in epidermal differentiation has yet to be unequivocally demonstrated. Assuming that this hypothesis is correct, the suprabasal location of RARs would explain why RA acts at least in part to inhibit suprabasal functions, and why culturing cells at an air–liquid interface enables suprabasal cells to differentiate via escape from the retinol-containing serum.

It is intriguing to speculate that RARs and retinoic acid may act directly to negatively control transcription of a number of differentiation-specific epidermal genes. Indeed, Blumenberg et al. (1990) have recently reported preliminary studies suggesting that expression of a reporter gene driven by the 5′ upstream sequences of several keratin genes is inhibited by retinoids, and that this inhibition is enhanced by cotransfection with RAR genes. Clearly, in coming years, this topic will be one of great interest. Coupled with the fact that retinoids can influence both cell proliferation and migration in basal cells as well as gene expression in terminally differentiating cells, the effects of retinoids on epidermal cells appear to be pleiotropic. Hence it will be important to elucidate whether the regulatory mechanisms involving retinoids change with relative stage of keratinocyte differentiation, or whether a single biochemical pathway is used. Are RARα and RARγ differentially expressed in epidermis? Are their mechanisms of action different? Do both transcriptional and posttranscriptional controls play a role in RA-mediated changes in keratinocyte protein expression? Are retinoids involved in the gate that controls commitment of an epidermal cell to undergo terminal differentiation? As future studies are conducted, the answers to these questions should become more apparent.

Calcium

When cultured in medium containing 0.05 mM calcium, mouse keratinocytes grow as a monolayer, because desmosome assembly is inhibited (Hennings et al., 1980). Upon a switch to high (1.2 mM) calcium, desmosomes form and cells stratify. After three to seven additional days in culture, squames containing cornified envelopes appear in the medium. These data indicate that at least some features of terminal differentiation can be induced by calcium in vitro. Calcium also seems to play a role in mediating keratinization in vivo: certain calcium ionophores have recently been shown to enhance action of TPA in promoting epidermal differentiation (Jaken and Yuspa, 1988).

While calcium is necessary for certain features of terminal differentiation (e.g., desmosome formation, stratification and transglutaminase activation) in human keratinocytes, its effects often seem more pronounced in regulating differentiation-specific changes in cellular architecture, than alterations in gene expression. Thus for example, when human cells are cultured in low calcium medium, withdrawal from the cell cycle and involucrin synthesis still occur (Watt, 1984). Similarly, while desmosomes cannot assemble in low calcium, desmosomal proteins are nevertheless synthesized in both low and high calcium medium (Watt et al., 1984; Duden and Franke, 1988). Finally, for human keratinocytes, high calcium medium does not induce appreciable levels of K1/K10 and filaggrin expression (Fuchs and Green, 1981; Fleckman et al., 1985). Mouse keratinocytes seem to differ from human cells in their sensitivity to calcium. Recently, it was discovered that substantial K1/K10 expression in mouse epidermal cells can be induced by a narrow window of calcium concentrations (Roop et al., 1987; Yuspa et al., 1989). In addition, Glick et al. (1990) showed that calcium induces a marked increase in TGFβ2 mRNA expression in mouse keratinocytes, thereby implicating calcium in both early and late stages of terminal differentiation. Hence, like other known regulators of epidermal differentiation, calcium seems to have pleiotropic effects, and furthermore, it differs from other known regulators in having a distinct positive influence on many aspects of terminal differentiation. While additional experiments will be necessary to elucidate the precise relation between calcium, TGF-βs and retinoids, it seems clear that calcium is intimately involved in the complex regulatory mechanisms underlying the balance between growth and keratinization.

Terminal Differentiation: Active or Passive?

Among the most fundamental of unresolved questions is when does an epidermal cell undergo a commitment to terminally differentiate? Is the commitment reached before a cell leaves the basal layer, or does it occur after a cell has left this layer? If commitment takes place in the basal layer, then the population of basal keratinocytes should be heterogeneous. Certainly, as judged by their cell cycling properties, this appears to be the case (Barrandon and Green, 1987; Potten and Morris, 1988). By this criterion, there seem to be at least two populations of basal cells. One population, stem cells, have a long lifespan, a long cycling phase and short S period (for review, see Potten and Morris, 1988). The second population, transit amplifying cells or paraclones, undergo only a limited number of divisions before terminally differentiating (Barrandon and Green, 1987). Moreover, paraclones seem to arise from stem cells, suggesting further that a sequence of events leading to terminal differentiation may take place before movement of a cell out of the basal layer (Barrandon and Green, 1987).

A priori, it might seem attractive to visualize terminal differentiation as a passive event, where an occupied basal layer provides the force to push a cell to the first suprabasal layer, and thereafter, its absence of contact with the basement membrane might trigger the cascade of events (including cell cycle withdrawal) leading to keratinization. While this notion seems plausible, this simple model does not account for why there are two to three layers of cells with biochemical and morphological characteristics of basal cells in squamous cell carcinoma cells cultured on floating lattices of collagen and fibroblasts (Stoler et al., 1988; Kopan and Fuchs, 1989). Moreover, it does not explain why certain features of epidermal differentiation can be induced in basal cells before their exit from the innermost layer (Watt, 1984; Roop et al., 1987; Choi and Fuchs, 1990). Thus, it seems more likely that cessation of cell division can be uncoupled from loss of basement membrane contact and is key to when a cell shifts to a program of gene expression leading to terminal differentiation. However, as indicated previously, cell cycle withdrawal does not seem to be sufficient for an irreversible commitment to terminal differentiation, and it could be that additional

critical events occur as cells leave the basement membrane. Further studies will be necessary to distinguish between these possibilities.

In summary, we are left with a picture whereby a keratinocyte becomes a terminally differentiating cell as a consequence of a series of biochemical checks and balances. There seems to be several early changes necessary for entry into the differentiation pathway, and among these are likely to be TGF-βs and other agents that slow or inhibit DNA synthesis and cell division. In normal epidermis, movement of a cell from its basement membrane may contribute to, but does not seem sufficient for, instigating a cascade of biochemical changes that culminate in irreversibly sealing the differentiative fate of a keratinocyte. Once a basal cell has left the innermost layer and its biochemical program has begun to change, it seems to be further influenced by retinoids, TGF-βs and calcium primarily in a fashion that appears to allow retailoring of the architecture of the keratinizing layers above. Further investigation will be necessary to determine whether this capacity to redesign the suprabasal program of differentiation affects basal as well as suprabasal cells.

Molecular Controls of Epidermal Gene Expression: Master Regulators of Keratinocyte Fate?

There is compelling evidence from other differentiation systems that expression of a few transcriptional regulatory genes are central to the manifestation and maintenance of the differentiative state (Blau et al., 1983; Lassar et al., 1986; Herr et al., 1988; Mangalam et al., 1989; Kemler and Schaffner, 1990). Perhaps the best understood master regulators of differentiation are the family of Myo D proteins, transcription factors that control expression of a large number of skeletal muscle genes (Davis et al., 1990). These proteins are expressed during early embryonic development at the time of myoblast differentiation, and their levels are elevated in skeletal muscle (Sassoon et al., 1989). Expression of Myo D in certain fibroblast cell lines in vitro appears to be sufficient to convert them into myoblasts (Lassar et al., 1986). While the Myo D family plays a central role in myoblast differentiation, regulation of these regulators involves a complex program of transcriptional and posttranslational controls, and moreover, there may be more to muscle differentiation than Myo D and its family (see for example Schafer et al., 1990). Hence for muscle, at least one family of central regulators controlling the balance of growth and differentiation are transcription factors, which are controlled by a dynamic group of intracellular and extracellular regulators.

Based on muscle and other differentiation systems, it seems that a knowledge of the major transcription factors controlling keratinocyte-specific gene expression will be of central importance in the quest for elucidating the molecular mechanisms underlying epidermal differentiation. While little is presently known about keratinocyte transcription factors, there are a number of keratinocyte genes that have already been isolated and characterized, and which serve as a foundation for pursuing these factors. These include genes encoding: (*a*) the basal keratins K5 (Lersch et al., 1989) and K14 (Marchuk et al., 1984); (*b*) the suprabasal keratins K1 (Johnson et al., 1985), K10 (Krieg et al., 1985; Rieger et al., 1985; Rieger and Franke, 1988), K6 (Tyner et al., 1985), and K16 (Rosenberg et al., 1988); (*c*) the cornified envelope protein, involucrin (Eckert and Green, 1986); and (*d*) filaggrin (Rothnagel and Steinert, 1990). Sequence comparisons have provided a few clues as to possible common regulatory elements involved in controlling keratinocyte-specific gene expression. One of these is the CK 8-mer sequence 5′ A A N C C A A A 3′, found upstream from a number of epidermal genes (Blessing et al., 1987; Cripe et al., 1987). Blessing et al. (1989) showed that a ~90-bp fragment containing a TATA box and a CK 8-mer sequence provided a 4× enhancement of expression of a CAT reporter gene, but Chin et al. (1989) were unable to demonstrate function for the CK 8-mer sequence in the E6/E7 promoter of a human papillomavirus genome. Hence, if a CK 8-mer-like sequence is involved in keratinocyte gene expression, it may act in concert either in multiple copies or with other regulatory elements in addition to a TATA box.

Recently, we identified a different sequence, 5′ G C C T G C A G G C 3′ located 5′ from the TATA box of the human K14 gene, that appears to act in conjunction with a distal element to control its transcription in keratinocytes (Leask et al., 1990). The sequence is also the target for the binding of an AP2-like factor, KER1, which is especially abundant in keratinocytes, and present in lower amounts in simple epithelial cells. While we do not yet know the extent to which KER1 regulates the expression of other keratinocyte-specific genes, it is intriguing to note that at least one gene, encoding the human differentiation-specific keratin K1, contains a sequence which competes for the binding of KER1 to the K14 gene (Leask et al., 1990). In addition, if KER1 is AP2, then AP2 may be generally used in controlling keratinocyte-specific expression, since classical AP2 sites exist in other keratinocyte genes, including the gene encoding K5, i.e., the partner to K14.

That KER1 might be important in controlling keratinocyte-specific gene expression is underscored by recent studies on the promoter of a keratin gene, XK81A1, expressed in the outer ectoderm after midblastula transition during *Xenopus* development (Snape et al., 1990). By using methylation interference and footprint analyses, Snape et al. (1990) have identified an imperfect palindrome 5′ A C C C T G A G G C T 3′ that binds a nuclear factor, KTF-1, enriched in embryonic ectodermal fractions. While preliminary studies have not revealed competition of the *Xenopus* sequence for binding of KER1 to the human K14 gene, nevertheless, the similarities between the sequences and KER1/KTF-1 binding properties suggest that mechanisms for epidermal gene expression in vertebrates might be distantly related (Leask et al., 1990). Interestingly, temporal expression of the *Xenopus* XK81A1 (Snape et al., 1990) and the human K14 gene (Kopan and Fuchs, 1989) coincide with commitment of a cell to keratinocyte development, suggesting the interesting possibility that one of the factors controlling the expression of these genes could be a candidate for a master regulator of keratinocyte gene expression.

While there is simply not enough known about the molecular mechanisms that act to regulate the expression of keratinocyte-specific genes, it seems likely that this knowledge will be essential to elucidating the missing steps involved in controlling keratinocyte fate and commitment to terminal differentiation. In the coming years, a major focus in the field of epidermal differentiation will be on keratinocyte-specific transcription factors as possible mediators of extracellular regulators and keratinocyte fate.

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