Ligands and Activators of Nuclear Hormone Receptors Regulate Epidermal Differentiation During Fetal Rat Skin Development

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Because a protective barrier is essential for life, the development of the epidermis and stratum corneum must be completed prior to birth. The epidermal permeability barrier is comprised of corneocytes embedded in a lipid enriched matrix. Recent studies from our laboratory, using an explant model of fetal rat skin development that closely parallels *in utero* development, have shown that hormones and other activators of members of the nuclear receptor family regulate permeability barrier ontogenesis by stimulating lipid metabolism and the formation of the extracellular lipid lamellae. Using this model we sought to determine whether these hormones and nuclear activators also regulate keratinocyte differentiation during fetal development. Profilaggrin/filaggrin and loricrin expression, assessed by *in situ* hybridization and by immunohistochemistry, were progressively increased during epidermal ontogenesis. Whereas profilaggrin/filaggrin and loricrin were not expressed at day 17 of gestation, by day 19 both were present in the upper layers of the epidermis and both became still more abundant by day 21. These developmental changes also occurred in fetal skin explants cultured *in vitro* for 4 d, although the expression levels did not appear as robust as *in utero*. Whereas neither profilaggrin/filaggrin nor loricrin were expressed in control explants cultured for 2 d, they were seen in explants treated with either thyroid hormone, glucocorticoids, or estrogens. In contrast, dihydrotestosterone treatment delayed the expression of profilaggrin/filaggrin and loricrin. Moreover, both clofibrate, a peroxisome proliferator-activated receptor-α ligand, and juvenile hormone III, a farnesoid X-activated receptor activator, markedly accelerated fetal epidermal differentiation, stimulating both profilaggrin/filaggrin and loricrin expression. Our results demonstrate that several hormones and activators of nuclear hormone receptors regulate epidermal differentiation during fetal development, affecting key constituents of both keratohyalin granules and the cornified envelope. Thus, a variety of ligands/activators of nuclear receptors accelerate not only permeability barrier ontogenesis, but also the expression of structural proteins essential for stratum corneum formation. ***Key words: dihydrotestosterone/estrogen/filaggrin/FXR/glucocorticoids/loricrin/PPARα/thyroid hormone. J Invest Dermatol 111:429–433, 1998***

The vectorial differentiation of keratinocytes in the epidermis is a multistep process that culminates in cornification and in the establishment of the permeability barrier to water transit (Fuchs, 1990; Jackson *et al*, 1993; Eckert *et al*, 1997). Cornification is characterized by the extensive cross-linking of loricrin and other structural proteins to form the cornified envelope (Fuchs, 1990; Mehrel *et al*, 1990; Steven *et al*, 1990; Hohl and Roop, 1993; Jackson *et al*, 1993; Eckert *et al*, 1997). The immediate precursors of corneocytes are the terminally differentiating keratinocytes of the stratum granulosum. These cells are characterized by the presence of keratohyalin granules, containing abundant quantities of profilaggrin and numerous epidermal lamellar bodies (Dale *et al*, 1985, 1993; Haydock and Dale, 1990; Elias and Menon, 1991). Following the secretion of the lipid and enzyme contents of lamellar bodies, a series of structural transformations leads to the formation of mature lamellar unit membranes in the interstices of the stratum corneum, resulting in the formation of the cutaneous permeability barrier (Jackson *et al*, 1993; Elias and Menon, 1991).

Because a competent barrier is essential for survival in a terrestrial environment, the development of the epidermis and stratum corneum is completed prior to term. Previous studies from our laboratory showed that a competent permeability barrier appears between days 20 and 21 of gestation (term = 22 d) in rats (Aszterbaum *et al*, 1992). Moreover, in an explant model of fetal rat skin development, which closely parallels *in utero* development, we showed that hormones and other ligands of members of the nuclear hormone receptor family regulate permeability barrier ontogenesis (Aszterbaum *et al*, 1993; Hanley *et al*, 1996a, b, 1997b, c). Specifically, thyroid hormone, glucocorticoids, and estrogen accelerate, whereas dihydrotestosterone delays permeability barrier formation (Hanley *et al*, 1996a, b). Furthermore, very recent studies demonstrated that activators of both peroxisome proliferator-activated receptor (PPAR)-α and farnesoid X-activated receptor (FXR) also accelerate permeability barrier develop-

Manuscript received January 5, 1998; revised April 17, 1998; accepted for publication April 29, 1998.

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Abbreviations: FXR, farnesoid X-activated receptor; PPAR, peroxisome proliferator-activated receptor.

ment in fetal rat skin explants (Hanley *et al*, 1997b). PPARα and FXR are nuclear hormone receptors that form heterodimers with RXR (Forman *et al*, 1995; Mangelsdorf *et al*, 1995). PPARα is activated by fatty acids and various drugs, such as clofibrate, and regulates numerous aspects of lipid metabolism (Schoonjans *et al*, 1996). FXR is activated by farnesol that is formed from intermediates in the cholesterol biosynthetic pathway, and by juvenile hormone III (Forman *et al*, 1995). The genes regulated by FXR have not yet been defined.

Although these studies demonstrate that the ontogenesis of the permeability barrier is regulated by hormones and PPARα and FXR activators, their importance in the regulation of the expression of structural proteins remains unknown. Recently, Bickenbach *et al* (1995) showed a parallel expression of loricrin with permeability barrier formation during fetal epidermal development in the mouse. These findings suggest that both ontogenesis of the extracellular lipid matrix of the stratum corneum, and cornified envelope formation may occur simultaneously. Yet, in intrauterine growth-retarded fetal rats the expression of cornification markers is decreased (Hoat *et al*, 1990), whereas the formation of the permeability barrier proceeds normally (Williams *et al*, 1993). Additionally, in mice who overexpress an retinoic acid receptor dominant-negative mutant in the suprabasal epidermis, keratinocyte cornification proceeds normally, but the formation of the extracellular lipid lamellae that comprise the permeability barrier is impaired (Imakado *et al*, 1995). Thus, these observations suggest that expression of structural proteins and permeability barrier formation may be regulated independently.

In this study we determined whether developmental events of keratinocyte differentiation are coordinately regulated with the establishment of the permeability barrier. We studied the effect of hormones (thyroid hormone, glucocorticoids, estrogen, and dihydrotestosterone) and activators of PPARα (clofibrate) and FXR (juvenile hormone III) on the expression of filaggrin and loricrin. Our results demonstrate that these hormones and nuclear receptor activators regulate not only permeability barrier formation, but also the expression of key structural proteins of epidermal differentiation.

MATERIAL AND METHODS

Organ culture Timed pregnant Sprague-Dawley rats (plug date = day 0) were obtained from Simonsen Laboratories (Gilroy, CA). A total of nine litters were used in this study. To establish the sequence of epidermal differentiation during *in utero* development, fetal skin was collected from fetuses on days 17, 19, and 21 of pregnancy. Tissue samples from at least five animals were used for each data point. For *in vitro* studies, full-thickness flank skin of 17 d old embryos was excised and incubated dermal side down on collagen membrane inserts (Transwell-COL, Costar, Cambridge, MA) for 2–4 d, in serum- and growth factor-free M199 tissue culture media, as described previously (Hanley *et al*, 1996a, b, 1997b). At the beginning of the culture dexamethasone (10 nM final concentration), thyroid hormone (10 nM final concentration), diethylstilbestrol (100 nM final concentration), dihydrotestosterone (100 nM final concentration), clofibrate (p-chlorophenoxyisobutyric acid, 300 μM final concentration), or juvenile hormone III (250 μM final concentration), all purchased from Sigma (St. Louis, MO), were added to the culture medium. Clofibrate was added to the medium bound to 0.5% bovine serum albumin. Dexamethasone, diethylstilbestrol, and dihydrotestosterone were dissolved in ethanol. Thyroid hormone and juvenile hormone III were dissolved in dimethylsulfoxide. Control explants were incubated in the presence of the appropriate vehicle.

The tissue samples were fixed in 4% formaldehyde (freshly prepared from paraformaldehyde) in phosphate-buffered saline for 24 h and were embedded in paraffin. Sections were collected on positively charged microscope slides (SuperfrostPlus, Fisher Scientific, Pittsburgh, PA) and stained with hematoxylin and eosin or used for *in situ* hybridization or immunohistochemistry. Photomicrographs were taken with a Microphot FX (Nikon) microscope, using a 20× planapochromate objective.

***In situ* hybridization** Digoxigenin-labeled RNA probes to detect profilaggrin and loricrin mRNA were made from linearized cDNA sequences as templates (Haydock and Dale, 1990; Mehrel *et al*, 1990), using reagent supplied by Boehringer (Indianapolis, IN). The hybridization of the DIG-labeled probes (Jowett, 1997) was detected by an anti-DIG antibody, conjugated to alkaline phosphatase (Boehringer). The activity of the alkaline phosphatase was revealed with BCIP/NBT substrate. A set of controls was used to establish the specificity of the hybridization. Probes of sense orientation served as controls to ensure the specificity of hybridization. Omitting the DIG-labeled probes resulted in no signal, indicating that only DIG-containing RNA hybrids were detected. The sense control loricrin probe resulted in no signal, indicating the specificity of hybridization with the anti-sense probe (not shown). Anti-sense profilaggrin probe resulted in staining not only in the upper epidermal layers, as expected, but in a variable staining of the basal cells at days 17 and 18, and in weak staining of dermis. The use of the sense control profilaggrin probe, however, showed that whereas the staining of the upper epidermis was due to specific RNA–RNA hybrid formation, the signal seen in the basal layer and in the dermis was a result of nonspecific interaction of the anti-sense probe with unrelated endogenous mRNA.

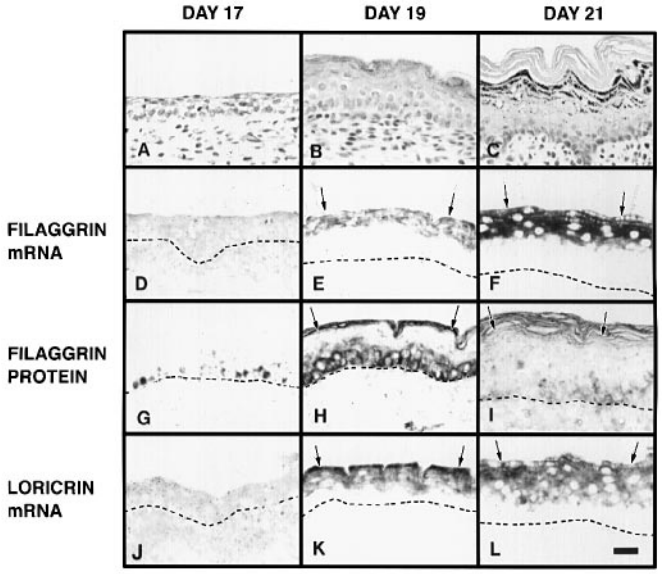

Figure 1. Profilaggrin/filaggrin and loricrin expression increases during *in utero* development of fetal rats. Hematoxylin and eosin stained sections of the epidermis at day 17 (*A*), day 19 (*B*), and day 21 (*C*). Epidermal expression of profilaggrin (*D*, *E*, *F*) and loricrin (*J*, *K*, *L*) mRNA was detected by *in situ* hybridization at day 17 (*D*, *J*), day 19 (*E*, *K*), and day 21 (*F*, *L*). Immunohistochemical localization of profilaggrin/filaggrin protein at day 17 (*G*), day 19 (*H*), and day 21 (*I*). The dotted line demarks the basal lamina of the epidermis. The *arrows* indicate the stratum granulosum/stratum corneum interface. Notice that whereas the mRNA are not detectable in the stratum corneum, profilaggrin/filaggrin protein is present both in the upper stratum granulosum and in the stratum corneum. *Scale bar*: 50 μm.

Immunohistochemistry A rabbit anti-peptide antibody specific for mouse loricrin (BabCo, Berkeley, CA) was found to cross-react with rat loricrin and was therefore used to detect loricrin. A rabbit antibody (gift from Dr. B. Dale), specific for rat profilaggrin and filaggrin, was used to localize profilaggrin and filaggrin. The binding of the first antibodies was detected by biotinylated anti-rabbit IgG, followed ABC reagent (Dako, Carpinteria, CA). Peroxidase activity was revealed with DAB as substrate. Omitting the first antibodies resulted in no signal, indicating the specificity of the immunodetection.

RESULTS

Characterization of profilaggrin/filaggrin and loricrin expression during *in utero* development and in fetal skin explants As described previously (Hanley *et al*, 1996a), the epidermis displayed dramatic changes during the last 4 d of gestation (from day 17 until day 21). Whereas on day 17 of gestation, the fetal epidermis consisted of only 2–3 cell layers, covered by a flattened layer of periderm (**Fig 1*A***), on day 19 the epidermis had more cell layers with the cells containing keratohyalin granules in the upper layers (**Fig 1*B***). A thin layer of stratum corneum was also present. On day 21 of gestation the epidermis was fully developed, with a mature basal, spinous, and granular layer, and the stratum corneum consisted of multiple layers of corneocytes (**Fig 1*C***). *In situ* hybridization showed that these morphologic changes were accompanied by changes in expression of profilaggrin and loricrin mRNA. Whereas expression of profilaggrin (**Fig 1*D***) and loricrin (**Fig 1*J***) mRNA was not observed at day 17 of gestation, by day 19 mRNA for both were present in the upper layers of the

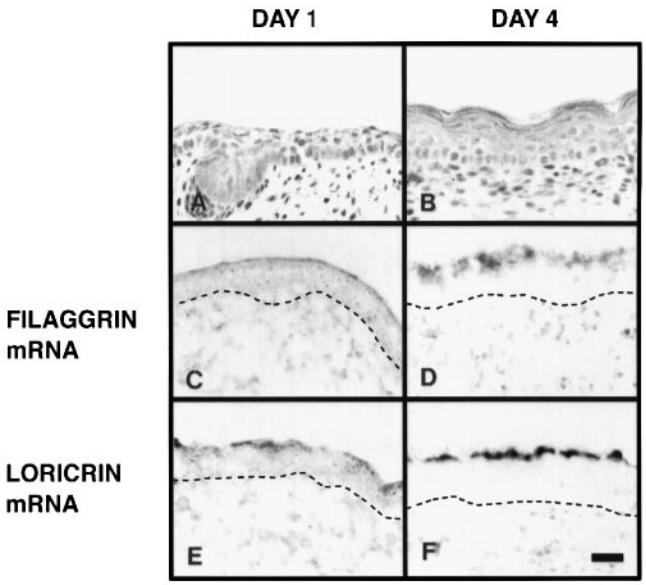

Figure 2. Development of fetal skin explants parallels *in utero* epidermal differentiation. Hematoxylin and eosin stained sections of the epidermis, collected on day 17 of *in utero* development, after 1 d (*A*) or 4 d (*B*) in culture. Expression of profilaggrin (*C*, *D*) and loricrin (*E*, *F*) mRNA was detected by *in situ* hybridization in explants cultured for 1 d (*C*, *E*) or 4 d (*D*, *F*). The dotted line demarks the basal lamina of the epidermis. *Scale bar*: 50 μm.

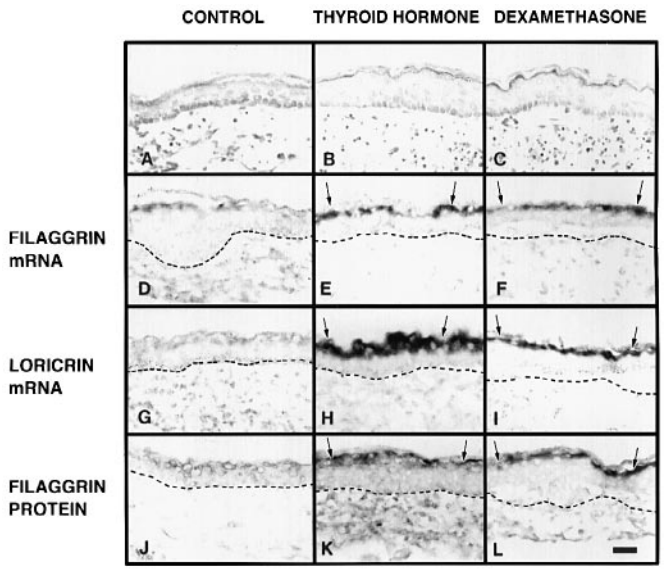

Figure 3. Thyroid hormone and glucocorticoids accelerate epidermal differentiation *in vitro*. Explants collected on day 17 of *in utero* development were cultured for 2 d with vehicle (control, left column) or in the presence of thyroid hormone (middle column) or dexamethasone (right column). Morphologic changes are shown in hematoxylin and eosin stained sections of control (*A*), thyroid hormone treated (*B*), or dexamethasone treated (*C*) explants. Expression of profilaggrin (*D*, *E*, *F*) and loricrin (*G*, *H*, *I*) mRNA was detected by *in situ* hybridization after 2 d in culture in control explants (*D*, *G*), or in the presence of thyroid hormone (*E*, *H*) or dexamethasone (*F*, *I*). The presence of profilaggrin/filaggrin proteins was detected by immunohistochemistry in control explants (*J*), or in thyroid hormone (*K*) or dexamethasone (*L*) treated explants. The dotted line demarks the basal lamina of the epidermis. The *arrows* indicate the stratum granulosum/stratum corneum interface. Notice that whereas the mRNA are not detectable in the stratum corneum, profilaggrin/filaggrin proteins are present both in the upper stratum granulosum and in the stratum corneum. *Scale bar*: 50 μm.

epidermis (**Figs 1*E, K***). Moreover, at day 21 of gestation mRNA for profilaggrin (**Fig 1*F***) and loricrin (**Fig 1*L***) became even more abundant. Not only did the number of cell layers expressing these messages increase, but also individual cells showed a greater staining intensity than earlier in gestation. The cells of the stratum corneum, however, did not contain profilaggrin or loricrin mRNA (**Figs 1*F, L***). Similar changes in the expression of profilaggrin/filaggrin and loricrin proteins were observed by immunohistochemical detection of these proteins with specific antibodies. Whereas at day 17 of gestation no detectable protein was observed (**Fig 1*G***), by day 19 the upper layers of the epidermis contained both profilaggrin/filaggrin (**Fig 1*H***) and loricrin, and by day 21 there was increased staining (**Fig 1*I***) in both granular cells and the stratum corneum (loricrin data not shown).

We next determined the time of appearance of these differentiation markers in our fetal skin explant model. After 1 or 2 d in culture (chronologic age 17 + 1 or 17 + 2 d), no detectable levels of expression of profilaggrin/filaggrin or loricrin were observed by either *in situ* hybridization or immunohistochemistry (**Figs 2*C, E***). By 4 d in culture (chronologic age 17 + 4 d), however, the epidermis developed further, with the appearance of both granular keratinocytes and a multilayered stratum corneum (**Fig 2*B***), as described previously (Hanley *et al*, 1996a, b). The suprabasal keratinocytes in the explants cultured for 4 d (chronologic age 17 + 4 d) expressed substantial amounts of profilaggrin and loricrin mRNA (**Figs 2*D, F***) as well as profilaggrin/filaggrin and loricrin proteins, although signal intensity was not as pronounced as *in utero* at 21 d of gestation.

Thyroid hormone and glucocorticoids accelerate profilaggrin/filaggrin and loricrin expression in fetal skin explants As described above, fetal skin explants after 2 d in culture expressed no detectable amounts of profilaggrin/filaggrin or loricrin; however, the addition of either thyroid hormone or glucocorticoids to the medium for 2 d resulted in the development of a stratified epidermis, with a prominent granular layer and a thin layer of corneocytes (**Figs 3*B, C***), as described previously (Hanley *et al*, 1996a). Moreover, profilaggrin and loricrin mRNA appeared in explants following either thyroid hormone (**Figs 3*D, G versus E, H***) or glucocorticoid (**Figs 3*D, G versus F, I***) treatment. Furthermore, the immunostaining for both profilaggrin/filaggrin (**Fig 3*J versus K, L***) and loricrin (not shown) increased with either thyroid hormone or glucocorticoid treatment. Yet, both of these hormones appeared to modulate loricrin expression more than profilaggrin/filaggrin expression (**Fig 3*E versus H*, and *F versus I***), and thyroid hormone, at these doses, appeared to have a more robust effect than glucocorticoid treatment (**Fig 3*E, H versus F, I***). Thus, in addition to stimulating permeability barrier development, both thyroid hormone and glucocorticoids stimulate the expression of structural proteins required for stratum corneum formation.

Estrogen accelerates, whereas dihydrotestosterone delays profilaggrin/filaggrin and loricrin expression in fetal skin explants We next studied the effect of the sex steroid hormones, estrogen and dihydrotestosterone, on epidermal keratinocyte differentiation in the *in vitro* fetal skin explant model. As described previously (Hanley *et al*, 1996b), *in vitro* estrogen treatment for 2 d (chronologic age 17 + 2 d) accelerated the appearance of a multilayered epidermis with granular keratinocytes and a thin layer of corneocytes (**Fig 4*B***). Profilaggrin and loricrin mRNA were readily seen in the outer epidermis of estrogen-treated explants (**Figs 4*E, H***). Furthermore, immunostaining for both profilaggrin/filaggrin and loricrin protein was also increased following estrogen treatment (not shown). In contrast, dihydrotestosterone treatment delayed development of the stratum corneum, even after 4 d (chronologic age 17 + 4 d) in culture (**Fig 4*C***). Following dihydrotestosterone treatment for 2 d (chronologic age 17 + 2 d), neither the mRNA nor the proteins for profilaggrin/filaggrin and loricrin were detectable (not shown). Although explants treated with dihydrotestosterone for 4 d (chronologic age 17 + 4 d) showed some signs of stratum corneum development, as well as profilaggrin and loricrin expression, the staining intensity was weaker than in control explants cultured for 4 d (**Figs 4*C, F, I***). Together, these observations indicate that both profilaggrin/filaggrin and loricrin expression are stimulated by estrogen treatment, whereas dihydrotestosterone delays expression of these structural proteins.

PPARα or FXR activators accelerate profilaggrin/filaggrin and loricrin expression in fetal skin explants As reported previously

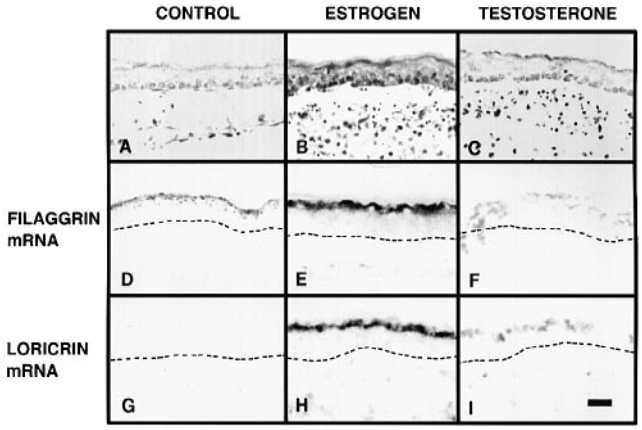

Figure 4. Estrogens accelerate whereas dihydrotestosterone delays epidermal differentiation *in vitro*. Explants collected on day 17 of *in utero* development were cultured for 2 d with vehicle (control, left column) or in the presence of estrogen (diethylstilbestrol; middle column), or for 4 d in the presence of dihydrotestosterone (right column). Morphologic changes are shown on hematoxylin and eosin stained sections of control (*A*), estrogen treated (*B*), or dihydrotestosterone treated (*C*) explants. Expression of profilaggrin (*D*, *E*, *F*) and loricrin (*G*, *H*, *I*) mRNA was detected by *in situ* hybridization in control explants (*D*, *G*), or in the presence of diethylstilbestrol (*E*, *H*) or dihydrotestosterone (*F*, *I*). The dotted line demarks the basal lamina of the epidermis. *Scale bar*: 50 μm.

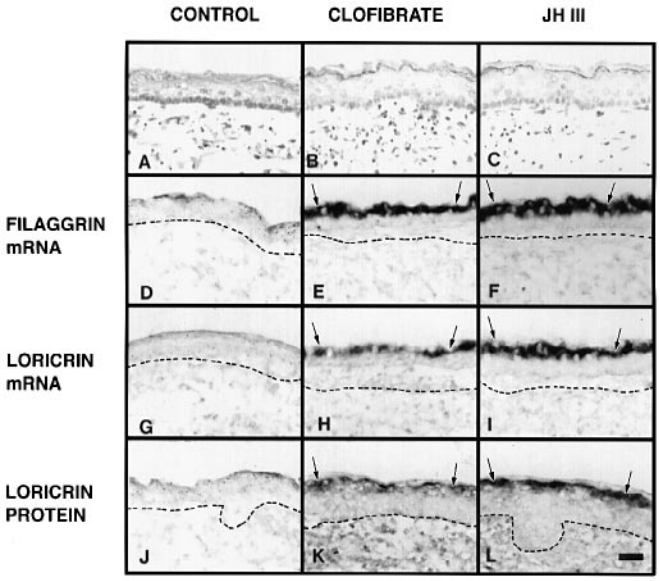

Figure 5. Activators of PPARα (clofibrate) and FXR (juvenile hormone III) accelerate epidermal differentiation *in vitro*. Explants collected on day 17 of *in utero* development were cultured for 2 d in the presence of vehicle (control, left column), clofibrate (middle column), or juvenile hormone III (right column). Morphologic changes in the epidermis, after 2 d in culture (control, *A*), or in the presence of clofibrate (*B*) or juvenile hormone III (*C*), are shown in hematoxylin and eosin stained sections. Expression of profilaggrin (*D*, *E*, *F*) and loricrin (*G*, *H*, *I*) was detected by *in situ* hybridization in control, untreated (*D*, *G*), and clofibrate (*E*, *H*) or juvenile hormone III (*F*, *I*) treated explants after 2 d in culture. Immunohistochemical localization of loricrin protein in control (*J*), and clofibrate (*K*) or juvenile hormone III (*L*) treated explants after 2 d in culture. The dotted line demarks the basal lamina of the epidermis. The *arrows* indicate the stratum granulosum/stratum corneum interface. Notice that whereas the mRNA are not detectable in the stratum corneum, loricrin protein is present both in the upper stratum granulosum and in the stratum corneum. *Scale bar*: 50 μm.

(Hanley *et al*, 1997b), addition of clofibrate, a PPARα activator, or juvenile hormone III, a FXR activator, to fetal skin explants for 2 d (chronologic age 17 + 2 d) markedly accelerated fetal epidermal development (**Fig 5*B*, *C***). Again, intense staining for both profilaggrin (**Fig 5*E*, *F***) and loricrin (**Fig 5*H*, *I***) mRNA occurred following 2 d treatment with either clofibrate (**Fig 5*E*, *H***) or juvenile hormone III (**Fig 5*F*, *I***). Furthermore, profilaggrin/filaggrin and loricrin protein expression increased in comparison with the control explants (**Fig 5*J*, *K*, and *L***). Clofibrate appeared to exert a more pronounced effect on profilaggrin mRNA expression than on loricrin expression (**Fig 5*E* *versus* *H***), whereas juvenile hormone III displayed an equally strong effect on both profilaggrin and loricrin expression (**Figs 5*F*, *I***). Finally, at the doses employed, both PPARα or FXR activators exerted a stronger effect on profilaggrin/filaggrin and loricrin expression than any of the hormones assessed in this study. Thus, activators of PPARα or FXR accelerate not only fetal permeability barrier ontogenesis (Hanley *et al*, 1997b), but also fetal epidermal development.

DISCUSSION

Adult mammalian epidermis is a stratified squamous epithelium, with a basal layer of proliferating cells surmounted by several layers of progressively differentiating, postmitotic suprabasal cells. The differentiation of keratinocytes in the epidermis is a complex process involving the sequential expression of structural proteins (Fuchs, 1990; Eckert *et al*, 1997). This study and those published previously by us (Aszterbaum *et al*, 1992, 1993; Hanley *et al*, 1996a, b, 1997b, c) and others (Bickenbach *et al*, 1995) suggest that during normal fetal development the formation of keratohyalin granules, cornified envelope, and the extracellular lamellar lipid membranes of the stratum corneum occur in parallel, suggesting coordinated regulation of these processes. In intrauterine growth-retarded fetal rats, however, expression of cornification markers is decreased and the formation of the permeability barrier proceeds normally (Hoath *et al*, 1990; Williams *et al*, 1993). In contrast, in mice that express an retinoic acid receptor dominant negative mutant in the suprabasal epidermis, cornification proceeds normally, but formation of the lamellar membranes is impaired (Imakado *et al*, 1995). These observations suggest that discordant regulation of epidermal keratinocyte differentiation could occur under certain circumstances.

In this study we demonstrate that several ligands/activators of nuclear hormone receptors (Isseman and Green, 1990; Forman *et al*, 1995; Mangelsdorf *et al*, 1995), which we previously showed to regulate permeability barrier formation, also regulate the expression of key cytoplasmic constituents of the corneocyte and cornified envelope synthesis. Specifically, in previous studies we demonstrated that glucocorticoids, thyroid hormone, estrogens, PPARα activators, and FXR activators all accelerate formation of the permeability barrier as measured by decreases in transepidermal water loss, the assembly of mature extracellular lamellar membranes in the stratum corneum, and increased activity of enzymes required for normal lipid processing (Hanley *et al*, 1996a, b, 1997b, c). In this study, we demonstrate that these same hormones and nuclear receptor activators also accelerate the expression of profilaggrin, a major protein constituent of keratohyalin granules, and its processing to filaggrin, and the expression of loricrin, a key structural protein of the cornified envelope. In our *in vitro* fetal skin explant model, the addition of these hormones and activators resulted in increased expression of both profilaggrin and loricrin mRNA and profilaggrin/filaggrin and loricrin protein, measured by *in situ* hybridization and by immunohistochemistry. Moreover, dihydrotestosterone, which in previous studies delayed the formation of the permeability barrier (Hanley *et al*, 1996b), here also delayed the expression of profilaggrin and loricrin. Our results demonstrate that these hormones and activators of nuclear hormone receptors (Isseman and Green, 1990; Forman *et al*, 1995; Mangelsdorf *et al*, 1995) coordinately regulate keratinocyte differentiation. This observation suggests that these nuclear hormone receptor ligands and activators, which are required for normal epidermal differentiation (Imakado *et al*, 1995; Saitou *et al*, 1995; Feng *et al*, 1997), probably affect early, fundamental processes. Whether these nuclear receptors directly increase profilaggrin and loricrin mRNA expression or act via stimulating other transcription factors remains to be determined.

We have demonstrated that a number of different hormones and nuclear receptor activators can stimulate epidermal differentiation

during fetal development. The physiologic role of each of these hormones and activators in regulating stratum corneum development and whether they do so independently or by effecting each other remains to be determined. In recent studies using animals deficient in specific hormones we have begun to address the importance of these hormones in fetal stratum corneum development. In Hyt/Hyt mice, which are hypothyroid due to a mutation in the TSH receptor (Beamer *et al*, 1981; Stein *et al*, 1991), stratum corneum formation is delayed but by birth the stratum corneum is normal (Hanley *et al*, 1997a). Similarly, in corticotropin releasing factor deficient mice, who are glucocorticoid deficient, stratum corneum formation is also delayed but again by birth the stratum corneum is normal (unpublished observations). This suggests that both thyroid hormone and glucocorticoids play an important role in stratum corneum ontogenesis but that neither hormone is absolutely essential, i.e., a normal stratum corneum will ultimately form, but develops at a slower rate. Interestingly, treatment of hypothyroid mice with glucocorticoids did not result in the usual stimulation of stratum corneum formation, suggesting that glucocorticoids may regulate stratum corneum development by modulating thyroid hormone response or that glucocorticoids require the euthyroid state in order to be effective (Hanley *et al*, 1997a). Further studies delineating the physiologic importance of these hormones and their interactions in regulating stratum corneum development are required.

In summary, this study demonstrates that hormones (glucocorticoids, thyroid hormone, and estrogen), PPARα activators (clofibrate), and FXR activators (juvenile hormone III) not only accelerate permeability barrier formation during fetal development but also accelerate profilaggrin/filaggrin and loricrin expression, indicating that these nuclear hormone receptor ligands and activators coordinately regulate keratohyalin granule, cornified envelope, and stratum corneum extracellular lamellar membrane ontogenesis.

We thank Dr. B. Dale (University of Washington), Dr. D. Roop (Baylor College of Medicine), and Dr. S. Yuspa (NIH) for gifts of plasmids and antibodies, used in this study. This work is supported by National Institutes of Health grants HD29707, PO39448, AR39639, and by a research grant from the Society of Pediatric Dermatology.

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