Permeability Barrier Disruption Coordinately Regulates mRNA Levels for Key Enzymes of Cholesterol, Fatty Acid, and Ceramide Synthesis in the Epidermis

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The extracellular lipids of the stratum corneum, which are comprised mainly of cholesterol, fatty acids, and ceramides, are essential for epidermal permeability barrier function. Moreover, disruption of the permeability barrier results in an increased cholesterol, fatty acid, and ceramide synthesis in the underlying epidermis. This increase in lipid synthesis has been shown previously to be due to increased activities of HMG-CoA reductase, acetyl-CoA carboxylase, fatty acid synthase and serine palmitoyl transferase, key enzymes of cholesterol, fatty acid, and ceramide synthesis, respectively. In the present study, we determined whether the mRNA levels for the key enzymes required for synthesis of these three classes of lipids increase coordinately during barrier recovery. By northern blotting, the steady-state mRNA levels for HMG-CoA reductase, HMG-CoA synthase, farnesyl pyrophosphate synthase, and squalene synthase, key enzymes for cholesterol synthesis, all increased significantly after barrier disruption by either acetone or tape stripping. Additionally, the steady-state mRNA levels of acetyl-CoA carboxylase and fatty acid synthase, required for fatty acid synthesis, as well as serine palmitoyl transferase, the rate-limiting enzyme of de novo ceramide synthesis, also increased. Furthermore, artificial restoration of the permeability barrier by occlusion after barrier disruption prevented the increase in mRNA levels for all of these enzymes, except farnesyl pyrophosphate synthase, indicating a specific link of the increase in mRNA levels to barrier requirements. The parallel increase in epidermal mRNA levels for the enzymes required for cholesterol, fatty acid, and ceramide synthesis may be due to one or more transcription factors that regulate lipid requirements for permeability barrier function in keratinocytes.
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The extracellular lipids of the stratum corneum, within which the lipid-depleted corneocytes are embedded, are essential for a competent permeability barrier (Grubauer *et al*, 1989). These lipids differ from the mainly polar membrane lipids of the nucleated cell layers in that they consist mainly of cholesterol, free fatty acids, and ceramides (Lampe *et al*, 1983a, b). Acute disruption of the barrier can be achieved by several treatments which remove the lipid, including solvents such as acetone and detergents such as sodium dodecyl sulfate (SDS), or by repeated tape stripping (Menon *et al*, 1985a; Proksch *et al*, 1990).

Barrier disruption induces metabolic changes in the underlying epidermis that result in the rapid return of lipid to the stratum corneum interstices leading to barrier recovery. These changes include stimulation of epidermal cholesterol, fatty acid, and ceramide synthesis (Menon *et al*, 1985a; Grubauer *et al*, 1987; Holleran *et al*, 1991a). Most importantly, inhibition of either cholesterol, fatty acid, or ceramide synthesis results in abnormal permeability barrier homeostasis indicating the key role these lipid synthetic pathways have in barrier homeostasis (Feingold *et al*, 1990; Holleran *et al*, 1991a, b; Mao-Qiang *et al*, 1993). The increase in cholesterol synthesis is attributed to an increase in the activity of 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase, an important enzyme for cholesterol synthesis, explicable both by an increase in the proportion of enzyme that is activated by dephosphorylation (Proksch *et al*, 1990) and by an increase in the steady-state mRNA levels of HMG-CoA reductase (Jackson *et al*, 1992), resulting in an increase in enzyme mass (Jackson *et al*, 1992). Likewise, the increase in fatty acid synthesis is attributed to increased activities of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), key enzymes of fatty acid synthesis (Ottey *et al*, 1995). Finally, the increase in ceramide synthesis is associated with increased serine palmitoyl transferase (SPT) activity, the rate-limiting enzyme of de novo ceramide synthesis (Holleran *et al*, 1991a). Whether barrier disruption stimulates ACC, FAS, and SPT activity by increasing mRNA levels is not known. Neither is it known whether mRNA levels for other key enzymes in the cholesterol synthetic pathway are stimulated.

Many of the proteins important for cholesterol metabolism, such as the low-density lipoprotein receptor, HMG-CoA reductase, HMG-CoA synthase, farnesyl pyrophosphate synthetase (FPPS), and squalene synthase, are regulated coordinately (Goldstein and Brown, 1990). This coordinate regulation has been shown to be due to transcription factors called sterol regulatory element binding proteins (SREBP) (Yokoyama *et al*, 1993; Shimano *et al*, 1996). The regulation of fatty acid synthesis proteins, ACC, and FAS has recently also been shown to be regulated

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Abbreviations: ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; FPPS, farnesyl pyrophosphate synthase; HMG-CoA, 3-hydroxy-3-methylglutaryl-Coenzyme A; SPT, serine palmitoyl transferase; SREBP, sterol regulatory element binding protein; TEWL, transepidermal water loss.

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by SREBP under some conditions (Bennett *et al*, 1995; Lopez *et al*, 1996); however, our laboratory and others have shown that several physiologic and pathophysiologic conditions can result in the discordant regulation of genes involved in cholesterol metabolism (Feingold *et al*, 1995). For example, following lipopolysaccharide or cytokine administration (TNFα, IL-1, TNFα + IL-1), HMG-CoA reductase mRNA levels in the liver of Syrian hamsters increase more than 10-fold without a significant increase in mRNA levels for the low-density lipoprotein receptor, HMG-CoA synthase, or FPPS (Feingold *et al*, 1995).

The purpose of this study was to determine (i) whether there is a coordinate increase in the epidermal mRNA levels of enzymes, other than HMG-CoA reductase, involved in cholesterol synthesis following acute barrier disruption, and (ii) whether the increase in activities of ACC, FAS, and SPT are attributed to increases in the mRNA levels for these enzymes.

MATERIALS AND METHODS

Animals and reagents Hairless male mice (Crl:SKHI-hrBR) were purchased from Charles River (Wilmington, MA). Molecular Biology grade chemicals were purchased from Sigma (St. Louis, MO) and Fischer (Fairlawn, NJ). [α-^{32}P] dCTP (3000 Ci per mmol, 10 mCi per ml) was purchased from New England Nuclear (Boston, MA). The Multiprime Labelling System was purchased from Amersham (Amersham, U.K.). Mini-spin columns (G-50) were purchased from Worthington (Freehold, NJ). Oligo(dT)-cellulose, type 77F, was purchased from Pharmacia (Uppsala, Sweden). Nytran Plus membrane was purchased from Schleicher and Schuell (Keene, NH). Spin-X centrifuge filters were purchased from Corning Costar (Cambridge, MA). cDNA for HMG-CoA reductase (pH Red-102, ATCC no. 57042) was purchased from the American Type Tissue Culture Collection (Rockville, MD). cDNA for rat HMG-CoA synthase (LA IIA) (Rosser *et al*, 1989) and FPPS (CR39) (Ashby and Edwards, 1989) were kindly provided by Dr. P.A. Edwards (University of California Los Angeles). cDNA for rat squalene synthase (Jiang *et al*, 1993) was kindly provided by Dr. I. Shechter (Uniformed Services, University of Health Services, Bethesda, DC). cDNA for rat ACC (Lopez-Casillas *et al*, 1989) was kindly provided by Dr. K-H. Kim (Purdue University, Lafayette, IN). cDNA for rat FAS was kindly provided by Drs C.M. Amy and S. Smith (Oakland Childrens Hospital, CA). cDNA for mouse SPT (LCB2) (Nagiec *et al*, 1996) was kindly provided by Dr. R.C. Dickson (University of Kentucky, KY). cDNA for rat cyclophilin cDNA (pCD15:8–1) was kindly provided by Dr. G. Strewler (Harvard Medical School, Boston, MA). Fuji RX film was used for autoradiography (Fischer Scientific, Fairlawn, NJ).

Acute barrier disruption Following Nembutal anesthetic the torso skin of 6–8-wk-old male hairless mice was treated by gently applying acetone-soaked cotton balls for 5–10 min as described in previous publications (Menon *et al*, 1985a) or by applying and removing cellophane tape (Tesa Tuck, New Rochelle, NY) 3–5 times successively. Controls for acetone perturbation of the barrier were treated with cotton balls soaked in 0.9% (wt/vol) sodium chloride. Untreated animals served as controls for tape strip barrier perturbation. The transepidermal water loss (TEWL) was measured immediately after treatment using a Meeco electrolytic water analyzer, as described previously (Menon *et al*, 1985a; Holleran *et al*, 1991a). Animals with a TEWL rate greater than 6 mg per cm^2 per h (normal $<$ 0.3 mg per cm^2 per h) after barrier disruption were included in this study. Occlusion of tape stripped mice was achieved by immediately inserting the mouse into a thumb of a powderless Latex glove as described previously (Grubauer *et al*, 1989).

Isolation of the epidermis Four h following barrier disruption, the animals were killed by Isoflurane anesthesia (Abbot, Chicago, IL) and the skin excised. The subcutaneous fat was removed by scraping with a scalpel blade and the skin was then placed in 10 mM ethylenediamine tetraacetic acid in calcium and magnesium free phosphate-buffered solution pH 7.4 for 35 min at 37°C (Jackson *et al*, 1992). The skin was blotted dry and the epidermis was removed by scraping with a scalpel blade. The epidermis was then snap frozen in liquid nitrogen and stored below –70°C.

Isolation of epidermal mRNA and northern blotting Total RNA was prepared by a variation of the guanidinium thiocyanate method (Chomczynski and Sacchi, 1987). Briefly, epidermis (0.2–0.4 g from the entire skin of two mice) was homogenized in 4 ml guanidinium thiocyanate solution (4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5%, wt/vol, N-lauroylsarcosine, 0.1 M 2-mercaptoethanol) using a ground glass hand-held homogenizer and sonicated twice for 50 s at 80% duty cycle output 2 (Sonifier cell disrupter, VWR, San Francisco, CA) standing on ice between bursts. Total RNA was purified and added to oligo(dT)-cellulose to obtain poly (A)$^+$ RNA. Quantitation was determined by measuring the absorbance at 260 nm. Equal amounts, 4–10 μg, poly (A)$^+$ RNA were applied to 1% (wt/vol) agarose-6% (vol/vol) formaldehyde gels and electrophoresed. The poly (A)$^+$ RNA was transferred electrophoretically onto Nytran plus membrane and fixed by baking. Hybridization was carried out overnight at 65°C in 0.75 M sodium chloride, 75 mM sodium citrate, 2% (wt/vol) SDS, 10% (wt/vol) Dextran sulfate, 2×Denhart's solution, and 100 mg per ml sheared salmon sperm DNA. Membranes were washed in 30 mM sodium chloride, 3 mM sodium citrate, 0.1% (wt/vol) SDS at 65°C for 30 min. Membranes were exposed to x-ray film at –70°C with intensifying screens. Quantitation of film, exposed in the linear range of sensitivity, was achieved using a Biorad (Hercules, CA) densitometer. The densitometry quantitation was adjusted for cyclophilin levels of the same sample determined on the same blot. Data in each experiment are expressed as fold of control, where the control equals one.

Statistics Statistical significance was determined using a two-tailed unpaired Student's t test, a one-tailed unpaired Student's t test was used where indicated. Results are expressed as mean ± SEM.

RESULTS

Effect of tape stripping on lipid synthetic enzyme mRNA levels To determine whether the increase in cholesterol synthesis after barrier disruption is due to an increase in only HMG-CoA reductase or to a coordinate increase in several enzymes in cholesterol synthetic pathway, we measured mRNA levels of HMG-CoA reductase, HMG-CoA synthase, FPPS, and squalene synthase. Four h after tape stripping there was a significant increase in the mRNA for all of these genes, indicating coordinate upregulation of cholesterol synthetic enzymes following barrier disruption (**Fig 1**).

Previous studies had demonstrated an increase in ACC, FAS, and SPT activity following barrier disruption. We next determined whether the increase in ACC, FAS, and SPT activities after tape stripping is accompanied by an increase in the levels of their respective mRNA. The mRNA levels of the enzymes of fatty acid synthesis, ACC, and FAS increased significantly, by 178% and 220%, respectively (**Fig 2**). Likewise, the steady-state mRNA levels of the rate-limiting enzyme of de novo ceramide synthesis, SPT (LCB2, 2.3 kb transcript), increased by 315% (**Fig 3**). Thus, barrier disruption by tape stripping results in a parallel increase in the steady-state mRNA levels for several key enzymes of cholesterol, fatty acid, and ceramide synthesis.

Effect of acetone treatment on epidermal lipid synthesis enzyme mRNA levels To determine whether the increase in mRNA levels is a specific response to barrier disruption, rather than a response to tape stripping, we next used the organic solvent, acetone, to disrupt the permeability barrier. When compared with tape stripping, acetone treatment resulted in a virtually identical increase in the mRNA levels for all of the key enzymes assessed (**Fig 4**). These results show that the increase in the mRNA levels is not unique for the method of barrier disruption, but occurs regardless of the method used for barrier disruption.

Effect of occlusion on mRNA levels following tape stripping To determine whether the changes in mRNA levels are due to barrier disruption rather than injury, we next provided an artificial barrier by Latex occlusion immediately following barrier perturbation. In previous studies, occlusion prevented the increase in lipid synthesis and the increases in activities of the lipid synthetic enzymes. As shown in **Fig 5**, occlusion also diminished the increase in mRNA levels for HMG-CoA reductase, HMG-CoA synthase, and squalene synthase. In contrast, FPPS mRNA levels were not effected by occlusion (**Fig 5**). The increased mRNA levels for key enzymes for fatty acid and ceramide synthesis also were reduced by occlusion (**Fig 5**). These results suggest that, with the exception of FPPS, the increase in mRNA levels that occurs following barrier disruption is due to barrier perturbation per se.

DISCUSSION

Previous studies have demonstrated that the extracellular lipids of the stratum corneum are essential for a competent epidermal permeability barrier (Grubauer *et al*, 1987; Feingold *et al*, 1990). Following acute

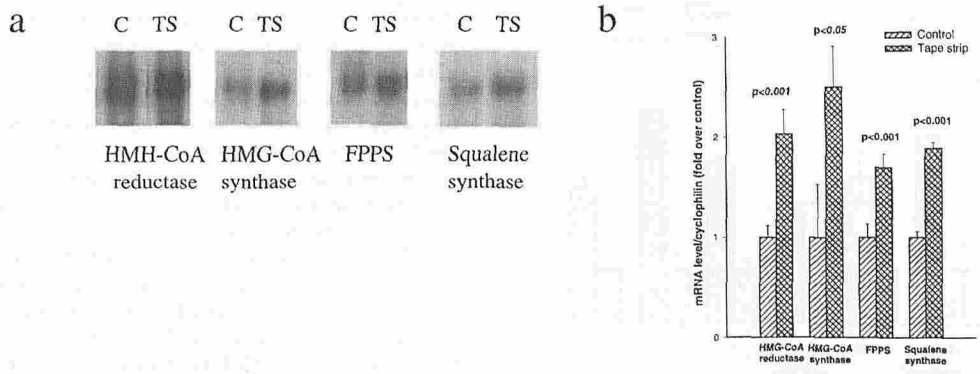

Figure 1. mRNA levels for enzymes required for cholesterol synthesis increase following tape stripping. Mice were tape stripped 3–5 times successively until a TEWL value of 6 mg per cm^2 per h was achieved. Poly $(A)^+$ RNA was isolated 4 h after barrier disruption and northern blotting was performed as in *Materials and Methods*. (*a*) Representative northern blots for cholesterol synthesis enzymes. C, control; TS, tape stripped. (*b*) Northern blot data representative of multiple experiments are presented as mean ± SEM and expressed *versus* fold of the animals control (i.e., 1.0) after correcting for loading using cyclophilin mRNA levels. Control n = 4–5, tape stripped n = 4–5, except FPPS control n = 10, tape stripped n = 10.

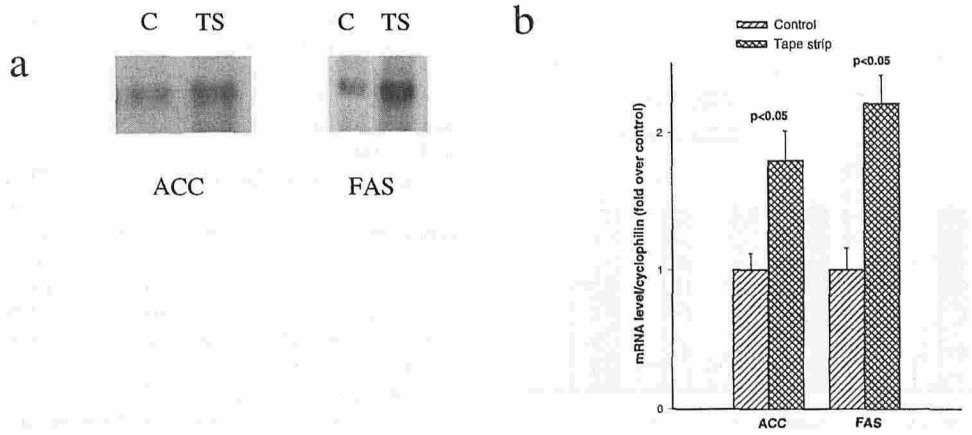

Figure 2. mRNA levels for enzymes required for fatty acid synthesis increase following tape stripping. Mice were tape stripped 3–5 times successively until a TEWL value of 6 mg per cm^2 per h was achieved. Poly $(A)^+$ RNA was isolated 4 h after barrier disruption and northern blotting was performed as in *Materials and Methods*. (*a*) Representative northern blots for ACC and FAS enzymes. C, control; TS, tape stripped. (*b*) Northern blot data representative of multiple experiments are presented as mean ± SEM and expressed *versus* fold of the control animals after correcting for cyclophilin. Control n = 4–5, tape stripped n = 4–5.

disruption of the permeability barrier there is a return of lipid to the extracellular space that restores barrier function. This homeostatic repair response includes a rapid secretion of a pool of preformed lamellar bodies from the outermost granular cells, an increase in lipid synthesis in the nucleated cell layers of the epidermis, and new lamellar body formation in the granular cell layers (Proksch *et al*, 1993). Moreover, the activities of HMG-CoA reductase, ACC, FAS, and SPT, enzymes required for de novo cholesterol, fatty acids, and ceramide synthesis, also increase shortly after barrier disruption (Proksch *et al*, 1990; Holleran *et al*, 1991a; Ottey *et al*, 1995). The increase in the activity of HMG-CoA reductase, ACC, FAS, and SPT following barrier disruption can also be prevented by provision of an artificial barrier, indicating that the increase in these enzymes is not a general injury response but is regulated by permeability barrier function. Inhibition of HMG-CoA reductase, ACC, and SPT after barrier disruption results in impaired barrier homeostasis (Proksch *et al*, 1990; Holleran *et al*, 1991b; Mao-Qiang *et al*, 1993).

In the present study we have demonstrated that following disruption of the permeability barrier there is a coordinate increase in several enzymes required for cholesterol synthesis (HMG-CoA reductase, HMG-CoA synthase, FPPS, and squalene synthase). Moreover, the increase in mRNA levels is independent of the method used to perturb the barrier and, with the exception of FPPS, can be diminished by providing an artificial permeability barrier. Additionally, we have also demonstrated that mRNA for ACC and FAS, the two rate-limiting enzymes for fatty acid synthesis, and for SPT, the rate-limiting enzyme that catalyzes the first step for ceramide synthesis, increase in the epidermis after barrier disruption. Because the increase in ACC, FAS,

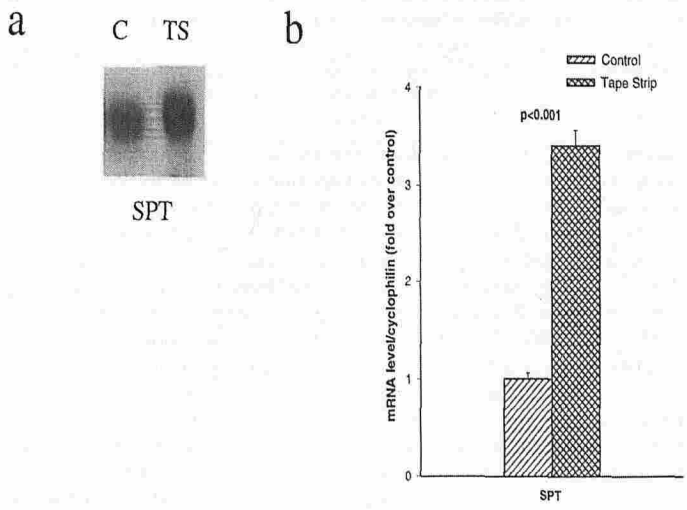

Figure 3. mRNA levels for SPT required for ceramide synthesis increase following tape stripping. Mice were tape stripped 3–5 times successively until a TEWL value of 6 mg per cm^2 per h was achieved. Poly $(A)^+$ RNA was isolated 4 h after barrier disruption and northern blotting was performed as in *Materials and Methods*. (*a*) Representative northern blot for SPT. C, control; TS, tape stripped. (*b*) Northern blot data representative of multiple experiments are presented as mean ± SEM and expressed *versus* fold of the control animals after correcting for cyclophilin. Control n = 5, tape stripped n = 5.

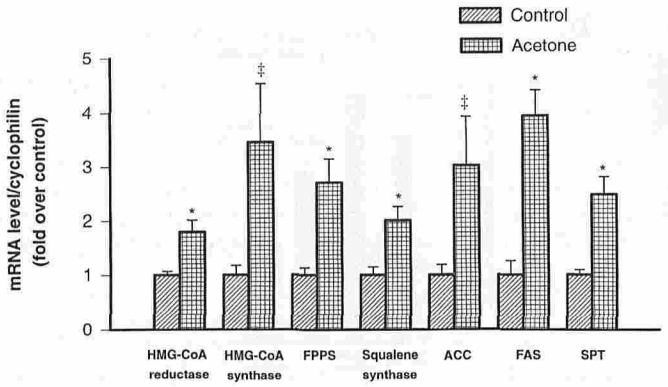

Figure 4. mRNA levels for enzymes required for cholesterol, fatty acid, and ceramide synthesis increase following acetone treatment. Mice were wiped with acetone soaked cotton swabs until a TEWL value of 6 mg per cm^2 per h was achieved. Poly $(A)^+$ RNA was isolated 4 h after barrier disruption and northern blotting was performed as in *Materials and Methods*. Northern blot data representative of multiple experiments are presented as mean ± SEM and expressed *versus* fold of the control animals after correcting for cyclophilin. Control n = 5, acetone n = 5. *$p < 0.05$ two-tailed Student's t test, ‡$p < 0.05$ one-tailed Student's t test.

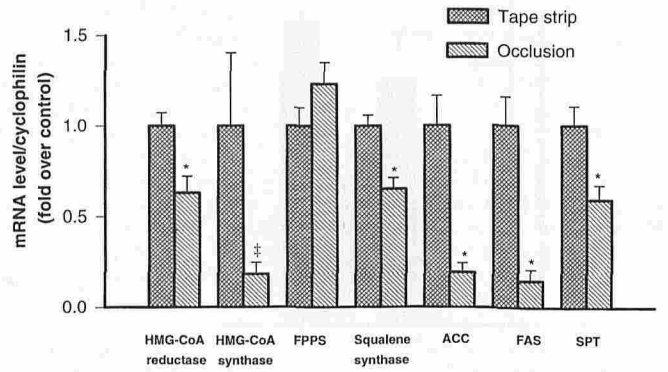

Figure 5. Effect of occlusion on mRNA levels for enzymes required for cholesterol, fatty acid, and ceramide synthesis. Mice were tape stripped 3–5 times successively until a TEWL value of 6 mg per cm^2 per h was achieved and occluded with Latex. Poly $(A)^+$ RNA was isolated 4 h after barrier disruption and northern blotting was performed as in *Materials and Methods*. Data are representative of multiple experiments and are presented as mean ± SEM and expressed *versus* fold of the control animals after correcting for cyclophilin. Tape stripped control n = 5–6, tape stripped occluded n = 5–6. *$p < 0.05$ two-tailed Student's t test, ‡$p < 0.05$ one-tailed Student's t test.

and SPT mRNA levels could be prevented by provision of an artificial permeability barrier, these changes are due to the permeability barrier requirements rather than a nonspecific response to injury.

The coordinate increase in the mRNA levels following disruption of the permeability barrier indicates that there could be regulation of these genes by transcription factors that bind to a shared regulatory element in the promoters of these genes. Sterol regulatory element binding proteins –1 and –2 (SREBP) are transcription factors that regulate the transcription of genes containing a functional sterol response element in their promoter sequence (Yokoyama *et al*, 1993; Hua *et al*, 1993). These include genes involved in: (i) cholesterol homeostasis, such as the low-density lipoprotein receptor, which we have shown previously increases after barrier perturbation (Jackson *et al*, 1992); (ii) cholesterol synthesis, such as HMG-CoA reductase, HMG-CoA synthase, FPPS, and squalene synthase; and (iii) fatty acid synthesis, i.e., ACC and FAS (Yokoyama *et al*, 1993; Jiang *et al*, 1993; Bennett *et al*, 1995; Vallett *et al*, 1996; Lopez *et al*, 1996; Ericsson *et al*, 1996). Precursor SREBP are ≈125-kDa proteins located in the membrane of the endoplasmic reticulum and nucleus. When sterol levels decrease, SREBP are cleaved by two proteases, releasing a mature ≈68-kDa *N*-terminal fragment that is then free to enter the nucleus and bind to the sterol response element and activate transcription (Wang *et al*, 1994). Acute disruption of the barrier may result in a decrease in sterol levels in keratinocytes because of an increased utilization of cholesterol to form extracellular lamellar membrane structures. This decrease in cholesterol concentration would increase the proteolytic cleavage of precursor SREBP to form mature SREBP, which would then increase the mRNA levels for fatty acid and cholesterol synthetic enzymes. At the present time assays to determine the activation of SREBP-2 in murine epidermis are not available, because murine SREBP-2 is not recognized by currently available (human) antibodies. In addition to SREBP, cholesterol and fatty acid synthetic enzymes are also regulated by other transcription factors such as AP-1, SP-1, NF-Y, and Red25 (Osborne *et al*, 1992; Vallett and Osborne, 1994; Jackson *et al*, 1995; Yieh *et al*, 1995). It remains to be determined whether SREBP or other transcription factors are activated following acute disruption.

It is not known which transcription factors regulate SPT transcription; however, SREBP-2 is unlikely to be a transcription factor for SPT. Preliminary studies from our laboratory have demonstrated that changes in the endogenous sterol levels in the epidermis result in a coordinate change in mRNA levels for SREBP-2 and cholesterol and fatty acid synthetic enzymes; however, SPT mRNA levels were not affected by changes in sterol levels.[1] Furthermore, following barrier disruption, the increase in SPT activity is delayed relative to the increase in fatty acid synthetic enzymes, ACC and FAS, and the cholesterol synthetic enzyme, HMG-CoA reductase. This would suggest that ceramide synthesis is not regulated by the same factors as cholesterol and fatty acid synthesis.

The exact molecular mechanisms by which the epidermal permeability barrier requirements regulate the expression of these lipid synthetic genes are unknown. A gradient in the concentration of calcium ions has been described in the epidermis, with the highest concentration of calcium ions in the nucleated layers of the outer epidermis and the lowest in the basal layer (Menon *et al*, 1985b). Following barrier disruption, the calcium gradient is abolished, returning in parallel with the formation of a functional barrier (Menon *et al*, 1994). Increasing the external concentration of calcium and potassium ions following barrier disruption prevents the increase in HMG-CoA reductase activity, reduces the return of lipid to the stratum corneum, and inhibits barrier recovery (Lee *et al*, 1992); however, it remains unknown whether calcium ions influence the expression of lipid synthetic genes and/or the activation of transcription factors such as SREBP in the epidermis.

In the present study, FPPS mRNA levels increase after barrier disruption, as do other cholesterol synthetic genes; however, the increase in FPPS mRNA levels is not diminished by occlusion following barrier perturbation. Thus, under these circumstances, FPPS may be regulated by factors in addition to those controlled directly by the permeability barrier. The expression of FPPS in other tissues is also not always coordinately regulated with HMG-CoA reductase and HMG-CoA synthase (Feingold *et al*, 1995). Sterol mediated suppression of a human leukemia cell line THP-1 can be reversed by the addition of a calcium ionophore A23187, which results in a rapid increase in mRNA of up to 40-fold for HMG-CoA reductase and 15-fold for HMG-CoA synthase with little or no change in FPPS mRNA levels (Wilkin and Edwards, 1992). In addition to providing substrate for cholesterol synthesis, FPPS is a key enzyme for the synthesis of mevalonate metabolites required for the prenylation of proteins, ubiquinone synthesis, and the glycosylation of proteins via dolichols. Prenylated proteins include protooncogenes and lamin that control cell division and may be involved in the increased cell division that occurs following barrier perturbation, a change that is also only partially prevented by occlusion (Proksch *et al*, 1991). Recent studies by our laboratory have shown that the prenylation of proteins is increased following barrier disruption, and that this increase is not blocked by

[1]Harris IR, Farrell AM, Grunfeld C, *et al*: Identification of sterol regulatory element binding protein in epidermis: modulation in parallel to changes in key enzymes of cholesterol and fatty acid synthesis. *J Invest Dermatol* 108:553, 1997 (abstr.).

occlusion (Denda *et al*, 1997). One could speculate that increased FPPS activity is required to provide substrate for the prenylation of proteins required for cells to progress through the G1 phase of the cell cycle or to undergo DNA synthesis. This would account for the increase in activity prior to the onset of DNA synthesis, which we have previously shown to occur 18–24 h after barrier disruption (Proksch *et al*, 1991).

In summary, following acute disruption of the epidermal permeability barrier there is a coordinate increase in the mRNA levels for cholesterol synthesis enzymes; HMG-CoA reductase, HMG-CoA synthase, FPPS, squalene synthase. There is also an increase in the mRNA levels for fatty acid synthesis enzymes, ACC and FAS, and SPT, the rate-limiting enzyme of de novo ceramide synthesis. Furthermore, with the exception of FPPS, we have demonstrated that the expression of these genes in the epidermis is regulated by epidermal permeability barrier function. The parallel increase in mRNA levels for these lipid synthetic enzymes suggests coordinate regulation by specific transcription factors that are activated following barrier disruption.

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