UVB irradiation up-regulates serine palmitoyltransferase in cultured human keratinocytes

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Abstract The enzyme serine palmitoyltransferase (SPT; EC 2.3.1.50), which catalyzes the first committed and rate-limiting step in sphingolipid synthesis, is up-regulated in the epidermis as part of the homeostatic repair in response to permeability barrier perturbation. Moreover, UVB exposure, which also perturbs the barrier, up-regulates sphingolipid synthesis, but the basis for this increase is not known. The recent isolation of cDNAs for SPT (i.e., *LCB1* and *LCB2*) allow molecular regulation studies to be performed. Therefore, we determined whether UVB exposure alters mRNA, protein, or activity levels for SPT in cultured human keratinocytes (CHKs) as a mechanism for regulation of epidermal sphingolipid synthesis. In CHK, transcripts for both *LCB1* (3.0 kb) and *LCB2* (2.3 kb) are evident by Northern blot analysis, and UVB exposure (23 mJ/cm^2) induces a delayed 1.8 to 3.3-fold increase in *LCB2* mRNA levels ($P < 0.01$) 48 h after treatment versus non-irradiated control cells. In contrast, neither *LCB1* nor a second *LCB2* transcript (8.0 kb) changed significantly. Likewise, Lcb2 protein levels (by Western blot analysis), as well as SPT activity, increase in parallel with the increased *LCB2* mRNA. Finally, incorporation of [^{14}C]-acetate into sphingolipids was increased significantly 48 h after UVB treatment. Together, these results demonstrate that CHKs respond to UVB by increasing sphingolipid synthesis, primarily through increases in both *LCB2* mRNA and protein levels, leading to increased SPT activity. These results demonstrate one mechanism (UVB) whereby SPT is regulated at the molecular level, and suggest further that epidermis up-regulates sphingolipid synthesis at both the mRNA and protein levels in response to UVB.—Farrell, A. M., Y. Uchida, M. M. Nagiec, I. R. Harris, R. C. Dickson, P. M. Elias, and W. M. Holleran. **UVB irradiation up-regulates serine palmitoyltransferase in cultured human keratinocytes. *J. Lipid Res.* 1998.** 39: **2031–2038.**

Supplementary key words barrier • ceramides • epidermis • keratinocytes • skin • sphingolipids • sphingosine • serine palmitoyltransferase • UVB

Ceramides, or acyl-sphingosines, are widely recognized as critical lipid species in the structure and function of the permeability barrier of mammalian epidermis, representing ≈40% of stratum corneum (SC) lipid content by weight (1). Serine palmitoyltransferase (SPT) (EC 2.3.1.50), the enzyme that catalyzes the initial committed step in de novo sphingolipid synthesis, is highly active both in murine epidermis and in cultured human keratinocytes (2, 3). Moreover, both SPT activity and sphingolipid synthesis increase during the process of barrier recovery after acute damage (4), and the increase in sphingolipid synthesis is required for permeability barrier homeostasis (5). Although these studies clearly demonstrated the importance of SPT activity for epidermal barrier function, the lack of both sequence information and antibodies directed against SPT limited further studies on the mechanism(s) by which enzyme expression is regulated. Recently, two genes necessary for SPT activity were identified in *Saccharomyces cerevisiae*: *LCB1* (long-chain base 1) (6) and *LCB2* (7). The isolation of cDNAs for the human *LCB2* (8) and *LCB1* (9) homologs now makes possible studies on the molecular regulation of SPT activity. Furthermore, the recent expression of human LCB2 in HEK cells (10) confirmed the role of this gene product in sphingolipid synthesis.

Acute exposure to ultraviolet light induces a wide variety of cutaneous responses, including the release of proinflammatory cytokines (11–13), induction of DNA damage repair mechanisms (14), alterations in keratinocyte proliferation (15, 16), stimulation of melanin production and redistribution (17), and induction of skin tumors. Moreover, a delayed abnormality in epidermal permeability barrier function also occurs after UVB (16, 18–21), which is dependent upon both thymocytes and epidermal hyperplasia (16).

Abbreviations: Cer, ceramide; CHKs, cultured human keratinocytes; SPT, serine palmitoyltransferase; TEWL, transepidermal water loss; LCB, long-chain sphingoid base; Sph, sphingosine; SPL, sphingolipids; 3KDS, 3-ketodihydrosphinganine; PBS, phosphate-buffered saline; DEPC, diethyl pyrocarbonate; HPTLC, high performance thin-layer chromatography.

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One mechanism leading to correction of the UV-induced perturbation may be the stimulation of ceramide synthesis. For example, increased stratum corneum lipids, with the appearance of novel ceramide species, have been reported after repeated sub-erythemal doses of UVB (22, 23). Recently, we demonstrated a delayed increase in SPT activity after acute, high-dose UVB exposure in vivo (24), which parallels the repair response in murine epidermis. In summary, whereas UV irradiation appears to up-regulate epidermal sphingolipid production, the mechanism(s) by which UVB mediates these process(es) have not been addressed.

The objectives of the present study were to determine: first, whether cultured human keratinocytes express mRNA transcripts for either *LCB1* and *LCB2*; second, whether SPT activity increases in cultured human keratinocytes (CHKs) in response to UVB, as occurs in intact murine epidermis (24); and third, whether changes in SPT activity and/or protein reflect alterations in mRNA, suggesting transcriptional/post-transcriptional control of this key enzyme in epidermal sphingolipid synthesis. We report here that UVB exposure of isolated CHKs, cultured in serum-free medium, increases SPT activity in a time-dependent manner. Moreover, the increase in mRNA for LCB2, but not LCB1, parallels an increase in enzyme activity and sphingolipid synthesis, suggesting that enzyme activity is, at least in part, regulated by antecedent changes in mRNA levels. These results represent one mechanism by which sphingolipid synthesis may be regulated in general, and specifically within the epidermis. Futhermore, as isolated keratinocytes up-regulate SPT activity, the epidermis appears to possess endogenous UVB-repair mechanisms, that include up-regulation of lipid production.

MATERIALS AND METHODS

Materials

Reagent-grade organic solvents, pyridoxal phosphate, dithiothreitol, palmitoyl-CoA, and standards for high-performance thin-layer chromatography (HPTLC) (including sphingomyelin, sphingosine, ceramides III and IV, and cerebrosides I and II) were obtained from Sigma Chemical Co. (St. Louis, MO). HEPES buffer was purchased from Fisher Scientific (Santa Clara, CA); [^{14}C]acetate (sp act 10 mCi/μmol) and [^{3}H]-l-thymidine (sp act 81 Ci/mmol) were from ICN Radiochemicals (Irvine, CA) and Amersham (Arlington Heights, IL), respectively. Protein reagents and bovine serum albumin were obtained from Bio-Rad (Richmond, CA). HPTLC plates (silica gel 60) were purchased from Merck (Darmstadt, FRG) (#5641).

Cultured human keratinocytes

Neonatal human foreskin keratinocytes (second passage) were grown to 90–100% confluence in serum-free medium (KGM; Clonetics, San Diego, CA) containing 0.07 mm $CaCl_2$ as described previously (2). The medium then was changed to KGM containing 1.2 mm $CaCl_2$, and after an additional 3 days, keratinocytes were irradiated with UVB (see below). All experiments were performed a minimum of three times in triplicate, with representative data presented.

Ultraviolet light (UVB) exposure and experimental protocol

Keratinocyte cultures were irradiated with a single dose of 23 mJ/cm^2 UVB (i.e., $\approx {}^{1}/_{10}$ the dose at which early effects on trypan blue exclusion were noted in preliminary studies), using FS-20/T12 bulbs (emission range: 280–340 nm; 305 nm max). Immediately prior to irradiation, the KGM medium was replaced with sterile phosphate-buffered saline (PBS) (pH 7.4, 37°C), and after irradiation, PBS was replaced with fresh growth medium. Non-irradiated controls were treated similarly. Cells were maintained at 37°C (5% CO_2) for various times (0 to 96 h) until harvesting for mRNA and enzyme assays. UVB exposure was quantitated using a Goldilux Ultraviolet radiometer (Oriel, Stratford, CT).

Isolation and analysis of mRNA

Poly (A+) RNA was isolated using a modification described previously (25). Briefly, keratinocytes were washed with PBS and lysed in 2 ml high salt buffer (0.5 m NaCl; 10 mm Tris-HCl, pH 7.5; 1 mm EDTA; 1% SDS) containing 200 μg/ml proteinase K. High molecular weight DNA was sheared, and proteinase K digestion was allowed to proceed at 37°C for 1 h. Oligo(dT)-cellulose (7.5 mg) was then incubated with the lysate for 1 h. After three washes in high salt buffer and one in low salt buffer (0.1 m NaCl; 10 mm Tris-HCl, pH 7.5; 1 mm EDTA; 1% SDS), poly (A+) RNA was eluted with diethyl pyrocarbonate (DEPC)-treated water. mRNA was ethanol precipitated overnight at −20°C, resuspended in DEPC-treated water, and quantified at 260 nm.

Aliquots of poly (A+) RNA (4 μg) were run on agarose/formaldehyde (0.8%/2 m) gels, transferred, and UV-crosslinked to Nytran Plus membranes (Schleicher & Schuell, Keene, NH). Probes for cyclophilin (0.95 kb), LCB1 (1.0 kb), and LCB2 (1.54 kb) were labeled with [α-^{32}P]dCTP by random priming (Amersham, UK). Blots were prehybridized at 42°C for 2–3 h, and hybridized with radiolabeled probes overnight at 42°C. Membranes were washed for 30 min at room temperature (2× SSPE, 0.1% SDS) and for 40 min at 60°C (0.1× SSPE, 0.1% SDS). Blots were exposed to X-ray film, and transcripts were quantified on autoradiographs by scanning densitometry.

Assay for serine palmitoyltransferase (SPT) activity

Our procedures for the isolation of microsomes and the subsequent quantitation of SPT from CHKs have been described in detail elsewhere (2, 4). Briefly, keratinocytes were lysed in low-salt buffer (50 mm Tris-HCl, pH 8.0; 0.1% NP-40; 150 mm NaCl), as described previously (26). Lysates were centrifuged (12,000 *g*, 5 min) to remove cell debris. Microsomal protein (50–100 μg) was incubated with 50 μm pyridoxal phosphate, 150 μm palmitoyl-coenzyme A, 1.0 mm [G-^{3}H]-l-serine (sp act 45–50,000 dpm/nmol) at 37°C (10 min). The reaction product, 3-ketodihydrosphinganine (3KDS), was isolated and counted using a Beckman LS-1800 scintillation counter. Protein content was determined by the Bradford method (27), using the Bio-Rad Protein assay kit (Bio-Rad, Richmond, CA), with bovine serum albumin as the standard. Enzyme specific activity is expressed as pmoles of 3KDS formed/min per mg microsomal protein.

Western immunoblotting

Rabbit polyclonal antibodies were raised against a fusion protein consisting of glutathione sulfotransferase (GST) and a 373 amino acid portion of human LCB2 (8) encoded by an EST clone (hbc 176; GenBank accession no. U15555). The fusion protein, produced in *Escherichia coli* and found in inclusion bodies, was purified by SDS-PAGE. Serum antibodies, immunoaffinity purified using fusion protein bound to a nitrocellulose membrane, were used for Western immunoblot analysis.

Protein samples, including both microsomal preparations (30

μg) and NP-40 lysates (9 μg), were separated by SDS-PAGE (8.5% total acrylamide concentration) under reducing conditions, and electrophoretically transferred to a PVDF membrane. Proteins were detected with a polyclonal serum directed against recombinant human Lcb2 using the ECL detection system (Amersham, UK). Molecular weight standards also are from Amersham.

Lipid synthesis

To assess rates of lipid synthesis, cultures were incubated for 3 h with [^{14}C]acetic acid, supplemented with additional cold acetate, in phosphate buffer to achieve a final acetate concentration of 500 μm (25 μCi/dish) (28). At the end of incubations, cultures were harvested manually by scraping with a rubber policeman in 1 ml of 0.05 m sodium phosphate buffer containing 2 m NaCl and 2 mm EDTA, pH 7.4. Cells were homogenized by brief sonication on ice using a sonic dismembranator (Fisher Scientific Model 300) at a relative output of 35%. Aliquots of the homogenate were taken for both measurement of DNA content (29) and lipid extraction, as described previously (4, 5). Briefly, polar and neutral lipid fractions were separated by thin-layer chromatography in three sequential solvent systems: *1*) chloroform–methanol–water 40:10:1 (by volume) to 2 cm, and again to 5 cm; *2*) chloroform–methanol–acetic acid 94:5:1 by volume) to the top. Lipid fractions were visualized under ultraviolet A light after spraying with 0.2% 8-anilino-1-naphthalene sulfonic acid, and identified by co-chromatography with known neutral and polar lipid standards (see Materials). Appropriate fractions were scraped directly into scintillation cocktail and quantitated. Each experiment was conducted in triplicate. Data are expressed as mean dpm (±SEM) incorporated into lipid fraction/μg DNA.

[^{3}H]thymidine incorporation

To assess the effects of UVB exposure on keratinocyte proliferation, we assessed [^{3}H]thymidine (1.0 μCi/ml; sp act = 81 Ci/mmol) incorporation into cultured human keratinocytes. Cells were treated with UVB as described above and incubated with [^{3}H]thymidine for 1 h beginning at 0, 2, 4, 6, 12, 24, 48, 72, or 96 h after UVB exposure. Incorporation of tritium into trichloroacetic acid (TCA)-precipitable materials was determined by scintillation spectrometry and reported as disintegrations per min (dpm) per mg DNA.

Statistical analysis

Statistical analyses of data were performed using either a two-tailed Student's *t*-test or a paired *t*-test.

RESULTS

Non-irradiated cultured human keratinocytes express three LCB transcripts

We first determined the baseline expression of *LCB1* and *LCB2* mRNAs in non-UVB-treated CHKs in order to compare the size of expressed transcripts with those described previously. Whereas the *LCB2* probe hybridizes with two transcripts (8 kb and 2.3 kb), the *LCB1* probe detects a single transcript (≈3.0 kb) in CHKs (**Fig. 1**). These results show that untreated CHKs express transcripts of similar size to those reported in human and mouse tissues (9, 10).

LCB1 and *LCB2* transcript levels change after UVB irradiation

We next determined the change in the level of the LCB mRNA transcripts at various time points after UVB irradiation. The dose of UVB was optimized in preliminary studies to 23 mJ/cm^2 (see Methods). A comparison of fold-changes versus untreated controls demonstrates that each LCB transcript decreased early after UVB irradiation; i.e., 4 h (Figs. 1 and 2). In contrast, by 24 h, levels of both the *LCB1* transcript and the larger *LCB2* transcript (8 kb) were increased

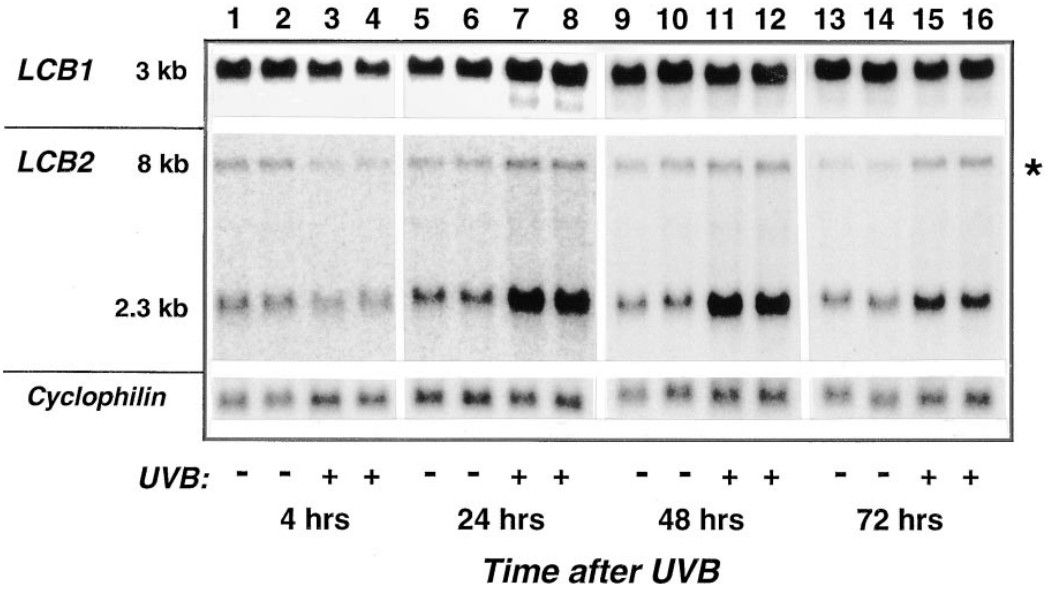

Fig. 1. Northern analysis of *LCB1* and *LCB2* transcripts in UVB-treated CHKs. CHKs were untreated (−) or irradiated with UVB (23 mJ/cm^2) (+) at time 0. Poly (A+) RNA was extracted at different times after exposure and hybridized with cDNA probes for *LCB1*, *LCB2*, or cyclophilin (see Methods). Duplicate samples from a representative experiment are shown; lanes 1–8 and 9–16 represent separate blots from the same experiment. Two transcripts hybridized with the *LCB2* probe (8 kb and 2.3 kb), while a single transcript (3 kb) was detected with the *LCB1* probe. LCB2 mRNA (2.3 kb) levels were increased at 24, 48, and 72 h, while no significant changes in LCB1 or cyclophilin levels were evident.

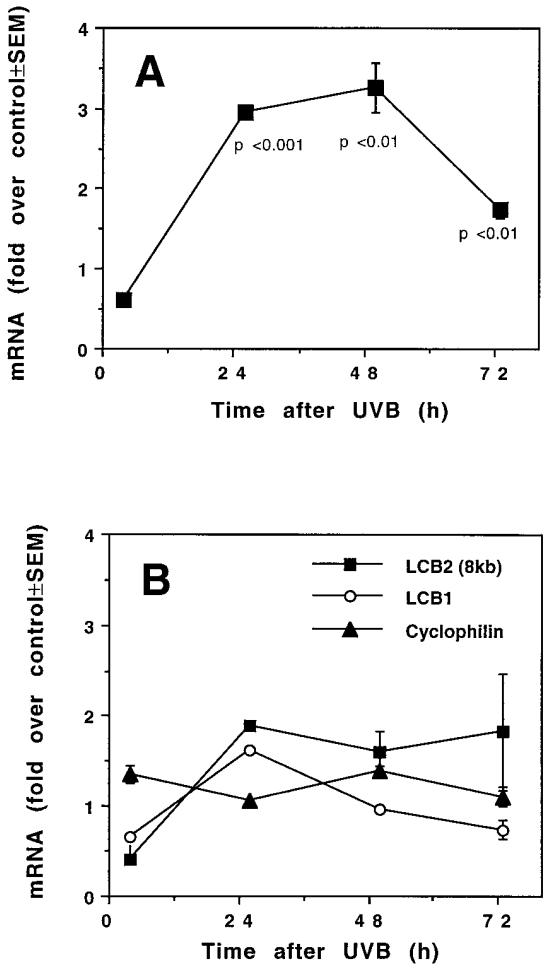

Fig. 2. Increased mRNA for LCB2 after UVB. mRNA levels after UVB irradiation are expressed as fold-increase over untreated control (±SEM, n = 3). The level of each LCB transcript decreased at 4 h after UVB irradiation (compared to untreated controls). Panel A: levels of the 2.3 kb *LCB2* transcript increased further to a maximum of 3.3-fold over control at 48 h ($P < 0.01$). Panel B: levels of the *LCB1* and 8 kb *LCB2* transcripts fluctuated, but were not significantly altered (i.e., P = NS) throughout the timecourse.

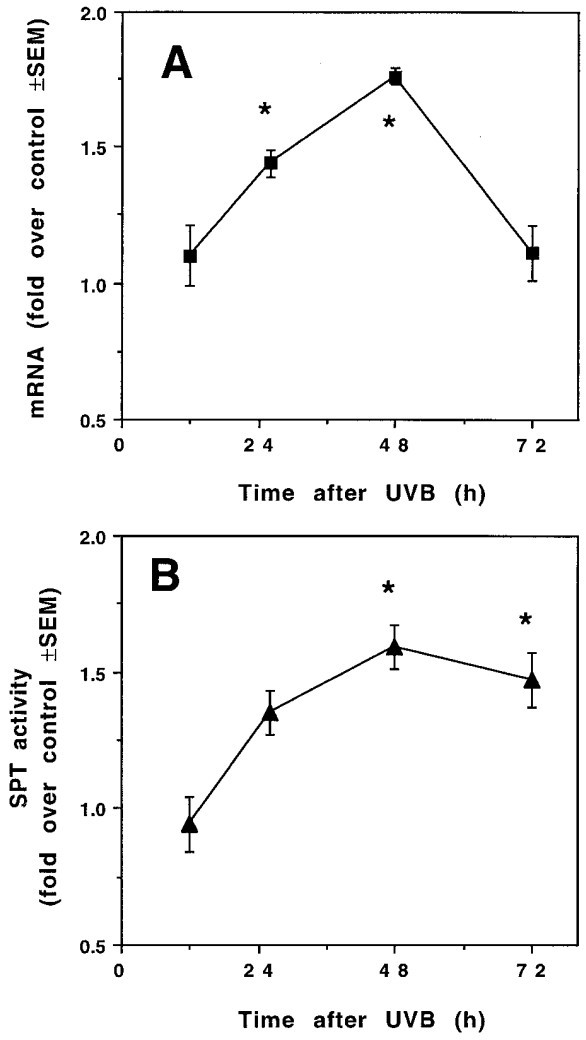

Fig. 3. SPT activity increases in parallel with *LCB2* transcript levels after UVB. CHKs were untreated or irradiated with UVB (23 mJ/cm^2). Panel A: Poly (A+) RNA was extracted and hybridized with a cDNA probe for *LCB2*. mRNA levels after UVB irradiation are expressed as fold-increase over untreated control (±SEM, n = 3). Levels of the 2.3 kb *LCB2* transcript increased after UVB irradiation, with a maximal increase to 1.7-fold over control after 48 h (*$P < 0.01$). Levels of the 8 kb *LCB2* and *LCB1* transcripts again were not significantly altered. B: SPT activity in microsomes was determined. Results are expressed as pmol 3KDS formed/min per mg protein (±SEM, n = 3). Microsomal SPT activity increased after UVB irradiation to ≈1.5-fold over control at 48 and 72 h (*$P <$ 0.01). Although activity was 1.3-fold over control at 24 h, this change was not statistically significant.

only modestly over control (Fig. 1 and **Fig. 2B**; P = NS). However, the 2.3 kb *LCB2* transcript was increased significantly, i.e., to a maximum of 3.3-fold over control by 48 h (Fig. 1 and Fig. 2A; $P < 0.01$). In contrast, cyclophilin mRNA levels, also presented as the ratio of UVB-treated to untreated controls at each time point, did not change significantly at any of the time points investigated (Fig. 2A). These results show changes in the levels of all three LCB transcripts after UVB, with significant increases in the 2.3 kb *LCB2* transcript at all timepoints (i.e., 24–72 h).

SPT activity increases in parallel with increased *LCB2* levels after UVB

To determine whether the changes in mRNA level for the 2.3 kb *LCB2* transcript correlated with alterations in SPT activity, we next performed Northern blot analyses and measured enzyme activity in parallel using UVB-treated CHK cells. As in the prior experiments, the level of the 2.3 kb *LCB2* transcript again increased after UVB irradiation (23 mJ/cm^2), reaching a maximum at 48 h (1.8-fold over untreated control; $P < 0.01$) (**Fig. 3A**; c.f., Fig. 2A). In contrast, mRNA levels for both the 8 kb *LCB2* and *LCB1* transcripts again did not increase significantly at 48 h (not shown). SPT activity in microsomal preparations increased in parallel after UVB irradiation; i.e., peaking at 1.6-fold over control at 48 h ($P < 0.01$) (Fig. 3B). Although enzyme activity increased 1.3- and 1.5-fold over control at 24 and 72 h, respectively, only the 72 h value achieved statistical significance. These results demonstrate that changes in the 2.3 kb *LCB2* transcript are reflected in

parallel or subsequent increases in SPT activity, which are sustained as mRNA levels decline (i.e., at 72 h).

Lcb2 protein increases in UVB-irradiated keratinocytes

To determine whether the increase in SPT activity was due to increased enzyme production, correlating with increased *LCB2* mRNA levels, we next performed Western blot analyses on microsomal preparations at various time points after UVB treatment. The major microsomal polypeptide, detected with anti-Lcb2 serum, is a 58 kDa species (**Fig. 4**), consistent both with the 63 kDa polypeptide size, predicted from the open-reading frame in the 2.3 kb mRNA transcript, and with previous studies of SPT in other cell types (10; R. Dickson and M. Nagiec, unpublished observations). Again, similar to both the increase in *LCB2* mRNA and SPT activity, the 58 kDa polypeptide (in CHK microsomes) increased at both 48 and 72 h (Fig. 4); i.e., 1.3 ± 0.07-fold and 1.9 ± 0.2-fold over controls at 48 and 72 h, respectively (n = 6; $P < 0.05$ for each). The level of a minor 35 kDa polypeptide, thought to be a proteolytic cleavage product (Fig. 4), also increased to 1.8- and 2.3-fold over controls at 48 and 72 h, respectively ($P < 0.03$). These studies demonstrate that the increased SPT activity after UVB exposure is related temporally not only to increased *LCB2* mRNA, but also to increased Lcb2 protein.

Incorporation of [^{14}C]acetate into sphingolipids increases after UVB

To determine whether the UVB-induced increase in SPT activity correlates with increased sphingolipid synthesis, we assessed acetate incorporation into total cellular ceramides (i.e., including glucosylceramide) after UVB. No significant change was evident immediately after UVB exposure (not shown), while at 24 h the difference (i.e., 1.25-fold increase) did not achieve statistical significance. However, by 48 h, a significant increase (1.35 ± 0.05-fold over control; $P \leq 0.01$; n = 3 for each) in acetate incorporation into total cellular ceramides was evident in UVB-treated versus non-irradiated controls. These results confirm that the increased SPT activity in CHKs is reflected in an increased production of ceramides by intact cultured keratinocytes.

Incorporation of [^{3}H]thymidine after UVB

Finally, as high doses of UVB produce a hyperplastic response in the epidermis (15, 16), we next investigated whether the increase in sphingolipid production reported above could be attributed to keratinocyte hyperproliferation. Thymidine incorporation into cellular DNA was measured in CHKs 0, 24, 48, and 72 h after exposure to UVB (23 mJ/cm^2). Although UVB exposure led to a moderate elevation of [^{3}H]thymidine incorporation (i.e., increased ≈30%) at 48 h (**Fig. 5**; $P < 0.005$), the total DNA content did not change at any of the timepoints investigated (not shown). These studies show that the observed increases in SPT activity may be due, in part, to increased production of sphingolipids required to sustain keratinocyte proliferation.

DISCUSSION

Previous studies by this laboratory have demonstrated the importance of sphingolipid synthesis for normal epi-

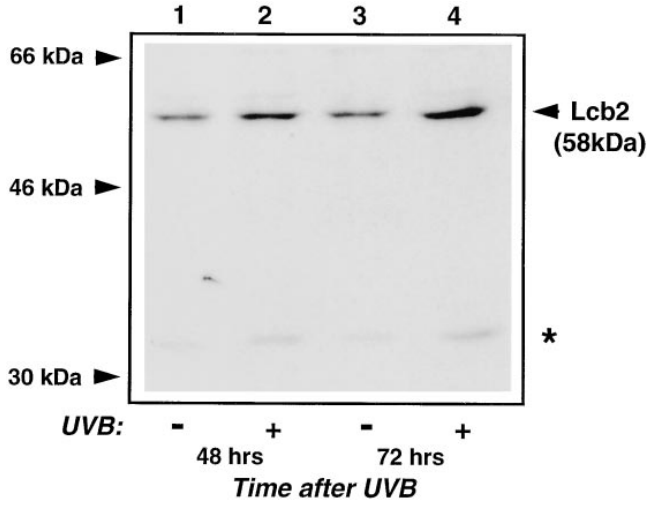

Fig. 4. Western analysis of Lcb2 protein in UVB irradiated keratinocytes. CHKs were treated as in Fig. 1. Microsomal proteins were separated by SDS-PAGE. Immunoreactive polypeptides were detected with a polyclonal antiserum directed against recombinant human Lcb2. The major microsomal polypeptide detected with anti-Lcb2 serum was 58 kDa. Levels of this polypeptide increased to 1.3- and 1.9-fold over control at 48 and 72 h, respectively (i.e., lanes 1 vs. 2, and 3 vs. 4), after UVB (n = 6; $P < 0.05$). Representative blot of duplicate experiments (total n = 6) is shown. *35kDA polypeptide likely represents a proteolytic product.

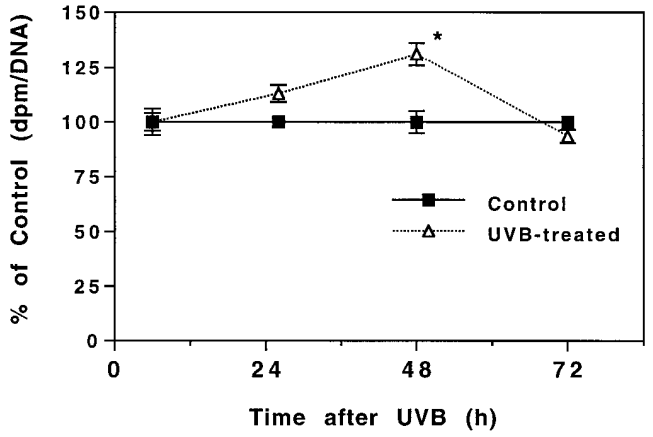

Fig. 5. Incorporation of [^{3}H]thymidine into DNA increases following UVB. CHKs were treated as in Fig. 1, and incubated (1 h) with [^{3}H]thymidine prior to (i.e., time 0), and at 24, 48, and 72 h after UVB exposure as described in Methods. Each point respresents the mean (dpm/mg DNA ± SEM) for 6 determinations (6 separate plates) relative to parallel, sham-irradiated controls at each time point; control values (approximately 6 × 10^2 dpm/mg DNA) for each time point were set to 100%. Thymidine incorporation was significantly increased only at 48 h (*30% over control; $P < 0.005$).

dermal barrier homeostasis (3–5). The production of sphingolipids is regulated within the epidermis through the activity of SPT (5), at least in part, at the mRNA level (30). In addition, we recently demonstrated that exposure of murine epidermis to UVB results in a delayed disruption of the permeability barrier (16, 21). Recovery from the UVB insult is paralleled by an increase in both epidermal sphingolipid synthesis and epidermal SPT activity in vivo (24). In the present study, we explored the regulation of sphingolipid synthesis in response to UVB. We show here that cultured human keratinocytes express a 3 kb *LCB1*, as well as both 2.3 kb and 8 kb *LCB2* transcripts. Although the exact role of each transcript is not known, the 2.3 kb *LCB2* transcript is thought to encode for active SPT enzyme, because the length and predicted amino acid sequence match prior reports in yeast (7), and this sequence contains the putative binding domain for pyridoxal phosphate-binding (7, 8), a required cofactor for SPT activity. Both the studies with Lcb-deficient *Saccharomyces* strains, in which absence of *LCB2* specifically correlated with SPT deficiency (7), and the recent cloning and expression studies (10) further demonstrate that *LCB2* encodes for mammalian SPT activity. Whereas the role of the longer *LCB2* transcript (8 kb), which also contains the putative pyridoxal phosphate-binding domain, remains to be determined, the *LCB1* transcript is thought to encode for either a catalytic or regulatory subunit of SPT in yeast (7, 8). However, recent studies do not support a regulatory function for LCB1 in mammalian cells (10). The results presented here, and our recent in vivo studies (30, 31), demonstrate that changes in *LCB2* mRNA levels are reflected by comparable alterations in SPT activity, providing further evidence for *LCB2* being a key determinant of keratinocyte SPT activity.

The data presented here also indicate that UVB regulates SPT activity at the transcriptional level in CHKs. An initial decrease in all three *LCB* transcript levels occurred shortly after (4 h) after UVB, while at later time points, the 2.3 kb *LCB2* transcript increased significantly, reaching a maximal increase after 48 h. SPT activity increased in parallel or slightly after the change in mRNA levels, with the greatest increase at 72 h. Moreover, the major polypeptide detected in microsomal preparations, using an antiserum directed against recombinant human Lcb2, displayed a molecular mass of 58 kDa, which approximates the deduced 63 kDa sequence of *LCB2*. This protein also increased significantly at both 48 and 72 h. In contrast, levels of neither the 8 kb *LCB2* nor the *LCB1* transcripts changed significantly. Finally, incorporation of [^{14}C]acetate into sphingolipids increased after UVB, suggesting that the increase in the activity of SPT, the rate-limiting enzyme in sphingolipid synthesis in the epidermis (4), results in increased sphingolipid synthesis. Furthermore, the increases in SPT mRNA, activity, and sphingolipid synthesis observed here (i.e., in the range of 1.4- to 3-fold over controls) are similar to those observed in vivo after acute insult to the permeability barrier (4), and represent significant changes to the overall production of ceramides in the epidermis.

Three possible explanations for the up-regulation of sphingolipid production by UVB need to be considered. First, these changes could be related to UVB-induced apoptotic events known to occur in keratinocytes (32), and in whole epidermis (i.e., sunburn cell formation) (33, 34) after UVB. Transient increases in ceramide levels, usually associated with an induction of sphingomyelin hydrolysis, are associated with the induction of apoptosis in a number of cell types (35, 36), including keratinocytes (37). However, an increase in ceramide synthesis also has been observed during anthracycline-induced apopotosis (38), and UVB exposure also has been shown to induce a transient increase in Cer levels (39). As these increases in ceramide levels are transient, they are ascribed to sphingomyelin hydrolysis rather than increased Cer production (35, 40). Our recent finding that TNFα and IL-1α induce a delayed increase in hepatic Cer synthesis (i.e., 24 to 48 h after treatment) suggest that increased cellular Cer levels may be affected by anabolic pathways in addition to the direct production via sphingomyelin hydrolysis. Therefore, it appears unlikely that the delayed increase in Cer synthesis after UVB, as reported here, represents an aspect of the apoptotic response in the epidermis, despite the occurrence of apoptosis in CHK irradiated under similar conditions.

Second, alterations in sphingolipid production might reflect changes in keratinocyte proliferation after UVB. Acute exposure to high-dose UVB in vivo leads to an early decrease in epidermal proliferation, followed by a sustained increase (15, 16, 21). The changes in sphingolipid production might therefore reflect alterations in epidermal growth kinetics after UVB. Indeed, we observed elevated thymidine incorporation rates at 48 h, but no significant change in DNA content (per plate) was observed after UVB. Further studies are needed to determine whether these changes in sphingolipid metabolism are tied to modulations in cell cycle kinetics.

A third potential explanation for the increase in sphingolipid synthesis after UVB involves the epidermal permeability barrier. CHKs produce abundant barrier-related lipids, including sphingolipids, even when studied under immersed, post-confluent conditions, as here (28). As ceramides represent critical lipid components of the epidermal permeability barrier, up-regulation of their synthesis by UVB may reflect an endogenous, protective response for maintenance of barrier homeostasis. For example, sphingolipid synthesis is up-regulated during repair of the barrier after a variety of acute insults, such as tape-stripping or solvent-extraction (3–5), and a delayed barrier defect also occurs after acute UVB exposure (16, 21). Indeed, epidermal ceramide content increases after UVB (22, 23). Moreover, the delayed increase in sphingolipid synthesis in CHKs is comparable to the kinetics for increased SPT activity after UVB exposure in vivo (24). Wilgram et al. (41) reported in human epidermis, and we have recently confirmed in murine epidermis (24), that the number of lamellar bodies increases after UVR, further evidence for an increase in barrier lipid synthesis. Thus, up-regulation of sphingolipid synthesis may represent a repair mechanism for the epidermis; i.e., to in-

crease production of critical barrier lipids. Yet, if this hypothesis were to be correct, then CHKs would have to be capable of responding to insults, such as UVB, even in the absence of an intact barrier. Presumably, the same as yet unidentified signals that trigger increased Cer synthesis are present both in CHK and intact epidermis. The present results, therefore, are consistent with the presence of a putative barrier repair response that is inherent to epidermal keratinocytes.

Prior studies have shown that certain cytokines, such as IL1α and TNFα, which are released from keratinocytes in response to epidermal UVR exposure (42), could initiate alterations in lipid metabolism in extracutaneous tissues. In addition, an increase in epidermal cytokines occurs in response to barrier abrogation, both by rapid migration from existing protein pools (43), and increased de novo cytokine production (44). As we recently showed that endotoxin (lipopolysaccharide; LPS), IL1α, and TNFα each increase SPT activity and LCB2 mRNA levels in Syrian hamster liver (31), it is plausible that both the UVB- and barrier-induced increases in sphingolipid production may be similarly regulated; i.e., by increased epidermal cytokine levels after barrier disruption/injury.

In conclusion, SPT activity increases in cultured human keratinocytes after exposure to UVB. Increased levels of a 2.3 kb *LCB2* transcript, but not an 8 kb *LCB2* or the *LCB1* transcript, parallel increased enzyme activity, suggesting that enzyme activity is, at least in part, regulated by *LCB2* mRNA levels, with subsequent formation of Lcb2 protein. These studies demonstrate that induction of sphingolipid synthesis may be regulated at the level of transcription, and that UVB could up-regulate sphingolipid synthesis, either directly or indirectly, at both the mRNA and protein levels.

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