Ultraviolet B (UVB) induces phosphorylation of histone H3 at serine 10, and mitogen-activated protein kinase inhibitors are involved in this signal transduction pathway. Here we provide evidence that Fyn kinase, a member of the Src kinase family, is involved in the UVB-induced phosphorylation of histone H3 at serine 10. UVB distinctly increased Fyn kinase activity and phosphorylation. Fyn kinase inhibitors 4-amino-5-(4-chlorophenyl)-7(t-buty1)pyrazol(3,4-d)pyrida and leflunomide, an Src kinase inhibitor, suppressed both UVB-induced phosphorylation of histone H3 at serine 10 and Fyn kinase activity and phosphorylation. UVB-induced phosphorylation of histone H3 at serine 10 was blocked by either a dominant-negative mutant of Fyn (DNM-Fyn) kinase or small interfering RNA of Fyn kinase. UVB-induced phosphorylation and activities of ERKs and protein kinase B/Akt were markedly inhibited by DNM-Fyn kinase. However, DNM-Fyn kinase did not inhibit UVB-induced phosphorylation of p38 MAPK or c-Jun N-terminal kinases. Active Fyn kinase phosphorylated histone H3 at serine 10 in vitro, and the phosphorylated Fyn kinase could translocate into the nucleus of HaCaT cells. These results indicate that Fyn kinase plays a key role in the UVB-induced phosphorylation of histone H3 at serine 10.

Experimental evidence suggests that solar ultraviolet irradiation is the most important environmental carcinogen leading to the development of malignant skin melanoma (1–3). UV irradiation results in suppression of the immune system (4) and chronic skin damage including premature aging (5). According to wavelength, UV is divided into UVA4 (UVA I, 340–400 nm; UVA II 320–340 nm), UVB (280–320 nm), and UVC (180–280 nm). UVA and UVB are recognized as the chief carcinogenic components of sunlight with relevance to human skin cancer (1, 2). UVB irradiation has the ability to stimulate oncogene c-fos promoter expression (6), to accumulate c-fos mRNA and c-Fos protein, and to increase activator protein-1 activation (7). In addition, it also induces tumor suppressor p53 (8–10) and p16 (INK4A-ARF) expression (8). By using oligonucleotide microarray analysis, researchers found that several biological processes, including basal transcription, splicing, and translation, as well as proteasome-mediated degradation, are globally affected by UVB irradiation in human keratinocytes (11). These results demonstrate the complexity of the UVB response. Various signal transduction pathways, including extracellular signal-regulated kinases (ERKs), p38 kinase, and c-Jun N-terminal kinases (JNKs), are involved in UVB-induced cell proliferation or apoptosis (1, 7, 12–14). We recently found that UVB induces phosphorylation of histone H3 at serine 10 or serine 28 through MAP kinases or mitogen- and stress-activated protein kinase 1 (MSK1) (15, 16). However, much less is known about the UVB-induced phosphorylation of histone H3, especially the upstream mediators in the signal transduction pathway of UVB irradiation.

Modification of histones is an important element in the regulation of gene expression, and histone H3 phosphorylation at serine 10 has traditionally been regarded as a marker for mitosis (17, 18). Increased phosphorylation of histone H3 at serine 10 was found in mitogen-stimulated and oncogene-transformed mouse fibroblasts (19). Phosphorylation of H3 at serine 10 corresponds with chromatin relaxation and gene expression in interphase, whereas in mitosis it is associated with chromosome condensation (20). Thus to identify the responsible kinases and the circumstances under which histone H3 at serine 10 becomes phosphorylated is important.

Fyn kinase is a member of the Src family of nonreceptor protein-tyrosine kinases and is associated with outgrowth during development and regeneration of the central and peripheral nervous systems (21, 22). Fyn kinase can interact with numerous other signal molecules, and it has diverse biological functions including signaling via the T cell receptor, regulation of brain function, and adhesion-mediated signaling (23). Recent research indicates that Fyn kinase is expressed in T cells, and it is the first signaling molecule to be activated downstream of the T cell receptor (24). Fyn kinase is also involved in epidermal growth factor receptor signaling and disrupts the function of the integrin α6β4 at hemidesmosomes and regulates normal keratinocyte migration and squamous carcinoma invasion (25). In fact, more and more evidence shows that Src kinase family members interact with MAP kinases in response to various stimuli. For example, activation of MAP kinase by cAMP requires an Src family kinase (SRC, YES, and FYN) that lies downstream of protein kinase A in the murine fibroblast cell line SYF (26). Reactive oxygen species-induced JNKs activation is mediated by Src kinase (27), and reactive oxygen species activate p90 ribosomal S6 kinase via Fyn kinase (28). Overall, Src kinases interact widely with serine/threonine kinases when cells are exposed to a variety of stimuli.

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1. The abbreviations used are: UVA, ultraviolet A; ERKs, extracellular signal-regulated kinases; MEKs, protein tyrosine kinases; MEM, minimal essential medium; FBS, fetal bovine serum; MSKs, mitogen- and stress-activated protein kinases; GSK, glycogen synthase kinase; PVD, polyvinylidene difluoride; PBS, phosphate-buffered saline; UVB, ultraviolet B; siRNA, small interfering RNA; PP2, 4-amino-5-(4-chlorophenyl)-7(t-buty1)pyrazol(3,4-d)pyrida; DNM-Fyn, dominant-negative mutant of Fyn; DMEM, Dulbecco’s modified Eagle’s medium; WT, wild type; MAP, mitogen-activated protein; IKK, IκB kinase.

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To explore further the mechanism of UVB-induced carcinogenesis, we used UVB to induce phosphorylation of histone H3 at serine 10. We found that UVB induced phosphorylation of histone H3 at serine 10 in a time- and dose-dependent manner and dramatically increased Fyn kinase activity. Fyn kinase inhibitors, PP2 and lefunomide, an Src kinase inhibitor, inhibited UVB-induced Fyn kinase activity and phosphorylation of histone H3 at serine 10 in a dose-dependent manner. Dominant-negative (DNM) Fyn kinase and siRNA Fyn kinase interrupted UVB-induced phosphorylation of histone H3 at serine 10. DNM-Fyn kinase inhibited UVB-induced ERKs and Akt1 activities and phosphorylation. This study indicates that Fyn kinase plays a key role in UVB-induced phosphorylation of histone H3 at serine 10 through regulation of Akt1 and ERKs.

**EXPERIMENTAL PROCEDURES**

**Materials**—Eagle’s minimal essential medium (MEM), L-glutamine, and Lipofectamine<sup>10</sup> 2000 reagent were from Invitrogen. Plasmids of pCMV5-wild type-huFyn and pCMV5-huFyn-K299M mutant vector were kindly provided by Dr. Marilyn D. Resh (Sloan-Kettering Institute for Cancer Research, New York). Antibodies to detect phosphorylation of histone 3 at serine 10 and total histone H3 protein, phosphorylation of ERKs, p38 MAP kinase, and JNKs antibodies and nonphospho-Fyn kinase antibody were from Upstate Biotechnology, Inc. (Lake Placid, NY). Phospho-Fyn (Thr-12) was from Santa Cruz Biotechnology (Santa Cruz, CA). Fetal bovine serum (FBS) was from Gemini Bio-Product (Calabasas, CA). Gentamicin sulfate was from BioWhittaker, Inc. (Walkersville, MD). The Akt kinase assay kit and ERKs kinase assay kit were from Cell Signaling Technology Inc. Polyvinylidene difluoride (PVDF) membrane was from Millipore (Bedford, MA). The Folin and Ciocalteus phenol reagent and modified Lowry protein assay kit (Calabasas, CA). Gentamicin sulfate was from BioWhittaker, Inc. (Walkersville, MD). The Akt kinase assay kit and ERKs kinase assay kit were from Cell Signaling Technology Inc. Polyvinylidene difluoride (PVDF) membrane was from Millipore (Bedford, MA). The Folin and Ciocalteus phenol reagent and modified Lowry protein assay kit were from Pierce. The sense and antisense oligonucleotides for siRNA of Fyn kinase were synthesized by Sigma.

**Cell Culture**—The JB6 Cl41 mouse epidermal cell line, dominant-negative (DN)-Fyn kinase, and wild type (WT) Fyn stably transfected JB6 cells were cultured as adherent monolayers in MEM supplemented negative (DN)-Fyn kinase, and wild type (WT) Fyn stably transfected kinase were synthesized by Sigma.

**Establishing the Stably Transfected DNM-Fyn-JB6 Cells**—By using plasmids pCMV5-wild type-huFyn and pCMV5-huFyn-K299M mutant vector, we established these stable transfections according to the protocol from Invitrogen. All of these cells were selected in media containing 500 µg/ml G418 for 2 weeks, and the G418 concentration was then decreased to 250 µg/ml and maintained. G418-selected cells were tested for phosphorylation and activity of Fyn kinase.

**FIG. 1.** UVB-induced phosphorylation of histone H3 at serine 10 in a time- and dose-dependent manner. HaCaT cells were starved for 48 h in 0.1% FBS/DMEM at 37 °C in a 5% CO<sub>2</sub> atmosphere. Cells were then incubated in fresh 0.1% FBS/DMEM for another 2 h before being treated with UVB and harvested at the indicated time (A) or treated with UVB at the indicated dose and harvested after treatment for 30 min (C). Acidic proteins were extracted as described under “Experimental Procedures.” By using Western blot analysis, phosphorylation of histone H3 at serine 10 was detected by using the Storm PhosphoImager analysis system (Amersham Biosciences). The level of total histone H3 protein (B and D) was determined as described under “Experimental Procedures.”

**Akt Kinase Assay**—The ability of DNM-Fyn kinase to inhibit UVB-induced phosphorylation of Akt was tested by using an Akt kinase assay kit (Cell Signaling Technology Inc.). In brief, cells were treated with UVB at various doses for the desired times. Cells were washed once with ice-cold PBS after removing the media, and then 0.5 ml of 1× ice-cold cell lysis buffer plus 1 mM phenylmethylsulfonyl fluoride was added to each plate, and the cells were scraped and transferred to fresh tubes. Cells were sonicated on ice four times for 5 s each and centrifuged for 10 min at 4 °C, and the supernatant fractions were transferred to other fresh tubes. Cell lysate protein (200 µg) and beads (20 µl) with immobilized Akt1 1G1 monoclonal antibody were added together by gently rocking for 3 h at 4 °C. These tubes were centrifuged for 1 min at 4 °C and washed twice with 500 µl of cell lysis buffer and then washed with 500 µl of 1× kinase buffer. The pellets were suspended in 20 µl of 1× kinase buffer supplemented with 200 µM ATP and 2 µM of GSK-3 fusion protein and incubated for 45 min at 30 °C. The reaction was terminated with 10 µl of 3× SDS sample buffer. The samples were denatured at 95–100 °C for 5 min before they were separated by 8% SDS-PAGE. The proteins were transferred to PVDF membranes, and Akt1 kinase activity was analyzed by Western blotting using a phospho-GSK-3α/β (serine 21/22) antibody.

**ERKs MAP Kinase Assay**—The ability of DNM-Fyn kinase to inhibit UVB-induced phosphorylation of ERKs was tested using a p44/p42 MAP kinase assay kit (Cell Signaling Technology Inc.). The treatment and lysis of cells were performed as described above. Cell lysate protein (200 µg) and immobilized phospho-p44/p42 MAP kinase (Thr-202/Tyr-204) antibody beads (20 µl) were added together by gently rocking for 3 h at 4 °C. These tubes were centrifuged and washed twice. The pellets were suspended in 20 µl of 1× kinase buffer supplemented with 200 µM ATP and 2 µg of Elk-1 fusion protein and incubated for 45 min at 30 °C. The reaction was terminated with 10 µl of 3× SDS sample buffer. The samples were denatured at 95–100 °C for 5 min before they were separated by 10% SDS-PAGE. The proteins were transferred to PVDF membranes, and ERK1/2 kinase activity was analyzed by Western blotting using a phospho-ERK1/2 (serine 38/29) antibody.

**ERKs Kinase Assay**—UVB-induced activation of Fyn kinase was detected by a 32P-labeled radioactive method. In brief, 200 µg of cell lysate protein was mixed with protein-A/G beads (20 µl) in advance for 1 h at 4 °C. The mixture was centrifuged at 12,000 rpm for 5 min at 4 °C, and the supernatant fraction was added to phospho-Fyn (Thr-12) antibody (20 µl) and gently rocked for 3 h at 4 °C. These tubes were centrifuged and washed twice. The pellets were suspended in 20 µl of 1× kinase buffer supplemented with 10 µl of diluted [γ-<sup>32</sup>P]ATP solution and 2.5 µl of Src substrate peptide (250 µM) and incubated for 30 min at 30 °C. A 20-µl aliquot was transferred onto PS1 paper and washed three times with 0.75% phosphoric acid for 5 min per wash and one time with acetone for 2 min. Radioactive incorporation was determined by scintillation counter. The same experiments were repeated three times.
Small Interfering RNA (siRNA) Against Fyn Kinase and Establishing the Stable Expression Cell Line—To study further the role of Fyn kinase in the regulation of phosphorylation of histone H3 at serine 10, we successfully “knocked down” Fyn kinase expression levels by the siRNA method. The sense oligonucleotide of Fyn kinase used for siRNA was 5'-TTTGCTCTCGGAAGGAGATTGGTTCAAGAGACAAATCTCTCCGAGCTGTTT TT-3', and the antisense is 5'-CTTAGAAAAACAGCTCGGAAGGAGATTGGTTCTCTTGAAACCAATCTCTTCGAGCTG-3'. In the sense sequence, the boldface letters indicate a Bbs1 endonuclease site, and italic underlined letters show the RNA loop. In the antisense sequence, the boldface letters show an XbaI endonuclease site. The ligated pair of oligonucleotides was inserted into the mU6pro vector. Annealing, ligation, and colony screening were done as described previously (30). The oligonucleotide synthesis and sequencing of the inserted sequences in the mU6pro vector were performed by Sigma. The reconstructed plasmid with mU6pro vector contained the siRNA Fyn kinase sequence and is named siRNA-Fyn-mU6pro. The plasmid siRNA-Fyn-mU6pro and pcDNA3.1 were stably transfected into JB6 Cl41 cells using the Lipofectamine™ 2000 reagent. The transfected cells were selected with G418 as described previously, and Fyn kinase expression level was confirmed by a Western blot assay using an anti-Fyn kinase antibody (Upstate Biotechnology, Inc., Lake Placid, NY).

### RESULTS

UVB Induces Phosphorylation of Histone H3 at Serine 10 in a Time- and Dose-dependent Manner—HaCaT keratinocytes were derived from adult human skin, and although spontaneously immortalized, they remain highly related to their normal cell counterparts, and it is an ideal cell line for studying the phosphorylation of histone H3 at serine 10. To study the role of Fyn kinase in this process, HaCaT cells were treated with UVB and harvested at the indicated times. We found that UVB-induced Fyn kinase activity increased in a time- and dose-dependent manner. The phosphorylation of histone H3 at serine 10 was also increased in a similar manner. These results suggest that Fyn kinase plays a role in the phosphorylation of histone H3 at serine 10 in response to UVB irradiation.
effects of UVB on human skin (31, 32). In this study, HaCaT cells were used to analyze the UVB-induced phosphorylation of histone H3 at serine 10. The time response study indicated that phosphorylation of histone H3 at serine 10 gradually increases from 15 to 30 min following treatment with UVB (4 kJ/m²) (Fig. 1A). The level of total histone H3 protein did not change (Fig. 1B). The dose-response study indicated that phosphorylation of histone H3 gradually increases after treatment with increasing doses of UVB (1, 2, or 4 kJ/m²) (Fig. 1C) with no effect on total histone H3 protein levels (Fig. 1D). These results indicate that phosphorylation of histone H3 at serine 10 is induced by UVB in a dose- and time-dependent manner.

**UVB Strongly Increases Fyn Kinase Activity and Phosphorylation of Fyn Kinase at Thr-12**—Our results also showed that UVB induced Fyn kinase activity in a time- (Fig. 2A) and dose-dependent (Fig. 2B) manner. We found that a 21-fold increase in Fyn kinase activity occurred in UVB-treated cells compared with untreated control cells (Fig. 2A). Fyn kinase activity reached a maximum level when cells were treated with 1 kJ/m² of UVB, and doses up to 4 kJ/m² produced a similar induction of activity. Because we already reported that 4 kJ/m² of UVB distinctly induced phosphorylation of histone H3 at serine 28 or serine 10 in JB6 cells (15, 16), a dose of 4 kJ/m² of UVB was used to further study the stress function of UVB. UVB induced phosphorylation of Fyn kinase (Thr-12) also in a time- and dose-dependent manner (Fig. 3, A and C), and non-phospho-Fyn kinase was unchanged (Fig. 3, B and D).

**Fyn Kinase Inhibitors Greatly Suppress UVB-induced Phosphorylation of Histone H3 at Serine 10**—The above data indicate that UVB induces phosphorylation of histone H3 at serine 10 and simultaneously induces Fyn kinase activity. Therefore, we hypothesized that Fyn kinase would play a key role in the UVB-induced phosphorylation of histone H3 at serine 10, and H3 phosphorylation would be changed by inhibition of Fyn kinase. In this study, we found that PP2, a Fyn kinase inhibitor (33), and leflunomide, an Src kinase inhibitor (34), markedly inhibited the UVB-induced phosphorylation of histone H3 at serine 10 in a dose-dependent manner (Fig. 4, A and C, respectively) but did not change the total histone H3 protein level (Fig. 4, B and D). This result not only confirms our hypothesis but also indicates that Fyn kinase plays an important role in UVB-induced phosphorylation of histone H3 at serine 10.

**Fyn Kinase Inhibitors Suppress Fyn Kinase Activity and Phosphorylation**—Because Fyn kinase inhibitors markedly suppressed UVB-induced phosphorylation of histone H3 at serine 10, we determined whether these inhibitors can affect Fyn kinase activity and phosphorylation under the same conditions. We found that either PP2 or leflunomide decisively inhibited UVB-induced Fyn kinase activity (Fig. 5A) and phosphorylation (Fig. 5B) in a dose-dependent manner.

**Dominant-negative (DNM) Mutant Fyn Kinase Inhibits UVB-induced Phosphorylation of Histone H3 at Serine 10**—To further explore the role of Fyn kinase in UVB-induced phosphorylation of histone H3 at serine 10, we used DNM-Fyn and WT-Fyn plasmids to establish stably expressed DNM-Fyn and WT-Fyn transfected JB6 cells. Our data show that phosphorylation of histone H3 at serine 10 induced by UVB was obviously inhibited in DNM-Fyn-transfected cells (Fig. 6C), but the total histone H3 protein level did not change (Fig. 6D). Meanwhile, DNM-Fyn also distinctly inhibited UVB-induced phosphorylation of Fyn kinase (Fig. 6A), but the total Fyn kinase protein level was also unchanged (Fig. 6B). These results indicate that Fyn kinase plays a very important role in mediating the phosphorylation of histone H3 at serine 10 induced by UVB.

**Small Interfering RNA (siRNA) Fyn Kinase Inhibits UVB-induced Phosphorylation of Histone H3 at Serine 10**—We also used the siRNA method to knock down the Fyn kinase expression level, and we then determined the effects on UVB-induced phosphorylation of histone H3 at serine 10. First, we confirmed the efficiency of siRNA for knocking down Fyn kinase level using Western blotting and an anti-Fyn antibody. Our results indicated that the Fyn kinase protein level decreased to 5% of that observed in control cells (Fig. 7A). β-Actin protein level was used to monitor equal protein loading in each well (Fig. 7B). These results indicate that the siRNA-Fyn kinase works well in vitro. Further results indicated that UVB increased phosphorylation of histone H3 at serine 10 in the “mock” mU6pro vector plus pcDNA3.1 vector-transfected cells but not in the siRNA-Fyn (mU6pro plus pcDNA3.1 vector) stably transfected cells in a time- and dose-dependent manner (Fig. 7, C and E). Total histone H3 levels were unchanged (Fig. 7, D and F).

**DNM Fyn Kinase Inhibits UVB-induced Phosphorylation of Akt (Serine 473) and ERKs (Thr-202/Tyr-204)**—Fyn kinase is involved in UVB-induced phosphorylation of histone H3 at serine 10; however, the signal transduction components mediated by Fyn kinase leading to that phosphorylation are unknown. We hypothesized that Fyn kinase is located upstream of the known UVB-stimulated signal transduction pathway,
and Fyn kinase can regulate several downstream kinases, which are then translocated into the nucleus where they phosphorylate histone H3 in vivo. We assessed the status of several MAP kinases and Akt phosphorylation in WT-Fyn cells and DNM-Fyn cells. We found that UVB only stimulated ERKs (Thr-202/Tyr-204) and Akt (serine 473) phosphorylation (Fig. 8, E and G) but not p38 MAP kinase or JNKs (Fig. 8, A and C) in WT-Fyn cells. ERKs and Akt were not affected by UVB in DNM-Fyn cells. The total protein levels of p38 MAP kinase, ERKs, JNKs and Akt were unchanged in either cell type (Fig. 8, B, D, F, and H).

**Fig. 5.** The Fyn kinase inhibitors, PP2 and leflunomide, repressed UVB-induced Fyn kinase activity and phosphorylation. HaCaT cells were pretreated with Fyn kinase inhibitor PP2 or leflunomide and then treated with UVB (4 kJ/m²) as described above. Fyn kinase activity was performed as described under “Experimental Procedures.” A, the phosphorylation of Fyn kinase at Thr-12 was analyzed by Western blot using a phospho-Fyn (Thr-12) antibody (B). Compared with UVB-treated cells, * indicates p < 0.01.

**Fig. 6.** Dominant-negative Fyn kinase inhibited UVB-induced phosphorylation of Fyn kinase and phosphorylation of histone H3 at serine 10. DNM-Fyn cells and wild type (WT) Fyn cells were treated with UVB (4 kJ/m²) after 48 h starvation in 0.1% FBS/MEM. At various times (5, 15, or 30 min) after UVB treatment, cell lysates were used to determine the phosphorylated or nonphosphorylated level of Fyn kinase (Thr-12) using a phospho-Fyn (Thr-12) or Fyn kinase antibody, respectively (A and B). Acidic proteins were extracted as described under “Experimental Procedures.” Phosphorylation of histone H3 at serine 10 was detected with a phospho-histone H3 (serine 10) antibody (C). The level of total histone H3 protein was detected with a nonphospho-histone H3 antibody (D).

**DNM-Fyn Kinase Inhibited Akt and ERKs Activity**—To confirm the results described above (Fig. 8), we further examined UVB-stimulated activity of ERKs and Akt in WT-Fyn and DNM-Fyn cells. A nonradioactive Akt and p44/p42 MAP kinase assay kit was used to test for Akt activity and ERKs activity, respectively. The Akt kinase substrate was GSK3, and the ERKs substrate was Elk1. Our results indicated that phosphorylation of GSK3-α and -β decreased in UVB-treated DNM-Fyn-JB6 cells (Fig. 9A). The nonphosphorylated protein level of GSK3 was unchanged (Fig. 9B). Phosphorylation of Elk1 dramatically decreased in UVB-treated DNM-Fyn-JB6 cells com-
pared with WT-Fyn-JB6 cells (Fig. 9C), and total Elk1 protein level was unchanged (Fig. 9D). These data showed that DNM-Fyn kinase also inhibited UVB-induced Akt and ERKs activity.

**Active Fyn Kinase Phosphorylated Histone H3 at Serine 10 in Vitro**—Furthermore, we tested whether Fyn kinase could directly phosphorylate histone H3 at serine 10 in vitro. We used pure histone H3 protein as the Fyn kinase substrate, and we incubated it with active Fyn kinase and then analyzed phosphorylation of histone H3 at serine 10 by Western blot. Our results showed that active Fyn kinase strongly phosphorylated histone H3 at serine 10 in vitro (Fig. 10A) in a dose-dependent manner. Totallab software was used to analyze the density value (Fig. 10B). The nonphosphorylated level of total histone H3 protein was unchanged (Fig. 10C). These data indicate that Fyn kinase can phosphorylate the histone H3 protein at serine 10 in vitro.

**Phosphorylated Fyn Kinase Is Translocated into the Nucleus**—To determine whether Fyn kinase can phosphorylate histone H3 in vivo, we first confirmed that the phosphorylated Fyn kinase (Thr-12) can be translocated to the nucleus from the cytosol in HaCaT cells. Thirty minutes after UVB treatment, phosphorylated Fyn kinase (Thr-12) increased in both the cytosol and nucleus (Fig. 11A). To confirm that the nuclear proteins were not mixed with cytosolic proteins, the samples were analyzed by Western blot using antibodies against p38 kinase (A), JNKs (C), Akt (E), and ERKs (G), respectively. Total p38 kinase, JNKs, Akt1, and ERKs protein levels were determined by Western blot using antibodies against p38 kinase (B), JNKs (D), Akt (F), and ERKs (H), respectively.

**DISCUSSION**

Fyn kinase is a member of the Src family of tyrosine kinases, and it has diverse biological functions, including signaling via the T cell receptor, regulation of brain function, and adhesion-mediated signaling (21–24). In the present study, we found that Fyn kinase plays a key role in the regulation of UVB-induced phosphorylation of histone H3 at serine 10.
studies show that UVB induces phosphorylation of histone H3 at serine 10 or serine 28 in JB6 cells, which is mediated through MAP kinases or mitogen- and stress-activated protein kinase (MSK1) (15, 16). However, little is known about the upstream mediators in UVB-induced phosphorylation of histone H3. To characterize further the carcinogenic effect of UVB and explore the mechanism of UVB-induced phosphorylation of histone H3 at serine 10, we used the HaCaT cell line. The HaCaT cell line is a spontaneously transformed human epithelial cell line derived from human adult skin, and it maintains full epidermal differentiation capacity (35). This cell line is obviously immortal and has a transformed phenotype in vitro but remains a nontumorigenic human keratinocyte (35). An early study indicates that sublethal doses of UVB produce a strong induction of c-jun and c-fos transcripts in HaCaT cells (36). Here we found that UVB strongly induced phosphorylation of histone H3 at serine 10 in human HaCaT cells in a dose- and time-dependent manner (Fig. 1). UVB also markedly increased Fyn kinase activity (Fig. 2) and phosphorylation (Fig. 3) in a time- and dose-dependent manner. Another study showed that UVB irradiation results in the up-regulation of the transcription factor activator protein-1 in HaCaT cells and specifically increased c-fos and JunD expression (37). Moreover, active immediate-early gene expression including that of the proto-oncogene c-fos is associated with phosphorylation of histone H3 at serine 10 (37, 38). Our results clearly indicate that UVB induces phosphorylation of histone H3 at serine 10 in the human keratinocyte HaCaT cell line, and Fyn kinase activity plays a key role in the UVB-induced phosphorylation of histone H3 at serine 10.

To confirm further that Fyn kinase is involved in UVB-induced phosphorylation of histone H3 at serine 10, PP2, a Fyn kinase inhibitor (33), and leflunomide, an Src family kinase inhibitor (34), were employed to suppress Fyn kinase activity. Our data indicate that PP2 and leflunomide also inhibited UVB-induced phosphorylation of histone H3 at serine 10 in a dose-dependent manner (Fig. 4). Under the same conditions, PP2 and leflunomide also inhibited UVB-induced Fyn kinase activity (Fig. 5 A) and phosphorylation at Thr-12 (Fig. 5 B). These results strongly illustrate that Fyn kinase is involved in UVB-stimulated phosphorylation of histone H3 at serine 10.

To exclude nonspecific characteristics of chemical inhibitors, we transfected WT-Fyn and DNM-Fyn plasmids into JB6 Cl41 cells, which are derived from mouse skin and are a well developed cell culture model for studying tumor promotion (39, 40). In the present study, we found that Fyn kinase-deficient cells inhibited UVB-induced phosphorylation of Fyn kinase at Thr-12 (Fig. 6 A) and phosphorylation of histone H3 at serine 10.
induced by UVB (Fig. 6C). Moreover, we further confirmed the importance of Fyn kinase function in UVB-induced phosphorylation of histone H3 at serine 10 by the siRNA method. Our results indicated that UVB-induced phosphorylation of histone H3 was also dramatically inhibited by siRNA Fyn kinase compared with control cells transfected with only the vector plasmid (Fig. 7, C and E). Fyn kinase has many functions. For example, Fyn is one of the signals involved in regulating myelination during development (41), and morphological differentiation of oligodendrocytes requires activation of Fyn tyrosine kinase (42). Here our results clearly indicate that Fyn kinase is a key mediator in regulating the UVB-induced phosphorylation of histone H3 at serine 10.

To explore the mechanism of Fyn kinase in the regulation of phosphorylation of histone H3 at serine 10, we determined the phosphorylation level of other kinases. All of these kinases are associated with phosphorylation of histone H3 at serine 10 (15, 16). Our results showed that DNM Fyn kinase inhibited UVB-induced ERKs and Akt1 phosphorylation (serine 473) (Fig. 8, E and G), but phosphorylation levels of p38 MAP kinase and JNKs were unchanged (Fig. 8, A and C) compared with wild type Fyn plasmid-transfected cells. Moreover, UVB-induced Akt1 (Fig. 9A) and ERKs activity (Fig. 9C) were both distinctly restrained in DNM-Fyn kinase cells. These data indicate that Fyn kinase regulates UVB-induced phosphorylation of histone H3 at serine 10 through Akt1 and ERKs. A previous study showed that Akt1 is involved in arsenite-induced phosphorylation of histone H3 at serine 10 (29). In the present study, we provide evidence indicating that Akt1 is also involved in UVB-induced phosphorylation of histone H3 at serine 10, and its upstream kinase is Fyn kinase. Recent studies indicate that Akt1 associates with and activates IkB kinase (IKK) (43–45). IKK-α kinase directly phosphorylates histone H3 in vivo, and histone H3 phosphorylation by IKK-α is critical for cytokine-induced gene expression (46). Moreover, our results indicate that Fyn kinase can also phosphorylate histone H3 protein at serine 10 in vitro (Fig. 10), and the phosphorylated Fyn kinase can be translocated to the nucleus from the cytosol (Fig. 11). These results strongly indicate that Fyn kinase probably phosphorylates histone H3 at serine 10 in vivo and also suggest that Fyn kinase may regulate UVB-induced phosphorylation of histone H3 at serine 10 by itself and through the Akt1/IKK-α pathway. On the other hand, DNM-Fyn kinase also inhibited UVB-induced phosphorylation of MSK1 (data not shown), which is involved in mediation of histone H3 phosphorylation and is downstream of ERKs (47). A recent study indicated that oxidase-dependent reactive oxygen species signaling and ERK1/2 phosphorylation are both controlled by Fyn kinase activation (48). Fyn kinase is still required for activation and phosphorylation of ERK1/2 stimulated by insulin-like growth factor-I (49) or thrombin (50). Fyn kinase regulates UVB-induced phosphorylation of histone H3 at serine 10 also through the Fyn/ERKs/MSK1 pathway.

The present study shows that Fyn kinase is involved in UVB-induced phosphorylation of histone H3 at serine 10 and that Fyn kinase is another important regulator that mediates the UVB-induced phosphorylation of histone H3 at serine 10. UVB also induces phosphorylation of histone H3 at serine 10 through a p38 MAP kinase pathway (15) (Fig. 12). This study provides powerful evidence that illustrates a probable mechanism of UVB in carcinogenesis in cells and animal models. However, many questions, including how UVB light enters into cells, still need to be answered.

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Fyn Kinase Regulates UVB-induced Phosphorylation of Histone H3

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