MAP Kinases Mediate UVB-induced Phosphorylation of Histone H3 at Serine 28*

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Histone H3 phosphorylation is closely related to chromatin remodeling and chromosome condensation. H3 phosphorylation at serine 28 is coupled with mitotic chromosome condensation in diverse mammalian cell lines. However, the pathway that mediates phosphorylation of H3 at serine 28 is unknown. In the present study, ERK1, ERK2, or p38 kinase strongly phosphorylated H3 at serine 28 in vitro. JNK1 or JNK2 were also able to phosphorylate H3 at serine 28 in vitro, but to a lesser degree. UVB irradiation markedly induced phosphorylation of H3 at serine 28 in JB6 C1 41 cells. PD 98059, a MEK1 inhibitor, and SB 202190, a p38 kinase inhibitor, efficiently repressed UVB-induced H3 phosphorylation at serine 28. Expression of dominant negative mutant (DNM) ERK2 in JB6 C1 41 cells totally blocked UVB-induced phosphorylation of H3 at serine 28. Additionally, DNM p38 kinase or DNM JNK1 partially blocked UVB-induced H3 phosphorylation at serine 28. Furthermore, UVB-induced H3 phosphorylation at serine 28 was inhibited in Jnk1−/− cells, but not in Jnk2−/− cells. These results suggest that UVB-induced H3 phosphorylation at serine 28 may be mediated by MAP kinases.
Histones are relatively small proteins with a very high proportion of positively charged amino acids (lysine and arginine); the positive charge helps the histones bind tightly to DNA, regardless of its nucleotide sequence (1). The five types of histones (H1, H2A, H2B, H3 and H4) fall into two main groups -- core histones and linker histones. Core histones are wrapped by DNA as octamers, consisting of two H2A-H2B dimers and a tetramer of H3-H4 (2-4). A number of studies previously reported that histones can be modified by acetylation and phosphorylation (5-7) and the subsequent function(s) of these modifications have begun to be understood (8-16). The histone modifications may alter chromatin structure by influencing histone-DNA and histone-histone contacts (17-19). The level of acetylated histone is regulated by histone acetyltransferases (HAT) and histone deacetylases (HDACs) (10, 20, 21). The p300/CBP HAT was described initially as a transcriptional coactivator that functions by interacting with a wide variety of enhancer-binding proteins (22) and the HDAC/Rpd3 family of HDACs is correlated with transcriptional regulatory proteins (23, 24).

The phosphorylation of histone H3 is thought to be a highly conserved event among eukaryotes and is probably involved in transcriptional regulation and chromosome condensation during mitosis and meiosis (15, 25, 26). Two phosphorylation sites are present in the N-terminal of histone H3, serine 10 and serine 28. Previous studies showed that H3 phosphorylation at serine 10 was associated with mitosis in diverse types of eukaryotic cells and with chromosome condensation during mitosis and meiosis (5, 10, 15, 27-29). H3 phosphorylation at serine 10 occurs concurrently with the transcriptional activation of the early genes c-fos and c-jun (25, 26) and induction of ras expression results in a rapid increase in H3 phosphorylation at serine 10 (30, 31). Various stimuli, including epidermal growth factor, 12-0-tetradecanoylphorbol-13-
acetate, anesomycin and okadaic acid and stresses such as UV irradiation, induce rapid H3 phosphorylation in mammalian cells (6, 25, 26, 32). The pathway responsible for mediating H3 phosphorylation at serine 10 depends on the type of stimulation (25, 26, 32) and phosphorylation of serine 10 in histone H3 is also functionally linked in vitro and in vivo to acetylation of histone at lysine 14 (33). H3 at serine 28 is phosphorylated during early mitosis and with mitotic chromosome condensation in various mammalian cell lines (34). However, the kinase that is responsible for H3 phosphorylation at serine 28 remains unknown. Here, we investigated the role of MAP kinases in phosphorylation of H3 at serine 28 in vitro and in vivo after UVB irradiation.

MATERIALS AND METHODS

Reagents and Antibodies—Minimum essential medium (MEM) and fetal bovine serum (FBS) were from Biowhittaker Biosciences; L-glutamine was from Life Technologies, Inc.; gentamicin was from Quality Biological; Bradford reagent was from Bio-Rad Laboratories; PD 98059 and SB 202190 were from Calbiochem-Novabiochem Co.; phenylmethylsulfonyl fluoride (PMSF) was from Sigma; pure histone H3 was from Boehringer Mannheim Inc.; antibody-conjugated alkaline phosphatase (AP) and antibodies for phosphorylated ERKs, p38 kinase, and JNKs were from New England Biolabs; antibody for H3 was from Upstate Biotechnology Inc; antibody for phosphorylated H3 at serine 28 was produced and identified as described previously (34); active ERK1, ERK2, p38 kinase, JNK1 and JNK2 were from Upstate Biotechnology Inc.

Phosphorylation Assay of Histone H3 In Vitro—Phosphorylation of histone H3 by activated ERK1, ERK2, p38 kinase, JNK1 or JNK2 was carried out as described previously (32, 35). In brief, pure histone H3 or chromatin of JB6 Cl 41 cells was incubated with ERK1, ERK2,
p38 kinase, JNK1 or JNK2 and 200 µM ATP in 50 µl kinase buffer (25 mM Tris, pH 7.5, 5 mM β-glycerolphosphate, 2 mM DTT, 0.1 mM Na3VO4, 10 mM MgCl2) for 45 min at 30 °C. The samples were resolved by 15% SDS-PAGE and phosphorylated H3 at serine 28 was detected by western blotting with a specific antibody (32, 34).

**UVB Irradiation**—Equivalent numbers of cells were seeded in 10-cm dishes and cultured in 5% FBS MEM until they reached 85% confluence and then were starved in 0.1% FBS MEM for 48 h. Cells were then incubated for 2 h in fresh 0.1% FBS MEM, after which time they were exposed to UVB and then cultured for additional time periods. Because the normal UVB lamp also generates a small amount of UVC light, the UVB irradiation was carried out in a UVB exposure chamber with a Kodak Kodacel K6808® filter that eliminates all wavelengths below 290 nm.

**Acid-soluble Protein Extraction**—Following UVB irradiation, the media were removed. The cultured cells were harvested and washed two times with cold phosphate buffered saline (PBS). Acid-solution protein extraction was carried out as described by the protocol of Upstate Biotechnology (www.upstatebiotech.com). In brief (32), acid-soluble proteins were extracted with lysis buffer [10 mM HEPES pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 1.5 mM PMSF, 0.5 mM dithiothreitol (DTT)] and then H2SO4 was added to a final concentration of 0.2 M (0.4 N) and the protein solutions were left on ice for 60 min. Supernatant fractions were transferred to fresh microfuge tubes after centrifugation at 14,000 rpm/10 min and precipitated on ice for 45 min with a final concentration of 20% trichloroacetic acid. These tubes were centrifuged at 14,000 rpm/10 min at 4 °C and the pellets were washed once with acidic acetone and then once with...
acetone. The protein concentration was measured by the Bradford method (36) and the acid-soluble proteins were stored at −20 °C.

Assay of Phosphorylated H3—Acid-soluble proteins were resolved by 15% SDS-PAGE after boiling for 5 min in SDS sample buffer. Resolved acid-soluble proteins were transferred to PVDF membranes. PVDF membranes were blocked with 5% nonfat dry milk in PBS for 1 h at room temperature and incubated overnight at 4 °C with the polyclonal antibody against H3 or the monoclonal antibody against phosphorylated H3 at serine 28. The second antibody against rabbit or rat IgG conjugated-AP, respectively, was incubated with the respective membrane for 4 h at 4 °C. Membrane-bound proteins were detected with chemiluminescence (ECF of Amersham Pharmacia Biotech) and analyzed using the Storm 840 Scanner (Molecular Dynamics Inc.).

RESULTS

Phosphorylation of Histone H3 In Vitro—Similar to phosphorylation of histone H3 at serine 10, phosphorylation of histone H3 at serine 28 plays a key role during early mitosis and coincides with the initiation of mitotic chromosome condensation (34). To determine the role of MAP kinases in mediating H3 phosphorylation at serine 28, we incubated pure histone H3 protein with each of the active MAP kinases (ERK1, ERK2, p38 kinase, JNK1 or JNK2) and 200 µM ATP (32, 35). Phosphorylated H3 at serine 28 was detected by a specific monoclonal antibody as before (32, 34). The results show that pure histone H3 at serine 28 was strongly phosphorylated by ERK1 (Fig. 1A), ERK2 (Fig. 1B) or p38 kinase (Fig. 1C) and to a comparatively lesser degree by JNK1 (Fig. 1D) or JNK2 (Fig. 1E) in vitro. Similar results were also found by using chromatin as substrate for these MAP kinases (data not shown).
Phosphorylation of Histone H3 at Serine 28 Following UVB Irradiation—Our previous study showed that UVB could induce phosphorylation of histone H3 at serine 10 in JB6 C1 41 cells (32). To investigate the signal transduction pathways responsible for H3 phosphorylation at serine 28 in vivo, we exposed mouse epidermal JB6 cells to UVB irradiation and then extracted acid-soluble proteins for detection of H3 phosphorylation at serine 28 by western blot with a specific antibody (32, 34). The results show that UVB strongly induced H3 phosphorylation at serine 28 (Fig. 2, A and B). The dose response study showed that H3 phosphorylation at serine 28 increased with UVB exposure from 1 to 6 kJ/m² (Fig. 2A). H3 phosphorylation at serine 28 was greater at 30 or 60 min than at 15 or 120 min following UVB irradiation (Fig. 2B). We found previously that phosphorylation of H3 at serine 10 was higher at 15 or 30 min than at 60 min (32), and the level of phosphorylated H3 at serine 28 was higher at 60 min compared to phosphorylation at serine 10 at 60 min (32) following UVB irradiation. This difference in the phosphorylation time courses between H3 at serine 28 and serine 10 suggests that phosphorylation of H3 at serine 28 and serine 10 may be mediated by different pathways. These results indicate that UVB-induced H3 phosphorylation at serine 28 is dose- and time-dependent.

Inhibition of UVB-induced Phosphorylation of Histone H3 at Serine 28 by PD 98059 and SB 202190—MAP kinases, including ERKs, p38 kinase and JNKs, are mediators of signal transduction from the cell surface to the nucleus. We previously showed that UVB strongly induced phosphorylation of ERKs, p38 kinase and JNKs in JB6 C1 41 cells (32). To determine the possible role of MAP kinases in mediating UVB-induced H3 phosphorylation at serine 28 in vivo, we first examined the influence of specific chemical inhibitors on UVB-induced H3
phosphorylation at serine 28 in JB6 C1 41 cells. PD 98059 is a specific inhibitor of the activation of MEK1 in vivo and in vitro (37-39). Previous studies demonstrated that PD 98059 specifically inhibits the activation and phosphorylation of ERKs (6, 37-40) and 50 µM PD 98059 totally blocks activation of ERKs (22), but not JNKs or p38 kinases (40, 41). Our results showed that 25 µM PD 98059 markedly inhibited UVB-induced phosphorylation of H3 at serine 28 (Fig. 3A). This result implies that ERKs may be involved in the UVB-induced phosphorylation of H3 at serine 28. SB 202190 is a specific inhibitor of p38 kinase (26, 40, 41) and pretreatment of cells with 0.5-4 µM SB 202190 almost totally blocked UVB-induced phosphorylation of H3 at serine 28 (Fig. 3B). A high concentration of SB 202190 (40 µM) can inhibit activation of ERKs, but 10 µM SB 202190 has almost no effect on ERKs phosphorylation (39). Therefore, we used low concentrations of SB 202190 (0.5-4 µM), which selectively blocks activation of p38 kinase, to inhibit p38-mediated H3 phosphorylation at serine 28. The above data indicate that UVB-induced phosphorylation of H3 at serine 28 may be mediated by ERKs and p38 kinase in vivo.

Inhibition of UVB-induced Phosphorylation of Histone H3 at Serine 28 by Expression of DNM ERK2, DNM p38 kinase and DNM JNK1—Previous studies showed that overexpression of dominant negative mutant (DNM) ERK2, DNM p38 kinase, or DNM JNK1 markedly inhibited activation of endogenous ERKs (35, 40, 42, 43), p38 kinase (44, 45) or JNKs (46), respectively. To identify the role of MAP kinases in UVB-induced H3 phosphorylation at serine 28 in vivo, we used cells expressing these mutant kinases to investigate the role of the different MAP kinases in UVB-induced H3 phosphorylation at serine 28. Compared to JB6 C1 41 cells (Fig. 4A), cells expressing DNM ERK2 totally blocked UVB-induced H3 phosphorylation at serine 28 at 60 min following UVB irradiation, and DNM p38 or DNM JNK1 also markedly
suppressed UVB-induced H3 phosphorylation at serine 28 by approximately 70-80% (Fig. 4, B and C). In contrast, phosphorylation of H3 at serine 28 at 60 min increased approximately 2-fold in UVB-treated JB6 C1 41 cells (Fig. 4, A-C). The inhibition of UVB-induced phosphorylation of H3 at serine 28 in DNM-ERK2, DNM-p38 and DNM-JNK1 cells also was dependent on UVB-dose (Fig. 5, A-C). However, inhibition of phosphorylation of H3 at serine 28 by DNM ERK2 cells (Fig. 5A) was stronger than that by DNM p38 (Fig. 5B) or DNM JNK1 cells (Fig. 5C).

Inhibition of Phosphorylation of Histone H3 at Serine 28 in Jnk1, but not Jnk2 Knockout Cells—We also used Jnk1 (Jnk1-/-) and Jnk2 (Jnk2-/-) knockout cells and Jnk wild-type (Jnk+/+) cells to examine the role of JNKs in UVB-induced H3 phosphorylation at serine 28. The results showed that UVB-induced H3 phosphorylation at serine 28 was blocked in Jnk1-/- cells (Fig. 6, A and C), but not in Jnk2-/- cells (Fig. 6, B and D) compared to Jnk1+/+ cells (Fig. 6, A-D). These experiments further confirmed that ERK1, ERK2, p38 kinase and JNK1 mediate UVB-induced phosphorylation of H3 at serine 28. In contrast, UVB-induced H3 phosphorylation at serine 10 was not affected in Jnk1-/- and Jnk2-/- cells (32).

DISCUSSION

Our present study indicates that UVB irradiation activates MAP kinases (ERKs, p38 kinase and JNKs) resulting in phosphorylation of H3 at serine 28. We found that active ERK1, ERK2 and p38 kinase strongly phosphorylated H3 at serine 28 while JNK1 and JNK2
phosphorylation of H3 at serine 28 was relatively weaker \textit{in vitro}. Further, our data showed that PD 98059 and SB 202190 and the expression of DNM ERK2, p38 kinase or JNK1 inhibited UVB-induced H3 phosphorylation at serine 28. UVB-induced phosphorylation of H3 at serine 28 was also blocked in \textit{Jnk1}⁻/⁻, but not in \textit{Jnk2}⁻/⁻ cells. These data clearly indicate that UVB-induced phosphorylation of H3 at serine 28 is mediated mainly through ERKs, p38 kinase and JNK1 pathways.

The covalent modification of the amino-terminal tails of histone H3 has emerged as an important mechanism in regulation of transcriptional activation and chromatin condensation. The best understood histone modification is acetylation of lysine residues of H3/H4, which is mediated by HATs and HDACs (19-22) and acetylation of H3/H4 is closely related to transcriptional regulation (10, 23, 24). However, mechanisms regarding phosphorylation of histone H3 at serine 10 and serine 28 have attracted a great deal of interest in recent years. Phosphorylation of histone H3 at serine 10 is tightly correlated with mitotic chromosome condensation and segregation in mammals (28, 47, 48), in Tetrahymena (15, 30), and Xenopus (27). Chromosome segregation is required for phosphorylation of histone H3 at serine 10 mediated by IpI1/aurora kinase and Glc7/PP1 in \textit{Saccharomyces cerevisae} and \textit{Caenorhabditis elegans} (11). Histone H3 at serine 10 is phosphorylated by NIMA kinase in \textit{A. nidulans} during mitosis (12). Histone H3 phosphorylation at serine 10 is closely related to transcriptional activation of mitogen-stimulated immediate-early response genes, such as \textit{c-fos} and \textit{c-jun} in mammalian cells (6, 25, 26). This mitogen-stimulated phosphorylation of histone H3 at serine 10 was shown to be mediated by RSK2 or MSK1 (25, 26), while UVB-induced histone H3 phosphorylation at serine 10 was found to be mediated by ERKs and p38 kinases (32). A recent
study showed that phosphorylation of histone H3 at serine 28 also occurred in mitotic chromosome condensation in mammalian cells (34). However, the pathway that mediates phosphorylation of histone H3 at serine 28 is unknown. In this study, we investigated the role of MAP kinases in mediating UVB-induced phosphorylation of histone H3 at serine 28. ERK2 was more effective than ERK1, p38 kinases or JNKs in phosphorylating histone H3 at serine 28 \textit{in vitro} (Fig. 1, A-E). PD 98059, a specific inhibitor of MEK1 (37-39), and SB 202190, a specific inhibitor of p38 kinase (26, 40, 41), inhibited UVB-induced phosphorylation of histone H3 at serine 28 in JB6 C1 41 cells (Fig. 3, A and B, respectively); and expression of DNM ERK2 completely blocked the phosphorylation of histone H3 at serine 28 (Fig. 4A). Inhibition of phosphorylation of histone H3 at serine 28 by expression of DNM ERKs (Fig. 4A) is more marked than inhibition by either DNM p38 kinase (Fig. 4B) or DNM JNK1 (Fig. 4C). This implies that ERKs may play a more important role in UVB-induced phosphorylation of histone H3 serine 28 than p38 kinase or JNKs. JNK1 and JNK2 also phosphorylated histone H3 at serine 28 \textit{in vitro}, but compared to JNK2, JNK1 phosphorylation of histone H3 at serine 28 was weaker (Fig. 1, D and E). Moreover, expression of DNM JNK1 inhibited UVB-induced phosphorylation of histone H3 at serine 28 (Fig. 4C, Fig. 5C), and phosphorylation of histone H3 was blocked in $\text{Jnk}1^{-/-}$ cells (Fig. 6, A and C), but not in $\text{Jnk}2^{-/-}$ cells (Fig. 6, B and D) compared to $\text{Jnk}^{+/+}$ cells (Fig. 6, A-D). These results indicate that JNK1 is indeed involved in UVB-induced phosphorylation of histone H3 at serine 28 \textit{in vivo}, but not in phosphorylation of histone H3 at serine 10 (32). The difference between phosphorylation of H3 at serine 28 and serine 10 by JNKs suggests that H3 phosphorylation at distinct sites in the N-terminal may be important in different physiological functions following UVB irradiation.
Our results also show that the highest peak of UVB-induced phosphorylation of histone H3 at serine 28 is at 60 min (Fig. 2B), while UVB-induced phosphorylation of histone H3 at serine 10 is highest at 30 min following UVB irradiation (32). As serine 10 of histone H3 is closer than serine 28 to the N-terminal tail of histone H3, serine 10 of histone H3 may be phosphorylated faster than serine 28 following UVB irradiation. This difference in phosphorylation time implies that outside serine residues of histone H3 are preferentially phosphorylated following UVB irradiation. This difference in phosphorylation time of H3 at serine 10 and serine 28 also suggested that MAP kinase may indirectly regulate phosphorylation of histone H3 at serine 28 through activation of as yet unidentified protein kinases. We are currently investigating the role of MSK1, a downstream kinase of MAP kinases, in the UVB-induced phosphorylation of H3 at serine 28 (26). Although serine 28 and serine 10 of histone H3 have identical surrounding sequences (that is, both are R-K-S) (10), our data indicate that the kinases responsible for phosphorylation of each of these serine residues of histone H3 are different. These phosphorylation responses to different signals are likely to have distinct effects on H3 function during chromatin remodeling and gene expression.

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FOOTNOTES

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1 The abbreviations used are: HATs, histone acetyltransferases; HDACs, histone deacetyltransferases; MEM, Eagle’s minimal essential medium; FBS, fetal bovine serum; AP, alkaline phosphatase; UVB, ultraviolet B; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated protein kinase; MAP, mitogen-activated protein; DNM, dominant negative mutant; PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol.
FIGURE LEGENDS

FIG. 1. Phosphorylation of H3 at serine 28 by active MAP kinases, ERK1, ERK2, p38 kinase, JNK1 or JNK2 occurs in vitro. ERK1, ERK2, p38 kinase, JNK1 or JNK2 were incubated with pure histone H3 protein at 30 °C for 45 min in the presence of 200 µM ATP to facilitate phosphorylation. Phosphorylated H3 at serine 28 was detected with a specific antibody (34). Total H3 protein was detected in a parallel blot with anti-histone H3 from Upstate Biotechnology. (A) H3 at serine 28 was phosphorylated by active ERK1. (B) H3 at serine 28 was phosphorylated by active ERK2. (C) H3 at serine 28 was phosphorylated by active p38 kinase. (D) H3 at serine 28 was phosphorylated by active JNK1. (E) H3 at serine 28 was phosphorylated by active JNK2.

FIG. 2. UVB induces phosphorylation of H3 at serine 28 in vivo. (A) Dose-response study: JB6 C1 41 CMV-neo cells were starved by incubating in 0.1% FBS MEM for 48 h at 37 °C in a 5% CO2 atmosphere. Cells were incubated for 2 h in fresh 0.1% FBS MEM, after which time they were exposed to 1, 2, 4 or 6 kJ/m² of UVB, and then incubated an additional 30 min. Phosphorylation of H3 at serine 28 was determined by western blot analysis of acid-soluble nuclear proteins resolved by SDS-PAGE using a specific antibody as in Fig. 1. Total H3 protein was detected in a parallel blot with anti-histone H3 from Upstate Biotechnology. (B) Time-course study: cells were treated as in (A) but were exposed to UVB (4 kJ/m²) and incubated an additional 15, 30, 60 or 120 min. Phosphorylation of H3 at serine 28 and total H3 protein were determined as indicated above. The arrows denote the position of phospho-H3 at serine 28 and
FIG. 3. **PD 98059 and SB 202190 inhibit the UVB-induced phosphorylation of H3 at serine 28 in JB6 C1 41 cells.** Cells were treated with various concentrations of PD 98059 or SB 202190 for 1 h and then exposed to UVB (4 kJ/m²). Phosphorylated and total histone H3 proteins were detected as indicated in Fig. 1. (A) PD 98059, a specific inhibitor for MEK1 kinase, blocked UVB-induced phosphorylation of H3 at serine 28. (B) SB 202190, a specific inhibitor for p38 kinase, blocked UVB-induced phosphorylation of H3 at serine 28. The arrows denote the position of phospho-H3 at serine 28 and total H3 protein.

FIG. 4. **UVB-induced phosphorylation of H3 at serine 28 is blocked by DNM ERK2, DNM p38 kinase or DNM JNK1.** Phosphorylated and total histone H3 proteins were detected as indicated in Fig. 1. (A) Phosphorylation of H3 at serine 28 was strongly induced by UVB in JB6 C1 41 CMV-neo cells, but UVB-induced H3 phosphorylation at serine 28 was completely blocked in JB6 C1 41 DNM ERK2 cells. (B) UVB-induced H3 phosphorylation at serine 28 was markedly blocked in JB6 C1 41 DNM p38 cells and (C) in JB6 C1 41 DNM JNK1 cells compared to JB6 C1 41 CMV-neo cells. The arrows denote the position of phospho-H3 at serine 28 and total H3 protein.

FIG. 5. **Dose-response of UVB-induced phosphorylation of H3 at serine 28 in JB6 C1 41, DNM ERK2, DNM p38, and DNM JNK JB6 C1 41 cells.** Cells of JB6 C1 41 and JB6 C1 41 DNM ERK2 (A), JB6 C1 41 DNM p38 (B), and JB6 C1 41 DNM JNK1 (C) were exposed to
UVB at doses of 1, 2, 4 or 6 kJ/m² and incubated an additional 30 min. Phosphorylation of H3 at serine 28 and total H3 protein were detected as indicated in Fig. 1. The arrows denote the position of phospho-H3 at serine 28 and total H3 protein.

FIG. 6. **UVB-induced phosphorylation of H3 at serine 28 is blocked in Jnk1⁻/⁻ cells, but not in Jnk2⁻/⁻ cells.** Jnks⁺/⁺, Jnk1⁻/⁻ and Jnk2⁻/⁻ cells were starved by incubating in 0.1% FBS DMEM for 48 h at 37 °C in a 5% CO₂ atmosphere. Cells were incubated for 2 h in fresh 0.1% FBS DMEM, after which time they were exposed to UVB (4 kJ/m²) and incubated an additional 15, 30 or 60 min for the time course studies (A and B), or exposed to UVB 1, 2, 4, kJ/m² for dose response studies (C and D). Phosphorylation of H3 at serine 28 and total H3 protein was determined by western blot analysis of acid-soluble nuclear proteins resolved by SDS-PAGE as described for Fig. 1. Phosphorylation of H3 at serine 28 was strongly induced by UVB in Jnks⁺/⁺ (A-D). Jnk1⁻/⁻ cells appear to markedly inhibit UVB-induced H3 phosphorylation at serine 28 (A and C), but Jnk2⁻/⁻ cells have little effect on UVB-induced H3 phosphorylation at serine 28 (B and D), compared to Jnks⁺/⁺ cells. The arrows denote the position of phospho-H3 at serine 28 and total H3 protein.
| Kinase (units) | 0   | 0.05 | 0.1 |
|---------------|-----|------|-----|
| Histone H3 (µg) | 8   | 8    | 8   |

|   |   |   |   |
|---|---|---|---|
| A | ERK1 | ![Image](phospho-H3-S28) | ![Image](H3) |
| B | ERK2 | ![Image](phospho-H3-S28) | ![Image](H3) |
| C | p38 kinase | ![Image](phospho-H3-S28) | ![Image](H3) |
| D | JNK1 | ![Image](phospho-H3-S28) | ![Image](H3) |
| E | JNK2 | ![Image](phospho-H3-S28) | ![Image](H3) |
| Condition                  | ± | + | + | + | + | + | + | + |
|---------------------------|---|---|---|---|---|---|---|---|
| UVB (4 kJ/m²)             |   |   |   |   |   |   |   |   |
| PD 98059 (µM)             | - | - | 12.5 | 25 | 50 | 100 |

---

| Condition                  | ± | + | + | + | + | + | + | + |
|---------------------------|---|---|---|---|---|---|---|---|
| UVB (4 kJ/m²)             |   |   |   |   |   |   |   |   |
| SB 202190 (µM)            | - | - | 0.5 | 1 | 2 | 4 |

---

**Figure 3**

Panel A: UVB (4 kJ/m²) with PD 98059 at various concentrations (12.5, 25, 50, 100 µM).

Panel B: UVB (4 kJ/m²) with SB 202190 at various concentrations (0.5, 1, 2, 4 µM).

- Phospho-H3-S28:
- H3
SP. Zhong-Fig. 4

A

| Time after UVB (4kJ/m²) | C1 41-neo | DNM-ERK2 |
|-------------------------|-----------|-----------|
| -                       | 15        | 15        |
| 15                      | 30        | 30        |
| 30                      | 60        | 60        |
| 60                      | 120       | 120       |

B

| Time after UVB (4kJ/m²) | C1 41-neo | DNM-p38 |
|-------------------------|-----------|---------|
| -                       | 15        | 15      |
| 15                      | 30        | 30      |
| 30                      | 60        | 60      |
| 60                      | 120       | 120     |

C

| Time after UVB (4kJ/m²) | C1 41-neo | DNM-JNK1 |
|-------------------------|-----------|----------|
| -                       | 15        | 15       |
| 15                      | 30        | 30       |
| 30                      | 60        | 60       |
| 60                      | 120       | 120      |

- phospho-H3-S28
- H3
A  

| UVB (kJ/m²) | C1 41-neo | DNM-ERK2 |
|------------|-----------|-----------|
| -          | 1         | 2         | 4         | 6 | - | 1 | 2 | 4 | 6 |

![Image A with bands labeled phospho-H3-S28 and H3]

B  

| UVB (kJ/m²) | C1 41-neo | DNM-p38 |
|------------|-----------|---------|
| -          | 1         | 2       | 4       | 6 | - | 1 | 2 | 4 | 6 |

![Image B with bands labeled phospho-H3-S28 and H3]

C  

| UVB (kJ/m²) | C1 41-neo | DNM-JNK1 |
|------------|-----------|----------|
| -          | 1         | 2        | 4        | 6 | - | 1 | 2 | 4 | 6 |

![Image C with bands labeled phospho-H3-S28 and H3]
**A**

| Time after UVB (minutes) | Jnks +/+ | Jnk1 -/- |
|--------------------------|----------|----------|
| -                        | 15       | 30       | 60       |

![Phospho-H3-S28 and H3 bands](image)

**B**

| Time after UVB (minutes) | Jnks +/+ | Jnk2 -/- |
|--------------------------|----------|----------|
| -                        | 15       | 30       | 60       |

![Phospho-H3-S28 and H3 bands](image)

**C**

| UVB (kJ/m²) | Jnks +/+ | Jnk1 -/- |
|-------------|----------|----------|
| -           | 1        | 2        | 4        |

![Phospho-H3-S28 and H3 bands](image)

**D**

| UVB (kJ/m²) | Jnks +/+ | Jnkd -/- |
|-------------|----------|----------|
| -           | 1        | 2        | 4        |

![Phospho-H3-S28 and H3 bands](image)
MAP kinases mediate UVB-induced phosphorylation of histone H3 at serine 28
Shuping Zhong, Yiguo Zhang, Cheryl Jansen, Hidemasa Goto, Masaki Inagaki and Zigang Dong

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