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Different Mechanisms of c-Jun NH_2-terminal Kinase-1 (JNK1) Activation by Ultraviolet-B Radiation and by Oxidative Stressors*

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Irradiation of mammalian cells with ultraviolet-B radiation (UV-B) triggers the activation of a group of stress-activated protein kinases known as c-Jun NH_2-terminal kinases (JNKs). UV-B activates JNKs via UV-B-induced ribotoxic stress. Because oxidative stress also activates JNKs, we have addressed the question of whether the ribotoxic and the oxidative stress responses are mechanistically similar. The pro-oxidants sodium arsenite, cadmium chloride, and hydrogen peroxide activated JNK1 with slow kinetics, whereas UV-B potentiated the activity of JNK1 rapidly. *N*-acetyl cysteine (a scavenger of reactive oxygen intermediates) abolished the ability of all oxidative stressors tested to activate JNK1, but failed to affect the activation of JNK1 by UV-B or by another ribotoxic stressor, the antibiotic anisomycin. In contrast, emetine, an inhibitor of the ribotoxic stress response, was unable to inhibit the activation of JNK1 by oxidative stressors. Although UV-A and long wavelength UV-B are the spectral components of the ultraviolet solar radiation that cause significant oxidative damage to macromolecules, the use of a filter to eliminate the radiation output from wavelengths below 310 nm abolished the activation of JNK1 by UV. Our results are consistent with the notion that UV-B and oxidative stressors trigger the activation of JNK1 through different signal transduction pathways.

Both in cultured mammalian cells and in the *in vivo* mouse skin experimental system, the nongermicidal intermediate wavelength ultraviolet part of the solar radiation (UV-B,[1] λ = 280–320 nm) elicits biological responses such as cytotoxicity, mutagenicity, carcinogenicity, and gene activation. Similar to the short wavelength ultraviolet radiation (UV-C, λ = 200–280 nm), UV-B produces oxygen-independent damage to DNA, RNA, and proteins. Similar to the long wavelength ultraviolet radiation (UV-A, λ = 320–400 nm), UV-B also induces oxidative damage to diverse cellular substrates. Murine fibroblasts exposed to UV-B generate superoxide anion radicals (O_2^-), which are, in turn, dismutated to hydrogen peroxide (H_2O_2) by superoxide dismutases (1). Thus generated, H_2O_2 further participates in the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow {}^{\cdot}OH + OH^- + Fe^{3+}$) to generate the highly reactive hydroxyl radical ($^{\cdot}OH$) (2). Apart from fibroblast cultures, the Fenton reaction has also been detected in UV-B-irradiated mouse skin (3). Hydroxyl radicals contribute to cellular damage by inducing lipid peroxidation. In addition to the generation of $^{\cdot}OH$, H_2O_2 can react with hypochlorous acid (HOCl) to generate singlet oxygen ($^1O_2$). Indeed, $^1O_2$ has been detected in UV-B irradiated mammalian cells (4). Furthermore, a substantial component of the oxygen-dependent damage to DNA (formation of the 7,8-dihydro-8-oxo-2′-deoxyguanosine lesion) in response to UV-B has been attributed to the generation of $^1O_2$ (5).

The cytoprotective, survival reaction of cells to UV-C and UV-B involves the rapid activation of the pre-existing transcription factor AP-1, a dimer composed of members of the c-Fos and c-Jun families of gene products. AP-1 activation is mediated by a profound (10–100-fold) increase in the activity of a group of related serine/threonine protein kinases collectively termed c-Jun NH_2-terminal kinases (JNKs) (reviewed in Ref. 6). JNKs phosphorylate and activate c-Jun, as well as the transcription factors TCF/Elk-1 and ATF-2 that positively regulate the expression of the c-*fos* and c-*jun* genes, respectively (reviewed in Ref. 7). The mechanisms of UV-C- and UV-B-induced activation of JNKs have been subjects of considerable investigation and debate over the last few years (8–11).

We have recently discovered a novel signaling pathway to JNK1 that is initiated in, or in close proximity to, the functional center of actively translating eukaryotic ribosomes (12). This center contains the 3′-end of 28 S rRNA and its proteinaceous environment and is responsible for aminoacyl-tRNA binding, peptidyl transfer, and ribosome translocation. This region of the 28 S rRNA is the target of the antibiotics anisomycin and blasticidin S and of the enzymatic ribotoxins ricin A chain and α-sarcin, all of which strongly activate JNK1 (see Ref. 12, and a detailed list of references therein). The activation of JNK1 by the foregoing agents was termed the ribotoxic stress response and is characterized by the absolute requirement for the presence of actively translating ribosomes at the moment of cellular encounter with the 28 S rRNA-acting antibiotic or ribotoxin. Cells whose ribosomes are not engaged in translational elongation fail to activate JNK1 in response to these agents. In contrast, the activation of JNK1 by nonribotoxic stressors, such as inflammatory cytokines, osmotic stress, and some DNA-damaging drugs, is intact in cells containing nontranslating ribosomes (12). Interestingly, both UV-C and UV-B require the presence of active ribosomes to activate JNK1; furthermore, nucleotide- and position-specific damage to the 3′-end of 28 S rRNA was detected in UV-C- or UV-B-irradiated cells (10). We concluded, therefore, that both UV-C and UV-B trigger the ribotoxic stress response that leads to the activation of JNK1. Our previous work, however, has not addressed the possibility that the ribotoxic stress response trig-

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[1] The abbreviations used are: UV-B, ultraviolet-B; UV-A, ultraviolet-A; UV-C, ultraviolet-C; JNK, c-Jun NH_2-terminal kinase; SAPK, stress-activated protein kinase; GSH, reduced glutathione; NAC, *N*-acetyl cysteine; IL-1α, interleukin-1α; MAP, mitogen-activated protein; ERK, extracellular signal-regulated kinase; SEK1/MKK4, SAPK/ERK kinase-1/MAP kinase kinase-4; EGF(R), epidermal growth factor (receptor); GST-Elk-1, glutathione *S*-transferase/Elk-1 fusion protein; CPD, cyclobutyl pyrimidine dimers.

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gered by UV-C- or UV-B may be mediated by UV-induced oxidative damage (for instance to RNA or protein components of ribosomes). Such a possibility is plausible because the damage to the 28 S rRNA incurred in response to UV-C involved guanosine-specific lesions (potentially 7,8-dihydro-8-oxo-guanosines) (10). Indeed, oxidative stressors are potent activators of JNK activity. Sodium arsenite, the carcinogenic form of trivalent arsenic (As^{3+}), has been found to induce elevated levels of ˙OH through depletion of cellular reduced glutathione (GSH) (13). Arsenite interacts directly with the sulfhydryl group of both GSH and proteins, leading to the formation of mixed protein-As-GS complexes (14, 15). Several groups have reported that arsenite is a potent activator of JNK activity (16–19). H_2O_2, a precursor of ˙OH (see above), was found to activate JNK as well (20–23). Cadmium chloride, an agent that, similar to sodium arsenite, depletes the cellular levels of reduced GSH (13), has also been found to activate JNK (19, 24).

In this investigation we have employed Rat-1 fibroblasts (the cells used initially to describe the ribotoxic stress response) to address the question of whether UV-B, on the one hand, and three oxidative stressors (sodium arsenite, cadmium chloride, and H_2O_2), on the other hand, share common signal transduction pathways to induce the activation of JNK1. We present several lines of evidence that suggest that UV-B does not activate JNK1 through oxidative damage. First, sodium arsenite, cadmium chloride, and H_2O_2 activated JNK1 with relatively slow kinetics, whereas UV-B potentiated JNK1 activity rapidly. Second, pretreatment of cells with *N*-acetyl cysteine (NAC, a potent scavenger of H_2O_2, ˙OH, and HOCl (25, 26) and a precursor for the biosynthesis of GSH (27–30)) at physiological pH abolished the ability of all oxidative stressors tested to activate JNK1, but failed to affect the activation of JNK1 by UV-B or by another ribotoxic stressor, the antibiotic anisomycin. Third, emetine, an immediate inhibitor of ribosomal translocation and of UV-B- and anisomycin-induced activation of JNK1 (10, 12), was unable to inhibit the activation of JNK1 by the oxidative stressors. Fourth, eliminating more than 90% of the spectral output below 310 nm (*i.e.* the wavelengths that produce more direct, oxygen-independent damage to macromolecules than oxidative damage) abolished the activation of JNK1 by UV. Taken together, these results strongly argue that, although oxidative damage plays a role in the long-term effects of UV-B, it does not participate in the immediate-early cellular response that involves the activation of JNK.

EXPERIMENTAL PROCEDURES

Anisomycin, sodium arsenite, cadmium chloride, and *N*-acetyl cysteine were from Sigma. Recombinant mouse IL-1α was from Genzyme (Cambridge, MA). H_2O_2 was from Fisher Chemicals (Fair Lawn, NJ). The cell culture and all experimental techniques employed in this work have been previously described in Iordanov *et al.* (12). Briefly, JNK1 activity was determined by a coupled immunoprecipitation/immunocomplex kinase assay using an anti-JNK1 antibody (Santa Cruz Biotechnology Inc., sc-474) to precipitate the active kinase and GST-Elk1 recombinant protein as a substrate for phosphorylation (12). The phosphorylated GST-Elk1 was quantified from dried gels using a Molecular Dynamics Phosphorimager and IP Lab Gel software (12). The activation of SEK1/MKK4 was determined in a Western blot procedure using an antibody directed against SEK1/MKK4 protein phosphorylated at Thr-223 (New England BioLabs Inc., Beverly, MA, 9151S). The antibody was used following the instruction of the manufacturer (12). After hybridization with the phospho-specific antibody, the same membrane was stripped, and total SEK1/MKK4 protein was detected through re-hybridization using the anti-SEK1/MKK4 antibody K-18 (Santa Cruz Biotechnology Inc.). The UV-B source and the method of UV-B irradiation of cells have been described in Iordanov *et al.* (10). The A-18 glass filter was from Eastman-Kodak.

For the NAC pretreatment of cells, cell culture medium was made to contain 30 mM NAC freshly before use. The pH of the NAC-containing medium was then adjusted with NaOH to the pH value of the medium without NAC. Either NAC-free or NAC-containing medium (after equilibrating in humidified incubator at 37 °C and 5% CO_2) was then used to exchange the old cell culture mediums 30 min before treatment with UV-B or another agent.

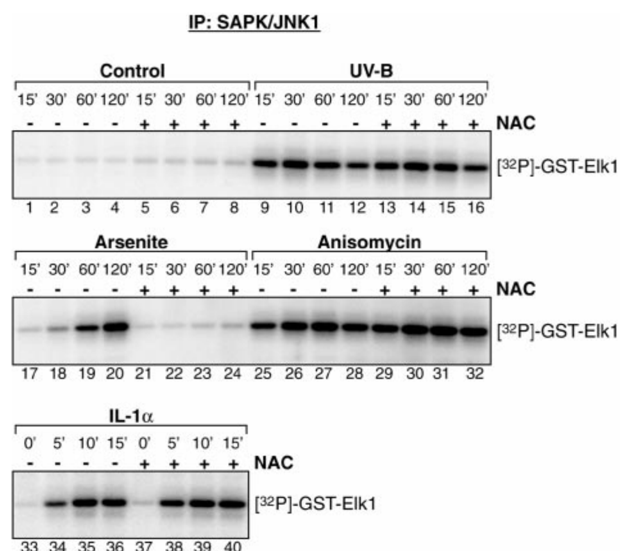

FIG. 1. **Kinetics of JNK1 activation in response to treatment with UV-B, sodium arsenite, anisomycin, or IL-1α and the effect of NAC on JNK1 activity.** For the experiment presented in this figure and for all the experiments presented hereafter, confluent Rat-1 cells were serum deprived for typically 24 h before treatment. The cells were left untreated or were pretreated as indicated (+) with NAC (30 mM) at physiological pH values for 30 min and then stimulated with UV-B (1200 J/m^2), sodium arsenite (200 μM), anisomycin (10 μg/ml), or IL-1α (25 ng/ml). At the indicated times (minutes) post-treatment, the cells were harvested, JNK1 was immunoprecipitated, and JNK1 activity was determined in immunocomplex kinase assays using recombinant GST-Elk1 fusion protein as a substrate for phosphorylation as described in Iordanov *et al*. (12). All NAC pretreatments were performed after the pH of the NAC-containing cell culture medium has been adjusted to physiological values.

RESULTS

Kinetics of JNK1 Activation by UV-B and by Oxidative Stressors—In Rat-1 cells irradiated with UV-B, the activity of JNK1 was markedly elevated as early as 15 min after the irradiation and remained elevated throughout the next 2 h, as determined in immunocomplex kinase activity assays (12) (Fig. 1, compare *lanes 1–4* with *lanes 9–12*). This kinetics of activation resembled that observed in anisomycin-treated cells (Fig. 1, compare *lanes 1–4* with *lanes 25–28*). In contrast, the activity of JNK1 in cells treated with sodium arsenite was not substantially elevated 15 min after the treatment and displayed a slow, graded, increase during the 2 h postincubation period (Fig. 1, compare *lanes 1–4* with *lanes 17–20*). Similarly, cadmium chloride-treated cells displayed a slow, graded, dose-dependent, increase in JNK1 activity (Fig. 2*B*, *lanes 1–8*, and not shown). The response of JNK1 in Rat-1 cells treated with H_2O_2 appeared to be complex; activation of JNK1 was not observed in cells treated with doses below 1 mM (not shown) as well as in cells treated with 10 mM H_2O_2 (Fig. 2*A*, *lanes 6–9*). Potent activation of JNK1 was observed only in cells treated with 1 mM H_2O_2 and, in addition, reproducibly only at 4 h after the treatment (Fig. 2*B*, *lanes 2–5*). These results demonstrate that three oxidative stressors (sodium arsenite, cadmium chloride, and H_2O_2) are slow activators of JNK1, whereas the ribotoxic stressors UV-B and anisomycin are rapid JNK1 activators.

Differential Effect of NAC on the Activation of JNK1 by Ribotoxic and by Oxidative Stressors—NAC exerts an antioxidant role via its dual capability to act both as a potent direct scavenger of H_2O_2, ˙OH, and HOCl (25, 26) and as a precursor

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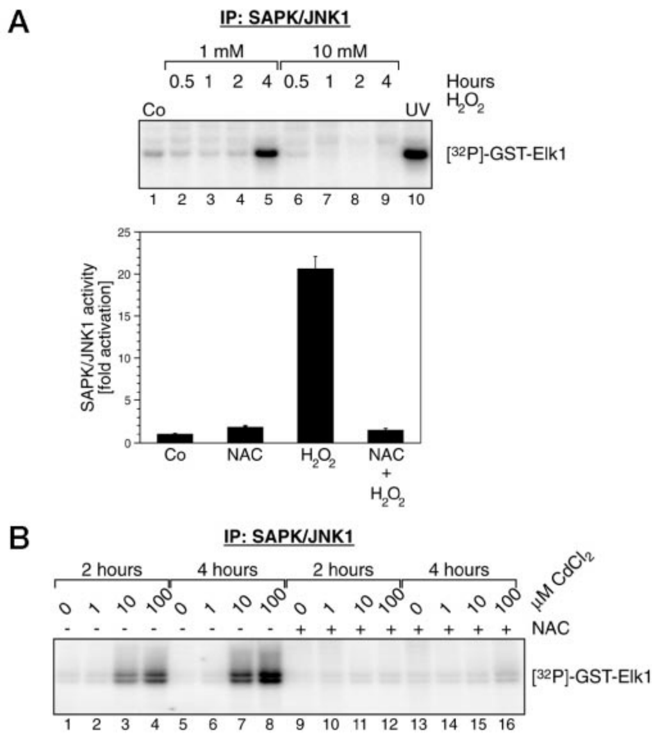

FIG. 2. **Kinetics and dose-dependence of JNK1 activation in response to treatment with H_2O_2 and cadmium chloride and the effect of NAC on JNK1 activity.** *A, top*, cells were treated with the indicated concentrations (1 mM or 10 mM) of H_2O_2 for the indicated times (0.5–4 h). JNK1 activity was determined as in Fig. 1. For comparison, the cells were irradiated with UV-B (1200 J/m^2), and JNK1 activity was determined 30 min later (*lane 10*). *A, bottom*, cells were pretreated where indicated with NAC as in Fig. 1 and then stimulated where indicated with 1 mM H_2O_2 for 4 h. JNK1 activity was determined as in Fig. 1. *Error bars*, standard deviation of the mean value obtained from experimental points in triplicates. *B*, cells were pretreated where indicated with NAC as in Fig. 1 and then stimulated where indicated with 1, 10, or 100 μM cadmium chloride for 2 or 4 h. JNK1 activity was determined as in Fig. 1. *IP*, immunoprecipitate.

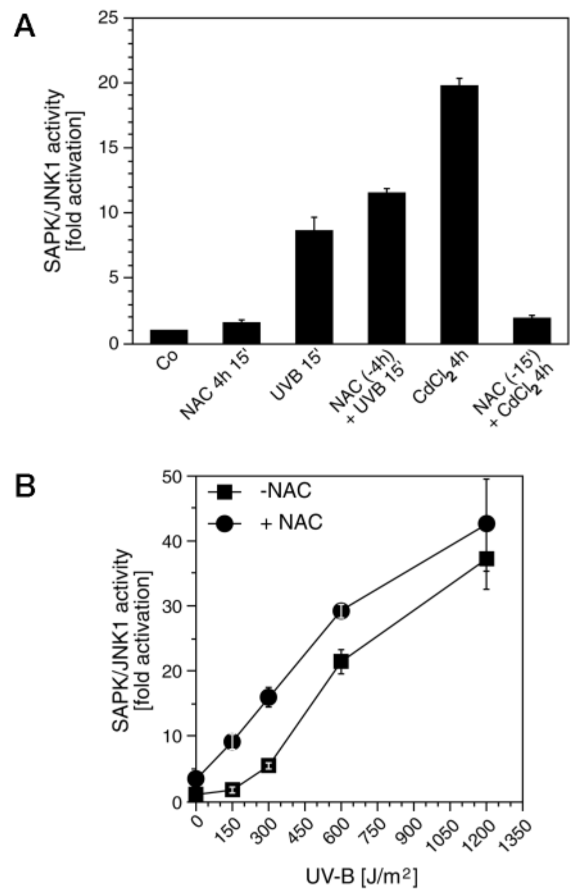

FIG. 3. **Lack of effect of NAC pretreatment on UV-B-induced JNK1 activity.** *A*, cells were pretreated where indicated with NAC as in Fig. 1 except that the UV-B stimulation (1200 J/m^2) was performed 4 h after the NAC pretreatment. JNK1 activity was determined 15 min after the irradiation. As positive control, the effect of NAC on the activation of JNK1 by cadmium chloride was studied in parallel. *Error bars*, standard deviation of the mean value obtained from experimental points in triplicates. *B*, cells were left untreated (■) or were pretreated with NAC as in Fig. 1 (●). Thirty min later, the cells were stimulated with 0, 150, 300, 600, or 1200 J/m^2 of UV-B, and JNK1 activity was determined 30 min after the irradiation. *Error bars*, standard deviation of the mean value obtained from experimental points in triplicates.

for the biosynthesis of GSH (27–30). To investigate whether UV-B-induced activation of JNK is dependent on reactive oxygen intermediates, we employed pretreatment of Rat-1 cells with NAC (30 mM) for 30 min before challenging the cells with UV-B or other stimuli. Because NAC acidifies the cell culture medium (not shown), we adjusted the pH of the medium containing NAC to physiological values (pH ~8.0 at atmospheric CO_2 concentrations; decreases to pH ~7.0 at 5% CO_2). Under these conditions, NAC specifically abolished the ability of sodium arsenite (Fig. 1, compare *lanes 17–20* with *lanes 21–24*), cadmium chloride (Fig. 2*B*, compare *lanes 1–8* with *lanes 9–16*), and H_2O_2 (Fig. 2*A*, *lower panel*) to activate JNK1. However, neither UV-B- nor anisomycin-induced JNK1 activities were affected by NAC pretreatment (Fig. 1, compare *lanes 9–12* with *lanes 13–16* and *lanes 25–28* with *lanes 29–32*). Furthermore, the activation of JNK1 in response to IL-1α was not affected by NAC (Fig. 1, compare *lanes 33–36* with *lanes 37–40*). We considered the possibility that NAC might have failed to inhibit rapid JNK activators (such as UV-B and anisomycin) because of the insufficient time of pretreatment with NAC (30 min, see Fig. 1). Rat-1 cells were therefore pretreated with NAC for 4 h and then challenged with UV-B for 15 min. Even under these conditions NAC failed to inhibit the activation of JNK1 by UV-B, whereas, in the same experiment, NAC completely inhibited cadmium chloride-induced JNK1 activity (Fig. 3*A*). Next, we considered the possibility that the failure of NAC to inhibit UV-B-induced JNK1 activity might result from the high (presumably, saturating) doses of radiation (1200 J/m^2 UV-B). However, NAC also failed to inhibit JNK1 activities induced by significantly lower does of UV-B radiation (150, 300, or 600 J/m^2; Fig. 3*B*). In fact, NAC slightly potentiated the UV-B-induced activity of JNK1, especially at lower UV-B doses (Fig. 3*B*). The ability of NAC to potentiate the activation of JNK1 by UV-B, however, might be because of the slightly elevated levels of JNK1 basal activity in the presence of NAC (Fig. 3*B*, see 0 J/m^2, and also see Figs. 2*A* and 3*A*, *graphs*).

Ribotoxic Stressors, Oxidative Stressors, and NAC Regulate the Activity of the JNK1 Cascade at a Level Upstream of MKK4/SEK1—We have recently reported that agents that induce ribotoxic stress activate the JNK1 cascade through signal transduction pathways that are independent (downstream) of cell surface cytokine receptors but are upstream of the dual specificity protein kinase MKK4/SEK1 (also known as JNK kinase-1, JNKK1) (10, 12). To investigate whether MKK4/SEK1 is activated by oxidative stressors as well, we monitored the phosphorylation of threonine 223 of this kinase, an event indicative of MKK4/SEK1 activation by upstream kinases such as MEKK1 (31, 32). Similar to the ribotoxic stressor UV-B (Fig. 4, *lanes 5–7*), both cadmium chloride and sodium arsenite induced a clear pattern of MKK4/SEK1 phosphorylation (Fig. 4, *lanes 11–14*, and not shown for sodium arsenite) as detected in immunoblot assays using an antibody specific for MKK4/SEK1

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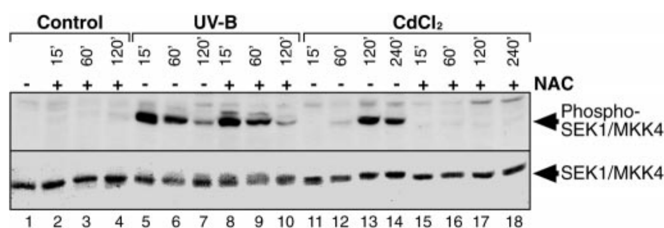

FIG. 4. **Phosphorylation of MKK4/SEK1 in response to treatment with UV-B or cadmium chloride and the effect of NAC on MKK4/SEK1 phosphorylation.** Cells were pretreated where indicated (+) with NAC for 30 min and then treated, as indicated, with UV-B (1200 J/m^2) or cadmium chloride (100 μM). At the indicated times after the treatments, the cells were lysed, and the phosphorylation status of SEK1/MKK4 was assessed in immunoblot analysis using an antibody directed against SEK1/MKK4 protein phosphorylated at threonine residue 223 as described in Iordanov *et al.* (12). The (phospho-Thr-223)MKK4/SEK1-specific signal (*top*) is indicated by an *arrow*. The same membrane was stripped and rehybridized with an antibody recognizing total MKK4/SEK1 protein (*bottom*).

protein phosphorylated at threonine 223. The kinetics of MKK4/SEK1 phosphorylation by either UV-B or cadmium chloride correlated closely with the kinetics of JNK1 activation by the same agents (compare Fig. 1, *lanes 9–12*, to Fig. 4, *lanes 5–7*, and Fig. 2*B*, *lanes 1–8*, to Fig. 4, *lanes 11–14*). In agreement with the results obtained using JNK1, NAC was unable to reduce the UV-B-induced MKK4/SEK1 phosphorylation (Fig. 4, compare *lanes 5–7* with *lanes 8–10*) but was very efficient in inhibiting the phosphorylation of the kinase induced by cadmium chloride and sodium arsenite (Fig. 4, compare *lanes 11–14* with *lanes 15–18*, and not shown for sodium arsenite). We concluded, therefore, that the ribotoxic stress- and the oxidative stress-induced signal transduction pathways to JNK1 are separate, but converge at, or upstream of, MKK4/SEK1.

Inability of Ribosomal Inactivation to Reduce the Responsiveness of JNK1 to Oxidative Stressors—We have previously demonstrated that the most characteristic feature of the ribotoxic stress-induced signaling to JNK1 is its absolute requirement for ribosomes actively engaged in translational elongation to elicit a signaling cascade to JNK1 (10, 12). Rat-1 cells, pretreated with emetine (and inhibitor of translational elongation), cannot activate JNK1 in response to ribotoxic stress, but possess a full capacity to activate JNK1 in response to cytokines, osmotic stress, and DNA-damaging agents (10, 12). The possible effects of ribosomal inhibitors on the oxidative stress-induced JNK1 activation have not, however, been investigated. Fig. 5 demonstrates the ability of emetine pretreatment to abolish the activation of JNK1 by UV-B. However, neither sodium arsenite- nor cadmium chloride-induced activation of JNK1 was inhibited by emetine pretreatment (Fig. 5). We concluded, therefore, that oxidative damage-induced signal transduction to JNK1 is ribosome-independent.

Failure of UV-B(Δ280–310 nm) to Activate JNK1—Both *in vitro* and in living cells, the ratio of oxidative damage (*e.g.* the 7,8-dihydro-8-oxo-2′-deoxyguanosine lesion, 8-oxodGuo) to direct damage (*e.g.* the cyclobutyl pyrimidine dimers, CPD) caused by UV increases with the wavelength. For instance, at equal doses, the 8-oxodGuo/CPD ratio for UV-A (320–400 nm)-irradiated HeLa cells was found to be approximately 1000-fold higher than that for UV-B- (280–320 nm) or UV-C (200–280 nm)-irradiated HeLa cells (33). If UV-induced oxidative damage is an important intermediate in the signal transduction pathways that lead to the activation of JNK1, the action spectrum of UV (measured by JNK1 activation) should be shifted toward wavelengths with high 8-oxodGuo/CPD ratios (*i.e.* UV-A). Because the UV-B source employed in our work has a substantial portion of UV-A spectral output (Fig. 6*A*), it became

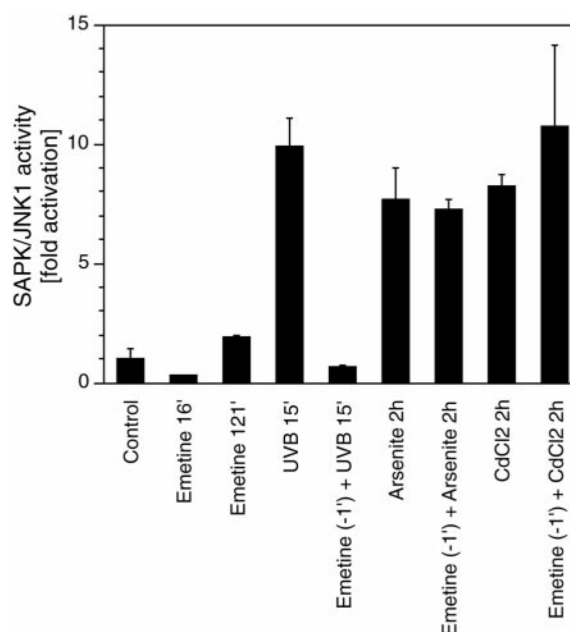

FIG. 5. **Effect of emetine on UV-B-, sodium arsenite-, or cadmium chloride-induced JNK1 activity.** Cells were pretreated, where indicated, with emetine (100 μg/ml) for 1 min before treatment with UV-B (1200 J/m^2), sodium arsenite (200 μM), or cadmium chloride (100 μM). JNK1 activity was determined 15 min after the UV-B irradiation or 2 h after the addition of sodium arsenite or cadmium chloride. *Error bars*, standard deviation of the mean value obtained from experimental points in triplicates.

possible to address this question experimentally. We observed that the A-18 glass filter from Eastman-Kodak retains more than 90% of the UV wavelengths shorter than 310 nm (Fig. 6*A*). By appropriately adjusting the irradiation time, we were able to deliver to cells equal doses of radiant energy containing either 100% of the original 280–310 nm spectral output (irradiation without the A-18 filter) or less than 10% of the original 280–310 nm spectral output (irradiation through the A-18 filter). Fig. 6*B* demonstrates that the use of the A-18 filter to eliminate more than 90% of the 280–310 nm spectral output abolished the ability of UV to activate JNK1 (compare *lanes 1–6* with *lanes 7–11*). Thus, the JNK1-activating spectral portion of the UV source used belongs to the wavelengths below 310 nm that have lower 8-oxodGuo/CPD ratios than UV-A. This finding is consistent with the previous results (Figs. 1–5) indicating that UV-B activates JNK1 through mechanisms that do not involve UV-induced oxidative damage.

DISCUSSION

Ultraviolet radiation and oxidative agents are relevant environmental hazards for eukaryotic organisms with cytotoxic, carcinogenic, and tumor-promoting properties. Whereas the biochemistry of reactive oxygen intermediates is relatively well understood, the modes of biological action of UV are considerably more complex, as they involve both oxygen-dependent and oxygen-independent (direct) damage to biomolecules. Although some of the biological activities of UV might be attributed to UV-induced oxidative stress, the question of the role of oxidative stress in the cellular transcriptional responses to UV (that involve the activation of cytoplasmic tyrosine- and serine/threonine-directed protein kinases) has not been extensively addressed. Recently, JNKs have emerged as important mediators of the transcriptional stress responses in mammalian cells to both UV and oxidative stressors. Because of their responsiveness to both UV and oxidative stressors, JNKs constitute biologically relevant end points to investigate the dependence of UV-induced signal transduction on UV-induced oxidative dam-

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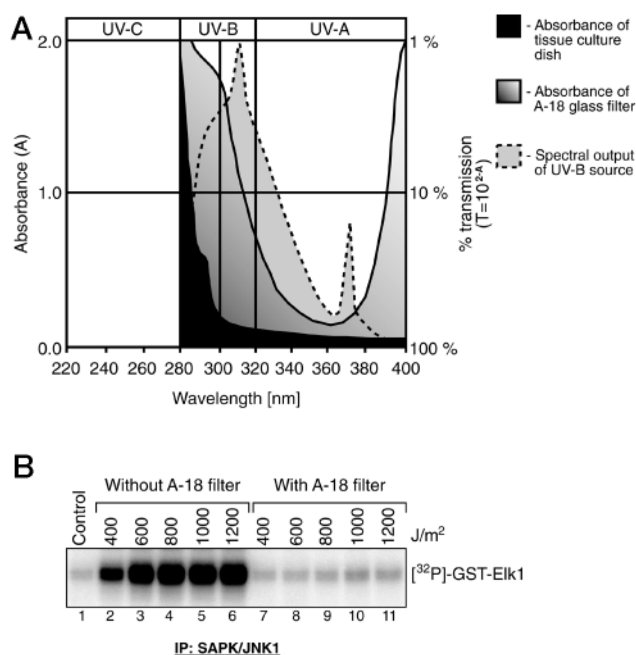

FIG. 6. **Elimination of the wavelengths below 310 nm and its effect on UV-B-induced JNK1 activity.** *A*, spectral characteristics of the UV radiation employed in this work. *Dashed line* and *dotted area*, spectral output of the UV source (as provided by the manufacturer, Fotodyne Inc., in *arbitrary units* on a linear scale); *black area*, absorbance of the plastic bottom of the tissue culture dish through which the cells were irradiated (left ordinate, linear scale). Note that the plastic efficiently blocks by ≥90% the transmission of wavelengths below ~285–290 nm (right ordinate, inverted logarithmic scale). *Shaded area*, absorbance of the A-18 glass filter (left ordinate). Note that the filter blocks by ≥90% the transmission of wavelengths below 310 nm (right ordinate). *B*, cells were irradiated with the indicated doses of UV-B in the absence, or in the presence, where indicated, of the A-18 glass filter. In the presence of the filter, approximately 10 times longer irradiation times were required to achieve equal doses of radiant energy. Thirty min after the end of the irradiation, cells were harvested and JNK1 activity was determined.

age. Previously, we have been able to demonstrate that an invariant requirement for the successful activation of JNK1 by either UV-C or UV-B is the presence, at the moment of UV irradiation, of ribosomes actively engaged in translational elongation (10). This finding placed UV in the distinct group of JNK1 activators that share the requirement for active ribosomes to activate JNK1 and that are collectively termed ribotoxic stressors (10, 12, 34). This group includes agents that either bind to the functional center of 28 S rRNA (anisomycin, blasticidin S, and gougerotin) or cause covalent damage to the functional center of 28 S rRNA (ricin A chain, abrin A chain, and α-sarcin). In contrast, multiple known activators of JNK1, such as signaling cytokines and hyperosmolarity, do not require the presence of active ribosomes to activate JNK1 (10, 12, 34). Despite the identification of UV as a ribotoxic stressor and of active ribosomes as essential mediators of the UV-induced signal transduction to JNK1, the possibility could not be ruled out that reactive oxygen intermediates, induced in response to UV irradiation of cells, cause damage to ribosomal components (RNA and/or proteins) thus triggering a ribosome-dependent signaling to JNK1. In the work presented, we have addressed this possibility experimentally by comparing the activation of JNK1 by ribotoxic (UV-B, anisomycin) and by oxidative (sodium arsenite, cadmium chloride, H_2O_2) stressors. The preponderance of experimental evidence presented in this work strongly supports a notion that reactive oxygen intermediates produced in the irradiated cells do not play a role in the activation of JNK1 by ribotoxic stressors.

With regard to the involvement of active ribosomes in UV-induced signal transduction, we have been able to demonstrate that active ribosomes are not required for the activation by UV of a related group of protein kinases, the extracellular signal-regulated kinase (ERKs) (10). Therefore, UV irradiation of cells generates at least two autonomous signaling cascades: one oxygen-independent, but ribosome-dependent that leads to the activation of JNKs; and another, ribosome-independent, that leads to the activation of ERKs. Interestingly, this second UV-stimulated signaling pathway seems to involve UV-induced oxidative damage. Knebel *et al*. (35) have presented results consistent with the notion that UV-C, UV-B, and UV-A cause oxidative damage to essential sulfhydryl groups in the catalytic pockets of protein phosphatases that dephosphorylate transmembranal receptor tyrosine kinases (such as the epidermal growth factor receptor, EGF-R). Decreased phosphatase activity, combined with high intrinsic kinase activity of the receptor tyrosine kinase, results in a net increase in the activity of the signal transduction pathways downstream of the respective receptor. Furthermore, Knebel *et al*. (35) present evidence that the activation by UV of the extracellular signal-regulated kinases (ERK) correlates with the UV-induced inhibition of EGF-R dephosphorylation. It must be noted, however, that of all the members of the MAP kinase superfamily (that also includes the JNKs and the p38 MAP kinase families of kinases), ERKs are kinases that are the weakest in their responsiveness to UV (10, 36). Taken together, our results and the work of Knebel *et al*. contribute to an emerging picture of the UV response through the MAP kinase superfamily of protein kinases in which the UV-induced oxidative damage plays a role in the activation of ERK family of kinases, but in which oxygen-independent and ribosome-dependent mechanisms predominate in the activation by UV of the stress-activated protein kinases of the JNK and p38 MAP kinase families.

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