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Ultraviolet Radiation Triggers the Ribotoxic Stress Response in Mammalian Cells*

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The ribotoxic stress response, which is conserved between prokaryotes and eukaryotes, is a cellular reaction to cytotoxic interference with the function of the 3′-end of the large (23 S/28 S) ribosomal RNA. The 3′-end of the large rRNA is directly involved in the three sequential steps of translational elongation: the aminoacyl-tRNA binding, the peptidyl transfer, and the ribosomal translocation. In mammalian cells, the ribotoxic stress response involves activation of the stress-activated protein kinase/c-Jun NH₂-terminal kinase and the p38 mitogen-activated protein kinase and transcriptional induction of immediate early genes such as c-*fos* and c-*jun*. Active ribosomes are essential mediators of the ribotoxic stress response. We demonstrate here that the transcriptional response of mammalian cells to ultraviolet radiation (UV response) displays the characteristics of a ribotoxic stress response, inasmuch as (i) the activation of stress kinases and gene expression in response to UV requires the presence of active ribosomes at the moment of irradiation; (ii) UV irradiation inhibits protein synthesis; and (iii) irradiation of cells with UV causes specific damage to the 3′-end of the 28 S rRNA. In contrast, the activation of the stress kinases by hyperosmolarity, by the DNA-cross-linking agent diepoxybutane, or by growth factors and cytokines does not depend on the presence of active ribosomes. Our results identify UV as a potential ribotoxic stressor and support the notion that some of the cellular signaling cascades in response to UV might be generated in the ribosome, possibly triggered by damage to rRNA.

Irradiation of mammalian cells with ultraviolet-C (UVC[1]; 200–280 nm) or ultraviolet-B (UVB; 280–320 nm) radiation transcriptionally activates immediate early genes such as c-*fos* and c-*jun*, both of which encode components of the transcription factor AP-1 (reviewed in Ref. 1). This transcriptional reaction of cells to UV (UV response; reviewed in Refs. 2 and 3)

[1] The abbreviations used are: UVC, ultraviolet-C; UVB, ultraviolet-B; MEF, mouse embryonic fibroblast; SAPK/JNK, stress-activated protein kinase/c-Jun NH₂-terminal kinase; p38/HOG1, mammalian homolog of the *Saccharomyces cerevisiae* high osmolarity glycerol response kinase-1; ERK, extracellular signal-regulated kinase; MAP, mitogen-activated protein; SEK1/MKK4, SAPK/ERK kinase-1/MAP kinase kinase-4; AP-1, activator protein-1; GST-Elk-1, glutathione *S*-transferase/Elk-1 fusion protein; IL, interleukin; IL-1, IL-1α; EGF, epidermal growth factor, TNF, tumor necrosis factor; S/R loop, α-sarcin/ricin loop; PTR, peptidyltransferase ring; DEB, diepoxybutane; CAT, chloramphenicol acetyltransferase.

promotes cell survival in mouse embryonic fibroblasts (MEFs), since MEFs with either c-*fos*⁻/⁻ or c-*jun*⁻/⁻ genotype are significantly more sensitive to cytotoxic UVC irradiation than their wild type counterparts (4). The transcriptional induction of c-*fos* by UV is mediated by phosphorylation of a member of the ternary complex factor family of transcription factors, either Elk-1 or Sap-1 (5, 6), each of which binds, together with the serum response factor, to the serum response element in the c-*fos* promoter (reviewed in Ref. 7). Several serine/threonine-proline motifs in the transactivation domains of TCFs are targets for phosphorylation by the proline-directed mitogen-activated protein (MAP) kinases (reviewed in Ref. 8). Consistent with this notion, all three families of MAP kinases, the extracellular signal-regulated kinases (ERK), the stress-activated protein kinases/c-Jun NH₂-terminal kinases (SAPK/JNK), and the p38/RK/Mpk2/CSBP/HOG-1 kinase (hereafter referred to as p38/HOG-1), are activated by UV irradiation (5, 9–12) and have been shown to mediate (albeit with different relative contributions in different cell types) the transcriptional activation of c-*fos* by UV (5, 13, 14). The serum response element is indispensable for the activation of the c-*fos* promoter by UV (15), but the full response of c-*fos* to UV requires also the cyclic AMP response element proximal to the TATA box (10). Consistently, the cyclic AMP response element-binding protein is also phosphorylated on the crucial serine residue 133 following UV irradiation by a p38/HOG-1-dependent cyclic AMP response element-binding protein kinase (10).

In mammalian cells, MAP kinases are activated by a broad spectrum of physiological stimuli and adverse environmental agents. In general, the ERK family of MAP kinases mediates the proliferative cellular response to mitogenic growth factors, whereas SAPK/JNK and p38/HOG-1 are thought to be important components of the cellular response to different kinds of stress, including hyperosmotic, genotoxic, and proinflammatory stresses (reviewed in Ref. 8). It is plausible that both the degree of incurred cellular damage and interactions with other stress-induced signaling pathways (*e.g.* activation of the p53 tumor supressor and of the c-Abl protein kinase; Ref. 16) allow SAPK/JNK and p38HOG-1 to determine the cell fate favoring either cell survival or apoptotic death.

Whereas specific cell surface receptors are undoubtedly the primary sensors for stress induced by proinflammatory cytokines, the nature of the receptive molecules sensing physical agents such as UV remains elusive. A major obstacle to the identification of the proper sensors is that in damaged cells physical agents produce a variety of molecular alterations, some of which are irrelevant to the generation of stress signals. UVB irradiation of HeLa cells causes clustering and multimerization of the receptors for epidermal growth factor (EGF), tumor necrosis factor-α (TNF-α), and interleukin-1α/β (IL-1) (17). Since the same effects were induced by hyperosmotic shock, it has been proposed that UV and hyperosmolarity use

similar growth factor/cytokine receptor-dependent signal transduction pathways to activate SAPK/JNK1 (17). However, one of us has previously reported that the p38/HOG-1-dependent phosphorylation of cyclic AMP response element-binding protein by UVC could not be prevented by a prior functional down-modulation of growth factor receptors (10). Here, we extend this observation to SAPK/JNK1 and provide evidence that the UV-induced activation of SAPK/JNK1 is independent of the action of UV on the TNF-α, IL-1, and EGF receptors.

UV-induced damage to DNA has long been considered a possible intermediate for the cellular responses to UV (reviewed in Refs. 2, 18, and 19). While the increased susceptibility of xeroderma pigmentosum patients (who are deficient in DNA repair synthesis after UV irradiation) to UV-induced skin cancer clearly establishes a connection between UV-induced DNA damage and UV carcinogenesis, the role of DNA lesions in the immediate early effects of UV (*e.g.* activation of SAPK/JNK1) is still debated (2). Enucleated HeLa cells are able to activate SAPK/JNK1 after UV irradiation, supporting therefore the notion that the UV sensing molecules and all of the components of the UV-induced signal transduction to SAPK/JNK1 must be extranuclear (20).

Still, the action spectra of UV (measured by gene activation, cytotoxicity, and carcinogenesis) strongly support the notion that the primary sensors for UV are molecules with UV absorption maxima identical to that of nucleic acids (2, 21). In view of this, RNA and ribonucleoprotein complexes are potential candidates for the role of UV sensors.

We have recently reported a novel stress signaling pathway in mammalian cells (ribotoxic stress response) (22) that is initiated at, or in close proximity to, the functional center of the 28 S ribosomal RNA and that leads to activation of SAPK/JNK1 and of its activator SEK1/JNKK/MKK4 (23–25; hereafter referred to as SEK1/MKK4). Damage to the α-sarcin/ricin loop (S/R loop) of the 28 S rRNA, which is induced by exposure of cells to the ribotoxic enzyme ricin A chain, or α-sarcin (hereafter referred to as ricin and sarcin, respectively), strongly activated SEK1/MKK4 and SAPK/JNK1 and transcriptionally induced the expression of c-*fos* and c-*jun* (22). Furthermore, the binding of peptidyltransferase inhibitors (*e.g.* anisomycin, blasticidin S, and gougerotin) to the adjacent peptidyl transferase center of the 28 S rRNA (26, 27) also potentiated the SAPK/JNK1 cascade (22). In summary, the ribotoxic stress response is characterized by the following features that allow its distinction from the cellular responses to genotoxic, hyperosmotic, and proinflammatory agents: (i) ribotoxic stressors interfere with translational elongation; (ii) ribotoxic stressors interfere with the function of the 3′-end (domains V and VI) of the 28 S rRNA by means of either covalent modifications (damage) to RNA or binding to the peptidyl transferase center of the 28 S rRNA; (iii) activation of stress kinases in response to ribotoxic stress requires the presence of active ribosomes at the moment of cellular exposure to the stressor (22). In the present work, we demonstrate that the response of mammalian cells to both UVC and UVB irradiation displays all three characteristics of a ribotoxic stress response and that this UV response is mechanistically different from the genotoxic, hyperosmotic, and proinflammatory stress responses.

EXPERIMENTAL PROCEDURES

Chemicals, Growth Factors, and Ribotoxins—Anisomycin, puromycin, cycloheximide, emetine, T-2 toxin, diepoxybutane, D-sorbitol, EGF (recombinant human), and abrin A (lectin from *Abrus precatotius*) were from Sigma. Pactamycin was a generous gift from The Upjohn Co. TNF-α (recombinant mouse) and IL-1α (recombinant mouse) were from Genzyme (Cambridge, MA). Puromycin and cycloheximide were dissolved freshly before use in double-distilled H_2O. Anisomycin, emetine, pactamycin, and T-2 toxin were dissolved in $(H_3C)_2SO$. In all of the

cases when $(H_3C)_2SO$ was used as a vehicle, corresponding control cells received the same amount of the vehicle alone (typically not more than 0.2% (v/v)). D-Sorbitol was dissolved in Dulbecco's modified Eagle's medium as a 3 M stock solution. All radiochemicals were from NEN Life Science Products.

Cell Culture—Rat-1 cells were maintained as described previously (28). The derivative cell line FC_2-Rat1 has been described by Rodland et al. (29). All experiments presented here were performed using confluent, quiescent cultures obtained through serum deprivation for typically 24 h. HeLa *tk⁻* cells and MEF cells were maintained as described for Rat-1 cells, except that the medium for MEFs was supplemented with 10% fetal bovine serum (HyClone, Logan, UT) instead of calf serum (HyClone).

Ultraviolet Sources and Irradiation—UVC irradiation was performed using the 254-nm source of a 3uv™ Transilluminator (UVP, Inc., Upland, CA). Irradiation was performed from above (17-cm distance), through serum-free culture medium. UVB irradiation was performed using a UV-transilluminator (model 3–3100, midrange bulb, half-maximal peak, 295–330 nm) from Fotodyne, Inc. (Hartland, WI) by placing the tissue culture dish on the transilluminator and irradiating through the plastic bottom of the culture dish (Sarstedt, Sparks, NV). The amount of UVC ($\lambda < 280$ nm) received by the cells using this mode of irradiation was less than 0.01%; the spectral transmission of the plastic tissue culture dish was determined spectrophotometrically (using a DU®-64 Spectrophotometer; Beckman Instruments) by measuring the absorbance in the range 210–400 nm. Measurements of radiant incidence were performed using a UVX digital radiometer and UVX-25 and UVX-31 Sensors (UVP) in accordance with the manufacturer's operating instruction manual, and irradiation times were subsequently calculated.

Immunoprecipitation of SAPK/JNK1, ERK, and p38/HOG-1 and Immunocomplex Kinase Assays—All immunoprecipitations and immunocomplex kinase reactions were performed as described for SAPK/JNK1 by Iordanov *et al.* (22). For immunoprecipitation of SAPK/JNK1, the antibody sc-474 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was used; for immunoprecipitation of ERK1, the antibody sc-93 (Santa Cruz Biotechnology) was used. The p38/HOG-1-specific serum was a generous gift from G. Johnson.

Western Blot Analysis of SEK1/MKK4 Phosphorylation—The analysis of threonine 223 phosphorylation of SEK1/MKK4 using the antibody 9151S (New England Biolabs Inc., Beverly, MA), was performed as described by Iordanov *et al.* (22).

Northern Blot Analysis of RNA—Rat-1 cells were harvested from two 10-cm tissue culture dishes per experimental point, and total cellular RNA was extracted, separated by electrophoresis, and analyzed in a Northern blot procedure as described previously (28). The hybridization probes for c-*fos* and cyclophilin were described by Pribnow *et al.* (30).

Measurement of Protein Synthesis—Incorporation of [³H]leucine was performed as described by Iordanov *et al.* (22). Determination of protein synthesis using FC_2-Rat1 cells and a CAT assay has been described by Rodland *et al.* (29).

Reverse Transcription of rRNA by Primer Extension—A detailed description of the method is provided in Ref. 22. The following primers (Genosys Biotechnologies, Inc., The Woodlands, TX) were used for mapping the UV-induced lesions in the 3′-end of the 28 S rRNA: 5′-C-GGCGCTGCCGTATCG-3′, 5′-CCCACAGATGGTAGCT-3′, 5′-CACAT-ACACCAAATGTC-3′, 5′-ATGGCAACAACACATC-3′, 5′-ACGTTCCCT-ATTAGTG-3′, 5′-CAATCCAACGCTTGGTG-3′, 5′-CGACATCGAAGG-ATCA-3′, 5′-TCGCTATGAACGCTTG-3′, 5′-CAGAAGGATCGTGAGG-3′, 5′-CTTAGGACACCTGCGT-3′, 5′-GAGTAGTGGTATTTCAC-3′, 5′-TTGACATTCTGAGCAC-3′, 5′-GCGACGCTTTCCAAG-3′, and 5′-TAG-GTTGAGATCGTTTC-3′.

PhosphorImager™ and Statistical Analyses—The quantification of all experiments based on detection of ³²P (immunocomplex kinase assays and primer extensions) was performed using the PhosphorImager™ apparatus and the IPLab Gel™ software from Molecular Dynamics, Inc. (Sunnyvale, CA). Statistical analyses were performed using the StatView™ software from Abacus Concepts, Inc. (Berkeley, CA).

RESULTS

Although some chemical stressors such as anisomycin, ricin, and palytoxin have been shown to activate SAPK/JNK1 through ribosome-mediated signal transduction pathways (22, 31), less is known concerning the mechanisms of stress kinase activation by physical agents such as ultraviolet radiation. We examined the possibility that UV irradiation of Rat-1 cells
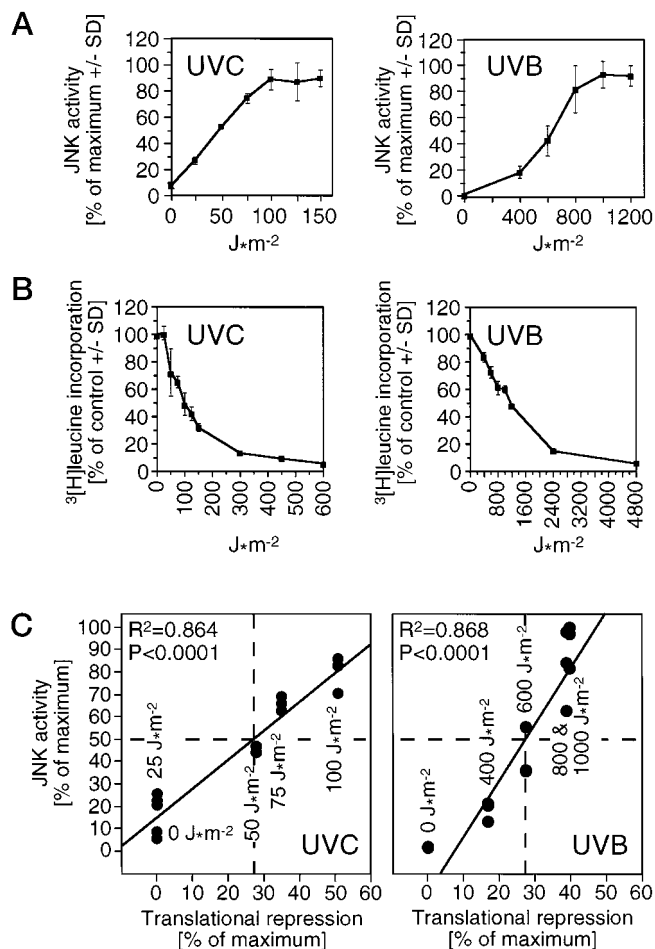
FIG. 2. **UVC-induced inhibition of CAT mRNA translation in FC$_2$-Rat1 cells.** Serum-deprived cells were stimulated for 2 h with EGF (40 ng/ml) in the presence of cycloheximide (25 μg/ml). Thereafter both EGF and cycloheximide were removed from the medium by extensive washout (indicated as *release*, $t = 0$ min). The accumulation of CAT protein was monitored by measuring CAT activity at 0, 60, 90, and 120 min after the release. UVC (150 J/m^2) was applied 60 min after the release (*arrow*). *Squares* represent CAT activity in the nonirradiated cells; *circles* represent CAT activity in the UV-irradiated cells. S.D. was obtained from experimental points in triplicates.

FIG. 1. **UV-induced activation of SAPK/JNK1 and inhibition of translation in rat fibroblasts.** *A*, dose dependence of the activity of SAPK/JNK1 in response to either UVC (*left*) or UVB (*right*). Rat-1 cells were irradiated with 25, 50, 75, 100, 125, or 150 J/m^2 of UVC or with 400, 600, 800, 1000, or 1200 J/m^2 of UVB, respectively. Cells were harvested 15 min after the irradiation, and the activity of SAPK/JNK1 was determined in immunocomplex kinase assays using an anti-SAPK/JNK1 antibody to precipitate SAPK/JNK1 and GST-Elk1 recombinant protein to serve as a substrate for phosphorylation (22). GST-Elk1 was used in all immunocomplex kinase assays (Figs. 3–5 and 9), including those in which the activity of either ERK1 or p38/HOG-1 was determined (Fig. 5, *B* and *C*). Graphic representation of the data from immunocomplex kinase assays is shown. S.D. was obtained from experimental points in triplicates. *B*, inhibition of [^{3}H]leucine incorporation as a function of the dose of UVC or UVB radiation. Rat-1 cells were irradiated with 25, 50, 75, 100, 125, 150, 300, 450, or 600 J/m^2 of UVC or with 400, 600, 800, 1000, 1200, 2400, or 4800 J/m^2 of UVB, respectively. Ten min later, the cells were pulse-labeled with [^{3}H]leucine for another 5 min, and the amount of [^{3}H]leucine incorporated into acid-insoluble material was determined (see "Experimental Procedures"). *C*, correlation between the translational repression caused by increasing doses of either UVC (*left*) or UVB (*right*) and the respective activities of SAPK/JNK1. The UV-induced translational repression was calculated using the data from *B* by applying the following formula: % of translational repression = (100 − [^{3}H]leucine incorporation (% of control)). The values for SAPK/JNK1 activity were from *A*. The correlations (R^2 values and p values) were calculated using StatView™ software.

could activate SAPK/JNK1 through ribosome-dependent pathways.

Translational Inhibition by UVC Irradiation—Rat-1 cells were irradiated with increasing doses of UVC, and SAPK/JNK1 activity was assayed 15 min later. SAPK/JNK1 activity increased linearly with the UVC dose up to 100 J/m^2, after which the activity of the kinase did not change significantly (Fig. 1*A*, *left*). The leveling of the rate of increase at doses of UVC higher than 100 J/m^2 reflected indeed saturation of the ability of
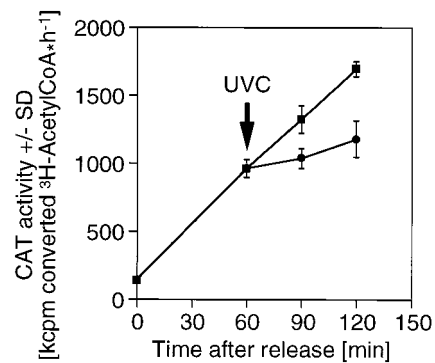
SAPK/JNK1 to be activated, since neither the amount of anti-SAPK/JNK1 antibody nor the substrate for the immunocomplex kinase assay was limiting (data not shown). Agents that act through ribotoxic signaling pathways have been shown to inhibit protein translation in the same dose range as their activation of stress kinases (22). Within the linear range response to UVC, there was a strong correlation ($R^2 = 0.864$, $p < 0.0001$, Fig. 1*C*, *left*) between activation of SAPK/JNK1 and translational inhibition (Fig. 1*B*, *left*), which was measured by incorporation of [^{3}H]leucine into protein. To confirm that the decrease in [^{3}H]leucine incorporation reflected a real inhibition of translation rather than a reduced uptake of [^{3}H]leucine by the irradiated cells, we applied an assay for protein synthesis that is independent of isotope uptake. A derivative of the parental Rat-1 cells, the cell clone FC$_2$-Rat1, bears a stably integrated chloramphenicol acetyl transferase (CAT) reporter gene under the control of the 1-kilobase promoter sequence of the human c-*fos* gene (29). The expression of the CAT mRNA is kept at very low levels in quiescent cells but can be induced up to 100-fold upon stimulation with EGF in the presence of cycloheximide (data not shown). The presence of cycloheximide prevents the translation of the CAT mRNA into CAT protein. Release from the cycloheximide-induced translational arrest (Fig. 2, $t = 0$ min) allows efficient translation of the accumulated CAT mRNA. The accumulation of CAT protein increased linearly within 2 h following the wash-out of cycloheximide as measured by a CAT activity assay (Fig. 2). Since EGF was also removed from the medium together with cycloheximide, the increase of CAT activity resulted solely from translation of the CAT mRNA accumulated before the wash-out. Irradiation of the cells with UVC (150 J/m^2) 60 min after the release from translational arrest resulted in a substantial decrease in CAT activity detectable both 30 and 60 min after the irradiation (Fig. 2). We therefore concluded that UVC irradiation of cells indeed interferes with the process of translation.

Active Ribosomes Mediate the UVC-induced Activation of the SAPK/JNK1—The activation of SAPK/JNK1 by ribotoxic stressors is abolished by immediate pretreatment of cells with ribosomal inhibitors that are unable to activate SAPK/JNK1 (22, 31). By contrast, activation of SAPK/JNK1 through cell surface receptors or by hyperosmotic stress does not require the presence of active ribosomes and is not blocked by ribosomal inhibitors (22, 31). To test the possibility that UVC-induced activation of SAPK/JNK1 is mediated through active ribo-
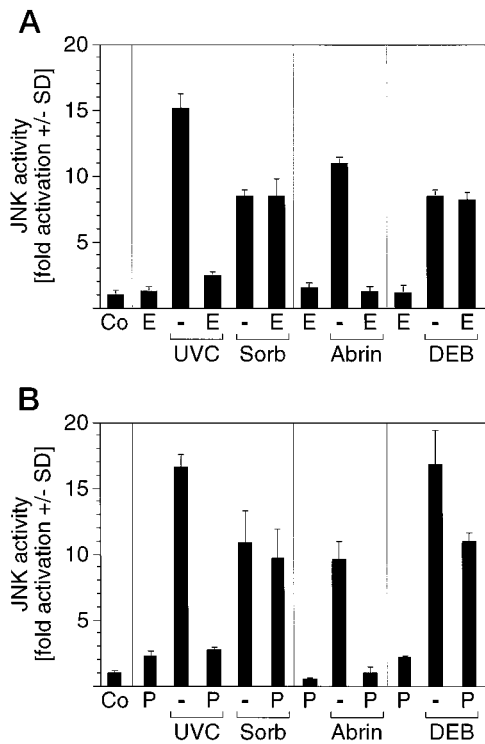
FIG. 3. **Specific inhibition of the activation of SAPK/JNK1 by the ribotoxic stressor abrin and by UVC in Rat-1 cells pretreated with either pactamycin or emetine.** *A*, cells were left untreated or were treated with UVC (150 J/m²), sorbitol (600 mM), lectin from *Abrus precatotius* (abrin, 1 μg/ml), or diepoxybutane (DEB, 1 mM), in the absence (−) or in the presence (*E*) of emetine (100 μg/ml) given 1 min prior to the addition of the respective agonist. Cells were harvested 15 min after the treatment with UVC or sorbitol, 60 min after the treatment with abrin, and 90 min after the treatment with DEB. Graphic representation of the data from immunocomplex kinase assays is shown. *Co*, control cells. S.D. was obtained from experimental points in triplicate. *B*, cells were treated as in *A*, except that, where indicated, pactamycin (*P*, 0.2 μg/ml) was added 30 min prior to the addition of the respective agonist. Graphic representation of the data from immunocomplex kinase assays is shown. *Co*, control cells. S.D. was obtained from experimental points in triplicates.

somes, we pretreated cells with pactamycin and emetine (antibiotics that fail to activate SAPK/JNK1 in Rat-1 cells) and assayed whether such pretreatment would inhibit the ability of UVC to activate SAPK/JNK1. For comparison, we studied the effects of the same drugs on the activation of SAPK/JNK1 by the ribotoxin abrin, an RNA *N*-glycosidase that specifically depurinates residue A^{4324} of the 28 S rRNA (32); by sorbitol, an inducer of the hyperosmotic stress response (33); or by a genotoxic agent, the DNA cross-linker diepoxybutane (DEB). Pretreatment of cells with either pactamycin or emetine abolished the activation of SAPK/JNK1 by abrin but had no significant effect on the activation of SAPK/JNK1 by sorbitol or by DEB (Fig. 3, *A* and *B*). Under the same conditions, both pactamycin and emetine pretreatments strongly suppressed the activation of SAPK/JNK1 by a saturating dose (150 J/m²) of UVC radiation (Fig. 3, *A* and *B*).

We employed emetine to rapidly inhibit translation (within 1 min after administration; Ref. 22) in order to determine the time interval in which the activation of SAPK/JNK1 by UV is dependent on the functional integrity of ribosomes. When given 1 min before, together with, or 1 min after the beginning of UVC irradiation (which itself lasted 30 s), emetine inhibited the activation of SAPK/JNK1 by ≥80% (Fig. 4). Thereafter, emetine became less effective in inhibiting the UV-induced activation of SAPK/JNK1. Similar time dependence of the sensitivity to emetine was obtained when SAPK/
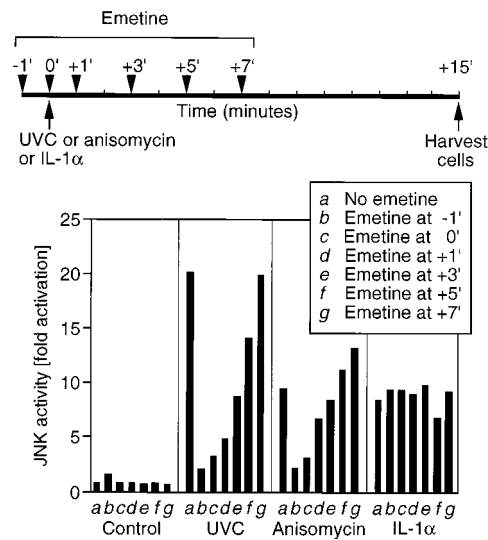

FIG. 4. **Differential effect of treatment with emetine on the activation of SAPK/JNK1 by UVC, anisomycin, or IL-1α.** Graphic representation of the data from immunocomplex kinase assays. Rat-1 cells were left untreated or were treated with UVC (150 J/m²), anisomycin (10 μg/ml), or IL-1α (25 ng/ml) under conditions of prior, contemporaneous, or subsequent treatment with emetine (100 μg/ml) as denoted in the *scheme* (*top*). SAPK/JNK1 activity was determined 15 min after the addition of the respective agonist.

JNK1 was activated by the peptidyltransferase inhibitor anisomycin (Fig. 4). However, whether given before or after stimulation of cells with IL-1α, emetine was unable to affect the activation of SAPK/JNK1 by this cytokine (Fig. 4; see "Discussion").

As with pactamycin and emetine, in Rat-1 cells pretreated with either cycloheximide or puromycin the ability of UVC to activate SAPK/JNK1 was inhibited by 94 and 88%, respectively (Fig. 5*A*, *lanes 4–6*). By contrast, pretreatment with cycloheximide or with puromycin failed to inhibit the activation of SAPK/JNK1 by IL-1α or hyperosmotic shock (data not shown).

Active Ribosomes Mediate the UV-induced Activation of the p38/HOG-1 Kinase, but Not the Activation of ERK—In addition to the activation of SAPK/JNK1, UVC also causes activation of the p38/HOG-1 stress kinase (10, 12, 13) and of the mitogen-activated protein kinases ERK1 and ERK2 (5, 10, 11). To investigate whether the activation of these kinases by UVC irradiation required active ribosomes, we pretreated Rat-1 cells with pactamycin and monitored the responsiveness of p38/HOG-1 and of ERK1 in immunocomplex kinase assays utilizing GST-Elk1 recombinant protein as a substrate for phosphorylation (see "Experimental Procedures"). The activation of p38/HOG-1 by UVC and anisomycin, but not by IL-1α, was strongly inhibited in pactamycin pretreated cells (Fig. 5*B*; compare *lanes 3* and *4* with *lanes 5* and *6* and *lanes 7* and *8*). To investigate the activation of ERK, extracts from UVC-treated cells (with or without pactamycin pretreatment) were split into halves, and SAPK/JNK1 and ERK activities were monitored in parallel (Fig. 5*C*). Both pactamycin (Fig. 5*C*, *top*, *lanes 17–20*) and UVC (Fig. 5*C*, *middle*, *lanes 22–25*) caused activation of ERK, and their effects were additive under conditions of pactamycin pretreatment (Fig. 5*C*, *bottom*, *lanes 27–30*). By contrast, the activation of SAPK/JNK1 by UVC was blocked in the pactamycin-pretreated cells (Fig. 5*C*, compare *lanes 7–10* to *lanes 12–15*), similar to the results shown in Fig. 3*B*.

Active Ribosomes Mediate the UV-induced Activation of SEK1/MKK4—Phosphorylation of SEK1/MKK4 at both serine and threonine residues within the motif VDS^{219}IAKT^{223}RD by upstream kinases such as MEKK1 (34) is indicative of SEK1/
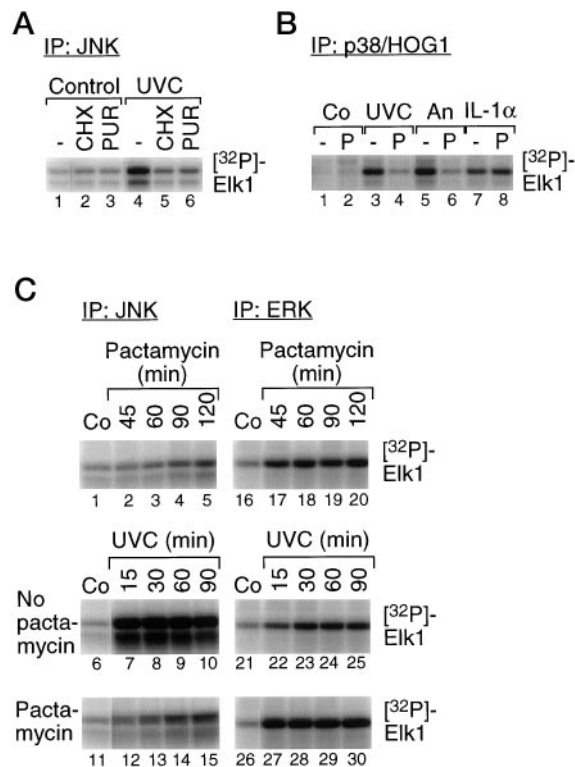
FIG. 5. **Effect of inhibitors of translation on the activation by UVC of SAPK/JNK1, p38/HOG-1, and ERK1.** *A,* inhibition of the UV-induced activation of SAPK/JNK1 by pretreatment of Rat-1 cells with either cycloheximide (*CHX*, 25 μg/ml) or puromycin (*PUR*, 75 μg/ml), each given 1 min prior to the irradiation with UVC (150 J/m^2). SAPK/JNK1 activity was determined 15 min postirradiation. *B,* specific inhibition of the activation of p38/HOG-1 by anisomycin and by UVC in Rat-1 cells pretreated with pactamycin. Cells were left untreated or were treated with UVC (150 J/m^2), anisomycin (*An*, 10 μg/ml), or IL-1α (25 ng/ml) in the absence (−) or in the presence (*P*) of pactamycin (0.2 μg/ml) given 30 min prior to the addition of the respective agonist. *Co,* control cells. *IP,* immunoprecipitation. The activity of p38/HOG-1 was determined 15 min after the addition of agonists. *C,* differential effect of pretreatment with pactamycin on the activation by UVC of SAPK/JNK1 and ERK. Rat-1 cells were left untreated or were pretreated, as indicated at the *left side* of each *part*, with pactamycin (0.2 μg/ml) for 30 min and then stimulated as indicated with UVC (150 J/m^2). Cells were harvested as indicated 15, 30, 60, or 90 min after the irradiation. The cell lysates were split into halves, and either SAPK/JNK1 (*left blots*) or ERK1 (*right parts*) was immunoprecipitated and, subsequently, assayed for activity. The *upper parts* represent the effect of pactamycin alone on the activity of either SAPK/JNK1 or ERK at all time points used in the *lower parts*. *Co,* control cells. *IP,* immunoprecipitation.

MKK4 activation (23, 25). To investigate whether the activation of SEK1/MKK4 by UV displays similar dependence on the functional integrity of ribosomes, we monitored SEK1/MKK4 phosphorylation in immunoblot analysis using an antibody directed against SEK1/MKK4 protein phosphorylated at threonine 223. Pretreatment of cells with either pactamycin (Fig. 6, *upper panel*) or emetine (Fig. 6, *lower panel*) had no effect on the phosphorylation of SEK1/MKK4 in response to hyperosmotic shock (induced either by NaCl (Fig. 6, *lanes 7* and *8*) or by sorbitol (Fig. 6, *lanes 15* and *16*)). Similarly, emetine pretreatment did not affect the phosphorylation of SEK1/MKK4 in response to DEB (data not shown). By contrast, the phosphorylation of SEK1/MKK4 in response to either anisomycin or UVC was completely abolished (Fig. 6, *lanes 3–6* and *11–14*).

UVC Irradiation Causes Nucleotide- and Site-specific Damage to 28 S rRNA in Vivo—Results presented in Figs. 1–6 demonstrate strong similarities between ribotoxic stressors (22, 31) and UVC in the activation of stress kinases. Ribotoxic stressors have been shown to interfere with translation by
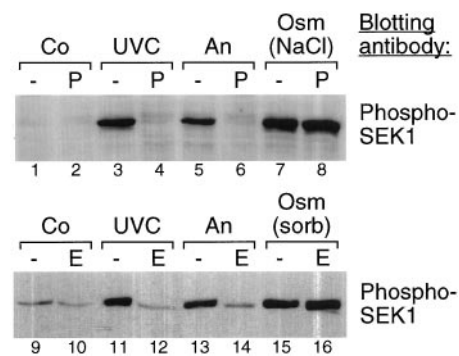

FIG. 6. **Specific inhibition of the phosphorylation of SEK1/ MKK4 by anisomycin and by UV in Rat-1 cells pretreated with either pactamycin or emetine.** Cells were left untreated or were treated as indicated with UVC (150 J/m^2), inducers of osmotic shock NaCl (*Osm (NaCl)*, 700 mM) or sorbitol (*Osm (sorb)*, 600 mM), or anisomycin (*An*, 10 μg/ml), in the absence (−) or in the presence of either pactamycin (*P*, 0.2 μg/ml, 30-min pretreatment) or emetine (*E*, 100 μg/m, 1 min pretreatment). Cells were harvested 15 min later, and the phosphorylation state of SEK1/MKK4 was assessed in Western blot using an antibody recognizing SEK1/MKK4 protein that is phosphorylated at threonine 223 as described by Iordanov *et al.* (22).

interacting with domains V and VI of the 28 S rRNA, either by noncovalent interaction with the peptidyl transferase ring (anisomycin, blasticidin S) or by specifically depurinating or cleaving a single nucleotide within the S/R loop. In search of a possible mechanism for the UVC-induced inhibition of translation, we investigated whether cells sustain UVC-induced RNA damage in the 3′-end of the 28 S rRNA. To this end, Rat-1 cells were irradiated with doses of UVC from 25 J/m^2 up to 600 J/m^2, and 15 min later total cellular RNA was prepared. The occurrence of UVC-induced lesions was detected by the lesion-induced arrest of a reverse transcriptase-driven primer extension (22) using altogether 14 oligonucleotide primers that bind to different regions within domains V and VI (see "Experimental Procedures"). The results of these studies are presented in Fig. 7 and are summarized as follows. (i) *In vivo* irradiation of cells caused detectable damage to 28 S rRNA in both domains V and VI at doses of UVC as low as 25 J/m^2 (exemplified here by the damage at position U^{4348}, Fig. 7, *A* and *B*). (ii) The lesions occurred at adjacent pyrimidine nucleotides (sequences 5′-UU-3′, 5′-CU-3′ or 5′-UC-3′), except for two lesions detected at guanine residues (G^{4110} and G^{4328}, Fig. 7, *A* and *E*). (iii) The lesions displayed site specificity, occurring reproducibly only at specific nucleotides within the region examined. For instance, multiple (U)$_2$-(U)$_4$ stretches were reproducibly spared from damage (*e.g.* see U^{4274}–U^{4278}; Fig. 7, *A* and *C*). (iv) Within domain V, several UVC-induced lesions were clustered in the "peptidyl transferase ring" (PTR; Ref. 35) and its adjacent helices 94 and 95; the lesions occurred at G^{4110}, U^{4118}, U^{4139}, U^{4155}, U^{4158}, C^{4162}, U^{4165}, and C^{4177} (Fig. 7, *A* and *F*). Outside this cluster, a strong UVC-sensitive site was detected at U^{4021} (Fig. 7, *A* and *D*). (v) Within domain VI, three UVC-sensitive sites were clustered in helix 100, which contains the S/R loop (U^{4316}, C^{4317}, and G^{4328}; Fig. 7, *A* and *E*). The only other UVC-sensitive sites detected were U^{4296} and U^{4348} (Fig. 7, *A* and *B*).

Although UVC radiation is known to produce damage to diverse cellular components, our data provide a potential explanation for the UVC-induced inhibition of translation. Damage by UVC to 28 S rRNA in both the PTR and the S/R loop would be expected to impair peptidyl transfer, aminoacyl-tRNA binding, and ribosomal translocation (see "Discussion").

Effects of UVB and UVC Are Similar—Although UVC radiation (200–280 nm) has been used in experimental systems to activate stress kinases and immediate-early genes (reviewed in
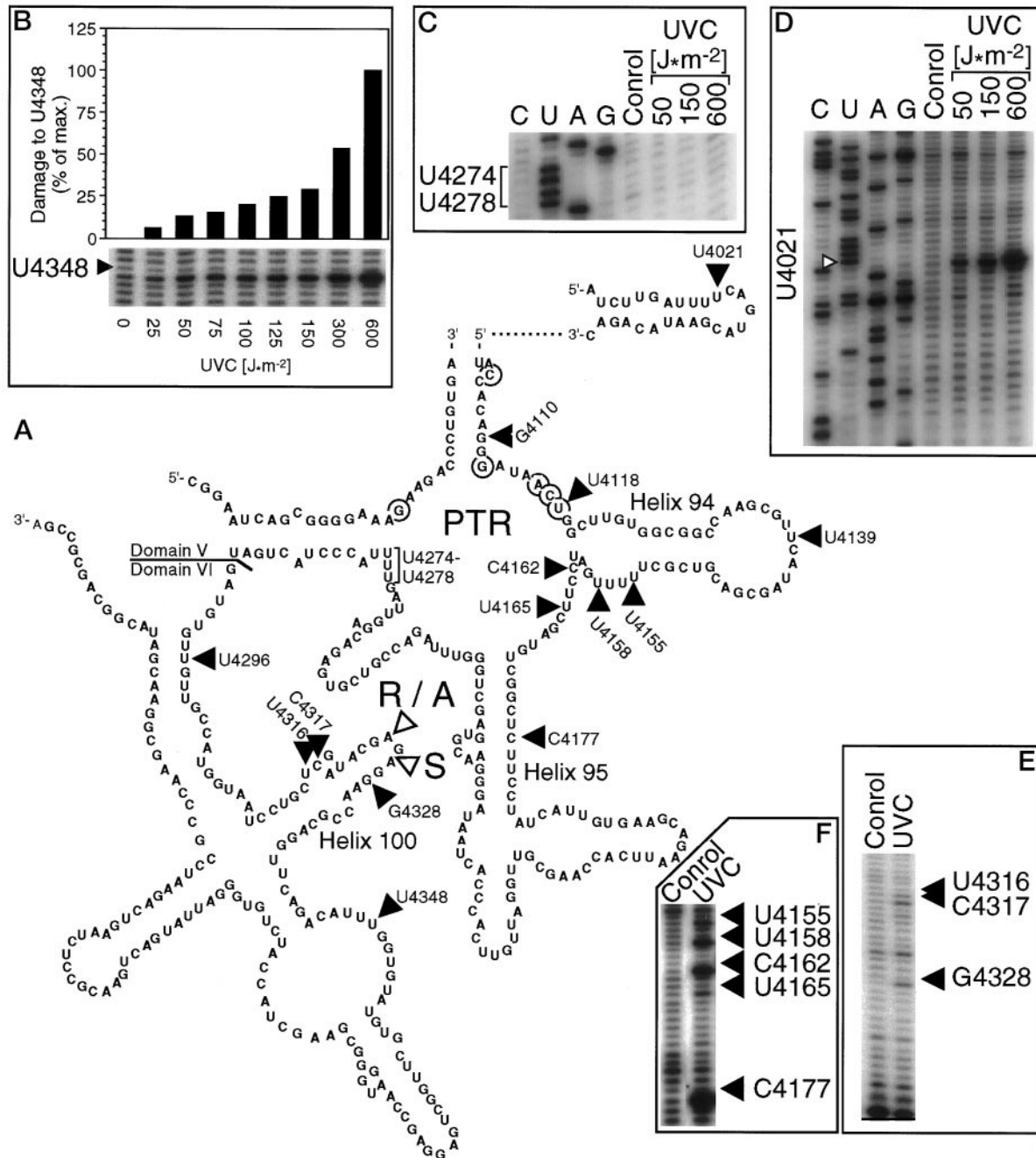
FIG. 7. ***In vivo* damage to the 3′-end of the 28 S rRNA (domains V and VI) incurred after UVC irradiation of Rat-1 cells.** Cells were treated as described in the text. *A,* secondary structure of the 3′-end of the 28 S rRNA (Ref. 22; modified from Ref. 38). The *black triangles* represent all UV-induced lesions detected in the region presented. Selected sites are presented in (*B–F*). The *empty circles* represent the nucleotides in the PTR involved in the binding of anisomycin (27, 43, 44). The *white triangles* represent the sites of action of ricin and abrin (*R/A*), and of sarcin (*S*), respectively, within the S/R loop (38). *B,* dose-dependent occurrence of the UVC-induced reverse transcriptase arrests, exemplified here by the arrest occurring at G^{4347}. The arrest is indicative of a lesion (*black triangles*) occurring at the *next* nucleotide U^{4348} located 3′ of G^{4347}. The same rule applies to all lesions detected here. The intensities of the G^{4347} band (obtained from PhosphorImager™ analysis) in the nonirradiated cells and after 600 J/m^2 of UVC were arbitrarily taken as 0 and 100% damage to U^{4348}, respectively. *C,* lack of UV-induced reverse transcriptase stops in the U_4 stretch 4274–4278. The nucleotide sequence in each region of interest (see, for instance, also *D*) was determined via reverse transcriptase-mediated RNA sequencing (22), shown in the first four lanes. *D,* damage to U^{4021} within the U_4 stretch 4018–4021. *E,* damage to helix 100 bearing the S/R loop. UVC was 600 J/m^2. *F,* some of the lesions incurred in the PTR and the adjacent helices 94 and 95. UVC was 600 J/m^2.

Ref. 2), UVB radiation (280–320 nm) is biologically relevant, since virtually all radiation below 280 nm is filtered out by the earth's atmosphere. UVB radiation has been shown to induce similar, if not identical, stress-induced responses as UVC (2, 17, 36), although comparable responses induced by UVB require the application of substantially more radiant energy (2, 17, 36). We investigated whether active ribosomes are required for UVB-mediated activation of stress kinases and whether a

dose-dependent relationship exists among UVB-mediated activation of stress kinases, inhibition of protein synthesis, and site-specific damage to 28 S rRNA.

Irradiation of cells with increasing doses of UVB resulted in an activation of SAPK/JNK1 activity that leveled at 1000 J/m^2 (Fig. 1*A*, *right*) and in a corresponding inhibition of protein translation (Fig. 1*B*, *right*) that correlated strongly ($R^2 = 0.864$, $p < 0.0001$) with SAPK/JNK1 activation (Fig. 1*C*, *right*). As
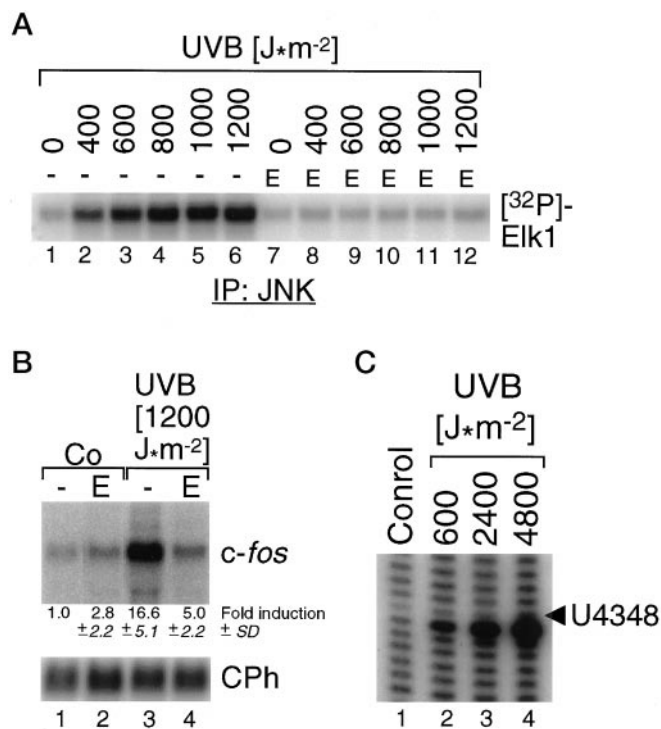
FIG. 8. **Effects of emetine pretreatment on the activation of SAPK/JNK1 and c-*fos* mRNA accumulation by UVB and UVB-unduced damage to the 28 S rRNA *in vivo*.** *A*, cells were left untreated or treated with the indicated doses of UVB, in the absence (−) or in the presence (*E*) of emetine (100 μg/ml) given 1 min prior to the irradiation. Cells were harvested 30 min postirradiation. *IP*, immuno-precipitation. *B*, cells were left untreated or were treated with 1200 J/m² of UVB, in the absence (−) or in the presence (*E*) of emetine (100 μg/ml) given 1 min prior to the irradiation. 30 min postirradiation, the cells were harvested, and the accumulation of c-*fos* mRNA was determined in Northern blot. Hybridization with a cyclophilin (*CPh*) probe (30) revealed similar amount of total RNA (10 μg) loaded in all lanes. *Co*, control cells. S.D. was obtained from three independent repetitions. *C*, cells were irradiated, and damage to RNA was determined as in Fig. 7, except that UVB at the indicated doses was applied.

with irradiation by UVC, the ability of UVB to activate SAPK/JNK1 was prevented by preincubation of cells in emetine (Fig. 8*A*), demonstrating the requirement for actively translating ribosomes in mediating the effects of UVB. Consistent with its effect on SAPK/JNK1 activation, pretreatment of cells with emetine also abrogated the ability of UVB to induce the accumulation of c-*fos* mRNA (Fig. 8*B*). As in the case of UVC, irradiation by UVB induced nucleotide-specific lesions in 28 S rRNA (Fig. 8*C*). Specific lesions in 28 S rRNA produced by UVC were also produced by UVB at doses as low as 600 J/m² (as exemplified in Fig. 8*C* by the lesion at U⁴³⁴⁸), although UVC damaged some sites that were not evident following UVB irradiation.

The Activation of SAPK/JNK1 by UV Is Independent of the Action of UV on Cell Surface Receptors—In HeLa cells, the receptors for EGF, IL-1, and TNF-α have been implicated in the activation of SAPK/JNK1 by UVB and hyperosmotic stress (17). Since our results in both Rat-1 (Figs. 3 and 8) and HeLa cells (data not shown; see Fig. 9*A*) demonstrate that UVC and UVB activate SAPK/JNK1 by mechanisms different from the ones used by hyperosmolarity and growth factor/cytokine receptors, we addressed this controversy experimentally in HeLa cells. We asked whether ribosomal arrest in these cells would affect the activation of SAPK/JNK1 by a single growth factor/cytokine (EGF, IL-1α, or TNF-α), by a combination of them (Σ; Fig. 9*A*), or by UVB. Pretreatment with emetine failed to inhibit the activation of SAPK/JNK1 in response to TNF-α or
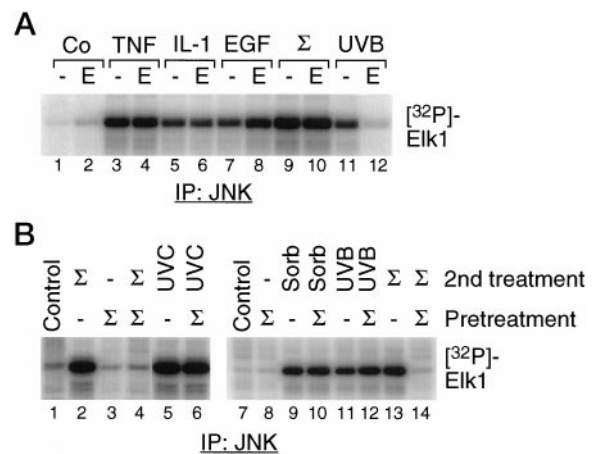

FIG. 9. **Independence of UV-activated SAPK/JNK1 from modulation by cell surface receptors.** *A*, HeLa cells were left untreated or were treated either singly with TNF-α (25 ng/ml), IL-1α (25 ng/ml), or EGF (40 ng/ml); with a combination of all three (Σ); or with UVB (600 J/m²), in the absence (−) or in the presence of emetine (*E*) given 1 min prior to the addition of the respective agonist. SAPK/JNK1 activity was determined 15 min later. *Co*, control cells. *IP*, immunoprecipitation. *B*, HeLa cells were pretreated as indicated with a combination of TNF-α (25 ng/ml), IL-1α (25 ng/ml), and EGF (40 ng/ml) (Σ) for 4 h and were then either left untreated or challenged as indicated with a second inducer: Σ again, sorbitol (*Sorb*, 600 mM), UVC (100 J/m²), or UVB (1200 J/m²). SAPK/JNK1 activity was determined 15 min after the second stimulation. *Co*, control cells. *IP*, immunoprecipitation.

IL-1α and even augmented the activation of the kinase in response to EGF (Fig. 9*A*, *lanes 3–8*). Furthermore, emetine did not inhibit the activation of SAPK/JNK1 by a combined growth factor/cytokine treatment (Fig. 9*A*, *lanes 9* and *10*). By contrast, as in Rat-1 cells, emetine pretreatment completely prevented UVB from activating SAPK/JNK1 (Fig. 9*A*, *lanes 11* and *12*). We then ensured a functional downmodulation of these receptors by pretreatment of HeLa cells with a combination of EGF, IL-1α, and TNF-α (Σ) for 4 h and tested whether SAPK/JNK1 was still activable by UV. The receptor downmodulation was complete, since a second treatment with a combination of EGF, IL-1α, and TNF-α was unable to activate SAPK/JNK1 (Fig. 9*B*, *lanes 2–4* and *lanes 8*, *13*, and *14*). Under these conditions, we applied a dose of UVC that caused an activation of SAPK/JNK1 comparable with that achieved by combined treatment with EGF, IL-1α, and TNF-α (Fig. 9*B*, compare *lanes 2* and *5*). Receptor down-modulation did not prevent UVC from activating SAPK/JNK1 (Fig. 9*B*, *lanes 5* and *6*); identical results were obtained using UVB (Fig. 9*B*, *lanes 11* and *12*) and sorbitol (Fig. 9*B*, *lanes 9* and *10*). Furthermore, results identical to those shown in Fig. 9, *A* and *B*, were obtained using Rat-1 cells (data not shown).

DISCUSSION

In this work, we tested the hypothesis that in mammalian cells ultraviolet radiation triggers a response similar to that elicited by agents such as anisomycin and ricin, whose ability to activate SAPK/JNK and p38/HOG1 have been shown to result from interactions with the 28 S rRNA molecule. This pathway of stress kinase activation has been termed the ribotoxic stress response (22).

Inhibition of Translation and rRNA Damage Induced by UV—Since both UVC and UVB inhibited translation in a dose-dependent manner (Fig. 1*B*), the ribosome appears to serve as an *in vivo* target for UV radiation. The ability of UVC irradiation, similar to emetine and cycloheximide, to arrest poly-

somes[2] supports the notion that UV acts on ribosomes predominantly by inhibiting translational elongation. Translational elongation consists of sequential repetitions of three translational steps: binding of aminoacyl-tRNA, peptidyl transfer, and ribosomal translocation. The involvement of the 3′-end (domains V and VI) of the 28 S rRNA in all three of these steps makes this part of the molecule a potential target for the action of UV on translation. Indeed, by using a reverse transcriptase primer extension assay, we were able to show that both domains V and VI incur UVC- and UVB-induced RNA damage *in vivo* (Figs. 7 and 8*C*). The UV-induced reverse transcriptase arrests occur predominantly at adjacent pyrimidine nucleotides in the 28 S rRNA (Fig. 7), suggesting that these arrests are likely to result from either cyclobutane dimers or 6–4 pyrimidine-pyrimidone photoproducts formed after UV irradiation (37). We have found that the UV-induced RNA lesions appear to be clustered in the two critical functional centers of the molecule: the PTR (involved in the peptidyl transfer) (26, 27) and the S/R loop (involved in both the aminoacyl tRNA binding and the ribosomal translocation) (38). Several lines of evidence support the notion that these lesions are likely to be inhibitory for translational elongation. Numerous inhibitors of the peptidyltransferase (*e.g.* anisomycin, blasticidin S, chloramphenicol, erythromycin, and amicetin) (27, 39) and some bacterial peptides (40) interfere with peptidyl transfer by binding directly to the PTR in the 28 S rRNA or to its highly conserved prokaryotic homolog in the 23 S rRNA. It appears reasonable to expect that the UV-induced lesions in the PTR (Fig. 7) would similarly hamper the peptidyltransferase reaction. Clusters of UV-sensitive sites were detected also in the S/R loop following irradiation by UV *in vivo* (Fig. 7). A single RNA-damaging event in the S/R loop, *i.e.* depurination of A^{4324} by ricin, renders the ribosome inactive (38). UV-induced damage to multiple sites within the S/R loop may similarly affect the function of the loop, thus inhibiting translation.

Unlike ribotoxins, whose interactions with the ribosomes are highly selective, physical agents such as UV and ionizing radiation produce a variety of structural alterations in diverse cellular locations. Absorption of UV radiation produces structural alterations in diverse nucleic acids and proteins and also can lead to oxidative damage via the production of reactive oxygen intermediates (36). The possibility cannot be excluded, therefore, that other UV-induced damages to the translational machinery (*e.g.* damage to tRNA; mRNA; or 5, 5.8, and 18 S rRNA) contribute to the overall ribotoxicity of UV. For example, we have detected UV-sensitive ribonucleotides in the large subunit 5 S rRNA.[3]

Correlations between the UV Dose and the Inhibition of Translation, rRNA Damage, and Activation of SAPK/JNK1— Biologically relevant (*i.e.* submaximal and within the linear rate of increase) levels of UV-induced activation of SAPK/JNK1 correlated with doses of either UVC or UVB that inhibited translation by less than 30% (Fig. 1*C*) and caused only moderate damage to rRNA (Figs. 7 and 8*C*). These results are in agreement with our previous observation that ribotoxic agents that activate SAPK/JNK1 (*e.g.* anisomycin) do so effectively without complete inhibition of protein synthesis (22). The functional implication of these findings is that low doses of a ribotoxic stressor would elicit a transcriptional response under conditions that allow sufficient translation of the induced mRNAs, thus ensuring the expression of stress-induced genes. Furthermore, the finding that for both UVC and UVB the dose

required to achieve half-maximal activation of SAPK/JNK1 corresponded to a UV irradiation that inhibited protein synthesis to the same extent (Fig. 1*C*) is consistent with the conclusion made by others (for a detailed discussion, see Ref. 2) that the primary sensors and the subsequent signal transduction events are the same for both UVC and UVB.

*Requirement for Actively Translating Ribosomes for the UV-induced Signaling to SAPK/JNK1 and to p38/HOG-1—*Ribotoxic stressors, which are among the most potent activators of SAPK/JNK1 activity (22), cause alterations in the structure of both the PTR and the S/R loop. We have previously shown that such alterations in the 28 S rRNA constitute the triggering events for the mammalian ribotoxic stress response (22). If UV acts as a bona fide ribotoxic stressor to elicit the transcriptional UV response, the ability of UV to activate stress kinases and gene expression should require the presence of active ribosomes at the moment of exposure to UV. The results presented in Figs. 3–6 demonstrated that the presence of actively translating ribosomes at the moment of UV irradiation is absolutely required for the UV-induced activation of SEK1/MKK4, SAPK/JNK1, and p38/HOG-1. Inhibition of the ribosomal function for as little time as 1 min prior to the UV irradiation obliterated the responsiveness of SAPK/JNK1 to UV, whereas blocking the ribosomal function later than 1 min after the irradiation was significantly less efficient in inhibiting the response of SAPK/JNK1 to UV (Fig. 4). These results demonstrate that the UVC-induced signaling to SAPK/JNK1 is sensitive to the functional state of ribosomes only for a short time after irradiation and then rapidly becomes independent of the presence of active ribosomes. In addition to the ribosomal inhibitors presented here (pactamycin, emetine, cycloheximide, and puromycin; Figs. 3–6), similar inhibition of the responsiveness of SAPK/JNK1 to UV was obtained using ribosomal inhibitors as diverse as diphtheria toxin and T-2 toxin (data not shown; see Ref. 22). The inhibition of UV-induced SAPK/JNK1 activation by pretreatment of cells with ribosomal inhibitors (Figs. 3 and 5*A*) was reproduced also in MEFs and HeLa cells (data not shown; see Fig. 9*A*, *lanes 11* and *12*), indicating a general cellular phenomenon not restricted to Rat-1 cells.

Phosphorylation of the kinase SEK1/MKK4, an upstream activator of both SAPK/JNK and p38/HOG-1 (23), also appeared to require the presence of actively translating ribosomes at the moment of application of UV or a ribotoxic stressor (Fig. 6). However, the responsiveness of this kinase to osmotic shock or DEB did not depend on the presence of active ribosomes. These results suggest that UVC and ribotoxic stress activate signal-transducing molecules upstream of SEK1/MKK4 through similar, if not identical, mechanisms and that these mechanisms are different from the ones utilized by osmotic stress and genotoxins.

*Requirement for Actively Translating Ribosomes for the UV-induced Accumulation of c-fos mRNA—*Our results (Fig. 5*C*) led us to conclude that the proximal signal transduction devices leading to the activation by UV of SAPK/JNK1 and p38/HOG-1, on one hand, and to the activation by UV of ERK, on the other hand, are different. Thus, the activation of ERK1 by UVC was not inhibited by ribosomal inactivation. In fact, we have reproducibly observed that pactamycin and other protein synthesis inhibitors potentiate ERK1 (Fig. 5*C* and data not shown). The slow kinetics of this activation is consistent with the idea of a disappearing negative regulator of ERK1 (such as MAP kinase phosphatase-1 or another phosphatase with a rapid turnover kinetics). Furthermore, the application of both pactamycin and UVC caused an additive activation of ERK1 (Fig. 5*C*), which is likely to result from the independent routes the two agents use to reach the kinase.

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[2] M. S. Iordanov, J. A. Pearson, and B. E. Magun, unpublished observations.

[3] M. S. Iordanov, D. Pribnow, and B. E. Magun, unpublished observations.

Taken together, our results suggest that the primary UV-absorbing events to generate signaling either to the stress kinases or to the ERK may be different (see below for discussion on growth factor receptors). However, since all three families of MAP kinases are activated by UV (Refs. 5 and 9–12; this work), it was relevant to investigate how the opposing effects that translational inhibitors exert on the UV-induced activation of the MAP kinase affect the induction of c-*fos* by UV. We were able to demonstrate that, in accordance with the effects of emetine on SAPK/JNK1 and p38/HOG-1, emetine pretreatment strongly inhibits the induction of c-*fos* in response not only to UV (Fig. 8*B*) but also to anisomycin (data not shown), whereas the induction of c-*fos* in response to EGF was unaffected (data not shown). These results support the conclusion that the induction of c-*fos* by ribotoxic stress and UV operate via SAPK and p38/HOG-1 and are ribosome-dependent but that signaling through ERK1 is ribosome-independent.

Lack of Evidence for the Involvement of Growth Factor/Cytokine Receptors in the UV-induced Signal Transduction to SAPK/JNK1 and p38/HOG-1—According to a recently proposed hypothesis put forward to explain the genesis of the UV response, UV irradiation of cells provokes a ligand-independent activation of multiple transmembrane growth factor/cytokine receptors (5, 17). A consequence of this hypothesis is that once functional activation of a receptor by UV has been achieved, the subsequent intracellular signal transduction events will be identical for UV and the respective growth factor or cytokine. Although probably only a fraction of the UV-activated receptors has been identified, it has been argued that in HeLa cells functional down-modulation of the receptors for EGF, TNF, and IL-1 is sufficient to render SAPK/JNK1 unresponsive to UVB (17), suggesting that most of the UV-induced signaling to SAPK/JNK1 is mediated by these receptors. The results shown in Fig. 9 do not support this hypothesis. Both in HeLa cells (Fig. 9*A*) and in Rat-1 cells (Fig. 4 and data not shown) ribosomal inactivation failed to inhibit the activation of SAPK/JNK1 by either a single growth factor/cytokine or a combination of them, whereas the UV-induced activation of SAPK/JNK1 was strongly suppressed by ribosomal inactivation. Similar to growth factors and proinflammatory cytokines, hyperosmotic conditions and the DNA-damaging agent DEB also did not require active ribosomes to activate SEK1/MKK4 and SAPK/JNK1 (Figs. 3 and 6). Furthermore, both in HeLa cells (Fig. 9*B*) and in Rat-1 cells (data not shown), we were unable to detect a reduced responsiveness of SAPK/JNK1 to UV following a combined down-modulation of the receptors for EGF, TNF, and IL-1. Taken together, our findings support the notion that UV uses proximal signaling pathways to SEK1/MKK4, SAPK/JNK1, and p38/HOG-1 that are different from the ones used by extracellular signaling polypeptides. On the other hand, our results are not in disagreement with the notion that the initial signal for the activation of ERK by UV irradiation may be generated by UV-induced activation of growth factor receptors (5, 10, 36).

In summary, the results presented in this work support the notion that, unlike genotoxic, hyperosmotic, and proinflammatory stressors, UV irradiation activates stress kinases and gene expression via UV-induced ribotoxic stress. The involvement of the 23 S/28 S rRNA in mediating the ribotoxic stress response is conserved between prokaryotes and eukaryotes (22). The antibiotics chloramphenicol and erythromycin bind to the same region of the 23 S rRNA in prokaryotes to which anisomycin binds in the 28 S rRNA of eukaryotic cells (27, 41). In *E. coli*, chloramphenicol and erythromycin elicit the bacterial cold shock response (42). This striking similarity in the ability of a conserved region of the 23 S/28 S rRNA to initiate cellular reactions in response to binding of inhibitors of the peptidyl transferase reaction, both in bacteria and in higher eukaryotes, demonstrates the presence of a universal and evolutionary conserved function of the ribosome in sensing stress and suggests that some of the ribosomal signal-transducing components acting immediately downstream of the 23 S/28 S rRNA may be conserved. The capacity of mammalian cells from diverse tissues to respond to UV radiation, a physical agent most somatic cells are unlikely to encounter, may represent a conserved response inherited from unicellular organisms that must mount productive responses following exposure to UV radiation.

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