The p38 mitogen-activated protein (MAP) kinase defines a subgroup of the mammalian MAP kinases that are induced in response to lipopolysaccharide, hyperosmolarity, and interleukin 1. p38 MAP kinase appears to play a role in regulating inflammatory responses, including cytokine secretion and apoptosis. Here we show that diverse classes of DNA-damaging agents such as cisplatinum, 1-β-D-arabinofuranosylcytosine, UV light, ionizing radiation, and methyl methanesulfonate activate p38 MAP kinase. We also demonstrate that cells deficient in c-Abl fail to activate p38 MAP kinase after treatment with cisplatinum and 1-β-D-arabinofuranosylcytosine but not after exposure to UV and methyl methanesulfonate. Reconstitution of c-Abl in the Abl−/− cells restores that response. Similar results were obtained for induction of the Jun-NH2-kinase/stress-activated protein kinase. These findings indicate that p38 MAP and Jun-NH2-kinase/stress-activated protein kinases are differentially regulated in response to different classes of DNA-damaging agents.

The mitogen-activated protein kinases (MAPks) are induced by diverse stimuli in the transduction of signals from the cell membrane to the nucleus. Three groups of MAPks have been identified: ERK (1–3), JNK/SAPK (4–7), and p38 MAPK (8–10). The MAPks are activated by phosphorylation on Thr and Tyr at Thr-Tyr motifs, which differ depending on the group (11). Each MAPK group has distinct upstream activators and substrate specificities (11). For example, the transcription factor c-Jun is a substrate of the JNK/SAPK pathway (4, 5), ATF2 is the target of p38 MAPK and JNK/SAPK (6, 12–14), and Elk1 is phosphorylated by all three groups of MAPks (12, 15, 16).

The JNK/SAPKs are activated by tumor necrosis factor (TNF), anisomycin, and UV light (4, 5). The demonstration that certain DNA-damaging agents activate p38 MAPK and JNK/SAPK (14, 30). Other studies have demonstrated that the c-Abl protein tyrosine kinase functions upstream to JNK/SAPK in the cellular response to IR, certain alkylating agents, and ara-C (23, 24). Transfection of activated forms of Abl has also been associated with stimulation of JNK/SAPK activity (24–26). The activation of JNK/SAPK by c-Abl-dependent mechanisms, however, may be limited to certain agents, since TNF-induced SAPK activity occurs in c-Abl-deficient cells (23).

The p38 MAPK pathway is activated by TNF and interleukin 1 (9, 10, 13). This pathway is also activated by endotoxin, osmotic stress, and heat shock (8, 9). Activation of p38 MAPK is mediated by phosphorylation on Thr and Tyr by MKK3 and MKK6 (12, 14, 27–29). MKK3 and MKK6 specifically activate p38 MAPK, whereas SEK1/MKK4 may activate both p38 MAPK and JNK/SAPK (14, 30). Other studies have demonstrated that the Rho GTPases and multiple PAKs regulate p38 MAPK and JNK/SAPK activation (31–33). Thus, some signals appear to be capable of activating both the p38 MAPK and JNK/SAPK pathways. Since certain DNA-damaging agents activate JNK/SAPK, the present studies have addressed the potential involvement of genotoxic stress in activation of p38 MAPK. The results demonstrate that diverse DNA-damaging agents activate p38 MAPK and that this response is mediated by c-Abl-dependent and -independent mechanisms.

### EXPERIMENTAL PROCEDURES

**Cell Culture and Reagents**—Human U-937 myeloid leukemia cells (American Type Culture Collection, Rockville, MD) were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mm L-glutamine. NIH3T3 and Abl−/− cells (23) were grown in DMEM containing 10% fetal bovine serum and antibiotics. Abl−/− cells were reconstituted with c-Abl by retroviral transfection as described (34). Cells (1 × 10^6/)100-mm culture dish) were plated 24 h before treating with ara-C (Sigma), CDDP (Sigma), and methyl methanesulfonate (MMS) (Sigma). Cells were treated with 10 or 20 gray IR at room temperature with a Gammacell 1000 (Atomic Energy of Canada, Ot-
tawa, Ontario, Canada) under aerobic conditions with a $^{137}$Cs source emitting at a fixed dose rate of 0.76 gray min$^{-1}$ as determined by dosimetry. Cells were also treated with 80 J/m$^2$ UV (UV Stratalinker 1800, Stratagene).

**Immune Complex Kinase Assays**—Cells were washed with PBS and lysed in 1 ml of lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, 1 mM diithiothreitol, and 10 μg/ml leupeptin and aprotinin) as described (24). Lysates were incubated with anti-SAP kinase (Santa Cruz Biotechnology, Santa Cruz, CA) or anti-p38 MAPK (16) antibodies for 1 h and then for 45 min after the addition of protein A-Sepharose. The immune complexes were washed three times with lysis buffer and once with kinase buffer and resuspended in kinase buffer containing $[^{32}]$P]ATP (6000 Ci/mmol; DuPont NEN) containing either GST-Jun (2–100) or GST-ATF2 (1–109). The reactions were incubated for 15 min at 30°C and were terminated by the addition of SDS sample buffer. The proteins were analyzed by 10% SDS-PAGE and autoradiography.

**Immunoprecipitation and Immunoblot Analysis**—Soluble proteins (100 μg) were incubated with anti-Abl antibody (Santa Cruz Biotechnology) for 1 h and precipitated with protein-A-Sepharose for an additional 1 h. The resulting immune complexes were washed three times with lysis buffer and once with kinase buffer and resuspended in kinase buffer containing (γ-$^{32}$P)ATP (6000 Ci/mmol; DuPont NEN) containing either GST-Jun (2–100) or GST-ATF2 (1–109). The reactions were incubated for 15 min at 30°C and were terminated by the addition of SDS sample buffer. The proteins were analyzed by 10% SDS-PAGE and autoradiography.

**RESULTS AND DISCUSSION**

Previous studies have demonstrated that certain classes of DNA-damaging agents, such as IR, CDDP, and ara-C, activate JNK/SAPK (17–19, 22–24). To determine whether p38 MAPK is activated by genotoxic stress, we treated NIH3T3 cells with CDDP and assayed anti-p38 MAPK immunoprecipitates for phosphorylation of GST-ATF2. The CDDP-treated cells exhibited an increase in p38 MAPK activity that was detectable at 1 h and reached maximal levels at 3 h (Fig. 1A). The CDDP-induced increases in p38 MAPK activity occurred in the absence of changes in p38 MAPK protein levels (Fig. 1A). Since p38 MAPK is activated, in part, by phosphorylation on Tyr(13), we assayed the anti-p38 MAPK immunoprecipitates for reactivity with an anti-Tyr(P) antibody. The results demonstrate increased tyrosine phosphorylation of p38 MAPK as a consequence of CDDP treatment (Fig. 1A). The results also demonstrate increased tyrosine phosphorylation of p38 MAPK as a consequence of ara-C treatment (Fig. 2B, upper panel). The results also demonstrate increased tyrosine phosphorylation of p38 MAPK as a consequence of ara-C treatment.
(Fig. 2B, bottom panel). Similar results were obtained in ara-C-treated NIH3T3 cells (Fig. 2C). In contrast, there was no detectable effect of IR on p38 MAPK activity in NIH3T3 cells (data not shown), whereas this kinase was induced in IR-treated U-937 cells (Fig. 2D, upper panel). Activation of JNK/SAPK by IR was more pronounced in U-937 cells compared to NIH3T3 cells (Fig. 2D, lower panel). Activation of JNK/SAPK by these agents (23, 24). Moreover, c-Abl is involved in activation of JNK/SAPK by these agents (23, 24). Consequently, we asked whether activation of p38 MAPK occurs by a c-Abl-dependent mechanism. To address this issue, we treated cells deficient in c-Abl (Abl<sup>−−</sup>) (34) with CDDP. In contrast to NIH3T3 cells, there was little effect of CDDP on p38 MAPK activity in Abl<sup>−−</sup> cells (Fig. 3A, left panel). c-Abl expression was reconstituted in Abl<sup>−−</sup> cells (designated Abl<sup>+</sup>), the level of c-Abl protein was approximately 20% of that in NIH3T3 cells (Fig. 3A, right panel). Treatment of Abl<sup>+</sup> cells with CDDP was associated with a 5-fold increase in p38 MAPK activity (Fig. 3A). We also treated Abl<sup>−−</sup> cells with CDDP for longer periods of time to test whether p38 MAPK is activated as a late response.
Activation of p38 MAP Kinase by c-Abl

Other studies have demonstrated that UV activates JNK/SAPK by damaging DNA (38, 40). Activation of JNK/SAPK in response to UV may, therefore, be regulated by at least two different mechanisms. In this context, our results demonstrate that UV-induced JNK/SAPK activation is mediated, at least in part, by a c-Abl-dependent mechanism.

Finally, MMS is a monofunctional alkylating agent that alkylates DNA and damages membrane proteins (41, 42). Therefore, MMS may also activate JNK/SAPK and p38 MAPK by DNA damage-independent mechanisms. Indeed, MMS induces JNK/SAPK and p38 MAPK activity in Abl-/{sup}−/−{sub} cells, perhaps by signals activated at the cell membrane. Taken together, these results demonstrate that activation of the stress response to diverse agents can be distinguished by c-Abl-dependent and -independent mechanisms.

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