Coinduction of c-*jun* Gene Expression and Internucleosomal DNA Fragmentation by Ionizing Radiation†

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ABSTRACT: Previous work has demonstrated that the cellular response to ionizing radiation includes transcriptional activation of the c-*jun* early response gene. The present studies demonstrate that this induction of c-*jun* expression is temporally related to the appearance of internucleosomal DNA fragmentation. These events were maximal at 6 h and transient after exposure to lethal doses (20 Gy) of ionizing radiation. We also demonstrate that *N*-acetyl-L-cysteine (NAC), an antioxidant, inhibits X-ray-induced c-*jun* expression and endonucleolytic DNA cleavage. These findings suggested that both events are mediated at least in part through the formation of reactive oxygen intermediates (ROIs). Since ROIs damage DNA and X-ray-induced DNA damage is associated with activation of poly(ADP-ribose) polymerase (ADPRP), we studied the effects of the ADPRP inhibitors 3-aminobenzamide (3-AB), nicotinamide, and theophylline. 3-AB blocked both X-ray-induced c-*jun* expression and internucleosomal DNA fragmentation. Similar findings were obtained with nicotinamide and theophylline. In contrast, 3-AB had little if any effect on induction of c-*jun* transcripts or DNA fragmentation induced by the alkylating agent mitomycin C. While c-*jun* expression is restricted to cells in G_1 and G_1/S phases, we have found that X-ray-induced c-*jun* transcripts are detectable throughout all phases of the cell cycle. The induction of internucleosomal DNA fragmentation by X-rays was also detectable throughout the cell cycle. Taken together, these results support the coinduction of c-*jun* transcription and internucleosomal DNA fragmentation by ionizing radiation. Similar studies were performed with H_2O_2 since this agent also results in the production of ROIs. While H_2O_2 induced c-*jun* expression by an NAC-sensitive mechanism, this event was not affected by 3-AB and was not associated with internucleosomal DNA fragmentation. These findings suggest that while activation of the c-*jun* gene and endonucleolytic DNA cleavage are coinduced by ionizing radiation, these events are differentially regulated by other ROI-mediated mechanisms.

Eukaryotic cells respond to ionizing radiation with cell cycle arrest and activation of DNA repair mechanisms. The associated DNA damage can result in mutagenesis, transformation, and cell death. The available evidence indicates that ionizing radiation induces such effects by direct interaction with DNA or through the formation of reactive oxygen intermediates (ROIs) which damage DNA (Hall, 1988). However, the signal transduction mechanisms that control the cellular response to ionizing radiation remain unclear. Recent studies have demonstrated that exposure to ionizing radiation is associated with induction of certain early response genes that code for transcription factors. The finding that ionizing radiation induces members of the *jun*/*fos* and *EGR*-1 gene families (Sherman et al., 1990a; Hallahan et al., 1991a; Datta et al., 1992a,b; Hallahan et al., 1993) has provided support for the involvement of nuclear signaling cascades. Other studies have shown that ionizing radiation induces expression and DNA binding of nuclear factor κB (NF-κB) (Brach et al., 1991). Moreover, recent work has demonstrated that levels of the tumor suppressor nuclear protein p53 increase in association with an arrest in G_1 phase that follows exposure to ionizing radiation (Kastan et al., 1991, 1992). The activation of transcription factors by ionizing radiation presumably constitutes the transduction of early nuclear signals to longer term changes that reflect the response to this agent. Indeed, p53 has been shown to be a sequence specific DNA-binding protein (Bargonetti et al., 1991; Kern et al., 1991) that regulates transcription of the GADD45 gene (Kastan et al., 1992). A variety of other genes induced by ionizing radiation include tumor necrosis factor (TNF), platelet-derived growth factor, fibroblast growth factor, and interleukin-1 (Hallahan et al., 1989; Witte et al., 1989; Woloschak et al., 1990). While TNF expression is regulated at the transcriptional level following exposure to ionizing radiation (Sherman et al., 1991), the trans-acting factors responsible for this effect remain unclear.

The c-*jun* gene, which is induced as an immediate early event by phorbol esters, serum, and growth factors (Quantin & Breathnach, 1988; Ryder & Nathans, 1988; Brenner et al., 1989; Wu et al., 1989), codes for a major form of the AP-1 transcription factor (Bohmann et al., 1987; Angel et al., 1988a; Chiu et al., 1988). AP-1 binds to the DNA consensus sequence $TGA^G/_CTCA$ (TRE) that regulates genes responsive to phorbol esters (Angel et al., 1987; Lee et al., 1987; Chiu et al., 1987). The affinity of *jun*/AP-1 binding to DNA is a function of the type of protein containing a leucine zipper and DNA binding domain that is present in the dimer complex (Mitchell & Tjian, 1989). This structure is shared by a family of transcription factors that include products of the *jun*-B and c-*fos* genes (Mitchell & Tjian, 1989). Certain insights are

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available regarding the signals that contribute to the regulation of c-*jun* and other members of the family. For example, the finding that phorbol esters activate c-*jun* transcription in diverse cell types has implicated the involvement of protein kinase C (PKC)-dependent mechanisms (Brenner et al., 1989; Angel et al., 1988a; Sherman et al., 1990b). Similar pathways may play a role in the induction of c-*jun* expression by ionizing radiation. In this context, both nonspecific inhibitors of PKC and prolonged exposure to phorbol esters block X-ray-induced c-*jun* expression (Hallahan et al., 1991a). However, recent studies have demonstrated that ionizing radiation induces c-*jun* transcription in an HL-60 myeloid leukemia cell variant, designated HL-525, which is deficient in PKC-mediated signal transduction (Datta et al., 1992a). The finding that these cells are resistant to phorbol ester-induced c-*jun* expression has suggested that X-rays and phorbol esters activate the c-*jun* gene by distinct mechanisms.

Other work has indicated that ionizing radiation induces c-*jun* expression through the formation of ROIs. Recent studies with *N*-acetyl-L-cysteine (NAC) have demonstrated that this antioxidant inhibits X-ray-induced increases in c-*jun* expression (Datta et al., 1992a). NAC counteracts the effects of oxidative stress by scavenging ROIs and increasing intracellular glutathione (GSH) (Aruona et al., 1989; Burgunder et al., 1989). Additional studies have demonstrated that NAC blocks activation of the c-*jun* gene by UV light (Devary et al., 1991) and the EGR-1 gene by ionizing radiation (Datta et al., 1993). Moreover, this antioxidant has been found to inhibit activation of NF-κB(Staal et al., 1990; Schreck et al., 1991). These findings have suggested that ROIs are involved in the activation of multiple nuclear signaling cascades. However, ROIs have extremely short half-lives and it is not clear whether activation of these cascades is directly or indirectly related to the formation of oxygen radicals. Since ROIs damage DNA in X-ray-treated cells, this damage may represent the signal responsible for induction of early response gene expression. Indeed, other DNA-damaging agents such as 1-β-D-arabinofuranosylcytosine (ara-C), cisplatin, and etoposide, which are not known to act through ROIs, also induce c-*jun* transcription (Kharbanda et al., 1990, 1991; Rubin et al., 1991).

The present studies demonstrate that induction of c-*jun* expression by lethal doses of ionizing radiation is temporally associated with internucleosomal DNA fragmentation. We have also found that both of these events are blocked by NAC, as well as inhibitors of poly(ADP-ribose) polymerase (ADPRP). Moreover, the results demonstrate that both c-*jun* expression and endonucleolytic DNA cleavage are coinduced in all phases of the cell cycle. Other studies with H_2O_2 suggest that these events are differentially regulated by other ROI-mediated pathways.

MATERIALS AND METHODS

Cell Culture. U-937 cells (American Type Culture Collection, Rockville, MD) were grown in RPMI 1640 medium containing 10% fetal bovine serum supplemented with 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. Irradiation (20 Gy) was performed at room temperature using a Gammacell 1000 (Atomic Energy of Canada Ltd., Ontario) with a ^{137}Cs source emitting at a fixed dose rate of 14.3 Gy/min. Dosimetry was calibrated by Nordian International (Kanata, Canada) using a Fricke dosimeter. The cells were also treated with 30 mM NAC (Sigma Chemical Co., St. Louis, MO), 5 mM 3-aminobenzamide (3-AB; Sigma), 10 μM mitomycin C (Sigma), 150 μM H_2O_2 (Sigma), 5 mM nicotinamide (Sigma) and 1 mM theophylline (Sigma).

Isolation and Analysis of RNA. Total cellular RNA was purified by the guanidine isothiocyanate–cesium chloride technique (Chirgwin et al., 1979). The RNA was analyzed by electrophoresis through 1% agarose–formaldehyde gels, transferred to nitrocellulose filters, and hybridized to the following ^{32}P-labeled DNA probes: (1) the 1.8 kb *Bam*HI/*Eco*RI insert of a human c-*jun* gene purified from a pBluescript SK(+) plasmid (Angel et al., 1988b); (2) the 1.5-kb *Eco*RI fragment of the murine *jun*-B cDNA from the p465.20 plasmid (Ryder et al, 1988); (3) the 0.9-kb *Sca*I/*Nco*I insert of a human c-*fos* gene purified from the pc-*fos*-1 plasmid (Curran et al., 1983); and (4) the 2.0-kb *Pst*I insert of a chicken β-actin gene purified from the pA1 plasmid (Cleveland et al., 1980). Hybridizations were performed at 42 °C for 24 h in 50% (v/v) formamide, 2 × SSC, 1 × Denhardt's solution, 0.1% SDS, and 200 μg/mL salmon sperm DNA. The filters were washed twice in 2 × SSC–0.1% SDS at room temperature and then in 0.1 × SSC–0.1% SDS at 60 °C for 1 h. Autoradiographic bands were scanned using a LKB Produkter (Bromma, Sweden) Ultroscan XL laser densitometer and analyzed with the Gelscan XL software package (version 1.21). Autoradiograms were developed at multiple exposure times to ensure that densitometric quantitation was in a linear range. Signal intensity was normalized to that for the actin control.

Analysis of DNA fragmentation. Cells (1×10^6) were harvested, washed, and incubated in 20 μL of 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 0.5% SDS, and 0.5 μg/mL proteinase K (Sigma) for 3 h at 50 °C. Ten microliters of 0.5 mg/mL RNase A was then added and the incubation continued for an additional 1 h. The digested samples were incubated with 10 μL of 10 mM EDTA (pH 8.0) containing 2% (wt/vol) low-melting-point agarose, 0.25% bromophenol blue, and 40% sucrose at 70 °C. The DNA was separated in gels containing 2% agarose/TAE (0.04 M Tris-acetate and 0.001 M EDTA, pH 8.0) buffer at 23 V for 16 h unless otherwise indicated and visualized by UV illumination after ethidium bromide staining.

The extent of fragmentation was quantitated by labeling cells with [6-^{3}H]thymidine (15 Ci/mmol) for 14 h. The cells were washed and resuspended in complete medium. After treatment, the cells were washed and lysed in 5 mM Tris-HCl, pH 8.0, 10 mM EDTA and 0.5% Nonidet P-40 at 4 °C for 3 h. The lysate was centrifuged at 13000*g* for 20 min at 4 °C, and supernatants containing low molecular weight DNA were assayed by scintillation counting. Pellets containing intact DNA were solubilized in 1 N NaOH, and the solution was neutralized with 1 N HCl. Percentage fragmentation was calculated by determining the fraction of radioactivity representing fragmented DNA with molecular weights of approximately 23 kb or less as determined by gel electrophoresis.

Centrifugal Elutriation. Cells in exponential growth phase or after treatment with ionizing radiation were subjected to centrifugal elutriation using the JE-5.0 elutriation system (Beckman Instruments, Inc., Palo Alto, CA). Approximately 2×10^8 cells were applied to the standard chamber (1600 rpm at 27 °C) using a digital flow controller (Cole-Parmer Instrument Co., Chicago, IL). The calibrated pump speed was increased from 10 to 30 mL/min. Enriched cell populations from different phases of the cell cycle were elutriated in 100-mL aliquots of RPMI 1640 medium containing 1% fetal bovine serum. Aliquots (1 mL) were fixed by adding 2 mL of ice-cold methanol for 60 min on ice. After

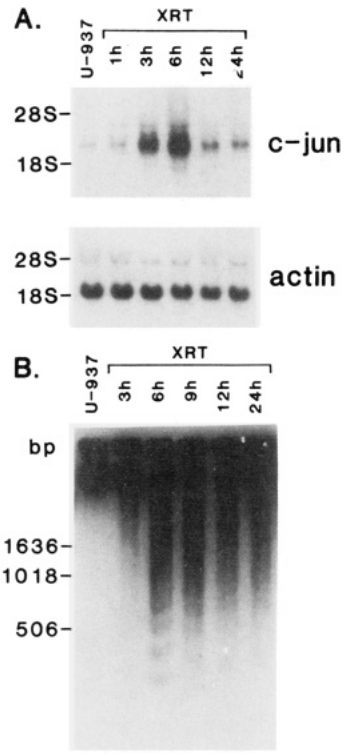

FIGURE 1: Induction of c-*jun* expression and internucleosomal DNA cleavage by ionizing radiation. U-937 cells were exposed to 20 Gy X-rays (XRT). (A) Total cellular RNA (20 μg) was isolated at the indicated times and hybridized to ^{32}P-labeled c-*jun* or actin probes. (B) DNA was isolated at the indicated times and monitored for DNA fragmentation by electrophoresis in 2% agarose gels.

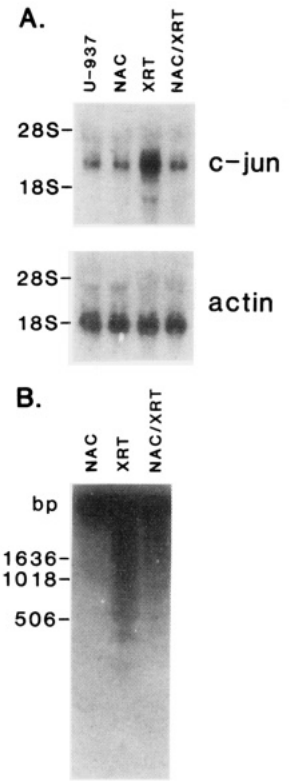

FIGURE 2: NAC inhibits induction of c-*jun* expression and internucleosomal DNA fragmentation by ionizing radiation. Cells were exposed to ionizing radiation (XRT) and/or 30 mM NAC. The NAC was added 30 min prior to irradiation and included in the medium for an additional 6 h. Cells treated with NAC alone were harvested after 6.5 h. (A) Total cellular RNA (20 μg) was hybridized to the ^{32}P-labeled c-*jun* and actin probes. (B) DNA fragmentation was monitored in 2% agarose gels.

centrifugation, the supernatant was removed and the cell pellet incubated with 500 μL of RNase (200 units/mL) and 500 μL of propidium iodide buffer in the dark at room temperature for 30 min. The stained cells were analyzed for DNA content on a FACScan (Becton, Dickinson & Co., Mountain View, CA) using CellFIT cell cycle analysis software.

RESULTS

Previous studies have shown that treatment of HL-60 cells with ionizing radiation is associated with transient induction of c-*jun* expression (Sherman et al., 1990a; Datta et al., 1992a). Similar findings were obtained in the present studies with U-937 cells. c-*jun* transcripts were at low levels in untreated U-937 cells and were increased following X-ray treatment Figure 1A. This increase was maximal at 6 h and transient, while there was little effect of ionizing radiation on expression of the actin gene (Figure 1A). Recent work has demonstrated that induction of c-*jun* expression by other DNA-damaging agents is associated with DNA fragmentation (Gunji et al., 1991; Rubin et al., 1991). Consequently, we isolated DNA for analysis in agarose gels. There was no detectable DNA fragmentation in untreated cells (Figure 1B). Moreover, there was no evidence of fragmentation at 1 h after exposure to X-rays. In contrast, DNA fragments at multiples of approximately 200 bp were present at 6 h after X-ray treatment (Figure 1B). This pattern of fragmentation is consistent with internucleosomal DNA cleavage, while longer intervals after ionizing radiation exposure were associated with a more diffuse pattern of degradation.

Recent studies have indicated that the effects of ionizing radiation on c-*jun* expression are mediated at least in part by the formation of ROIs (Datta et al., 1992a). The role of ROIs was similarly addressed in the present work with the antioxidant NAC. Exposure of U-937 cells to 30 mM NAC had little if any effect on constitutive levels of c-*jun* transcripts (Figure 2A). However, this agent blocked X-ray-induced increases in c-*jun* transcripts by over 80% Figure 2A. Similar preparations of U-937 cells were analyzed for DNA fragmentation. NAC alone had no detectable effect on DNA fragmentation (Figure 2B). Exposure of these cells to ionizing radiation was associated with endonucleolytic cleavage and this effect was blocked by NAC (Figure 2B). Taken together, these findings supported a temporal relationship between induction of c-*jun* expression and DNA fragmentation and that both of these events are induced through formation of ROIs.

Ionizing radiation damages DNA directly or through the formation of ROIs (Hall, 1988). The DNA strand breaks resulting from X-ray-induced damage function as a cofactor for activation of ADPRP (Benjamin & Gill, 1980). Consequently, we asked whether inhibition of ADPRP and thereby poly-ADP-ribosylation of chromosomal proteins would alter induction of c-*jun* expression and internucleosomal DNA cleavage. In addressing this issue, we used 3-aminobenzamide (3-AB), an inhibitor of ADPRP and DNA repair (Milam and Cleaver, 1984). Treatment of U-937 cells with 3-AB alone had little effect on c-*jun* mRNA levels Figure 3A. However, this agent inhibited X-ray-induced increases in c-*jun* expression by over 75% (Figure 3A). Similar findings were obtained in three separate experiments (mean percent inhibition ± SE: 79.3 ± 5.2). In contrast, 3-AB had no detectable effect on induction of c-*jun* transcripts by the bifunctional alkylating agent mitomycin C (Figure 3A). Other studies have demonstrated that ionizing radiation induces additional members

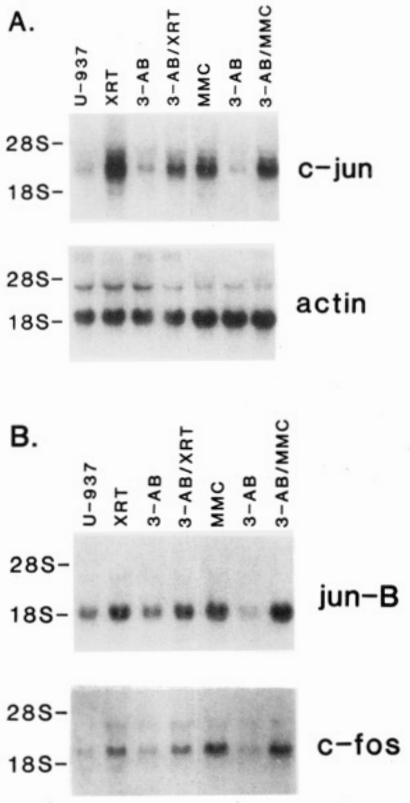

FIGURE 3: Effects of 3-AB on ionizing radiation- and mitomycin C-induced *jun*/*fos* expression. Cells were exposed to ionizing radiation (XRT), 10 μM mitomycin C (MMC), or 5 mM 3-AB. The 3-AB was added 30 min prior to irradiation or mitomycin C treatment and included in the medium for an additional 3 or 12 h. The irradiated cells were harvested at 3 h and the mitomycin C-treated cells at 12 h. Total cellular RNA (20 μg) was isolated and hybridized to (A) the ^{32}P-labeled c-*jun* and actin probes and (B) the *jun*-B and c-*fos* probes.

of the *jun* gene family, including *jun*-B and c-*fos* (Sherman et al., 1990a). Similar effects were obtained in the present studies with U-937 cells (Figure 3B). However, in contrast to the findings with c-*jun*, 3-AB had little effect on induction of these genes by ionizing radiation (Figure 3B). Moreover, induction of *jun*-B and c-*fos* by mitomycin C was unaffected by 3-AB (Figure 3B). These results indicated that inhibition of ADPRP decreases X-ray-induced expression of the c-*jun* gene. In order to confirm and extend these findings, we performed similar experiments with other ADPRP inhibitors, nicotinamide, and theophylline (Pekala & Moss, 1983). Nicotinamide blocked X-ray-induced increases in c-*jun* transcripts by over 90% Figure 4. Similar findings were obtained with theophylline, while both of these agents had little effect on actin mRNA levels (Figure 4).

The finding that inhibitors of ADPRP partially block induction of c-*jun* expression by ionizing radiation prompted an analysis of 3-AB on the associated DNA fragmentation. This agent had no detectable effect on DNA integrity when used alone and partially blocked internucleosomal DNA cleavage induced by ionizing radiation (data not shown). In contrast, 3-AB had little effect on mitomycin C-induced DNA fragmentation (data not shown). Using an assay to quantitate the extent of DNA cleavage, the results of five experiments demonstrated approximately 50% DNA fragmentation at 6 h after ionizing radiation exposure Figure 5. Treatment with both 3-AB and X-rays decreased the extent of fragmentation by half to 25% (Figure 5). While treatment with mitomycin C resulted in a similar level of DNA fragmentation compared to X-rays, 3-AB had no significant effect when combined

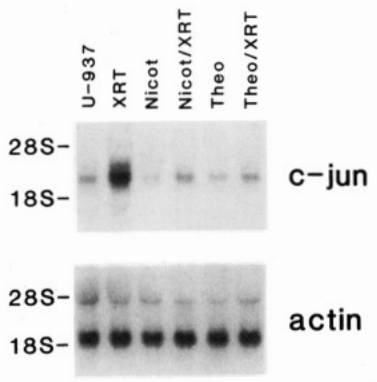

FIGURE 4: Nicotinamide and theophylline inhibit induction of c-*jun* expression by ionizing radiation. Cells were exposed to ionizing radiation (XRT), 5 mM nicotinamide (Nicot), and 1 mM theophylline (Theo). Nicotinamide and theophylline were added 30 min prior to irradiation and included in the medium for an additional 3 h. Total cellular RNA (20 μg) was hybridized to the c-*jun* and actin probes.

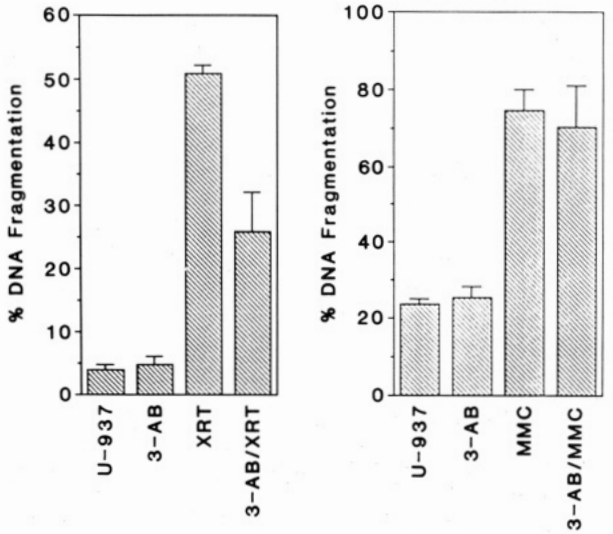

FIGURE 5: Effects of 3-AB on ionizing radiation and mitomycin C-induced DNA fragmentation. Cells were labeled with [^{3}H]-thymidine for 14 h and then treated with ionizing radiation (XRT), mitomycin C (MMC), and/or 3-AB. The 3-AB was added 30 min before irradiation or mitomycin C treatment. Cells were harvested 6 h after irradiation and at 12 h after adding mitomycin C. The cells were lysed and analyzed for fragmented and high molecular weight DNA. The results are expressed as percent DNA fragmentation (mean ± SD of five separate experiments).

with this agent (Figure 5). Taken together, these results indicated that 3-AB inhibits induction of both c-*jun* expression and internucleosomal DNA fragmentation induced by ionizing radiation. Similar studies were performed with the other ADPRP inhibitors. Nicotinamide had no effect on DNA fragmentation in otherwise untreated U-937 cells, while this agent blocked X-ray-induced DNA fragmentation by 45% Figure 6. Treatment with X-rays and theophylline decreased the extent of fragmentation by 80% compared to that with X-rays alone (Figure 6). These results indicated that diverse ADPRP inhibitors block the induction of internucleosomal DNA fragmentation by ionizing radiation.

The c-*jun* gene is activated during the G_0/G_1 transition in proliferating fibroblasts (Ryseck et al., 1988; Carter et al., 1991). Similar findings were obtained in U-937 cells subjected to centrifugal elutriation. Cell cycle distribution of the elutriated cells was determined by flow cytometry. Cells in G_1 and G_1/S phases had detectable levels of c-*jun* transcripts, while those in S, S/G_2 and G_2/M exhibited little if any

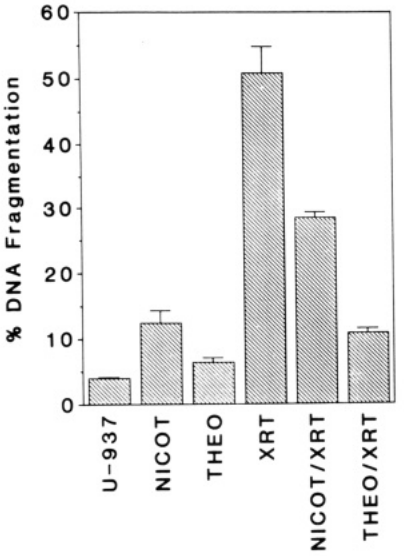

FIGURE 6: Effects of nicotinamide and theophylline on ionizing radiation-induced DNA fragmentation. Cells were treated with ionizing radiation (XRT), 5 mM nicotinamide (NICOT), and 1 mM theophylline (THEO). Nicotinamide and theophylline were added 30 min prior to irradiation and were included in the medium for an additional 6 h. The cells were lysed and analyzed for fragmented and high molecular weight DNA. The results are expressed as percent DNA fragmentation (mean ± SD of five experiments).

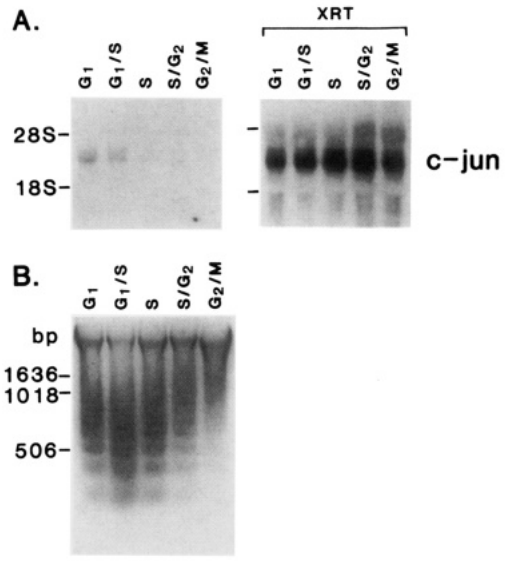

FIGURE 7: Cell cycle distribution of ionizing radiation-induced c-*jun* expression and internucleosomal DNA cleavage. Untreated cells and cells treated with ionizing radiation were separated after 6 h by centrifugal elutriation. The fractions were analyzed by flow cytometry to determine cell cycle distribution. (A) Total cellular RNA (20 μg) from the indicated phase of the cell cycle was hybridized to the c-*jun* probe. Actin hybridization signals demonstrated equal loading of the lanes. (B) Cells were treated with ionizing radiation and after 6 h were separated into the indicated phases of the cell cycle. DNA was analyzed for fragmentation in agarose gels.

expression Figure 7A. Similar studies were performed on cells exposed to ionizing radiation for 6 h and then separated by elutriation. Under these experimental conditions, c-*jun* expression was detectable throughout the cell cycle including G_2/M phase (Figure 7A). These findings indicated that while c-*jun* expression is restricted to G_1/S in untreated U-937 cells, this restriction is lost following exposure to ionizing radiation. DNA from the elutriated fractions was also analyzed in agarose gels. There was no detectable DNA fragmentation in untreated U-937 cells (data not shown). In contrast, a pattern

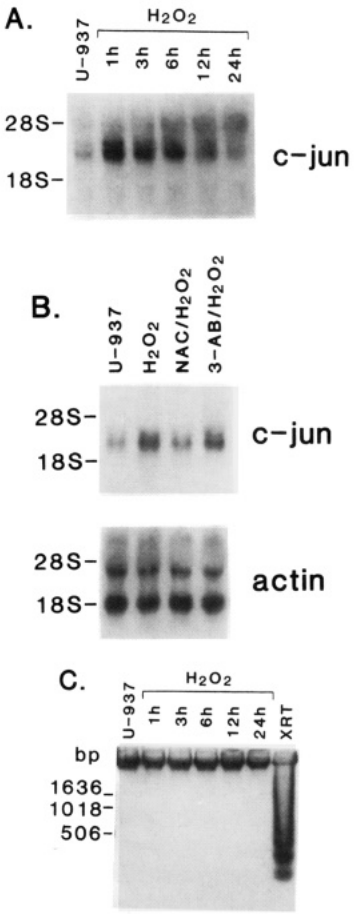

FIGURE 8: Effects of H_2O_2 on c-*jun* expression and internucleosomal DNA fragmentation. Cells were treated with 150 μM H_2O_2 for the indicated times. (A) Total cellular RNA (20 μg) was hybridized to the ^{32}P-labeled c-*jun* probe. Hybridization to the actin probe demonstrated equal loading of the lanes. (B) Cells were exposed to H_2O_2 alone for 2 h and with NAC or 3-AB. NAC and 3-AB were added 30 min prior to H_2O_2 exposure and were included in the medium for an additional 2 h. Total cellular RNA (20 μg) was hybridized to the c-*jun* and actin probes. (C) DNA from cells treated with H_2O_2 for the indicated times was analyzed by electrophoresis in 2% agarose gels at 117 V for 4 h. DNA from irradiated cells was also included as a positive control in the analysis.

of internucleosomal DNA cleavage was present in all phases of the cell cycle following irradiation (Figure 7B).

Since H_2O_2 also acts as a DNA-damaging agent through the production of ROIs, we performed similar experiments in U-937 cells treated with this agent. H_2O_2 rapidly increased c-*jun* mRNA levels in these cells Figure 8A. The increase in c-*jun* expression was detectable at 1 h and transient (Figure 8A). Analysis of the cells by centrifugal elutriation at 6 h of H_2O_2 treatment demonstrated induction of c-*jun* transcripts in G_1, S and G_2/M phases of the cell cycle (data not shown). Moreover, induction of c-*jun* transcripts by H_2O_2 was blocked by treatment of the cells with NAC (Figure 8B). Thus, H_2O_2-induced c-*jun* expression is similar in certain respects to that induced by ionizing radiation. However, in contrast to the inhibition of X-ray-induced c-*jun* expression by 3-AB, this agent had little if any effect on H_2O_2-induced c-*jun* transcripts (Figure 8B). Furthermore, analysis of H_2O_2-treated U-937 cells for DNA integrity demonstrated no detectable DNA fragmentation (Figure 8C). Similar results were obtained with 300 μM H_2O_2 (data not shown). These findings indicated that while c-*jun* expression is coinduced with internucleosomal DNA cleavage following ionizing radiation, these events can be differentially activated by other ROI-mediated mechanisms.

DISCUSSION

Recent work has demonstrated that ionizing radiation activates transcription of the c-*jun* gene (Sherman et al., 1990a; Datta et al., 1992a). This gene is induced as an immediate-early event in response to phorbol esters, serum and growth factors (Quantin & Breathnach, 1988; Brenner et al., 1989; Wu et al., 1989). These and other studies have supported a role for *jun*/AP-1 in G_0/G_1 and G_1/S phase progression (Ryseck et al., 1988; Carter et al., 1991). In contrast, treatment with ionizing radiation is associated with cell cycle arrest, activation of DNA repair and, in the event of irrepairable damage, cell death. Thus, activation of c-*jun* expression in X-ray-treated cells would appear to be related to events distinct from those associated with stimulation of quiescent cells. Indeed, issues that need to be addressed regarding induction of the c-*jun* gene by ionizing radiation include elucidation of the signaling mechanisms responsible for activation of c-*jun* transcription. Moreover, it is not clear what role if any c-*jun* plays in controlling the cellular response to ionizing radiation.

Certain insights are available regarding intracellular signals that contribute to induction of c-*jun* expression by ionizing radiation. Previous studies have suggested that activation of the c-*jun* gene by X-rays is mediated by a PKC-dependent mechanism (Hallahan et al., 1991a). Treatment with phorbol esters to down-regulate PKC results in marked attenuation of c-*jun* activation by X-rays. X-ray-induced c-*jun* expression is inhibited by H7, a nonspecific inhibitor of PKC, but not by HA1004, a more selective inhibitor of cyclic nucleotide-dependent protein kinases (Hallahan et al., 1991a). Furthermore, exposure of cells to ionizing radiation is associated with induction of a PKC-like activity (Hallahan et al., 1991a). However, other studies have shown that ionizing radiation is an effective inducer of c-*jun* expression in HL-525 cells which are deficient in PKC-mediated signaling and respond to phorbol esters with an attenuated increase in c-*jun* transcripts (Datta et al., 1992a). Nonetheless, treatment of these cells with phorbol esters for prolonged periods or with H7 also inhibits X-ray-induced increases in c-*jun* transcripts. These findings have indicated that ionizing radiation induces c-*jun* expression by a pathway distinct from that activated by phorbol esters and that this event involves a protein kinase possibly related to PKC. Other studies have suggested that ionizing radiation activates a protein tyrosine kinase and that this event is required for activation of c-*jun* expression (Chae et al., 1993). Thus, multiple signals involving both serine/threonine and tyrosine protein phosphorylation may be involved in induction of the c-*jun* gene by ionizing radiation.

The present work as well as as previous studies (Datta et al., 1992a) further implicate the involvement of ROIs in X-ray-induced c-*jun* expression. NAC counteracts the effects of ROIs and blocks activation of the c-*jun* gene by ionizing radiation. In contrast, NAC had no detectable effect on induction of c-*jun* expression by other DNA-damaging agents, such as mitomycin C (data not shown) and ara-C (Datta et al., 1992a) which are not known to mediate their cytotoxic effects through ROIs. These findings have indicated that NAC is a selective inhibitor of X-ray-induced c-*jun* transcription, presumably through scavenging of ROIs and increasing intracellular glutathione. ROIs damage DNA in irradiated cells, and this damage may represent the common event with other genotoxic agents, such as ara-C, UV light, certain alkylating agents, and etoposide, which also induce c-*jun* transcription (Devary et al., 1991; Kharbanda et al., 1990, 1991; Rubin et al., 1991). Since ionizing radiation-induced DNA strand breaks contribute to activation of ADPRP (Brown et al., 1984), we asked whether inhibition of this enzyme would affect induction of c-*jun* expression. The finding that 3-AB, as well as two other inhibitors of ADPRP, blocks X-ray-induced increases in c-*jun*, but not *jun*-B or c-*fos*, transcripts supports the involvement of this enzyme in signaling that contributes to induction of c-*jun* expression. ADPRP results in ADP-ribosylation of nuclear proteins and contributes to X-ray induced DNA repair (Brown et al., 1984). As such, induction of the c-*jun* gene may be involved in repair of DNA strand breaks. However, since DNA repair is a rapid event, the increases in c-*jun* transcripts at 3–6 h after ionizing radiation exposure fail to adequately support this possibility. Alternatively, c-*jun* expression may be coinduced with specific genetic pathways that contribute to cell lethality. In any event, the results suggest that ADPRP is involved in an X-ray-induced signaling cascade that confers activation of c-*jun* expression.

The role of *jun* in the response to ionizing radiation and other DNA-damaging agents remains unclear. However, previous studies with ara-C supported the temporal association between induction of c-*jun* expression and internucleosomal DNA fragmentation (Gunji et al., 1991). Similar findings have been obtained with etoposide (Rubin et al., 1991) and in the present studies with ionizing radiation. Clonogenic survival studies of U-937 cells exposed to various doses of ionizing radiation has demonstrated a lethal $dose_{50}$ (LD_{50}) of 2 Gy. Exposure of U-937 cells to 1 or 5 Gy has no detectable effect on c-*jun* mRNA levels or DNA fragmentation by 6 h, while treatment with 10 Gy results in the induction of both events at 6 h (data not shown). Exposure to 20 Gy also resulted in the induction of both c-*jun* expression and internucleosomal DNA cleavage. The present studies extend this relationship by demonstrating that both events are (1) inhibited by NAC, (2) blocked by ADPRP inhibitors, and (3) detectable in all phases of the cell cycle. Indeed, to our knowledge, this is the first demonstration that c-*jun* expression is not restricted to G_0/G_1 and that c-*jun* transcripts are detectable throughout the cell cycle following exposure to a genotoxic agent. Other work has demonstrated that c-*jun* is induced during internucleosomal DNA fragmentation and programmed cell death following withdrawal of growth factors from lymphoid cells (Colotta et al., 1992). Moreover, antisense oligonucleotides directed against c-*jun* block induction of programmed cell death (Colotta et al., 1992). Since induction of internucleosomal DNA cleavage can in part be prevented by the presence of RNA and protein synthesis inhibitors, it has been postulated that transcription and translation are required for this event. Thus, induction of genes, such as c-*jun*, that encode for transcription factors may represent one of the signals that contribute to internucleosomal DNA cleavage and potentially other aspects of programmed cell death.

The present results further indicate that the cellular response to ROIs differs with respect to the inducing agent. Ionizing radiation predominantly induces hydroxyl radical and superoxide production, while H_2O_2 exposure is primarily associated with formation of peroxides (Limoli & Ward, 1993). Hydroxyl radicals are capable of damaging deoxyribose moieties, and peroxides can oxidize DNA bases. Perhaps related to the different ROIs associated with ionizing radiation and H_2O_2 is the finding that induction of c-*jun* expression in H_2O_2-treated U-937 cells is considerably more rapid than that in irradiated cells. Moreover, in contrast to ionizing radiation, H_2O_2-induced c-*jun* expression was unaffected by 3-AB and was not associated with internucleosomal DNA fragmentation. These results support the involvement of

different mechanisms in X-ray- and H_2O_2-induced c-*jun* gene expression. In this context, studies in bacteria have demonstrated distinct adaptive responses to superoxide and H_2O_2 (Demple & Amabile-Cuevas, 1991). For example, while the soxRS gene products exert positive transcriptional control over the response to superoxide, the oxyR gene product functions in the activation of genes which constitute the response to H_2O_2 (Demple & Amabile-Cuevas, 1991). The present findings with ionizing radiation and H_2O_2 suggest that, in addition to bacteria, mammalian cells exhibit differential responses to ROIs in terms of c-*jun* expression and internucleosomal DNA fragmentation.

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