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Regulation of c-*jun* Gene Expression in HL-60 Leukemia Cells by 1-β-D-Arabinofuranosylcytosine. Potential Involvement of a Protein Kinase C Dependent Mechanism†

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ABSTRACT: 1-β-D-Arabinofuranosylcytosine (ara-C) is an effective chemotherapeutic agent that incorporates into DNA and results in DNA fragmentation. Recent work has demonstrated that ara-C transiently induces expression of the c-*jun* immediate early response gene. The present studies in HL-60 myeloid leukemia cells extend these findings by demonstrating that the increase in c-*jun* mRNA levels at 6 h of ara-C treatment is regulated by a transcriptional mechanism. In contrast, the subsequent down-regulation of c-*jun* expression is controlled by a posttranscriptional decrease in the stability of the c-*jun* transcripts. Previous work in phorbol ester treated cells has indicated that c-*jun* expression is regulated by the activation of protein kinase C. The present results demonstrate that protein kinase C activity is increased in ara-C-treated cells. This increase was maximal at 60 min and remained detectable through 6 h of ara-C exposure. Moreover, the induction of c-*jun* transcripts by ara-C was inhibited by the isoquinolinesulfonamide derivative H7, but not by HA1004, suggesting that this effect is mediated by protein kinase C. Ara-C-induced c-*jun* expression was also inhibited by staurosporine, another inhibitor of protein kinase C. Taken together, these results indicate that the cellular response to ara-C includes the activation of protein kinase C and that ara-C potentially induces c-*jun* transcription by a protein kinase C dependent signaling mechanism.

The compound 1-β-D-arabinofuranosylcytosine (ara-C)[1] is one of the most effective agents in the treatment of acute myelogenous leukemia (Frei et al., 1969). Ara-C incorporates into leukemic cell DNA (Kufe et al., 1980; Major et al., 1981). The extent of (ara-C)DNA formation correlates with the inhibition of DNA synthesis and the loss of clonogenic survival (Kufe et al., 1980; Major et al., 1981, 1982; Kufe et al., 1984). More recent studies have demonstrated that the inhibitory effects of ara-C are related to both incorporation into DNA and sequence of the DNA template (Townsend & Cheng, 1987: Ohno et al., 1988). Moreover, the inhibition of DNA synthesis by ara-C is associated with DNA fragmentation and endonucleolytic cleavage (Fram & Kufe, 1982; Gunji et al., 1991). These findings are in concert with the conformational and hydrogen-bonding differences of the arabinose sugar moiety altering reactivity of the 3′ terminus and slowing DNA chain elongation (Sundaralingam, 1975; Cozzarelli, 1977). The precise mechanism(s) of action of ara-C and the basis for selectivity against leukemic cells, however, remain unclear.

The c-*jun* protooncogene has been implicated in the regulation of cellular growth and differentiation. This gene is induced as an immediate early event following treatment of fibroblasts with serum, growth factors, and phorbol esters (Ryder & Nathans, 1988; Quantin & Breathnach, 1988; Brenner et al., 1989; Wu et al., 1989; Pertovaara et al., 1989; Ryseck, et al., 1988). The c-*jun* gene codes for the major form of the transcription factor AP-1 (Bohmann et al., 1987; Angel et al., 1987, 1988a; Chiu et al., 1988; Lee et al., 1987). Jun homodimers bind to a heptameric DNA consensus sequence $TGA^{G}/_{C}TCA$ (TRE) that regulates the transcription of genes responsive to growth factors and phorbol esters (Angel et al., 1987, 1986; Lee et al., 1987; Chiu et al., 1987). The DNA binding affinity of Jun/Ap-1 is modulated by the formation of complexes with other factors that contain a leucine zipper and a region rich in basic amino acids (Chiu et al., 1988; Kouzarides & Ziff, 1988; Halazonetis et al., 1988; Rauscher

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[1] Abbreviations: ara-C, 1-β-D-arabinofuranosylcytosine; TRE, phorbol ester responsive element; CHX, cycloheximide; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; STSP, staurosporine.

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et al., 1988; Nakabeppu et al., 1988). This multigene family of leucine zipper transcription factors includes the *jun*-B, *jun*-D, c-*fos*, *fos*-B, and *fra* genes (Mitchell & Tjian, 1989; Bohmann et al., 1988). While the Jun proteins can bind to the TRE as homodimers, the Fos proteins are unstable as homodimers and therefore fail to interact with this site. In contrast, Jun/Fos heterodimers exhibit a higher affinity for the TRE than that achieved with Jun homodimers (Chiu et al., 1988; Nakabeppu et al., 1988; Halazonetis et al., 1988).

Recent studies have demonstrated that the c-*jun* expression is induced during treatment of human myeloid leukemia cells with ara-C (Kharbanda et al., 1990a; Henschler et al., 1991). The basis for this effect is unclear, although the results have indicated that ara-C induces c-*jun* expression at least in part by a transcriptional mechanism (Kharbanda et al., 1990a; Henschler et al., 1991). The present findings demonstrate that the transient appearance of c-*jun* transcripts is regulated by both transcriptional and posttranscriptional mechanisms. We also demonstrate that ara-C activates protein kinase C and that transcriptional regulation of the c-*jun* gene by this agent may be controlled by a protein kinase C dependent mechanism.

Materials and Methods

Cell Culture. HL-60 leukemia cells (American Type Culture Collection, Bethesda, MD) were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin (GIBCO Laboratories, Grand Island, NY). The cells were treated with varying concentrations of ara-C (Sigma Chemical Co., St. Louis, MO), 5 μg/mL actinomycin D (Sigma), 10 μg/mL cycloheximide (Sigma), 50 μM H7 (Seikagaku America, Inc., Rockville, MD), 50 μM HA1004 (Seikagaku), and 50 μM staurosporine (Sigma).

Isolation and Analysis of RNA. Total cellular RNA (20 μg) was purified by the guanidine isothiocyanate/cesium chloride method (Chirgwin et al., 1979), 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* DNA purified from a pBluescript SK(+) plasmid (Angel et al., 1988a) and (2) the 2.0-kb *Pst*I insert of a chicken β-actin gene purified from the pA1 plasmid (Cleveland et al., 1980). The hybridizations were performed for 24 h at 42 °C in a solution of 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.

Run-On Transcription Assay. Nuclei were isolated from 10^8 cells by lysis in 0.5% NP-40, and the ^{32}P-labeled nuclear RNA was prepared as described (Sherman et al., 1990a). The labeled RNA was hybridized to the following digested DNAs: (1) the 2.0-kb *Pst*I fragment of the chicken β-actin gene (Cleveland et al., 1980) and (2) the 1.8 kb-*Bam*HI/*Eco*RI fragment of the human c-*jun* cDNA (Angel et al., 1988a). The digested DNAs were run in 1% agarose gels and transferred to nitrocellulose filters. Hybridizations were performed with 10^7 cpm of ^{32}P-labeled RNA/mL in 4× SSC/5 mM EDTA/0.4% SDS/5× Denhardt's solution/40% formamide-/100 μg/mL yeast tRNA for 72 h at 42 °C. The filters were then washed in 2x SSC/0.1% SDS at 37 °C for 30 min, 10 μg/mL RNase A in 2× SSC at 37 °C for 20 min and 0.1× SSC/0.1% SDS at 42 °C for 30 min.

Protein Kinase C Assays. HL-60 cells (10^7) were pelleted and resuspended in 0.4 mL of ice-cold TEM buffer (20 μM Tris-HCl, pH 7.5, 0.5 mM EDTA, 0.5 mM EGTA, and 10 mM β-mercaptoethanol) containing 0.5% Triton X-100, 25 μg/mL, leupeptin, and 25 μg/mL aprotinin. The cells were disrupted in a Dounce homogenizer, incubated for 30 min on ice, and then spun in a microcentrifuge for 2 min to remove cellular debris. The supernatant was assayed for protein concentration with use of the Bio-Rad protein assay (Richmond, CA) and then applied to DEAE-cellulose ion-exchange columns (Whatman DE52; 0.5 g/column). The DEAE eluate (2 mL of 0.2 M NaCl in TEM) was used directly or after dilution in TEM to obtain a range of enzyme concentrations. Protein kinase C activity was determined as described (Yasuda et al., 1990; Protein Kinase C Assay System, GIBCO BRL, Grand Island, NY). The partially purified protein extract in TEM was incubated for 5 min at 30 °C in phospholipid (phosphatidylserine and phorbol ester in Triton X-100 mixed micelles; GIBCO BRL), [γ-^{32}P]ATP, and protein kinase C synthetic peptide from meylin basic protein (GIBCO BRL). All assays were also performed in the presence of a protein kinase C inhibitor peptide (GIBCO) to ensure specificity of the phosphorylation reaction. The samples were dried on phosphocellulose, washed in 1% H_3PO_4, and assayed by scintillation counting. Protein kinase C activity was determined as described in the Protein Kinase C Assay System (GIBCO BRL).

Results

Low to undetectable levels of c-*jun* mRNA were present in untreated HL-60 cells (Figure 1). In contrast, treatment with 5 × 10^{-6} M ara-C was associated with an increase in c-*jun* transcripts (Figure 1A). This induction was detectable at 6 h of ara-C exposure and was followed by decreases in c-*jun* mRNA levels at 12 h. HL-60 cells were also exposed to 10^{-5} M aphidicolin, an inhibitor of DNA polymerase α that is not incorporated into DNA (Huberman, 1981). While aphidicolin also resulted in complete inhibition of HL-60 cell growth (data not shown), this agent had little if any effect on c-*jun* expression (Figure 1A). A more detailed time course was performed to further define the kinetics of ara-C-induced c-*jun* expression. c-*jun* transcripts were increased by 4 h, reached maximal levels by 6 h, and were then progressively down-regulated in the continued presence of drug (Figure 1B).

In order to examine the basis for this induction and down-regulation of c-*jun* expression, we studied the transcriptional and posttranscriptional control of c-*jun* mRNA levels by ara-C. Nuclear run-on assays were performed to determine the rates of c-*jun* transcription during both the ara-C-induced increase and the down-regulation of c-*jun* mRNA levels. Nuclear RNA isolated from cells treated with ara-C for 6 and 24 h was hybridized to actin and c-*jun* DNAs (Figure 2). The actin gene was constitutively activated in untreated HL-60 cells, and its transcription was unaffected by ara-C treatment (Figure 2). While a low level of c-*jun* gene transcription was observed in untreated HL-60 cells, this rate was increased 4-fold following treatment with ara-C for 6 h (Figure 2). Moreover, exposure to this agent for 24 h was associated with an 8-fold increase in the c-*jun* transcription rate (Figure 2). Taken together, these findings indicated that the down-regulation in c-*jun* expression is controlled by a posttranscriptional mechanism.

The stability of c-*jun* transcripts was studied by treating HL-60 cells with ara-C for 6 h and then adding actinomycin D to inhibit further transcription. With this approach, c-*jun* mRNA levels were increased at 6 h and then progressively declined in the presence of actinomycin D (Figure 3). The half-life of c-*jun* mRNA as determined by densitometric scanning was 64 min (Figure 3). Similar stability studies were

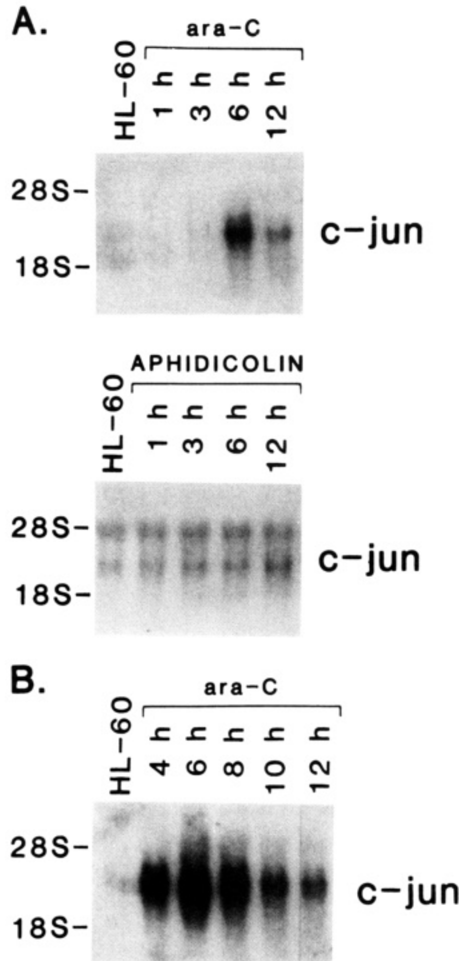

FIGURE 1: Effects of ara-C and aphidicolin on c-*jun* expression in HL-60 cells. HL-60 cells were treated with 5×10^{-6} M ara-C or 10^{-5} M aphidicolin for the indicated times. Total cellular RNA (20 μg) was purified and hybridized to the ^{32}P-labeled c-*jun* probe. Hybridizations to a labeled chicken β-actin probe demonstrated equal loading of the lanes.

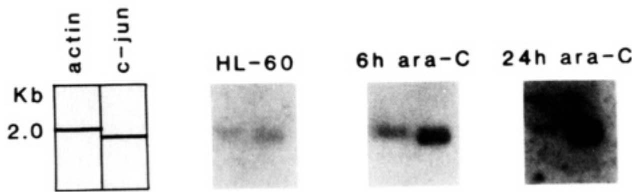

FIGURE 2: Effects of ara-C on rates of c-*jun* transcription. HL-60 cells were treated with 5×10^{-6} M ara-C for 6 and 24 h. Nuclei were isolated, and newly elongated ^{32}P-labeled transcripts were hybridized to 2 μg of β-actin and c-*jun* DNA inserts after restriction enzyme digestion and Southern blotting. As compared to that for the β-actin gene, c-*jun* gene transcription was increased 4- and 8-fold following treatment with ara-C for 6 and 24 h, respectively.

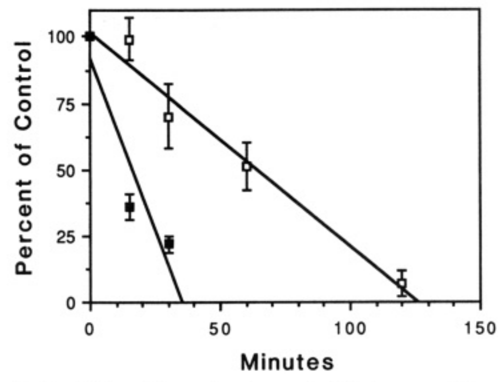

FIGURE 3: Stability of the c-*jun* transcript during ara-C treatment. HL-60 cells were treated with 5×10^{-6} M ara-C for 6 (□) and 24 (■) h. Actinomycin D was then added to inhibit further transcription. At the indicated times after the addition of actinomycin D, total cellular RNA was isolated and hybridized to the ^{32}P-labeled c-*jun* and β-actin DNA probes. Signal intensity as determined by densitometric scanning was determined for the c-*jun* hybrids and normalized to that for actin. The results represent the mean ± average deviation of two separate experiments. The half-life calculated by the methods of least-squares was 64 min ($R = 0.99$) for 6-h ara-C-treated cells and 18 min ($R = 0.97$) for cells treated with ara-C for 24 h.

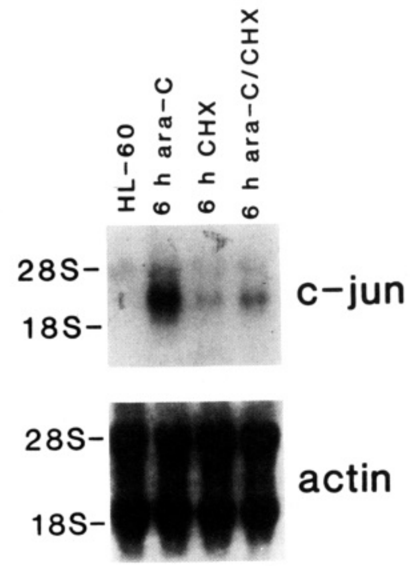

FIGURE 4: Requirement of protein synthesis for ara-C-induced c-*jun* expression. HL-60 cells were treated for 6 h with 5×10^{-6} M ara-C, 10 μg/mL, cycloheximide (CHX), or a combination of both agents. Total cellular RNA (20 μg) was hybridized to the c-*jun* and β-actin DNA probes.

performed by adding actinomycin D at 24 h of ara-C exposure. The half-life of c-*jun* mRNA under these experimental conditions was 18 min. These results indicated that the downregulation in c-*jun* mRNA levels is related to a decrease in the stability of these transcripts.

While these studies indicated that ara-C regulates c-*jun* mRNA levels by both transcriptional and posttranscriptional mechanisms, little is known about the signaling events responsible for this effect. In order to determine whether ara-C-induced c-*jun* expression requires protein synthesis, HL-60 cells were also treated with cycloheximide (CHX). Although ara-C treatment was associated with increased c-*jun* mRNA levels, a similar 6-h exposure to CHX had little effect on the expression of this gene (Figure 4). Moreover, treatment with both ara-C and CHX resulted in a decrease in the levels of c-*jun* transcripts compared to that obtained with ara-C alone (Figure 4). These findings indicated that the induction of c-*jun* expression by ara-C requires the synthesis of a labile protein.

Previous studies have demonstrated that 12-*O*-tetradecanoylphorbol-13-acetate (TPA), an activator of protein kinase C (Nishizuka, 1988), induces c-*jun* expression in HL-60 cells (Sherman et al., 1990a). Moreover, other agents that activate this enzyme similarly induce c-*jun* transcripts in these cells (Sherman et al., 1990a). Consequently, studies were performed to investigate whether protein kinase C is involved in ara-C-induced c-*jun* expression. In order to address this issue, we first studied the effects of ara-C on protein kinase C activity in HL-60 cells. These studies were performed with use of a synthetic peptide derived from myelin basic protein that has been reported to act as a specific substrate for this enzyme (Yasuda et al., 1990). The level of enzyme activity in un-

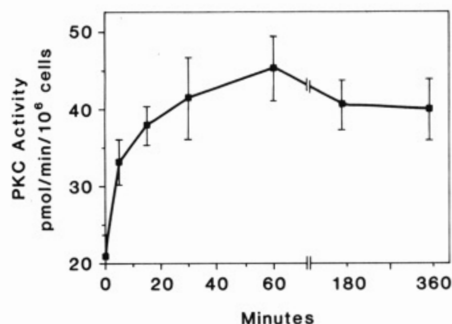

FIGURE 5: Activation of protein kinase in ara-C-treated cells. HL-60 cells were treated with 5×10^{-6} M ara-C. At the indicated times, cells were harvested, lysed, and assayed for phosphorylation of a synthetic peptide from myelin basic protein. Six replicates were performed in each experiment. The results represent the mean ± standard deviation of two separate experiments. Incubation of a pseudosubstrate inhibitor in the reactions at each time point demonstrated a decrease in enzyme activity to less than 10% of control.

treated HL-60 cells was increased after treatment with 5×10^{-6} M ara-C (Figure 5). This increase was detectable by 15 min and was maximal by 60 min (Figure 5). Moreover, phosphorylation of the synthetic peptide remained above that in control cells by 6 h (Figure 5). Similar experiments were performed in the presence of a pseudosubstrate inhibitor of protein kinase C, and under these experimental conditions the level of enzyme activity was less than 10% that obtained in the absence of inhibitor (data not shown). Taken together, these findings indicated that the treatment of HL-60 cells with ara-C is associated with activation of protein kinase C.

The effects of ara-C treatment on protein kinase C activity prompted further studies to determine the relationship, if any, between this event and the induction of c-*jun* expression. This issue was addressed by treating HL-60 cells with H7, an isoquinolinesulfonamide derivative that inhibits protein kinase C (Hidaka et al., 1984). H7 had no effect on c-*jun* mRNA levels in otherwise untreated cells (Figure 6A). In contrast, treatment with H7 was associated with nearly complete abrogation of ara-C-induced c-*jun* mRNA levels (Figure 6A). Since H7 can also inhibit other protein kinases, we used similar concentrations of HA1004, a more selective inhibitor of cyclic nucleotide dependent protein kinases (Asano & Hidaka, 1984). HA1004 had little effect on c-*jun* mRNA levels in control and ara-C-treated cells (Figure 6A). While these findings suggested the involvement of protein kinase C, similar studies were performed with staurosporine, another inhibitor of this enzyme (Tamaoki et al., 1987; Vegesna et al., 1988). Staurosporine had no effect on c-*jun* mRNA levels in control HL-60 cells and blocked the induction of c-*jun* expression by ara-C (Figure 6B). These findings suggested that ara-C induces c-*jun* expression by a protein kinase dependent mechanism.

Activation of protein kinase C in TPA-treated HL-60 cells is associated with the transcriptional induction of c-*jun* mRNA levels (Sherman et al., 1990a). Further studies were therefore performed to determine whether ara-C-induced activation of protein kinase C is involved in the transcriptional control of this gene. Nuclear run-on assays were performed with use of ara-C-treated cells in the presence and absence of H7. The treatment of HL-60 cells with ara-C resulted in a 2.5-fold increase in the rate of c-*jun* gene transcription (Figure 7). Treatment with both H7 and ara-C had little effect on the constitutive transcription of the actin gene. In contrast, H7 inhibited the induction on c-*jun* transcription by ara-C (Figure 7). These results suggested that a protein kinase dependent mechanism controls ara-C-induced transcription of the c-*jun* gene.

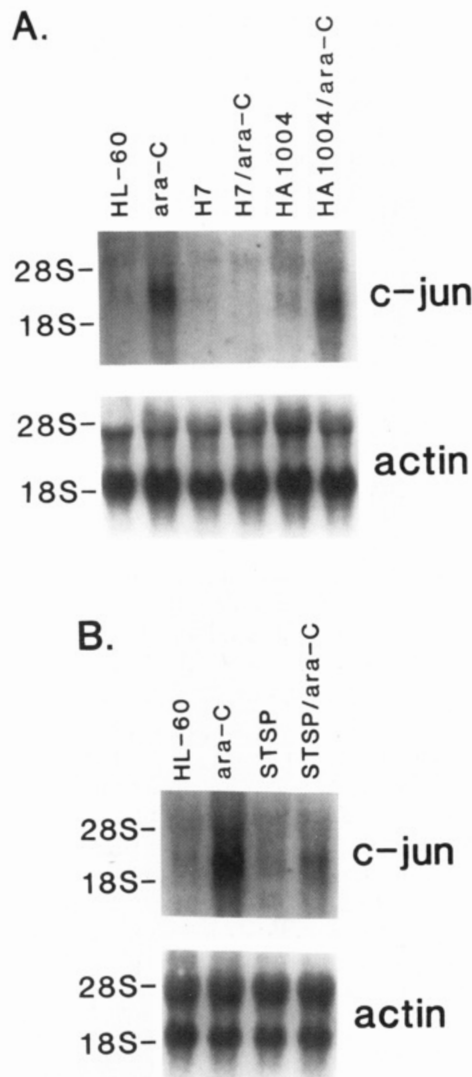

FIGURE 6: Effects of kinase inhibitors on ara-C-induced c-jun expression. (A) HL-60 cells were treated for 6 h with 5×10^{-6} M ara-C, 50 μM H7, 50 μM HA1004, and a combination of H7 or HA1004 with ara-C. (B) Cells were treated for 6 h with 5×10^{-6} M ara-C, 50 μM staurosporine (STSP), and a combination of these agents. Total cellular RNA (20 μg) was isolated and hybridized to the ^{32}P-labeled c-*jun* and β-actin probes.

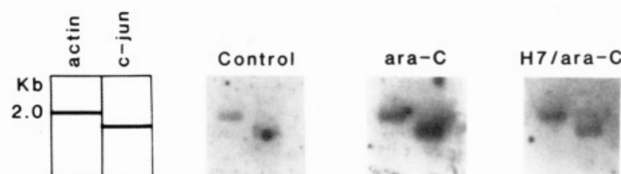

FIGURE 7: Inhibition of ara-C-induced c-*jun* transcription by H7. HL-60 cells were treated with 5×10^{-6} M ara-C or 50 μM H7/ara-C. Nuclei were isolated after 6 h, and newly elongated ^{32}P-labeled transcripts were hybridized to the β-actin and c-*jun* DNA inserts. As compared to the β-actin gene, c-*jun* gene transcription was increased 2.5- and 0.7-fold in ara-C treated and H7/ara-C-treated cells, respectively.

DISCUSSION

Previous studies have demonstrated that ara-C induces the expression of the c-*jun* gene in human KG-1 myeloid leukemia cells (Kharbanda et al., 1990a; Henschler et al., 1991). These findings are not restricted to KG-1 cells since similar results were obtained in other myeloid leukemia cell lines, including U-937, THP-1, and KG-1a (Kharbanda et al., 1990a). The increase in c-*jun* mRNA levels is transient during ara-C

treatment. Similar findings have been obtained in quiescent fibroblasts treated with growth factors or phorbol esters, although the effects of these agents are more rapid (<30 min) compared to that for ara-C (Ryder & Nathans, 1988; Quantin & Breathnach, 1988; Brenner et al., 1989; Wu et al., 1989; Pertovaara et al., 1989; Ryseck et al., 1988). The present results demonstrate that both transcriptional and posttranscriptional mechanisms are responsible for transient c-*jun* expression in ara-C-treated cells. While an increase in the rate of c-*jun* transcription was associated with the appearance of these transcripts at 6 h, the subsequent down-regulation of c-*jun* expression was controlled by a posttranscriptional mechanism. In this regard, c-*jun* transcription was increased (~2-fold) at 24 h despite the decline in c-*jun* mRNA levels. In contrast, the half-life of c-*jun* transcripts at 6 h ($t_{1/2}$ = 64 min) was 3–4-fold longer than that obtained at 24 h ($t_{1/2}$ = 18 min). This decline in c-*jun* mRNA stability at 24 h is thus in concert with the decrease in expression of this gene. Transcriptional and posttranscriptional regulation of c-*jun* mRNA levels has also been demonstrated in TPA-treated HL-60 cells (Sherman et al., 1990a). However, while TPA similarly induces c-*jun* transcripts at 6 h, down-regulation of c-*jun* expression was more rapid in cells treated with ara-C.

Although ara-C slows DNA replication, the rapid induction of c-*jun* transcripts by ara-C occurred in the absence of similar changes during treatment with a cytostatic concentration of aphidicolin. However, in contrast to the inhibition of DNA polymerase α by aphidicolin (Huberman, 1981), ara-C incorporates into DNA and can thereby alter DNA structure as well as induce strand termination. DNA strand breaks and internucleosomal cleavage are also associated with ara-C treatment (Fram & Kufe, 1982; Gunji et al., 1991). Consequently, studies have been performed with other DNA-damaging agents to determine whether alterations in chromatin structure are associated with c-*jun* expression. Indeed, recent findings have demonstrated that treatment of HL-60 cells with ionizing radiation is associated with transcriptional activation of the c-*jun* gene (Sherman et al., 1990b). Other work has demonstrated that expression of the related c-*fos* gene is increased following treatment with genotoxic agents (Stein et al., 1989), and similar findings have been obtained in ara-C-treated KG-1 (Kharbanda et al., 1990b) and HL-60 (unpublished data) cells. Since the affinity of Jun/AP-1 binding to the TRE is enhanced by dimerization with Fos (Kouzarides & Ziff, 1988; Halazonetis et al., 1988; Rauscher et al., 1988; Nakabeppu et al., 1988; Zerial et al., 1989), coexpression of these genes during DNA damage may represent activation of nuclear signal pathways that control specific gene transcription.

The present results also demonstrate that treatment of HL-60 cells with ara-C is associated with the activation of protein kinase C. This effect was maximal at 60 min of ara-C exposure and thus represents a relatively rapid response to this agent. In these studies, a specific protein kinase C substrate was used to assay for changes in enzyme activity. Moreover, while it is possible that these findings might be attributable to a related protein kinase, inhibition of ara-C-induced activity by a pseudosubstrate of protein kinase C supports activation of this enzyme. Protein kinase C, which consists of a family of isozymes, is activated by diacylglycerol (Nishizuka, 1988). Since diacylglycerol is generated by the hydrolysis of membrane phospholipids, such as phosphatidylinositol or phosphatidylcholine, further studies on the potential induction of these pathways by ara-C should provide insights into the signaling events activated by this agent. Ara-C can interfere with CDP–choline metabolism (Sasvari-Szekely et al., 1989) and thus might result in alterations of phospholipid hydrolysis. Alternatively, signaling pathways may be activated as a consequence of ara-C-induced DNA damage (Fram & Kufe, 1982; Gunji et al., 1991). Of relevance to this potential mechanism is the finding that other DNA-damaging agents, such as ionizing radiation, also activate signaling events that include protein kinase C (unpublished data).

Activation of protein kinase C is associated with increases in c-*jun* expression. For example, treatment of epithelial cells with TPA results in stimulation of a c-*jun* promoter/reporter gene by an autoregulatory mechanism involving the AP-1 protein (Angel et al., 1988). The c-*jun* gene is also transcriptionally activated following TPA treatment of human fibroblasts (Brenner et al., 1989). In addition to TPA, other agents that activate protein kinase C, such as bryostatin 1, similarly increase c-*jun* transcription in HL-60 cells (Sherman et al., 1990a). While distinct transduction pathways may conceivably regulate c-*jun* expression, the available findings in those models indicate that activation of protein kinase C is at least one signal responsible for the induction of c-*jun* transcription. The present findings that H7 and staurosporine, but not HA1004, inhibit the induction of c-*jun* transcripts by ara-C support, but do not directly establish, the involvement of this enzyme in transducing signals initiated by ara-C. Indeed, ara-C could potentially activate a distinct protein kinase that is sensitive to these agents and is responsible for the induction of c-*jun* expression.

Finally, other studies in cells treated with ionizing radiation, another genotoxic agent, have similarly demonstrated the induction of c-*jun* transcription (Sherman et al., 1990b). Thus, alterations in DNA structure may activate protein kinase dependent signaling events that in turn control specific gene transcription. In this context, ara-C-induced c-*jun* expression has been detected in association with internucleosomal DNA fragmentation (Gunji et al., 1991). Previous studies have also demonstrated that ara-C induces differentiation of myeloid leukemia cell lines (Lotem & Sachs, 1974; Takeda et al., 1981; Griffen et al., 1982; Mitchell et al., 1986). Activation of the c-*jun* gene by other agents that induce myeloid differentiation (Sherman et al., 1990a) has raised the possibility that c-*jun* expression in ara-C-treated cells may contribute to the appearance of a terminally differentiated phenotype. However, further studies are needed to determine whether the cellular response to DNA-damaging agents relates to induction of differentiation.

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