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# 1-β-D-Arabinofuranosylcytosine Activates Serine/Threonine Protein Kinases and c-*jun* Gene Expression in Phorbol Ester-Resistant Myeloid Leukemia Cells

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## SUMMARY

1-β-D-Arabinofuranosylcytosine (ara-C) is an effective antileukemic agent that misincorporates into DNA. Recent studies have demonstrated that ara-C treatment is associated with transient induction of the c-*jun* early response gene. The present studies have examined the effects of ara-C on c-*jun* expression in a phorbol ester-resistant variant of the HL-60 myeloid leukemia cell line, designated HL-525, that is deficient in protein kinase C (PKC)-mediated signal transduction and fails to respond to 12-*O*-tetradecanoylphorbol-13-acetate with induction of c-*jun* transcripts. The results demonstrate that treatment of HL-525 cells with ara-C is associated with transcriptional activation of the c-*jun* gene. We also demonstrate that ara-C treatment is associated with activation of a PKC-like activity. Partial purification of this $Ca^{2+}$-independent activity has demonstrated phosphorylation of synthetic peptides derived from (a) amino acids 4–14 of myelin basic protein and (b) the pseudosubstrate region of PKC (amino acids 19–31), with substitution of $Ala^{25}$ with serine. The finding that the ara-C-induced activity is inhibited by the pseudosubstrate PKC(19–36) supports the activation of a PKC-like enzyme. Because PKC can act upstream of the mitogen-activated protein (MAP) kinases, we studied the effects of ara-C treatment on MAP kinase activity. The results demonstrate that MAP kinase is activated in ara-C-treated cells and that the kinetics of this activation are similar to those of the PKC-like activity. Because 12-*O*-tetradecanoylphorbol-13-acetate has little, if any, effect on the PKC-like and MAP kinase activities in HL-525 cells, these findings suggest that ara-C activates a distinct signaling cascade that may contribute to induction of the c-*jun* gene.

Ara-C is the most effective agent in the treatment of human acute myelogenous leukemia (1). Certain insights are available regarding the mechanism of action of ara-C; however, the basis for the selectivity of this agent against leukemic cells remains unclear. Ara-C is a potent inhibitor of DNA replication (2–4) that incorporates into elongating strands (4–7). The extent of (ara-C)DNA formation correlates with inhibition of DNA synthesis (4) and loss of clonogenic survival (6). Other work has suggested that these effects are related to incorporation of ara-C at specific sites in the DNA template (8, 9). Moreover, incorporation of ara-C into DNA is associated with DNA fragmentation and endonucleolytic DNA cleavage (10, 11). Although these findings have provided an explanation for the lethal effects of ara-C, recent studies have indicated that the cellular response to this agent involves a cascade of signaling events that includes induction of early response gene expression (12–15).

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Early response genes are induced by diverse mitogenic signals, in the absence of *de novo* protein synthesis. Certain early response genes code for transcription factors involved in nuclear signaling events. One family encoding nuclear proteins with a leucine zipper DNA-binding motif includes the c-*jun*, *jun*-B, *jun*-D, c-*fos*, *fos*-B, and *fra* genes (16). Although ara-C has been shown to activate transcription of both the c-*jun* and *jun*-B genes (12, 13), studies on the regulation of c-*jun* by other agents, such as phorbol esters, have provided certain insights into the signaling pathways that activate this gene. The c-*jun* gene codes for the major form of the transcription factor AP-1 that binds to the heptameric DNA sequence $TGA^{G}/_{C}TCA$ (16). The demonstration that AP-1 regulates transcription of genes induced by phorbol esters is in concert with the finding that these agents also activate c-*jun* gene expression. For example, studies have shown that TPA stimulates expression of a c-*jun* promoter/reporter gene and that Jun/AP-1 positively autoregulates the c-*jun* gene (17). TPA also induces c-*jun* expression during monocytic differentiation of HL-60 myeloid leukemia

**ABBREVIATIONS:** ara-C, 1-β-D-arabinofuranosylcytosine; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; PKC, protein kinase C; MAP, mitogen-activated protein; MBP, myelin basic protein; kb, kilobase(s); SSC, standard saline citrate; SDS, sodium dodecyl sulfate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

cells (18). Similar results have been obtained in HL-60 cells treated with other agents, such as bryostatin-1, that activate PKC (18). Taken together with the demonstration that growth factors known to activate PKC also induce c-*jun* expression (19, 20), these findings have indicated that signaling events involving Jun/AP-1 are controlled at least in part by activation of this enzyme.

The present studies have asked whether ara-C induces c-*jun* expression by pathways different from those activated during TPA treatment of HL-60 cells. To address this issue, we have used a phorbol ester-resistant myeloid leukemia cell line designated HL-525 (21). HL-525 cells are deficient in the PKC $\alpha$ and $\beta$ isoforms (22, 23). Recent studies have demonstrated that TPA has little if any effect on c-*jun* expression in HL-525 cells (24). Moreover, TPA activates serine/threonine MAP kinase activity in HL-60, but not HL-525, cells (24). The present results demonstrate that ara-C induces transcription of the c-*jun* gene in HL-525 cells and that this event is associated with activation of PKC-like and MAP kinase activities.

## Materials and Methods

**Cell culture.** The phorbol ester-resistant subclone of HL-60 cells, designated HL-525 (21), was grown in RPMI 1640 medium containing 15% fetal bovine serum with 1 mM L-glutamine, 100 units/ml penicillin, and 100 $\mu$g/ml streptomycin. The cells were treated with $10^{-5}$ M ara-C (Sigma Chemical Co., St. Louis, MO) and 32 nM TPA (Sigma). These cells have remained resistant to TPA-induced c-*jun* expression and differentiation (22, 24) in long term culture (>100 passages).

**Isolation and analysis of RNA.** Total cellular RNA was purified by the guanidine isothiocyanate-cesium chloride technique (25). The RNA (20 $\mu$g) was analyzed by electrophoresis through 1% agarose-formaldehyde gels, transferred to nitrocellulose filters, and hybridized to the following $^{32}$P-labeled DNA probes: (a) the 1.8-kb *Bam*HI/*Eco*RI insert of a human c-*jun* gene purified from a pBluescript SK(+) plasmid (26) and (b) the 2.0-kb *Pst*I insert of a chicken $\beta$-actin gene purified from the pA1 plasmid (27). Hybridizations were performed as described (15). Autoradiographic bands were scanned using a LKB Produkter (Bromma, Sweden) Ultroscan XL laser densitometer and were analyzed with the Gelscan XL software package (version 1.21). Signal intensity was determined in a linear range and normalized to that for the actin control.

**Nuclear run-on assays.** Nuclei were isolated from $10^8$ cells by lysis in a 0.5% Nonidet P-40 solution, and the $^{32}$P-labeled nuclear RNA was prepared as described (18). The labeled RNA was hybridized to the following digested DNAs: (a) the 2.0-kb *Pst*I fragment of the chicken $\beta$-actin gene (27) and (b) the 1.8-kb *Bam*HI/*Eco*RI fragment of the human c-*jun* cDNA (26). 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 $\mu$g/ml yeast tRNA, for 72 hr at 42°. The filters were then washed with 2× SSC/0.1% SDS at 37° for 30 min, with 10 mg/ml RNase A in 2× SSC at 37° for 20 min, and with 0.1× SSC/0.1% SDS at 42° for 30 min. Signal intensity was determined by laser densitometry.

**Subcellular fractionation.** Subcellular fractionation was performed as described (28). Cells were washed twice with cold phosphate-buffered saline and resuspended in 1 ml of hypotonic lysis buffer (1 mM EGTA, 1 mM EDTA, 10 mM $\beta$-glycerophosphate, 1.0 mM sodium orthovanadate, 2 mM $MgCl_2$, 10 mM KCl, 1 mM dithiothreitol, 40 $\mu$g/ml phenylmethylsulfonyl fluoride, 10 $\mu$g/ml leupeptin, 10 $\mu$g/ml aprotinin, pH 7.2). After swelling on ice for 30 min, the cells were disrupted by Dounce homogenization (25 strokes). The supernatant above the sucrose cushion was collected and centrifuged at 150,000 × *g* for 30 min at 4°, to collect the soluble fraction.

**Analysis of ara-C-induced PKC-like activity.** The soluble cell extract was diluted with 0.1 M NaCl in buffer A (20 mM Tris·HCl, pH 7.4, 0.5 mM EDTA, 0.5 mM EGTA, 10 mM $\beta$-mercaptoethanol) and applied to a Q-Sepharose Fast Flow column (Pharmacia, Piscataway, NJ). Protein was eluted with 0.4 M NaCl/buffer A. PKC activity was assayed for 15 min at 30° in reactions containing 20 mM Tris·HCl, pH 7.4, 5 mg/ml synthetic peptide derived from amino acids 4–14 of MBP (GIBCO/BRL), 20 $\mu$M [$\gamma$-$^{32}$P]ATP, 10 mM $MgCl_2$, and 0.4 mM dithiothreitol. The eluates were adjusted to 0.1 M NaCl/buffer A and applied to a Mono Q column (HR 10/10; Pharmacia). Protein was eluted with a linear 0.1–0.6 M NaCl/buffer A gradient and the fractions were again assayed for MBP(4–14) phosphorylation.

**Immune complex MAP kinase assays.** Soluble cell lysates were prepared as already described and were precleared by incubation with preimmune rabbit serum and Protein A-Sepharose for 1 hr at 4°. The supernatant fraction was then incubated with anti-MAP kinase antiserum (provided by J. Kyriakis and J. Avruch, Massachusetts General Hospital, Boston, MA) (29) and Protein A-Sepharose for 2 hr at 4°. The immunoprecipitates were washed twice with lysis buffer, three times with LiCl buffer (0.5 M LiCl, 100 mM Tris, pH 7.6), and once with assay buffer (20 mM Tris·HCl, pH 7.2, 50 mM $\beta$-glycerophosphate, 1.5 mM EGTA, 1 mM dithiothreitol, 0.03% Brij-35). The immune complexes were then suspended in 20 $\mu$l of reaction buffer (20 mM Tris·HCl, pH 7.2, 10 mM $MgCl_2$) containing 10 $\mu$g of MBP and 1 $\mu$Ci of [$\gamma$-$^{32}$P]ATP. The reaction was allowed to proceed for 15 min at 30° and was terminated by the addition of SDS-polyacrylamide gel electrophoresis sample buffer. The proteins were separated by electrophoresis in an SDS-15% polyacrylamide gel and were analyzed by autoradiography.

**c-Jun Y peptide phosphorylation assays.** Cell extracts were applied to Q-Sepharose Fast Flow columns that had been previously equilibrated with 0.15 M NaCl in buffer B (20 mM Tris, pH 7.4, 0.5 mM EDTA, 0.5 mM EGTA, 10 mM $\beta$-mercaptoethanol, 0.5 mM sodium orthovanadate, 40 $\mu$g/ml phenylmethylsulfonyl fluoride, 10 $\mu$g/ml leupeptin, 10 $\mu$g/ml aprotinin). After the columns were washed with 0.15 M NaCl/buffer B, protein was eluted with 0.55 M NaCl/buffer B. Each fraction was assayed for phosphorylation of the c-Jun Y peptide (amino acids 56–69, NSDLLTSPDVGLLK) by incubating 5–25 $\mu$l of the fraction with 1 $\mu$Ci of [$\gamma$-$^{32}$P]ATP, 20 mM Tris·HCl, pH 7.5, 10 mM $MgCl_2$, 50 $\mu$M ATP, and 2.5 $\mu$g of Y peptide, in a 50-$\mu$l reaction, for 15 min at 30°. Aliquots (25 $\mu$l) of the reactions were spotted onto P81 phosphocellulose discs (GIBCO/BRL, Gaithersburg, MD). The discs were washed twice with 1% $H_3P0_4$ and twice with water before counting.

## Results

Previous work has demonstrated that treatment of HL-60 cells with TPA is associated with induction of the c-*jun* gene (18). Other studies have shown that the effects of TPA on c-*jun* expression are attenuated in TPA-resistant HL-525 cells (22, 23). These findings are in concert with the demonstration that HL-525 cells are defective in TPA-induced PKC-mediated signaling events (21). Because recent studies have also suggested that ara-C induces c-*jun* transcripts by a PKC-dependent mechanism (15), we sought to determine the effects of ara-C on c-*jun* expression in HL-525 cells. A low level of c-*jun* expression was detectable in untreated HL-525 cells, whereas exposure to $10^{-5}$ M ara-C was associated with increases in c-*jun* mRNA levels that were maximal at 6 hr and returned nearly to baseline at 24 hr (Fig. 1). Both the kinetics and magnitude of this response were comparable to those reported for ara-C-treated HL-60 cells (15).

Nuclear run-on assays were performed to determine whether transcriptional mechanisms contribute to the induction of c-*jun* expression. Nuclear RNA isolated from HL-525 cells that had been treated with ara-C for 6 hr was hybridized to actin and c-*jun* DNAs. The actin gene was constitutively activated

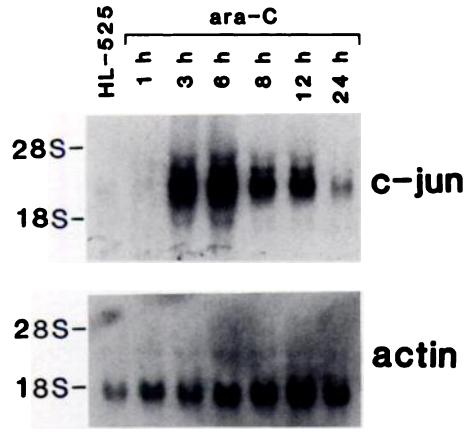


**Fig. 1.** Ara-C induction of c-*jun* expression in HL-525 cells. HL-525 cells were treated with $10^{-6}$ M ara-C. At the indicated times, total cellular RNA (20 μg) was isolated and hybridized to the $^{32}$P-labeled c-*jun* and actin probes.

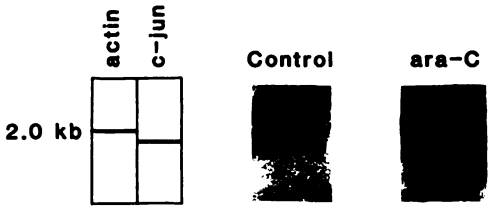


**Fig. 2.** Activation of c-*jun* transcription in ara-C-treated HL-525 cells. Cells were treated with ara-C for 6 hr. Nuclei were isolated, and newly elongated $^{32}$P-labeled transcripts were hybridized to 2 μg of actin and c-*jun* DNA inserts after restriction enzyme digestion and Southern blotting. *Solid lines in the schema*, relative positions of the inserts.

in untreated HL-525 cells and its transcription was unaltered by ara-C treatment (Fig. 2). In contrast, a low level of c-*jun* transcription was detectable in untreated HL-525 cells and this rate was increased 8-fold in ara-C-treated cells (Fig. 2). These findings indicated that ara-C induces c-*jun* expression in HL-525 cells at least in part by transcriptional mechanisms.

The finding that ara-C, but not TPA, induces c-*jun* transcription in HL-525 cells indicated the involvement of a signaling cascade distinct from that activated by phorbol esters. Other studies in HL-60 cells have demonstrated that ara-C treatment is associated with induction of a PKC-like activity, as determined by phosphorylation of a synthetic peptide derived from MBP (amino acids 4–14) (15). Consequently, we asked whether similar findings would be obtained in the TPA-resistant HL-525 cells. Extracts of HL-525 cells treated with ara-C were partially purified by Q-Sepharose Fast Flow chromatography and assayed for MBP(4–14) phosphorylation. Increases in MBP kinase activity were detectable at 3 hr and maximal (2.5–3.0-fold) at 6 hr of ara-C treatment (Fig. 3). This activity was further purified by adsorption to Mono Q anion exchange beads and then elution with a linear NaCl gradient. The ara-C-induced MBP kinase activity was detectable in an initial peak that was eluted with 0.3 M NaCl (Fig. 4). The activity in this peak was approximately 6-fold higher in ara-C-treated cells, compared with control or TPA-treated cells (Fig. 4). In contrast, other, less prominent, peaks of MBP kinase activity exhibited little if any increase after ara-C treatment (Fig. 4). The finding that similar results were obtained in the presence

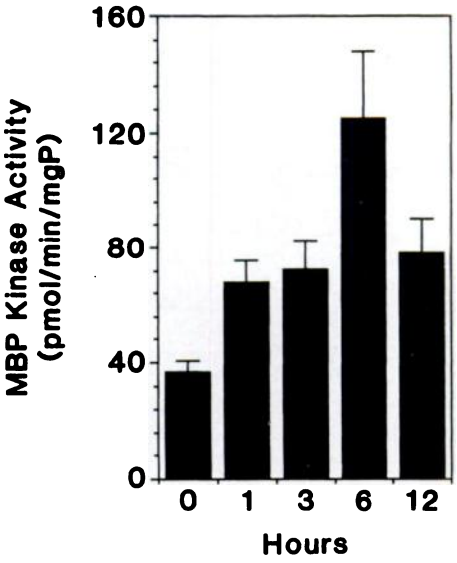


**Fig. 3.** Association of ara-C treatment with induction of MBP kinase activity. HL-525 cells were treated with ara-C for the indicated times. The cells were lysed and the soluble fraction was applied to a Q-Sepharose column. Proteins eluting with 0.4 M NaCl were assayed for protein kinase activity using MBP(4–14) as substrate.

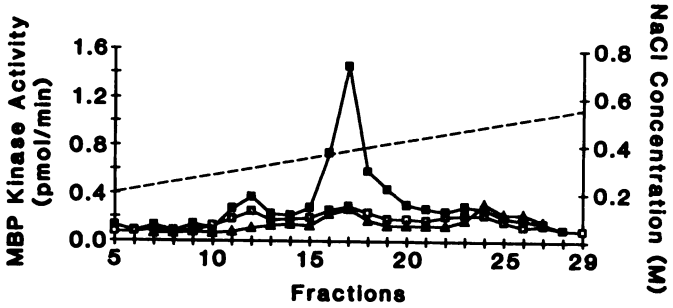


**Fig. 4.** Partial purification of MBP kinase activity in ara-C-treated cells. Soluble fractions from untreated cells (□), cells treated with ara-C for 6 hr (■), and cells treated with 32 nM TPA for 3 hr (▲) were separated on Q-Sepharose and then applied to Mono Q columns. Protein was eluted with a 0.1–0.6 M NaCl gradient. Fractions were monitored for MBP(4–14) phosphorylation. Treatment of cells with TPA for 3 or 6 hr yielded similar results (data not shown).

or absence of $Ca^{2+}$ supported the partial purification of a $Ca^{2+}$-independent protein kinase.

The MBP(4–14) peptide serves as a specific phosphate acceptor for PKC (Refs. 29 and 30 and PKC assay system from GIBCO/BRL, Grand Island, NY). We therefore assayed the ara-C-induced activity with other known substrates of the PKC family. For example, replacement of Ala$^{25}$ with serine in the pseudosubstrate region (amino acids 19–31) of PKC has been shown to produce a specific PKC phosphorylation site (30). The [Ser$^{25}$]PKC(19–31) peptide also served as a substrate for the ara-C-induced protein kinase (Table 1). In contrast, there was little if any phosphorylation of the c-Jun Y peptide (Table 1). Furthermore, the PKC pseudosubstrate region (amino acids 19–36), which acts as a substrate antagonist (30), inhibited the activity of the ara-C-induced protein kinase (Table 1). This activity was also blocked by the indole carbazole staurosporine, a nonspecific inhibitor of PKC (31, 32) (Table 1). These findings indicated that ara-C treatment of HL-525 cells is associated with activation of a PKC-like protein kinase.

Recent studies in TPA-treated myeloid leukemia cells have

TABLE 1

**Phosphorylation of synthetic substrates by the ara-C-induced protein kinase activity, after partial purification by Q-Sepharose and Mono Q chromatography**

| Substrate | Inhibitor | Phosphorylation |
|---|---|---|
| | | *pmol/min* |
| MBP(4–14), 50 μM | None | 21.2 |
| [Ser$^{25}$]PKC(19–31), 50 μM | None | 11.1 |
| Y peptide, 50 μM | None | 0.02 |
| MBP(4–14), 50 μM | PKC(19–36), 2 μM | 5.1 |
| MBP(4–14), 50 μM | PKC(19–36), 20 μM | 0.9 |
| MBP(4–14), 50 μM | Staurosporine, 10 nM | 3.7 |

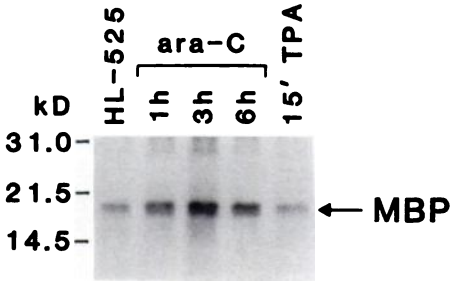


**Fig. 5.** Activation of MAP kinase by ara-C. Cells were treated with either ara-C or TPA for the indicated times. Total cell lysates, using equal amounts of protein, were immunoprecipitated with anti-MAP kinase antiserum and Protein A-Sepharose. Immune complexes were assayed for phosphorylation of MBP by gel electrophoresis and autoradiography. The MBP signal had an apparent molecular mass of 18.5 kDa. Treatment of HL-60 cells with TPA for 15 min is associated with increases in MAP kinase activity (24).

demonstrated that PKC can function upstream in the activation of MAP kinase (24). Consequently, we asked whether activation of the PKC-like activity in ara-C-treated HL-525 cells is associated with stimulation of MAP kinase, as determined by immune complex kinase assays with MBP as the substrate. As a control, lysates of HL-525 cells treated with TPA were subjected to immunoprecipitation with an anti-MAP kinase antibody. Assays of anti-MAP kinase immune complexes from ara-C-treated HL-525 cells demonstrated an increase in MBP phosphorylation (Fig. 5). This stimulation of MAP kinase activity was detectable at 1 hr and persisted through 6 hr of ara-C exposure (Fig. 5). In contrast, anti-MAP kinase immune complexes from the TPA-treated HL-525 cells exhibited no detectable increase in MBP phosphorylation (Fig. 5). These findings indicated that ara-C induces MAP kinase by a mechanism distinct from that activated by TPA treatment.

Other studies have demonstrated that MAP kinase specifically phosphorylates serine residues in the amino-terminal domain of c-Jun and thereby positively regulates the *trans*-acting activity of this protein (33). A similar activity has been identified that phosphorylates Ser$^{63}$ and Ser$^{73}$ in c-Jun (34, 35). Recent cloning of the gene encoding this activity has demonstrated identity with the pp46/55 member of the MAP kinase family (36, 37). Studies were therefore performed to determine whether activation of MAP kinase in HL-525 cells corresponds to phosphorylation of the synthetic Y peptide (Ser$^{63}$) derived from c-Jun. Treatment of HL-525 cells with ara-C was associated with an increase in Y peptide phosphorylation at 3 hr, and the increase reached maximal levels (2.5–3.0-fold) at 6 hr (Fig. 6). Longer exposures to ara-C resulted in subsequent declines

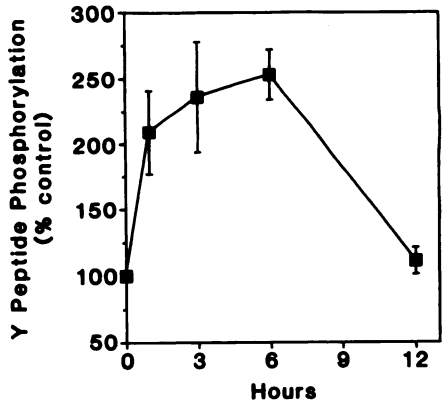


**Fig. 6.** Induction of c-Jun Y peptide phosphorylation during ara-C treatment. HL-525 cells were treated with ara-C for the indicated times. The soluble fraction was applied to Q-Sepharose columns and MAP kinase was eluted with 0.55 M NaCl. Kinase activity was assayed using c-Jun Y peptide as substrate. The results represent the mean ± standard error of two experiments (each performed in duplicate) and are expressed as a percentage of activity in control cells.

in this activity. Activation of MAP kinase in ara-C-treated HL-525 cells was thus temporally similar to induction of the PKC-like activity and the c-*jun* gene.

## Discussion

Previous work has demonstrated that treatment of human myeloid leukemia cells with ara-C is associated with activation of the c-*jun* gene (12, 15). Other studies using phorbol ester treatment of these cells have indicated that c-*jun* transcription is regulated by the activation of PKC (18). These findings suggested that ara-C-induced c-*jun* expression is also regulated by a PKC-dependent mechanism. Indeed, studies in HL-60 cells have shown that ara-C treatment is associated with increased phosphorylation of the PKC substrate MBP(4–14) and that this activity is inhibited by nonspecific PKC inhibitors (15). The present work was performed with phorbol ester-resistant HL-525 cells to determine whether ara-C induces c-*jun* expression by a pathway independent of that activated by phorbol esters. Although TPA treatment of HL-525 cells has little effect on c-*jun* expression (22–24), the present results demonstrate that ara-C induces c-*jun* transcripts in HL-525 cells and that the level of this induction is similar to that in ara-C-treated HL-60 cells (15). Other studies in HL-60 cells have shown that ara-C-induced increases in c-*jun* mRNA levels are regulated at the transcriptional level (15). Similar results were obtained in the present studies with HL-525 cells. These findings in TPA-resistant HL-525 cells indicated that ara-C induces c-*jun* transcription by a mechanism distinct from that activated during phorbol ester treatment.

The PKC family consists of at least 10 subspecies, including α, β, γ, δ, ε, ζ, and η (38). Group A PKC isoforms (α, β, and γ) require $Ca^{2+}$ for activity, whereas group B and C isoforms are active in the absence of this cation (38). Because the HL-525 variant expresses relatively low levels of PKC-α and PKC-β, compared with HL-60 cells, these isoforms have been suspected of mediating the induction of certain events, such as c-*jun* expression, by phorbol esters in HL-60 cells (22, 23). Indeed, other work has supported the involvement of PKC-α in TPA-induced differentiation of HL-60 cells (39), whereas more recent studies have implicated PKC-β in the activation of c-*jun*

expression in TPA-treated HL-525 cells (24). The present findings demonstrate that ara-C treatment of HL-525 cells is associated with the induction of a $Ca^{2+}$-independent PKC-like activity that is not inducible by TPA exposure. In this regard, the ara-C-induced protein kinase phosphorylated specific PKC substrates such as MBP(4–14) and [$Ser^{25}$]PKC(19–31) (30, 40, 41). Moreover, this activity was inhibited by the PKC pseudosubstrate and the PKC inhibitor staurosporine. Previous work in HL-60 cells has demonstrated that staurosporine blocks ara-C-induced c-*jun* transcription (15). These results and the finding that staurosporine also inhibits ara-C-induced c-*jun* expression in HL-525 cells (data not shown) support the involvement of a PKC-like activity in induction of the c-*jun* gene by ara-C. Further identification of the ara-C-induced PKC-like activity by purification to homogeneity requires additional study, due to low levels of this protein in HL-525 cells.

Several serine/threonine protein kinases have been identified that participate in the regulation of cell growth. MAP kinase is required for the transduction of certain growth signals, whereas the role of this enzyme in the response to genotoxic agents is unknown. Studies have indicated that MAP kinase and other serine/threonine kinases are involved in the positive and negative regulation of c-Jun (33–35, 42–44). In experiments using anti-MAP kinase (pp42/44) immune complexes and MBP as substrate, the present results demonstrate that MAP kinase is activated in ara-C-treated HL-525 cells. These findings are in contrast to an absence of MAP kinase activation in TPA-treated HL-525 cells (Fig. 5 and Ref. 24). Two serine residues ($Ser^{63}$ and $Ser^{73}$) in the amino-terminal A1 *trans*-activation domain of c-Jun that are phosphorylated in response to various mitogens, activated *ras*, and phorbol esters have been identified as substrates for pp42/44 (45), as well as the related pp46/55 (34–37), MAP kinase. Although pp42/44 and pp46/55 are members of the MAP kinase family on the basis of nucleic acid homology, MBP is an excellent substrate for pp42/44 and not for pp46/55 (36, 37). Another kinase that phosphorylates the amino terminus of c-Jun has been identified (42); however, it is not known whether this kinase phosphorylates sites, such as $Ser^{63}$ and $Ser^{73}$, that are phosphorylated *in vivo*. Because activation of c-Jun contributes to induction of c-*jun* gene transcription through AP-1 binding (17), stimulation of MAP kinase (pp42/44 and/or pp46/55) activity may be necessary for increases in c-*jun* mRNA levels. Indeed, previous studies have demonstrated that treatment with ara-C is associated with a post-translational modification of c-Jun and enhancement of c-Jun/AP-1 activity (46). Moreover, binding of activated c-Jun to the AP-1 site in the c-*jun* gene promoter confers ara-C inducibility of this gene (46). Thus, the finding that MAP kinase-mediated phosphorylation of c-Jun Y peptide is also increased in association with induction of c-*jun* transcription supports the activation of this cascade in ara-C-treated HL-525 cells.

The incorporation of ara-C into replicating DNA results in DNA strand breaks (10). Indeed, the available evidence indicates that ara-C acts predominantly, if not exclusively, by incorporation into DNA. Consequently, termination of DNA strand elongation by the incorporated arabinosyl moiety and accumulation of breaks may represent initial signals that stimulate a cascade of events. Previous work has demonstrated that other agents, such as UV light and ionizing radiation, induce c-*jun* transcription through activation of c-Jun/AP-1 binding (47–49). The UV response appears to be generated by signals initiated at or near the plasma membrane (50). Thus, a variety of agents that damage cells by distinct mechanisms appear to be capable of stimulating events that include activation of c-Jun/AP-1 and c-*jun* transcription. The present studies demonstrate that the response to ara-C includes induction of PKC-like and MAP kinase activities and that these kinases may function as upstream signals in transcriptional activation of the c-*jun* gene.



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