Regulation of c-jun Gene Expression by cAMP in HL-60 Myeloid Leukemia Cells*

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Previous studies have demonstrated that expression of the c-jun proto-oncogene is induced by phorbol esters and other agents that activate protein kinase C. The present work has examined the involvement of cAMP-dependent signaling mechanisms in the regulation of c-jun gene expression. Low levels of c-jun transcripts were detectable in untreated HL-60 myeloid leukemia cells. In contrast, treatment of these cells with 8-bromoadenosine 3',5'-cyclic monophosphate was associated with increases in c-jun expression that were maximal at 3 h and then declined to pretreatment levels. Similar findings were obtained with N6,2'-O-dibutyryl adenosine 3',5'-cyclic monophosphate and 8-(4-chlorophenylthio)-adenosine 3',5'-cyclic monophosphate, but not with 8-bromoguanosine 3',5'-cyclic monophosphate. c-jun transcripts were also increased with agents, such as prostaglandin E2 and forskolin, that increase intracellular cAMP levels. The effects of these agents on c-jun expression were associated with activation of cAMP-dependent protein kinase. Moreover, inhibition of this kinase activity with the isoquinolinesulfonamide derivative H8 was associated with a block in the induction of c-jun expression by cAMP. Nuclear run-on analysis further demonstrated that while c-jun transcription is at low levels in untreated HL-60 cells, treatment with cAMP analogs is associated with an increase in the transcriptional rate of this gene. Taken together, these findings suggested that, in addition to activation of protein kinase C, stimulation of cAMP-dependent protein kinase activity is also involved in the transcriptional induction of c-jun gene expression. The present results similarly demonstrate that c-fos gene transcription is induced in HL-60 cells through a mechanism involving cAMP-dependent protein kinase activity. Since heterodimers of the Jun and Fos proteins have been shown to bind to the phorbol ester-responsive element (AP-1-binding site), the present findings indicate that cAMP-induced signaling events may also regulate gene transcription through formation of Fos/Jun heterodimers and that interaction between phorbol ester- and cAMP-dependent pathways could occur through induction of the c-jun gene in these cells.

The c-jun proto-oncogene has been implicated in the regulation of cellular proliferation (1). This gene is induced as an immediate early event during serum treatment of NIH3T3 cells (2). c-jun expression is also regulated at the transcriptional and posttranscriptional levels in BALB/c 3T3 fibroblasts exposed to serum, platelet-derived growth factor, and fibroblast growth factor (3). Other studies have demonstrated that epidermal growth factor activates c-jun gene expression in rat fibroblasts (4), while tumor necrosis factor induces this gene in human fibroblasts (5). The c-jun gene is also expressed in PC12 pheochromocytoma cells stimulated with nerve growth factor (6) and in several cell lines exposed to transforming growth factor-β (7).

The c-jun gene codes for the major form of the transcription factor AP-1, which is composed of several polypeptides ranging in size from 40 to 44 kDa (6-12). The AP-1 polypeptides bind to a DNA sequence motif (TRE) that regulates transcription of genes responsive to phorbol esters (9, 12-14). This DNA-binding site is also involved in the activation of other genes that are induced in response to the expression of transforming proteins (15-18). The affinity of Jun/AP-1 for DNA binding is related to dimerization with other transcription factors that contain a leucine zipper and a region rich in basic amino acids (19). For example, heterodimers between the Jun protein and the product of the c-fos proto-oncogene bind more efficiently to the TRE than c-jun homodimers (20).

The finding that AP-1 regulates transcription of genes induced by phorbol esters is in concert with the demonstration that these agents also activate c-jun gene expression. In this regard, treatment of human fibroblasts with 12-O-tetradecanoylphorbol-13-acetate (TPA) is associated with increased c-jun expression (5). Other studies have demonstrated that TPA stimulates expression of a c-jun promoter/reporter gene by an autoregulatory mechanism in epithelial cells (21). More recent work has shown that TPA induces c-jun expression in human myeloid leukemia cells (22). Similar findings in HL-60 leukemia cells have been obtained with other agents that activate protein kinase C (22). Taken together with the findings that certain growth factors known to activate protein kinase C also induce c-jun expression (5, 7), these results have suggested that signaling events involving Jun/AP-1 are controlled through the activation of this enzyme.

The present studies have examined the possibility that other signal transduction pathways might be involved in the regulation of c-jun expression.

The abbreviations used are: TRE, phorbol ester response element; TPA, 12-O-tetradecanoylphorbol-13-acetate; 8-Br-cAMP, 8-bromoadenosine 3',5'-cyclic monophosphate; dBr-cAMP, N6,2'-O-dibutyryladenosine 3',5'-cyclic monophosphate; 8-Br-cGMP, 8-bromoguanosine 3',5'-cyclic monophosphate; 8-CPT-cAMP, 8-(4-chlorophenylthio)-adenosine 3',5'-cyclic monophosphate; PGE2, prostaglandin E2; IBMX, 3-isobutyl-1-methylxanthine; H8, N-[2-(methylamino)ethyl]-5-isouquinolinesulfonamide; kb, kilobase(s); SDS, sodium dodecyl sulfate; EGTA, ethylenediaminetetraacetic acid.
induction of c-jun gene expression. In this regard, previous work has shown that a CAMP analog transiently induces c-jun transcripts in rat FC12 cells (12). The present results demonstrate that analogs of CAMP and agents that increase intracellular levels of this nucleotide induce c-jun expression in HL-60 cells. The results also indicate that this effect is mediated through the activation of CAMP-dependent protein kinase. The finding that CAMP similarly regulates c-fos gene expression in these cells suggests that CAMP-induced signaling events may involve several members of this family of transcription factors.

MATERIALS AND METHODS

Cell Culture—HL-60 promyelocytic leukemia cells (American Type Culture Collection, Bethesda, MD) were grown in RPMI 1640 medium supplemented with 15% heat-inactivated fetal bovine serum (GIBCO), 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. The cells were grown in the presence of 8-bromoadenosine 3',5'-cyclic monophosphate (8-Br-cAMP, Sigma), N6,2'-O-dibutyryladenosine 3',5'-cyclic monophosphate (dBi-cAMP, Sigma), 8-bromoguanosine 3',5'-cyclic monophosphate (8-BG-cAMP, Sigma), 8-(4-chlorophenyl)adenosine 3',5'-cyclic monophosphate (8-CP-cAMP, Sigma), protaglandin E2 (PGE2, Sigma), forskolin (Sigma), 3-isobutyl-1-methylxanthine (IBMX, Sigma) and N-[2-(methylamino)ethyl]-5-isouquinolinesulfonamide (HS) (Seikagaku, Tokyo, Japan).

Isolation and Analysis of Total Cellular RNA—Total cellular RNA was purified by the guanidine isothiocyanate-cesium chloride method (23), analyzed by electrophoresis through 1% agarose-formaldehyde gels, transferred to nitrocellulose filters and hybridized to one of the following DNA probes: 1) the 1.8-kb BamHI/EcoRI insert of a human c-jun DNA probe purified from a pBlueScript SK(+) plasmid (10); 2) the 0.9-kb ScaI/NcoI insert of a human c-fos gene purified from the pc-fos-1 plasmid (24); and 3) the 2.0-kb PsiI insert of a chicken β-actin gene purified from the pA1 plasmid (25). Hybridizations were performed at 42 °C for 24 h in 50% (v/v) formamide, 2 × SSC (standard sodium citrate), 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.

Run-on Transcription Assay—Nuclei were isolated from 106 cells by lysing 5% Nonidet P-40 and 32P-labeled different RNA prepared as described (22). The labeled RNA was hybridized to the following digested plasmid DNAs: 1) the 2.0-kb PsiI fragment of the chicken β-actin gene (25); 2) the 1.1-kb BamHI insert of the human β-globin gene (26); 3) the 1.8-kb BamHI/EcoRI fragment of the human c-jun DNA from the pBlueScript SK(+) plasmid (10); and 4) the 3.1-kb NcoI/Xhol insert of the human c-fos gene from the pc-fos-1 plasmid (24). The digested DNAs were run in 1% agarose gels and transferred to nitrocellulose filters. Hybridizations were performed with 105 cpm of 32P-labeled RNA/ml in 4 × SSC, 5 mM EDTA, 0.4% SDS, 5 × Denhardt’s solution, 40% formamide, and 100 μg/ml yeast tRNA for 3 d at 42 °C. The filters were washed in 2 × SSC, 0.1% SDS at room temperature and then in 0.1 × SSC, 0.1% SDS at 42 °C for 30 min.

CAMP-dependent Protein Kinase Assay—The CAMP-dependent protein kinase assay was performed as described with certain modifications (27). HL-60 cells (4 × 106) were treated with the indicated agents for 10 min, washed with phosphate-buffered saline, and resuspended in 300 μl of sonication buffer containing 2 mM EGTA, 25 mM Tris-HCl, pH 7.6, 0.2 mM sodium orthovanadate, 2 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 0.1% Triton X-100, and 0.5 mM IBMX. The cells were sonicated and the lysate centrifuged at 12,500 × g for 5 min at 4 °C. The supernatant (40 μl) was incubated for 10 min at 30 °C in 20 μl of reaction buffer containing 75 mM Tris-HCl, pH 7.5, 6 mM EGTA, 30 mM MgCl2, 0.6 mM sodium orthovanadate, 0.6 mM ATP, 3 μg of histone type VI-S (Sigma) and 1 μCi of [γ-32P]ATP (5000 Ci/mmol, Amersham Corp.), in the presence or absence of 200 μM H8. The reaction was stopped by adding 6 μl of 30 mM EDTA. The proteins were separated by electrophoresis in a 15% SDS-polyacrylamide gel and exposed to x-ray film. Autoradiographic bands were quantitated using an LKB Ultrascan XL laser densitometer.

RESULTS AND DISCUSSION

Effects of CAMP Analogs on c-jun Expression in HL-60 Cells—Agents that increase CAMP levels in HL-60 cells induce a program in which these cells express monocyte-specific antigens, α-naphthyl butyrate esterase, and certain functional properties of phagocytes (28, 29). Furthermore, CAMP levels increase during monocyte differentiation as a result of increases in adenylate cyclase and decreases in CAMP phosphodiesterase activity (30). The effects of CAMP on c-jun expression in HL-60 cells was first studied using membrane-permeable analogs of this cyclic nucleotide. c-jun mRNA was at low to undetectable levels in untreated HL-60 cells. In contrast, exposure to 1 mM 8-Br-cAMP in the presence of IBMX, a phosphodiesterase inhibitor, was associated with increased expression of 2.7-kb c-jun transcripts (Fig. 1A). This increase was first detectable at 1 h and reached maximum levels by 3 h. Longer exposures were associated with a progressive down-regulation by 12 h to that in untreated cells (data not shown). There was little if any effect of either 8-Br-cAMP or IBMX alone on c-jun expression. Moreover, these agents had no detectable effect on actin mRNA levels (Fig. 1A). Other studies indicated that the effects of 8-Br-cAMP are concentration-dependent (Fig. 1B). Taken together with the finding that similar concentrations of 8-Br-cGMP and IBMX have no detectable effect on c-jun transcripts (data not shown), these results suggested that CAMP may play a role in regulating expression of this gene.

Studies were also performed with other membrane-permeable CAMP analogs. While the C-8 position of the adenine ring is modified in 8-Br-cAMP, dBt-cAMP represents an analog with modification at the C-6 position. Exposure of HL-60 cells to 1 mM dBt-cAMP and IBMX was also associated with increases in c-jun transcripts that were first detectable at 0.5 h (Table I). Maximal increases in c-jun expression were observed at 6 h of exposure to these agents and followed by down-regulation at 12 h (Table I and data not shown). As in the studies with 8-Br-cAMP, exposures to dBt-cAMP alone had little effect on c-jun mRNA levels (data not shown).

![Fig. 1. Effects of 8-Br-cAMP on c-jun mRNA levels in HL-60 cells.](image-url)

- **A.** HL-60 cells were treated with 1 mM 8-Br-cAMP and 0.5 mM IBMX for the indicated times. Cells were also incubated with each of these agents alone for 3 h. Total cellular RNA (20 μg) was analyzed (22) and hybridized to 32P-labeled c-jun and actin DNA probes. **B.** HL-60 cells were treated with varying concentrations of 8-Br-cAMP and 0.5 mM IBMX. After 3 h, total cellular RNA (20 μg) was isolated and hybridized to the 32P-labeled c-jun probe. Similar hybridizations to the labeled actin probe demonstrated equal loading of the lanes.
Similar findings were also obtained with the C-8-thio analog, 8-CPT-cAMP. For example, treatment of HL-60 cells with 0.1 and 1.0 mM 8-CPT-cAMP in the presence of IBMX was associated with increased levels of c-jun mRNA (Table I). Thus, c-jun expression was increased by both C-8 analogs that selectively bind to site 1 and C-6 analogs that selectively interact with site 2 of cAMP-dependent protein kinases (31).

**Table I**

<table>
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<th>Drug</th>
<th>0 h</th>
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<th>1 h</th>
<th>3 h</th>
<th>6 h</th>
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<td>ND</td>
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</table>

Involvement of cAMP-dependent Protein Kinase Activity in the Regulation of c-jun Expression—The findings that cAMP induces c-jun expression in HL-60 cells could be related to the activation of cAMP-dependent protein kinase activity. cAMP binds to the regulatory subunits of type I and II cAMP-dependent protein kinase and activates these enzymes to phosphorylate certain histones in vitro (37). Studies were therefore performed to determine the effects of cAMP analogs on the ability of HL-60 cell extracts to phosphorylate histone VII-S. The cells were treated with 1 mM 8-Br-cAMP or dBt-cAMP in the presence of IBMX for 10 min. While histone VII-S was phosphorylated by extracts of untreated HL-60 cells, there was over a 2-fold increase in kinase activity after treatment with the CAMP analogs (Fig. 2). Forskolin treatment of these cells was also associated with a 1.6-fold increase in histone VII-S phosphorylation (Fig. 2). Furthermore, H8, a preferential inhibitor of cyclic nucleotide-dependent kinases (38), blocked the increases in kinase activity induced by both cAMP analogs and forskolin (Fig. 2). These findings indicated that these agents activate cAMP-dependent protein kinase in HL-60 cells.

In order to study the potential role of cAMP-dependent protein kinase activity in the regulation of c-jun expression, HL-60 cells were exposed to the cAMP analogs for 3 h with 50 mM H8 added during the last 0.5 h of incubation. H8 blocked 8-Br-cAMP-induced increases in c-jun expression by 99 ± 1% (mean ± standard deviation of three separate experiments normalized to actin mRNA levels). In contrast, while H8 also inhibits protein kinase C (38), the finding that TPA-induced c-jun expression was inhibited by 57 ± 7% (mean ± average deviation of two separate experiments normalized to actin mRNA levels) is in concert with the selective effects of this agent against cAMP-dependent protein kinases.

**Effects of cAMP on c-fos Gene Expression in HL-60 Cells—**

The c-jun protein contains a conserved DNA-binding domain with high affinity for the TRE (9, 12–14). Furthermore, heterodimers of the Jun and Fos proteins bind more efficiently to the TRE than Jun homodimers (20). Previous work has demonstrated that cAMP increases c-fos mRNA levels in macrophages (39). Consequently, further studies were performed to determine whether cAMP also induces c-fos expression in HL-60 cells. While c-fos transcripts were at low levels in untreated HL-60 cells, treatment with 8-Br-cAMP was associated with rapid increases in c-fos expression that were maximal by 3 h (Table II). Moreover, longer exposures were associated with down-regulation of c-fos mRNA levels (data not shown). The effects of 8-Br-cAMP on c-fos expression were concentration-dependent over a range of 0.1 to 10 μM (data not shown). Similar effects were observed with other cAMP analogs. For example, dBt-cAMP and 8-CPT-cAMP also rapidly induced c-fos transcripts (Table II). Other studies performed with PGE2 and forskolin similarly demonstrated rapid increases in c-fos mRNA levels that were maximal at 3 h of exposure (Table II). These findings indicated that cAMP also induces c-fos expression in HL-60 cells and that this induction could involve a cAMP-dependent protein kinase. Indeed, while treatment with 8-Br-cAMP or forskolin for 0.5 h was associated with increased c-fos mRNA levels, the ad-
been identified thus far in CAMP-responsive genes. The 8-
activation by CAMP to certain mammalian gene promoters (40).
This CAMP-responsive element differs from the AP-1-binding
sequence (CCGCAGGC) in the c-jun gene acts as a binding
element that might contribute to the effects of CAMP on c-jun
expression in HL-60 cells. The CAMP-induced increase in c-
fos expression is another potential mechanism in that en-
hanced formation of Fos/Jun heterodimers could activate c-
jun transcription through TIRE binding. c-jun expression is
present at low but detectable levels in uninduced HL-60 cells
and thus could play a role in positive autoregulation following
induction of c-fos expression.

The induction of c-jun expression by CAMP appears to be
cell-type specific. For example, there are no detectable
changes in c-jun expression after incubation of HeLa TK- or
HepG2 cells with forskolin (10). In contrast, another study
has demonstrated that 8-Br-cAMP transiently increases c-
jun mRNA levels in PC12 cells (6). Since c-fos expression is
also increased by CAMP in PC12 cells (41), CAMP inducibility
of the c-jun gene may be dependent on c-fos expression in
specific types of cells. The finding that CAMP induces the
expression of these genes also suggests that signaling by the
formation of Fos/Jun heterodimers can occur as a result of
activating either protein kinase C or the CAMP-dependent
protein kinase pathways. In HL-60 cells, activation of protein
kinase C is associated with induction of c-fms, macrophage
colony-stimulating factor, and tumor necrosis factor expres-
sion (24-44), while CAMP acts in the negative regulation of
these genes (44, 45). Therefore, although CAMP and TPA
both induce c-jun expression in HL-60 cells, the effects of
these agents on the subsequent expression of genes involved
in monocytic differentiation are distinct.

REFERENCES
Acad. Sci. U. S. A. 84, 3316-3319
Nature 334, 535-537
85, 8464-8467
J. Biol. Chem. 264, 9000-9005
8. Bohmman, D., Bos, T. J., Admon, A., Nishimura, T., Vogt, P. K.,
9. Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Rahms-
49, 729-739
10. Angel, P., Allegrato, E. A., Okino, S., Hattori, K., Boyle, W. J.,
11. Chiu, R., Boyle, W. J., Meek, J., Smeal, T., Hunter, T., and Karin,
13. Chiu, R., Imagawa, M., Imbra, R. J., Buckoven, J. R., and Karin,
15. Imler, J. L., Schatz, C., Wasylyk, C., Chatton, B., and Wasylyk,
2475-2483
18. Sistonen, L., Holta, E., Maek, T. P., Keskis-Oja, J., and Alitalo,
875-885
22. Sherman, M. L., Stone, R. M., Datta, R., Bernstein, S. H., and
23. Chirgwin, J. M., Przybyle, A. E., MacDonald, R. J., and Rutter,
Regulation of c-jun Gene Expression

W. J. (1979) Biochemistry 18, 5294-5299
Regulation of c-jun gene expression by cAMP in HL-60 myeloid leukemia cells.
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