Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7247–7251, August 1992
Cell Biology

Phorbol ester-induced amino-terminal phosphorylation of human JUN but not JUNB regulates transcriptional activation

CHRISTOPHER C. FRANKLIN*, VERONICA SANCHEZ*, FRED WAGNER*, JAMES R. WOODGETT†, AND ANDREW S. KRAFT*‡

*Division of Hematology/Oncology, University of Alabama, Birmingham, AL 35294; and †Ludwig Institute for Cancer Research, 91 Riding House Street, London W1P 8BT, United Kingdom

Communicated by Eugene P. Cronkite, March 30, 1992

ABSTRACT **Phorbol ester tumor promoters activate gene transcription by regulating both the synthesis and posttranslational modification of the activator protein 1 (AP-1) transcription factor. c-Jun and JunB are components of the mammalian AP-1 complex. Here we demonstrate that in U-937 human leukemic cells, phorbol esters stimulate the phosphorylation of the amino terminus of human c-Jun (JUN) but not human JunB (JUNB). Mutational analysis indicates that serine-63 and -73, which reside within the putative regulatory domain of JUN, are required for both constitutive and phorbol 12-myristate 13-acetate-inducible N-terminal JUN phosphorylation. To determine the functional role of this N-terminal phosphorylation, we prepared several chimeric proteins containing the N-terminal 84 amino acids (positions 5–89) of human JUN or murine JUNB fused to the yeast GAL4 DNA-binding domain. This region was found to be sufficient for the phorbol ester-inducible transcriptional activity of JUN, but not JUNB. This induction was abolished by the mutation of serine-63 and -73 to leucine residues. Thus, we propose that phorbol esters enhance the trans-activation potential of JUN, but not JUNB, by the phosphorylation of the N-terminal regulatory domain of JUN.**

Phorbol esters such as phorbol 12-myristate 13-acetate (PMA) induce the differentiation of U-937 human leukemic cells to macrophages (1). This differentiation is accompanied by a rapid increase in the expression of the protooncogenes *JUN*, *JUNB*, and *FOS* (human c-*jun*, *junB*, and c-*fos*) (2, 3) and the protein products JUN and FOS (2, 4). The JUN and FOS family of proteins are components of the activator protein 1 (AP-1) transcription factor (5–7). U-937 cells contain two members of the JUN family of proteins, JUNB and JUN, which appear to have different biologic functions. Although both proteins exhibit similar DNA-binding properties (8), JUN is a more potent transcriptional activator than JUNB (9, 10). Cotransfection studies also indicate that JUNB represses the trans-activation and the transformation and immortalization potential of JUN (9, 10). These negative regulatory effects are thought to be the result of variations within the N-terminal activation domains of JUN and JUNB as judged by domain-swapping studies (9). This functional antagonism suggests that the integral regulation of both JUN and JUNB may influence the trans-activation potential of JUN.

JUN activity is regulated at both the transcriptional and posttranscriptional levels (11–14). PMA-induced JUN binding to the PMA-response element (called TRE) occurs rapidly and is unaffected by the inhibition of protein synthesis, suggesting that phorbol esters stimulate a posttranslational modification of preexisting JUN protein (11). In cultured fibroblasts and epithelial cells, PMA enhances JUN binding by inducing the site-specific dephosphorylation of serine and threonine residues proximal to the C-terminal DNA-binding domain of JUN (13). Other DNA-binding proteins such as CREB and MYB are inducibly phosphorylated, and this posttranslational modification regulates not only DNA binding but also the transcriptional activity of these nuclear factors (15–17). HRAS is thought to activate JUN in a similar manner, by stimulating the phosphorylation of the JUN N-terminal activation domain (18). While it is unknown whether HRAS induces the phosphorylation of JUNB, HRAS does not stimulate JUNB transcriptional activity (18). Such functional differences suggest that the differential phosphorylation of JUN and JUNB may regulate their trans-activation potential in response to a variety of stimuli.

In this study we demonstrate that PMA treatment of U-937 cells stimulates the phosphorylation of JUN but not JUNB. Using site-directed mutagenesis we identify Ser-63 and -73 within the regulatory domain of JUN as the *in vivo* sites of phosphorylation. *In vivo* transcriptional assays utilizing chimeric proteins containing the N terminus of JUN fused to the DNA-binding domain of the yeast GAL4 transcriptional enhancer demonstrate a PMA-inducible transcriptional activation domain within the N-terminal 84 amino acids (positions 5–89) of JUN. Mutation of Ser-63 and -73 abolishes this transcriptional activation. In contrast, the corresponding N-terminal region of JUNB is not capable of PMA-inducible transcriptional activity. These results suggest that PMA enhances JUN but not JUNB transcriptional activity by stimulating the phosphorylation of its N-terminal regulatory domain.

MATERIALS AND METHODS

Cell Labeling and Immunoprecipitation. U-937 cells (5–10 × 10^6/ml) were labeled for either 6–12 hr with 1 mCi (37 MBq) of [^{32}P]orthophosphate per ml or 1–4 hr with 0.1 mCi of Tran^{35}S-label per ml in minimal essential medium containing 10% (vol/vol) dialyzed bovine calf serum and lacking either phosphate or methionine, respectively. After cell treatment, crude nuclei were isolated by hypotonic lysis where indicated (19). Native lysates were prepared by lysis in radioimmunoprecipitation assay (RIPA) buffer (10 mM Tris·HCl, pH 7.4/1% deoxycholate/1% Nonidet P-40/0.1% SDS/150 mM NaCl) containing 0.5 mM phenylmethylsulfonyl fluoride, 100 μM sodium vanadate, 50 mM NaF, 0.5 mM benzamidine, and 1 μg each of pepstatin A, leupeptin, and aprotinin per ml. Denatured lysates were prepared by boiling for 5 min in 50 mM Tris·HCl, pH 8.0/0.5% SDS/5 mM dithiothreitol, followed by a 1:4 dilution in RIPA buffer lacking SDS. Lysates were clarified by centrifugation, and the supernatant was precleared twice with protein A-Sepharose beads. JUN,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PMA, phorbol 12-myristate 13-acetate; AP-1, activator protein 1; CAT, chloramphenicol acetyltransferase; RIPA, radioimmunoprecipitation assay.

‡To whom reprint requests should be addressed.

JUNB, and JUND were immunoprecipitated with rabbit antisera at 1:500 dilution. Antisera specific for JUN, JUNB, and JUND were raised against bacterially expressed JUN fusion proteins and exhibited no cross-reactivity (ref. 20 and data not shown). Immune complexes were collected with protein A-Sepharose beads, washed extensively, and resolved on SDS/10% polyacrylamide gels.

Plasmids and Transfections. Site-specific mutations were accomplished by cloning the full-length human JUN cDNA into the phage M13 vector. The Amersham oligonucleotide-directed *in vitro* mutagenesis system was used to change Ser-63 and then Ser-73 to leucine residues (TCG to CTG). To create constructs encoding truncated proteins, a termination codon was placed at amino acid 234 of both the native and double-mutant cDNAs (ACA to TGA). The cDNAs containing the termination codon were then cloned into the *Hin*dIII/*Xba* I site of the pRC-CMV expression vector (Invitrogen, San Diego).

GAL4-JUN cDNA fusion constructs were prepared by cloning PCR products encoding various regions of the N terminus of either the native or double-mutant JUN (amino acid residues 5–89, 5–143, or 5–200) into the *Bam*HI/*Xba* I site of pSG424, which encodes amino acids 1–147 of GAL4 (21). Because JUNB contains an internal *Bam*HI site, a PCR product encoding the N terminus of JUNB, corresponding to amino acids 5–89 of JUN, was inserted into the *Eco*RI/*Xba* I site of pSG424. The G_5CAT reporter plasmid contains five GAL4 DNA-binding sites located upstream of the chloramphenicol acetyltransferase (CAT) reporter gene (21).

U-937 cells ($2–3 \times 10^7$ cells) were transfected by electroporation. Cells were washed twice with ice-cold phosphate-buffered saline (PBS), resuspended in 0.5 ml of PBS, and electroporated with 30 μg of total DNA at a setting of 250 V and 250 μF. Cells were placed on ice 10 min before and after electroporation. Cells were cultured in Dulbecco's modified Eagle's medium containing 10% bovine calf serum until further manipulation.

RESULTS

PMA Stimulates the Phosphorylation of JUN in U-937 Cells. To determine whether PMA induces the phosphorylation of JUN in U-937 cells, we immunoprecipitated JUN from cells labeled with [^{32}P]orthophosphate. While JUN from untreated cells appeared as a single phosphorylated band of 39 kDa on SDS/PAGE, PMA treatment stimulated a hyperphosphorylation of JUN protein, which migrated as several bands of retarded electrophoretic mobility (Fig. 1*A*). These retarded bands were not observed upon treatment of the immunoprecipitate with calf intestinal alkaline phosphatase (Fig. 1*A*, lanes CiP), and this effect was blocked by coincubation with a phosphatase inhibitor (Fig. 1*A*, lanes CiP + inhib.). Similar results were observed upon immunoprecipitation and phosphatase treatment of JUN from cells labeled with Tran^{35}S-label (Fig. 1*B*). These data suggest that the retarded mobility of JUN after PMA treatment is caused by an increase in JUN phosphorylation. The PMA-induced JUN mobility shift was observed in both native and denatured immunoprecipitates (Fig. 1*C*), indicating that the retarded bands represent authentic JUN protein and not coimmunoprecipitation of additional proteins induced by PMA treatment. We have examined the phosphorylation of JUN in U-937 cells in response to other agents that induce the differentiation of leukemic cells, stimulate the transcription of JUN, and activate PMA-response elements (22, 23). Both bryostatin 1, a non-phorbol-ester tumor promoter and protein kinase C activator, and okadaic acid, an inhibitor of phosphatases 1 and 2A, stimulated the appearance of retarded JUN protein, whereas agents that activate protein kinase A had no effect (Fig. 1*C*). Pretreatment with okadaic acid potentiated the effect of PMA, causing an increased proportion of JUN to migrate as highly retarded bands (Fig. 1*C*). These results suggest that the activity of phosphatases as well as kinases play a significant role in mediating the phosphorylation state of JUN in U-937 cells.

PMA Induces the Synthesis but Not the Phosphorylation of JUNB in U-937 Cells. To determine whether PMA induces the synthesis or phosphorylation of other JUN protein family members, JUN, JUNB, and JUND were immunoprecipitated from ^{35}S-labeled U-937 cells treated with PMA for various times. Whereas no JUND could be detected (data not shown), both JUN and JUNB were markedly increased in 4 hr (Fig. 2*A*). Unlike JUN, however, the increase in JUNB synthesis was not accompanied by a retarded mobility upon SDS/PAGE (Fig. 2*A*). PMA induction of the highly retarded JUN bands was evident as early as 15 min and persisted for

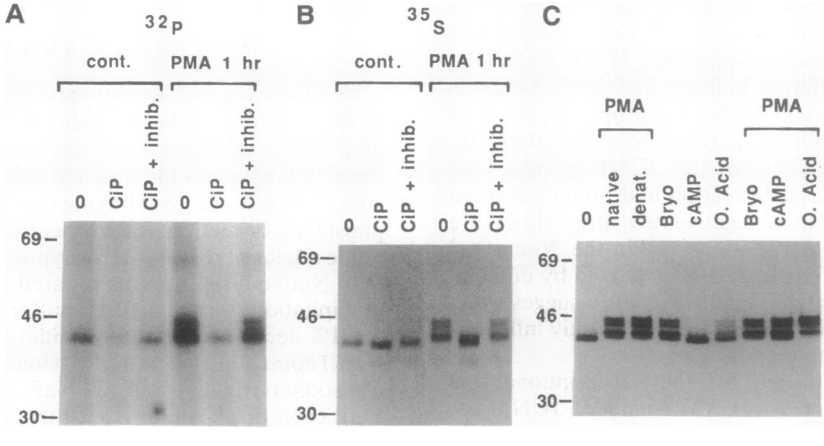

FIG. 1. Phorbol ester-induced phosphorylation of JUN. (*A*) U-937 cells were labeled with [^{32}P]orthophosphate for 6–12 hr and treated in the absence (lanes cont.) or presence (lanes PMA) of 0.1 μM PMA for 1 hr, and JUN was immunoprecipitated from denatured nuclear lysates. Washed immunoprecipitates were split into three aliquots and incubated for 1 hr at 37°C in the absence (lanes 0) or presence of 5U calf intestinal alkaline phosphatase (lanes CiP) with or without phosphatase inhibitor (10 mM *p*-nitrophenyl phosphate; inhib.). (*B*) The experiment was identical to those in *A* except that U-937 cells were labeled with Tran^{35}S-label for 2 hr and treated for the last hour of labeling with 0.1 μM PMA. JUN was immunoprecipitated from denatured nuclear lysates, and CiP treatment was carried out as in *A*. (*C*) U-937 cells were labeled for 2 hr with Tran^{35}S-label (lane 0) and treated for 1 hr with 0.1 μM PMA (lanes PMA), 1 μM bryostatin 1 (lane Bryo), 100 ng of okadaic acid per ml (lane O. Acid), or 100 μM isobutylmethylxanthine/10 μM forskolin/0.5 mM dibutyryl cAMP (lane cAMP). In the last three PMA lanes, cells were pretreated with bryostatin, cAMP, or okadaic acid for 15 min prior to the 1-hr treatment with PMA. JUN was immunoprecipitated from native RIPA lysates except where indicated (denatured). Molecular mass is shown in kDa.

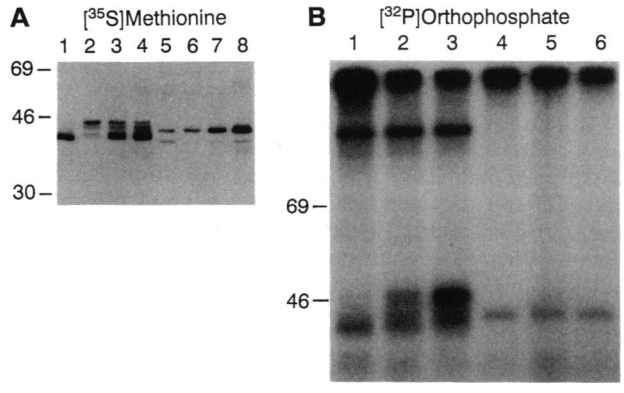

FIG. 2. PMA induces the synthesis (*A*) but not the phosphorylation (*B*) of JUNB. (*A*) U-937 cells were labeled for 4 hr with Tran^{35}S-label ([^{35}S]methionine) and were untreated (lanes 1 and 5) or treated with 0.1 μM PMA for various periods of time prior to cell lysis: 15 min (lanes 2 and 6), 2 hr (lanes 3 and 7), or 4 hr (lanes 4 and 8). At each time point, native crude nuclear lysates were prepared. Extracts were split into equal aliquots and immunoprecipitated with either JUN (lanes 1–4) or JUNB (lanes 5–8) antisera. The immunoprecipitates were resolved on a SDS/10% polyacrylamide gel. (*B*) U-937 cells were labeled for 6–12 hr with [^{32}P]orthophosphate and were untreated (lanes 1 and 4) or were treated with PMA for 15 min (lanes 2 and 5) or 1 hr (lanes 3 and 6), and denatured nuclear lysates were prepared. The lysates were split, and JUN (lanes 1–3) and JUNB (lanes 4–6) were immunoprecipitated as above. Molecular mass is shown in kDa.

at least 4 hr. To exclude the possibility that JUNB is inducibly phosphorylated but no gel retardation occurs, U-937 cells were labeled with [^{32}P]orthophosphate and treated with PMA, and JUN and JUNB were immunoprecipitated. While JUNB from untreated cells appeared as a phosphorylated band of 42 kDa on SDS/PAGE, PMA had little effect on the level of JUNB phosphorylation for up to 1 hr of treatment (Fig. 2*B*).

Mutation of Ser-63 and -73 Abolishes Both Constitutive and PMA-Induced N-Terminal JUN Phosphorylation. In contrast to recent results in HeLa cells (13), phosphopeptide mapping of JUN from U-937 cells indicates that PMA stimulates the phosphorylation of the *x* and *y* peptides, which reside within the N-terminal regulatory domain of JUN (24). The sites of phosphorylation in the *x* and *y* peptides have been mapped *in vitro* to Ser-73 and Ser-63, respectively (24). To determine whether PMA stimulates the phosphorylation of these N-terminal sites *in vivo*, we prepared a cDNA construct encoding a truncated form of JUN by placing a termination codon at amino acid 234 [JUN-(1–234)], allowing us to distinguish between exogenous and endogenous JUN protein upon SDS/PAGE. A mutated construct was also prepared in which Ser-63 and Ser-73 in the truncated protein were mutated to leucine residues—namely, [Leu^{63,73}]JUN-(1–234). Immunoprecipitation of the native truncated JUN protein from untreated ^{32}P-labeled cells revealed a single phosphorylated band of ≈30 kDa, which became hyperphosphorylated and retarded in its gel mobility upon PMA treatment (Fig. 3*A*). However, similar immunoprecipitates from cells transfected with [Leu^{63,73}]JUN-(1–234) contained no phosphorylated bands at 30 kDa in either untreated or PMA-treated cells (Fig. 3*A*). To rule out the possibility that the double-mutant protein was not being expressed or was unstable, cells were labeled with Tran^{35}S-label, and JUN protein levels were analyzed by

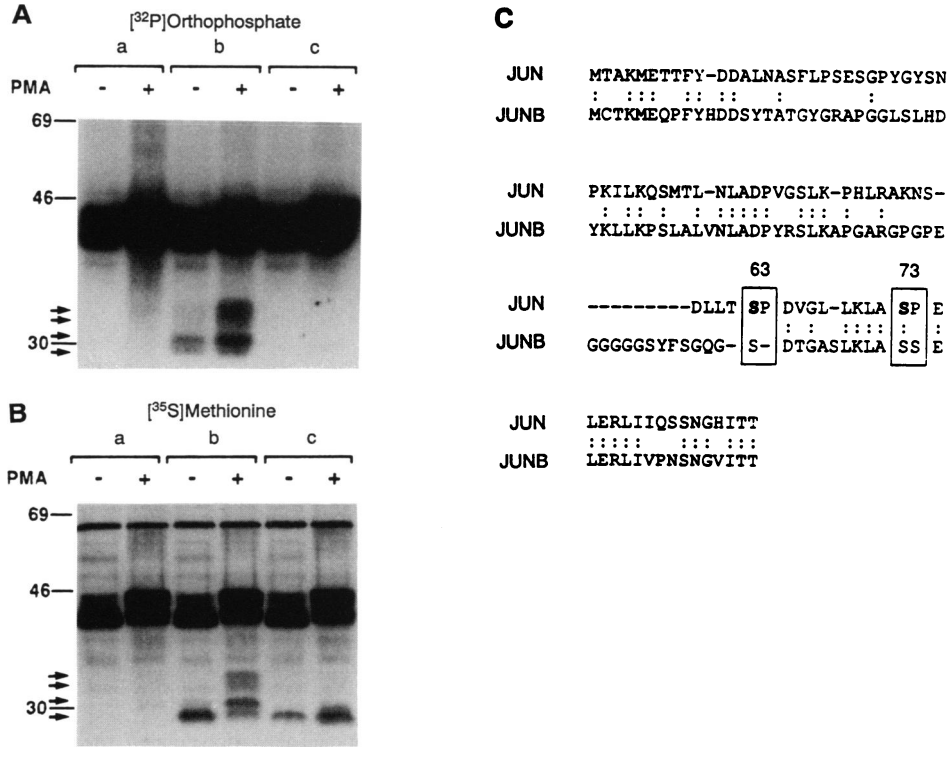

FIG. 3. Ser-63 and Ser-73 are required for the constitutive and PMA-inducible N-terminal phosphorylation of JUN. (*A*) U-937 cells were transiently transfected with either vector alone (lanes a), or vector containing JUN-(1–234) (lanes b), or [Leu^{63,73}]JUN-(1–234) (lanes c). Two days after transfection the cells were labeled for 6–12 hr with [^{32}P]orthophosphate. Half of the cells (lanes +) were treated with 0.1 μM PMA for 1 hr. JUN was immunoprecipitated from whole-cell native RIPA lysates and resolved on a SDS/10% polyacrylamide gel. (*B*) U-937 cells were transfected as in *A*, and 2 days later the cells were labeled for 4 hr by using 0.1 mCi of Tran^{35}S-label per ml. Cells were treated, and JUN was immunoprecipitated as described above. (*C*) Sequence comparison of the N terminus of JUN (6) and JUNB (25). Putative JUN phosphorylation sites are in boldface letters, and the corresponding JUNB sequences are boxed. Double dots indicate identity between JUN and JUNB. Molecular mass is shown in kDa.

immunoprecipitation. Similar amounts of the 30-kDa protein were detected in untreated cells transfected with either the native or double-mutant JUN constructs (Fig. 3*B*). While PMA stimulated the appearance of the retarded forms of the native truncated JUN protein, PMA had no effect on the gel mobility of the double-mutant JUN protein. These effects are site-specific, as similar mutations at residues proximal to these sites (Ser-48 or -58) did not alter the PMA-induced phosphorylation profile of the truncated JUN protein (data not shown). These data indicate that Ser-63 and -73 are necessary for both the constitutive and PMA-inducible phosphorylation of the JUN N terminus *in vivo* and also the appearance of the retarded JUN bands upon SDS/PAGE.

Ser-63 and -73 Are Required for PMA-Inducible JUN Transcriptional Activity. To address the functional role of this N-terminal phosphorylation, we prepared several cDNA constructs encoding chimeric proteins containing the N terminus of human JUN or murine JUNB fused to the DNA-binding domain of the yeast GAL4 transcriptional activator (21) (Fig. 4*A*). A CAT reporter gene containing five copies of the GAL4 DNA binding domain was cotransfected with these fusion constructs (21). While PMA treatment of cells cotransfected with an expression vector containing only the GAL4 DNA-binding domain (pSG424) did not stimulate transcription from the reporter gene, treatment of cells cotransfected with the GAL4-JUN expression vector encoding amino acids 5–89 of JUN led to a 12-fold increase in CAT activity (Fig. 4*B*). Mutation of Ser-63 and -73 to leucine residues within this fusion construct abolished the PMA-induced transcriptional activity. The inhibitory effect of these mutations on PMA-induced JUN transcriptional activity was evident in constructs spanning either 143 or 200 amino acids of the JUN N terminus (Fig. 4*B*). Again, similar mutations of serine residues proximal to these sites within the GAL4-JUN construct had no effect on PMA-inducible transcription (data not shown). To examine whether the N terminus of JUNB could also function as a PMA-inducible transcriptional activator, the corresponding N-terminal amino acids of JUNB were fused to the GAL4 DNA-binding domain. In contrast to the stimulatory effect on the JUN fusion protein, PMA was unable to stimulate transcription from the GAL4-JUNB fusion (Fig. 4*B*).

The relative levels of the GAL4-JUN fusion proteins were measured to determine whether differential expression or stability of the fusion proteins may account for the differences in transcriptional activity. Due to the low level of

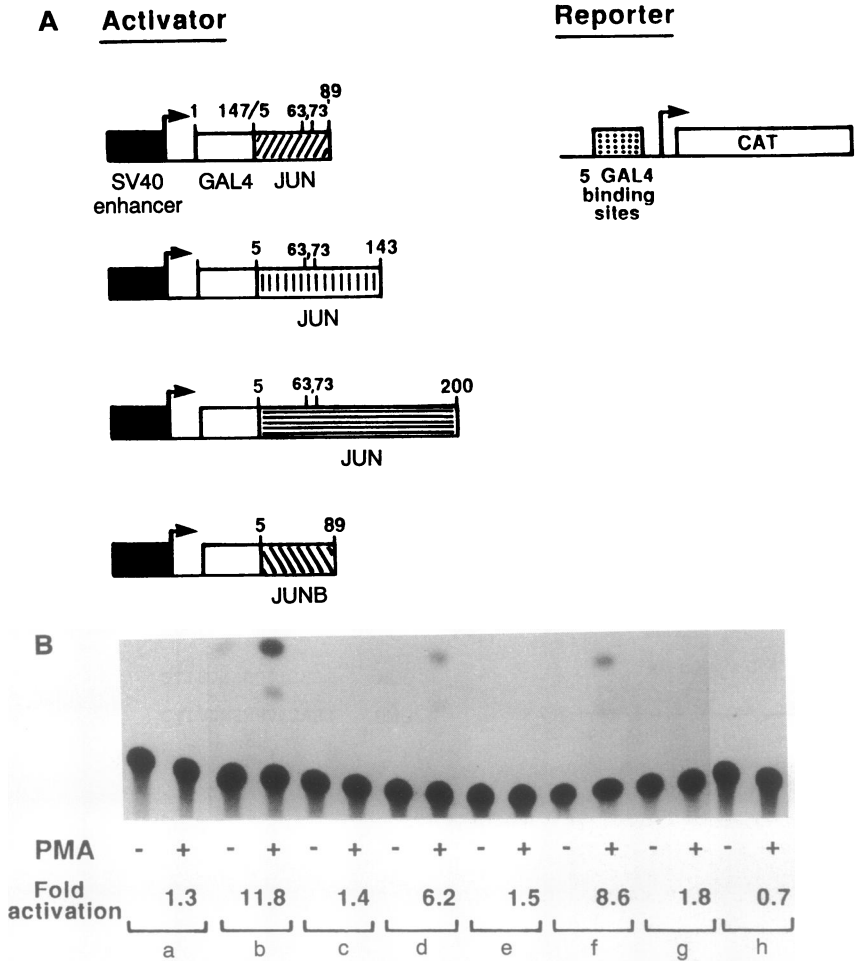

FIG. 4. The N terminus of JUN but not JUNB contains a PMA-inducible transcriptional activation domain. (*A*) Diagram demonstrating the GAL4-JUN fusion constructs and the reporter gene used to measure *in vivo* transcriptional activity. Mutations affecting Ser-63 → Leu and Ser-73 → Leu changes were introduced in GAL4-JUN fusion cDNA constructs encoding amino acids 5–89, 5–143, or 5–200 of JUN. The GAL4-JUNB fusion protein contains the N-terminal residues of JUNB that correspond to amino acids 5–89 of JUN. (*B*) U-937 cells were cotransfected with 20 μg of GAL4-JUN expression vector, 5 μg of G_5CAT reporter plasmid, and 5 μg of a β-galactosidase construct. The cells were split after 24 hr, and half (lanes +) were treated with 0.1 μM PMA for 24 hr. The cells were lysed, and CAT activity was determined as described (2). Lanes: a, pSG424 lacking JUN cDNA; b–h, vector containing inserts encoding JUN-(5–89) (lanes b), [$Leu^{63,73}$]JUN-(5–89) (lanes c), JUN-(5–143) (lanes d), [$Leu^{63,73}$]JUN-(5–143) (lanes e), JUN-(5–200) (lanes f), [$Leu^{63,73}$]JUN-(5–200) (lanes g), and JUNB-(5–89) (lanes h). The fold-activation values shown are averages of at least two independent experiments and represent the fold increase in CAT activity over that in untreated cells. Transfection efficiency was found to be similar with all constructs as determined by β-galactosidase activity. SV40, simian virus 40.

expression of the fusion constructs in U-937 cells, expression was measured in human Hep G2 cells, which exhibit a similar pattern of PMA-inducible JUN phosphorylation (ref. 24 and data not shown). Hep G2 cells were transfected with the various GAL4-JUN cDNA constructs, and the products were labeled with Tran^{35}S-label. Immunoprecipitation of the fusion proteins with anti-GAL4 antisera demonstrated comparable levels of expression (data not shown). These data indicate that the lack of inducible transcriptional activity described for the double-mutant GAL4-JUN and the GAL4-JUNB constructs cannot be attributed to low protein expression or protein instability.

DISCUSSION

We have demonstrated that PMA treatment of U-937 cells induces the phosphorylation of JUN but not JUNB. Mutation of Ser-63 and -73 abolishes both the constitutive and PMA-inducible N-terminal phosphorylation of JUN *in vivo*. Identical mutations also inhibit the PMA-inducible JUN transcriptional activity of several GAL4-JUN constructs spanning various lengths of the JUN N terminus. These findings provide strong evidence that the site-specific phosphorylation of JUN at Ser-63 and -73 positively regulates JUN transcriptional activity. These transcriptional studies corroborate and extend recent results obtained in mouse F9 cells, in which similar mutations abolished the constitutive JUN trans-activation of a CAT reporter gene containing multiple upstream AP-1 sites (24). Thus, Ser-63 and -73 are necessary for both the constitutive and PMA-inducible transcriptional activity of mammalian c-Jun.

We have recently shown that an N-terminal JUN protein kinase isolated from U-937 cells copurifies with mitogen-activated protein (MAP) kinase pp42 over three columns and a 5000-fold purification (B. J. Pulverer, K. Hughes, C.C.F., A.S.K., S. Leevers, and J.R.W., unpublished data). *In vitro* analyses also indicate that both pp42/44 and pp54 MAP kinases phosphorylate JUN on Ser-63 and -73 (24). While certain regions of JUN and JUNB exhibit a high degree of amino acid homology, the N termini of JUN and JUNB are only 50% identical (25). In JUN but not JUNB Ser-63 and -73 are followed by proline residues (Fig. 3*C*), which are part of the MAP kinase phosphorylation motif (26). These sequence variations within the N terminus of JUN and JUNB may dictate their ability to be inducibly phosphorylated by phorbol esters. This differential phosphorylation may account for the activation of JUN but not JUNB in response to both PMA (Fig. 4*B*) and HRAS (18).

Although the transcriptionally active domains of JUNB have not been mapped, JUN contains a transcriptional repressor domain δ (amino acids 40–66) and two transcriptional activation domains A1 (amino acids 90–186) and A2 (amino acids 238–257) (27–29). Deletion of the δ domain enhances the transcriptional activity of the A1 and A2 activation domains both *in vivo* and *in vitro*, indicating that the δ domain negatively regulates the A1 and A2 domains (27–30). Our results, however, show that amino acids 5–89 of JUN alone are sufficient to activate transcription *in vivo*. These findings indicate the presence of an N-terminal activation domain (A3) within the regulatory domain of JUN. The N-terminal phosphorylation of JUN induced by PMA and HRAS (18) may relieve some latent inhibitory function associated with this regulatory region. Both SRC and RAS have been shown to relieve such an inhibitory activity in certain cell types, although these studies did not address the issue of JUN phosphorylation (30). Phosphorylation could either cause a release of a putative inhibitory factor from the δ domain or induce a change in the tertiary structure of the N terminus, allowing for the activation of transcription. Amphipathic α-helices are important motifs in proteins that regulate transcription (31). However, analysis of amino acids 5–89 of JUN in either the phosphorylated or dephosphorylated form does not suggest such a structure. Phosphorylation may impart a net negative charge to the N terminus of JUN, creating an acidic activation domain similar to that described for other transcriptional activating proteins, such as GCN4, GAL4, and VP16 (31). PMA (13) and HRAS (18) also stimulate the dephosphorylation of sites proximal to the C-terminal DNA-binding domain of JUN, which positively regulates JUN/AP-1 binding (13). Thus, the induction of JUN transcriptional activity *in vivo* during the differentiation of leukemic cells and transformation of fibroblasts involves the coordinate regulation of at least two distinct phosphorylation events.

We thank R. Bravo for providing the JUNB and JUND antisera, M. Ptashne for the GAL4 constructs, D. Nathans for the JUNB plasmid, and I. Sadowski for the GAL4 antisera. This work was supported by National Cancer Institute Grant CA42533 to A.S.K. C.C.F. is supported by Postdoctoral Training Grant CA09128 from the National Cancer Institute, and V.S. is supported by Predoctoral Training Grant RCD 9054714 from the National Science Foundation.

1. Harris, P. & Ralph, P. (1985) *J. Leukocyte Biol.* **37**, 407–422.
2. William, F., Wagner, F., Karin, M. & Kraft, A. S. (1990) *J. Biol. Chem.* **265**, 18166–18171.
3. Datta, R., Sherman, M. L., Stone, R. M. & Kufe, D. (1991) *Cell Growth Differ.* **2**, 43–49.
4. Mitchell, R. L., Zokas, L., Schreiber, R. D. & Verma, I. M. (1985) *Cell* **40**, 209–217.
5. Bohmann, D., Bos, T. J., Admon, A., Nishimura, T., Vogt, P. K. & Tjian, R. (1987) *Science* **238**, 1386–1392.
6. Angel, P., Allegretto, E. A., Okino, S. T., Hattori, K., Boyle, W. J., Hunter, T. & Karin, M. (1988) *Nature (London)* **332**, 166–171.
7. Rauscher, F. J., III, Cohen, D. R., Curran, T., Bos, T. J., Vogt, P. K., Bohmann, D., Tjian, R. & Franza, B. R., Jr. (1988) *Science* **240**, 1010–1016.
8. Nakabeppu, Y., Ryder, K. & Nathans, D. (1988) *Cell* **55**, 907–915.
9. Chiu, R., Angel, P. & Karin, M. (1989) *Cell* **59**, 979–986.
10. Schutte, J., Viallet, J., Nau, M., Segal, S., Fedorko, J. & Minna, J. (1989) *Cell* **59**, 987–997.
11. Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Rahmsdorf, H. J., Jonat, C., Herrlich, P. & Karin, M. (1987) *Cell* **49**, 729–739.
12. Lamph, W. W., Wamsley, P., Sassone-Corsi, P. & Verma, I. M. (1988) *Nature (London)* **334**, 629–631.
13. Boyle, W. J., Smeal, T., Defize, L. H. K., Angel, P., Woodgett, J. R., Karin, M. & Hunter, T. (1991) *Cell* **64**, 573–584.
14. Angel, P., Hattori, K., Smeal, T. & Karin, M. (1988) *Cell* **55**, 875–885.
15. Yamamoto, K. K., Gonzalez, G. A., Biggs, W. H., III, & Montminy, M. R. (1988) *Nature (London)* **334**, 494–498.
16. Gonzalez, G. A. & Montminy, M. R. (1989) *Cell* **59**, 675–680.
17. Luscher, B., Christenson, E., Litchfield, D. W., Krebs, E. G. & Eisenman, R. N. (1990) *Nature (London)* **344**, 517–522.
18. Binetruy, B., Smeal, T. & Karin, M. (1991) *Nature (London)* **351**, 122–127.
19. Adunyah, S. E., Unlap, T. M., Wagner, F. & Kraft, A. S. (1991) *J. Biol. Chem.* **266**, 5670–5675.
20. Kovary, K. & Bravo, R. (1991) *Mol. Cell. Biol.* **11**, 2451–2459.
21. Sadowski, I. & Ptashne, M. (1989) *Nucleic Acids Res.* **17**, 7539.
22. Stone, R. M., Sariban, E., Pettit, G. R. & Kufe, D. W. (1988) *Blood* **72**, 208–213.
23. Thevenin, C., Kim, S.-J. & Kehrl, J. H. (1991) *J. Biol. Chem.* **266**, 9363–9366.
24. Pulverer, B. J., Kyriakis, J. M., Avruch, J., Nikolakaki, E. & Woodgett, J. R. (1991) *Nature (London)* **353**, 670–674.
25. Ryder, K., Lau, L. F. & Nathans, D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1487–1491.
26. Erickson, A. K., Payne, D. M., Martino, P. A., Rossomando, A. J., Shabanowitz, J., Weber, M. J., Hunt, D. F. & Sturgill, T. W. (1990) *J. Biol. Chem.* **265**, 19728–19735.
27. Baichwal, V. R. & Tjian, R. (1990) *Cell* **63**, 815–825.
28. Bohmann, D. & Tjian, R. (1989) *Cell* **59**, 709–717.
29. Abate, C., Luk, D. & Curran, T. (1991) *Mol. Cell. Biol.* **11**, 3624–3632.
30. Baichwal, V. R., Park, A. & Tjian, R. (1991) *Nature (London)* **352**, 165–168.
31. Ptashne, M. (1988) *Nature (London)* **335**, 683–689.