Nucleic Acids Research, Vol. 20, No. 4 897–902

Upstream regions of the c-*jun* promoter regulate phorbol ester-induced transcription in U937 leukemic cells

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Received August 15, 1991; Revised and Accepted January 6, 1992 EMBL accession no. X59744

ABSTRACT

To understand the mechanism by which phorbol esters (PMA) stimulate c-*jun* transcription in human leukemic cell line U937, we have mutated specific enhancer sequences within the c-*jun* promoter. We find in the region of DNA from −132 to +170 containing Sp1, C-TF and AP-1 sequences that mutation of the AP-1 sequence alone is not sufficient to abrogate transcription, and mutation of the Sp1 sequence increases transcription 4-fold. Although mutation of the CTF site had no effect, CTF and AP-1 mutations together totally abrogate PMA-induced transcription. In comparison mutations of either of these sites alone or together in a construct containing −1639/+740 of the c-*jun* promoter had no effect on transcription. Because this data suggested the possibility of other upstream control regions, we sequenced the promoter from −142 to −1639. This sequence demonstrates a greater than 70% homology between human, and mouse c-*jun* promoters for the region from −142 to −441, and a second AP-1-like site in the −183 to −192 region. Mutation of this site did not influence transcription by PMA. By making constructs containing varying portions of the promoter, we have identified the region between −142 and −711 to be responsible for mediating PMA-induced c-*jun* transcription.

INTRODUCTION

The addition of phorbol esters to U937 human leukemic cells induces the differentiation of these cells towards macrophages (1–3). Initial events in the induction of differentiation by phorbol esters include activation of protein kinase C (4), stimulation of phosphorylation of specific membrane and cytosolic proteins (5–7), and translocation of this enzyme to the membrane (8). These events are followed by induction of specific mRNAs and proteins, such as lipocortin 1 (9) and the cell cycle protein kinase $p58^{GTA}$ (10), repression of mRNA synthesis, for example, c-*myc* and c-*myb* (11–13) and an inhibition of DNA synthesis. Within 24–48 hours after addition of PMA the cells have stopped dividing, are adherent to plastic, and are capable of undergoing a macrophage-like oxidative burst in response to stimulants such as the f-met-leu-phe peptide (3). It remains unclear how activation of protein kinase C mediates this complex chain of events. To unravel the earliest events in this differentiation pathway, we have focused on the steps by which stimulation of protein kinase C leads to increases in transcription of the early response gene, c-*jun*. The c-*jun* protooncogene has been shown to encode a leucine zipper containing protein which forms homodimers or binds the product of the c-*fos* gene to yield a heterodimer (14–17). This heterodimer is then capable of binding to AP-1 DNA enhancer sequences (5′TGACTCA3′) (16–19) and stimulating transcription of genes located downstream from these sites (20).

Previously, we have demonstrated that the control of AP-1 enhancer activity in U937 cells is complex (21). We have demonstrated that, as in fibroblast models, phorbol esters or granulocyte–macrophage colony stimulating factor (22), both of which activate protein kinase C in U937 cells, stimulate increases in transcription of c-*jun* and c-*fos* mRNA, followed by increases in both proteins. These increases lead to activation of gene transcription mediated by AP-1 enhancer sequences. In contrast to these results obtained with PMA, the addition of either calcium ionophore, ionomycin, or a single dose of diacylglycerol to these cells stimulated an increase in c-*jun* and c-*fos* mRNA but little change in c-*jun* or c-*fos* protein or AP-1 enhancer activity. Multiple doses of diacylglycerol given every two hours stimulated repeated increases in c-*jun* mRNA, eventual increases in Jun protein, and activation of AP-1 enhancer sequences. These data suggest a complex transcriptional and translational control of Jun protein levels and activity and that stimulation of c-*jun* transcription can occur in the absence of activation of the AP-1 enhancer sequence.

In an examination of the c-*jun* promoter, Angel *et al.* identified Sp1, CTF, and AP-1 binding sites within the region from −130/+170 (23). This c-*jun* AP-1 site (5′TGACATCAT3′) differs from the collagenase AP-1 binding site (5′TGAGTCAT3′) containing an additional base. Mutation of this c-*jun* demonstrates that this AP-1 site plays a regulatory role in response to both cotransfected c-*jun* and induction by PMA (23).

To examine further the regulation of c-*jun* transcription by phorbol esters we have transfected U937 cells with plasmids

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containing the −132/+170 or −1639/+740 regions of the c-*jun* promoter upstream from a chloramphenicol acetyl transferase (CAT) recorder gene. Mutations were made in the Sp1, CTF, or AP-1 sequences to evaluate the role of each site within either the small or large region of the c-*jun* promoter. We find that in the segment −132/+170, mutation of the AP-1 sequence only partially decreases transcription induced by phorbol esters, C-TF mutation has no effect, and mutation or deletion of the Sp1 site markedly increases transcription. Double mutations demonstrate that combining the CTF and AP-1 mutations completely abrogates transcription and that the CTF mutation markedly diminishes the stimulatory effect of the Sp1 mutation. In the −1639/+740 segment, however, mutation of these regions had little effect on transcription. To further investigate the control of transcription, we sequenced the upstream region of the c-*jun* promoter to −1639 and made CAT constructs spanning a region from −1639/−142. We have identified a segment from −711/−142 which plays an important role in regulating c-*jun* transcription. A second AP-1-like site has been identified from −183/−191, and mutation of this site did not affect transcription induced by PMA. These data suggest that other regions in addition to the −191/−183 upstream (uAP-1) and −63/−72 downstream AP-1 (dAP-1) sites mediate c-*jun* transcription induced by PMA.

MATERIALS AND METHODS

Cell culture — U937 human monocytic cells were obtained from the American Type Tissue Culture bank and were maintained in Dulbecco's Modified Eagle's medium (DMEM) with 10% iron-supplemented calf serum and 50 U/ml of penicillin and streptomycin in a 5% CO_2 atmosphere. The constructs −1639/+740 c-*jun* in pBLCAT3 (23) and −132/+170 c-*jun* in pBLCAT3 (23) were a kind gift of Dr M.Karin, University of California San Diego, San Diego, CA.

DNA transfection and CAT assays — U937 cells were transfected by the DEAE−dextran procedure (24). Cells were washed twice in cold Dulbecco's PBS and once in isotonic Tris-HCl, pH 7.8. For each transfection, 4 μg of plasmid was mixed with 250 μg of DEAE−dextran in a total volume of 500 μl. Then 1×10^7 washed cells were incubated in this mixture for 30 min at 37°C, pelleted and washed with DMEM plus 10% calf serum containing 5 units/ml of heparin and washed again without heparin. The cell pellet was resuspended in 10 ml of DMEM plus serum and incubated for 12 hr. Cells were then treated with 100 nM PMA for 24 hr, lysed, and CAT assays were performed as previously described (21).

Gel mobility shift assay — The gel mobility shift assay was performed as previously described (22). One picomole of each double stranded probe (collagenase (col) AP-1 5′CTAGTGATG-AGTCAGCCG-3′, uAP-1 5′-AGCGGAGCATTACC-TCATC-3′, CTF 5′-ACGCGAGCCAATGGGAAG-3′ or dAP-1 5′-GATCTTGGGGTGACATCATGG-3′) was labelled with reverse transcriptase at 37°C for 30 min in a 50 μl reaction mixture containing 50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 5 mM DTT, 50 mM KCl, 10 μM each of dATP, dGTP, and dTTP, 50 μg BSA, 100 μCi (α^{32}P)-dCTP, and 40 units reverse transcriptase. Free probe was removed by centrifugation at 1000 rpm for 45 sec on a Sephadex G-50 column. Cold competitors were either CTF, col AP-1, uAP-1, or dAP-1. Binding was carried out in a 20 μl reaction mixture containing 20 mM Hepes, pH 7.9, 50 mM KCl, 1 mM $MgCl_2$, 0.5 mM DTT, 4% Ficoll, 2 μg poly dI/dC, 5 μg protein extract, and 5000 cpm of labelled oligonucleotide. Antibodies were incubated with the binding reaction for 2 hr at 4°C prior to the addition of labelled probe. The reaction mixture was electrophoresed on a 6% non-denaturing polyacrylamide gel in 0.25×TBE for 3 h at 150 V. The gel was vacuum dried and exposed to XAR-5 film.

Sequencing the c-jun promoter and site-directed mutagenesis — To construct the −132/+170 mutant c-*jun*-CAT in which the binding sites for dAP-1, CTF, or Sp1 are mutated, the region from −132 to +170 was inserted between the HindIII and PstI sites of M13mp18 and single-stranded (SS) DNA was prepared. Mutant oligonucleotides (mdAP-1 5′-ATAGCCCATGGTGGAT-CCCCAAGGCCTTC-3′, mCTF 5′-GGCCTTCCTATCGATT-CGCGTCGCT-3′, mSp1 5′-GTCGCTCTCAGGTCTAGAAT-TCTAGACGTCACCATGGA-3′) were annealed to SS DNA and mutagenesis was carried out as outlined by the manufacturer (Amersham, Arlington Heights, IL). Site-specific mutagenesis was confirmed by dideoxy DNA sequencing. Double mutations were constructed, using M13mp18 already containing the single mutants. Mutation of dAP-1, CTF, and Sp1 sites in the −1639 to +740 region was accomplished by cloning this region of DNA into the HindIII/XbaI sites in the pSelect vector and using the Altered Sites in Vitro Mutagenesis Kit (Promega) with the oligomers as noted above. This kit was also used to create the uAP-1 mutation by cloning the HindIII/BamHI −441/−142 region of pCJun 7 into the pSelect vector and using the oligomer muAP-1 (5′CTCACGGGAATTCCATATGCTCCGCTG-3′) to create the mutation. All mutated clones were selected by direct dideoxy sequencing.

Sequencing of the upstream region of the c-*jun* promoter from −1639 to −142 was accomplished by digesting the sequence −1639/+740 in pBLCAT3 (23) with NcoI at −142. This site was then blunt-ended, and the −1639/−142 region was removed with HindIII. This region was cloned into M13mp18 at the HindIII/PstI sites where the PstI site had been blunt ended. The non-coding strand was sequenced using the dideoxy method as outlined in the Sequenase Kit (US Biochem.). The coding strand was then sequenced in the opposite direction in −1639/+740 pBLCAT3 plasmid.

CAT constructs from the upstream c-jun promoter — The −1639/−142 pBLCAT2 was constructed by cutting −1639/+740 pBLCAT3 with NcoI. The ends were blunt-ended, and the fragment was removed by digestion with HindIII. To ligate this fragment into pBLCAT2, the XbaI site was blunt-ended and the vector was cut with HindIII. The 1.5 kb fragment was then ligated. To construct pCJ1, the BamHI fragment of pBLCAT2 −1639/−142 was cloned into the BamHI site of pTATACAT (29). To facilitate cloning of different regions of the promoter into the HindIII/BamHI sites of the pTATACAT vector, PCR was done using 5′ primers containing HindIII site and 3′ primers with a BamHI site. pCJ2 was made by using primers 2 (5′) and 7 reverse (R) (3′); pCJ3 by using primers 3 (5′) and 7R (3′); pCJ4 by using primers 3 (5′) and 6R (3′); pCJ5 by using primers 3 (5′) and 4R (3′); pCJ6 by using primers 4 (5′) and −7(3′); pCJ7 by using primers 5 (5′) and 7R (3′); pCJ8 by using primers 5 (5′) and 6R (3′); and pCJ9 by using primers 6 (5′) and 7R (3′). The primers spanned the following coding sequences: 2 −1214/−1195, 3 −973/−952, 4

−711/−687 4R −690/−713, 5 −441/−424, 6 −241/−224 6R −224/−241, and 7R −142/−163, respectively. To rule out the creation of mutations by PCR, the plasmids were sequenced using the Sequenase Kit (U.S. Biochemicals).

RESULTS

The role of the AP-1, CTF, and Sp1 sites on TPA-induced cJun promoter activity

The segment of the c-*jun* promoter from −132/+170 has been shown to contain only three sequences, AP-1, Sp1, and CTF, which are capable of binding proteins and regulating transcription. To determine the potential role of these three enhancer binding sequences in controlling c-*jun* transcription, we have mutated each site individually (Fig. 1) or in pairs in a short segment of the c-*jun* promoter −132/+170 and cloned these mutants into a plasmid containing a CAT recorder gene, pBLCAT3 (23). Gel mobility shift studies were carried out using control or mutant oligomers (see Methods) to examine whether these mutations blocked binding of protein factors to these sequences. In each case, mutation of these sequences inhibited normal binding of these three factors (data not shown). The plasmids containing the mutations were transfected into U937 cells and CAT activity determined. The presence of the AP-1 mutation decreased PMA-induced transcription from this promoter sequence to 75% of control activity, while the CTF-1 mutation had little effect. Mutating the Sp1 site gave a 4.2-fold increase in CAT activity stimulated by PMA, suggesting that the binding of this factor represses transcription in this short segment of the c-*jun* promoter. Mutating both the CTF and AP-1 sites almost completely abrogated transcription, decreasing CAT activity to 10% of control (Fig. 1). The presence of a CTF mutation in combination with the Sp1 mutation markedly decreased the stimulation of transcription seen with the Sp1 mutation alone.

To examine whether treatment of U937 cells with phorbol esters increased the level of CTF protein capable of binding to DNA, gel mobility shifts were done using labelled CTF or AP-1 oligomers. U937 cells were treated with PMA for 30 min, 1 hr, 3 hrs, 6 hrs, and 24 hrs and nuclear extracts were made from each sample. Treatment with PMA increased the level of protein binding to the col AP-1 sequence. This binding was inhibited by 100× unlabelled collagenase (col) AP-1 but not by unlabelled CTF. In comparison, protein binding to the CTF oligomer, present at time zero and competed by 100-fold excess of unlabelled CTF oligomer but not by unlabelled dAP-1, did not increase with PMA treatment. Previously, we have found that PMA treatment of U937 cells does not increase the amount of protein capable of binding to Sp1 sites (22). These findings suggests that PMA treatment of U937 cells does not increase the amount of CTF or Sp1 binding protein in these cells.

To examine the role of these three protein-binding sequences in the regulation of the c-*jun* transcription, we mutated each of these sequences in a larger portion of the c-*jun* promoter, −1639/+740. Unlike the effect of these mutations in the −132/+170 construct, mutation of the AP-1, CTF or CTF and AP-1 sites had little effect on CAT recorder gene activity (Fig. 1). This finding suggests that sequences in other regions of the promoter might play an important role in regulating transcription of c-*jun* in response to PMA. To evaluate this possibility, the

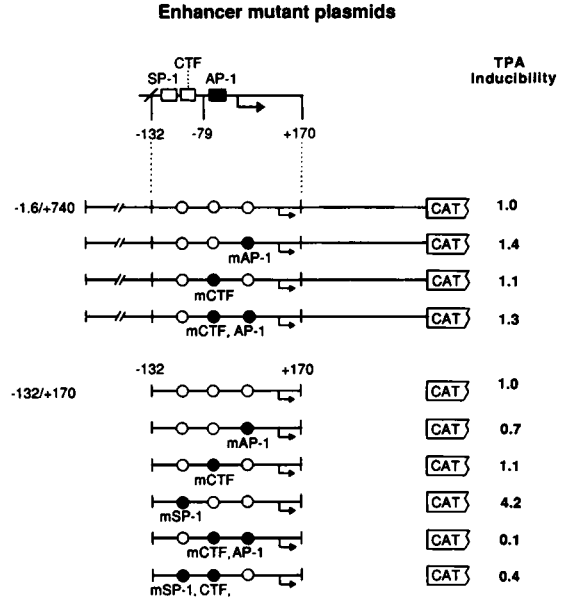

Figure 1. Quantitation of PMA-induced enhancer activity in −132/+170 and −1639/+740 mutants. Three separate experiments were done as described in Methods. Silica gel plates were scraped and unacetylated and acetylated derivatives quantitated by scintillation counting. The% acetylation for PMA-treated cells was quantitated by dividing the acetylated derivatives by the total radioactivity. TPA inducibility for each plasmid was then calculated by dividing the% acetylation induced by PMA by the untreated value. The TPA inducibility shown is the result of dividing the value obtained in the mutant plasmid by that determined for the control unmutated plasmid. The value shown is the average of experiments done in triplicate.

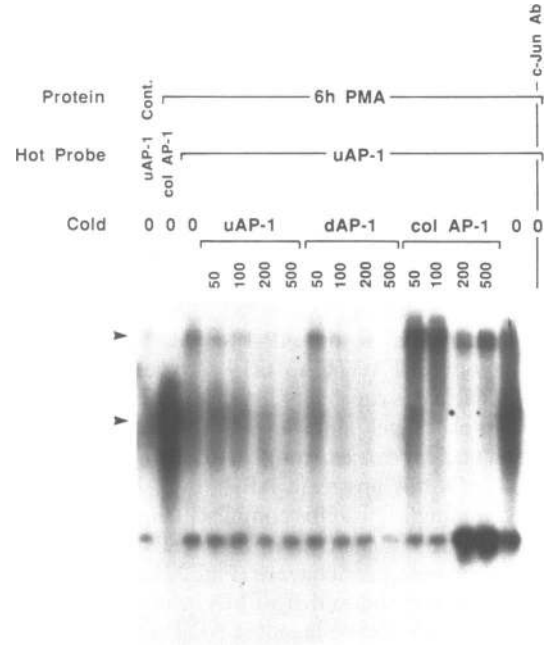

Figure 2. The upstream AP-1 binds two protein complexes in the U937 nuclear extract. Nuclear extracts were made from control cells or cells treated for 6 hrs with PMA. The protein extracts were incubated with either labelled uAP-1 or col AP-1 probe. This labelled probe was competed with excess uAP-1, dAP-1 or col AP-1. c-Jun antibody (Ab) was added 2 hrs prior to adding the radioactive probe.

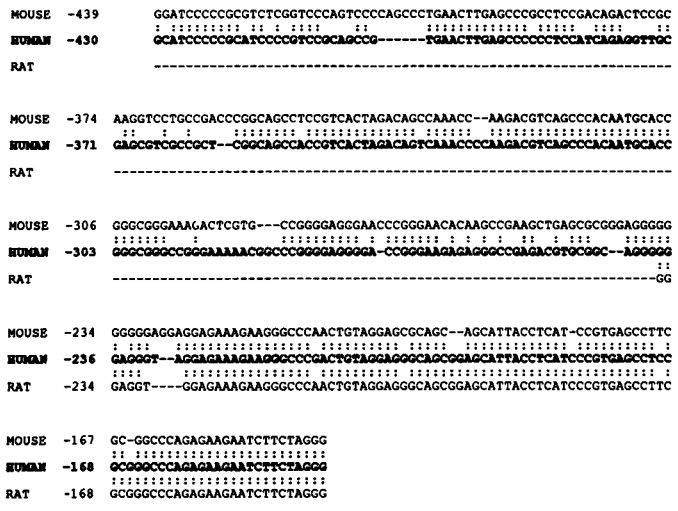

Figure 3. Alignment of the human, mouse and rat upstream c-*jun* promoter.

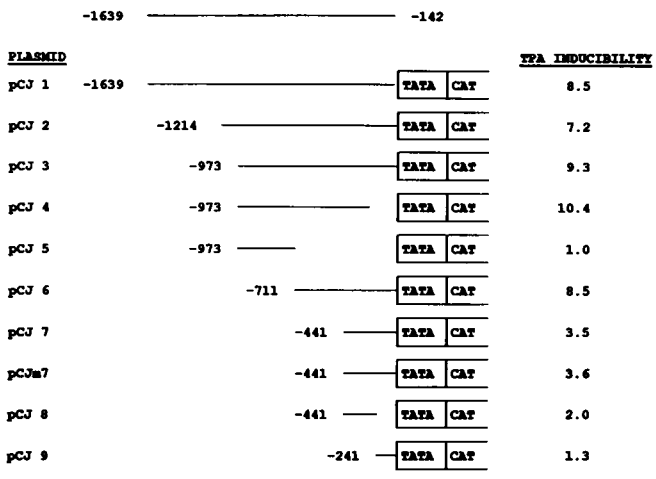

Figure 4. Quantitation of PMA-induced activity in the region from −1639/−142. Silica gel plates representing triplicate experiments done as in Fig. 1 were scraped, counted, and the% acetylation calculated by dividing the cpm in acetylated derivatives by the total cpm. TPA inducibility was calculated by dividing the PMA-induced% acetylation for each construct by the control (untreated) value for the same construct. The value shown is the average of triplicate determinations.

−1639/+740 construct was cleaved at −142, removing the enhancer binding sites between −132 and +170 and the TATA box, and cloned into pBLCAT2 which contains a minimal thymidine kinase promoter. Transfection of this plasmid into U937 cells and treatment with PMA demonstrated that the upstream region from −1639 to −142 enhanced transcription over 2-fold when compared to control (data not shown). However, the plasmid containing the thymidine kinase promoter alone was also markedly stimulated by PMA. These data suggest that the upstream region of the c-*jun* promoter, which lacked the −63/−72 AP-1 (downstream AP-1, dAP-1) binding site, also contains important control regions.

Sequence analysis of −1639/−142 demonstrates a second AP-1-like site and strong similarity to mouse and rat c-*jun* promoters

To examine the upstream region for additional PMA regulatory elements, it was necessary to sequence the region of c-*jun* from −1639 to −142. This was accomplished by cloning this segment into M13mp18 and sequencing it in both directions. Scanning of this sequence for possible control sequences demonstrates a second AP-1-like element from −183 to −191 (5′TTACCTC-A3′), which is similar to the AP-1 sequence identified in the SV-40 promoter (5′TTAGTCA3′) (19). To demonstrate that this SV-40-like sequence is capable of binding to Jun/Fos heterodimer protein complexes, gel shift analysis was done, using this sequence and nuclear extract from control or PMA-treated cells. In untreated cells this upstream AP-1 (uAP-1) (5′TTACCTC-A3′) binds a single protein complex (Fig. 2 lane 1). In comparison, extracts of nuclei from U937 cells treated with PMA bind two complexes to this sequence (Fig. 2, lane 3). This second complex, which was induced by PMA, ran identically with a complex which bound the collagenase AP-1 (col AP-1) sequence (5′TGAGTCA3′) (Fig. 2, lane 2). Increasing amounts of unlabelled competitor uAP-1 markedly decreased the upper band but had a lesser effect on the lower band (Fig. 2). Excess unlabelled downstream AP-1 sequence (dAP-1) (5′TGACATC-A3′) competed the upper and lower complex with equal efficiency. The col AP-1 sequence inhibited binding to the lower band but, even at 500-fold excess cold competitor, was not able to inhibit the upper band formation. Preincubation with an antibody to cJun blocked binding formation of both bands (Fig. 4 last lane). Preincubation with antibodies to Jun B or Jun D did not inhibit either band, and antibodies to c-Fos protein only abrogated the lower band (data not shown). These data demonstrate that this uAP-1 sequence binds Jun and that binding to this upstream site is inducible by PMA. This upstream site may have a different affinity for various heterodimers i.e Jun/cyclic AMP response element binding protein (25, 26), distinct from the collagenase AP-1 site.

Comparison of the human c-*jun* sequence to the mouse (27) and rat (28) demonstrates a high degree of homology (Fig. 3). Experiments with the murine c-*jun* sequence promoter has led to the identification of three retinoic acid response elements (27). Two of these three sequences 5′GAGCCCcCCTCCatCAG3′ and 5′AAGACGTCAGCCCACAATGCACCGGGCGGGCCGG-GAA3′, in the region between −390 and −290 are almost completely conserved between human and mouse.

Transcriptional activation by PMA is mediated by elements within the region −711/−142

To examine the specific regions within the sequence −1639/−142 which mediate transcriptional activation by PMA, nine plasmids were constructed, spanning various regions of the promoter in a vector containing a TATA box and the CAT recorder gene (29) (Fig. 4). Unlike the vector used earlier (pBLCAT2) in these studies which contains a minimal thymidine kinase promoter, this vector was not transcribed in untreated cells and did not respond to PMA. Although there were small differences in CAT activity among plasmids made from the −1639 and −711 region, the −1639/−142 and −711/−142 constructs both gave approximately an 8.5-fold increase in CAT activity when the cells were treated with PMA (compare pCJ1 to pCJ6) (Figs 4 and 5). Shortening this region to −441/−142 (pCJ7) decreased by 2-fold CAT activity with only minimal

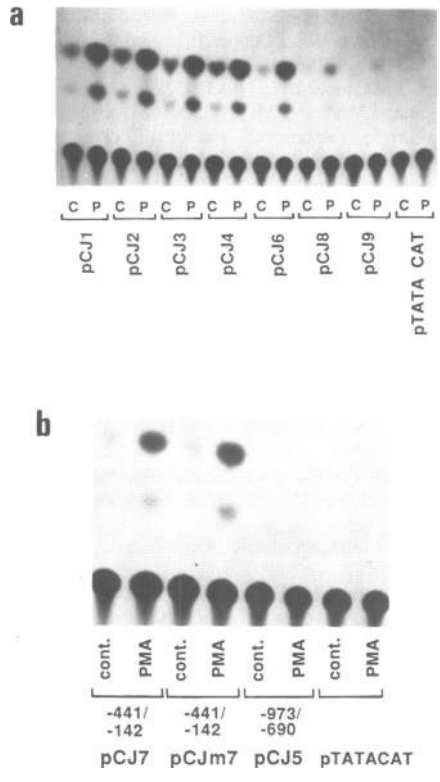

Figure 5. a) The effect of varying portions of the −1639/−142 c-*jun* promoter region on transcription induced by PMA. U937 cells were transfected with plasmids encoding varying portions of the c-*jun* upstream region or the plasmid backbone (pTATA CAT). The cells were divided into two groups and one remained untreated (control) while the other was treated with PMA (P). The CAT shown is representative of this experiment performed in triplicate. **b)** Mutation of the uAP-1 site did not alter PMA induction of the −441/−142 c-*jun* fragment The pTATA-CAT, pCJ7 or pCJm7, containing the uAP-1 mutation (see Methods), was transfected into cells as described in Fig. 1. The cells were divided, and one half remained untreated (Cont.) while the other half was treated with PMA (0.1 μM).

activity observed when the construct was shortened to −241/−142 (pCJ9) (Figs 4 and 5). Cloning of the region from −711/−441 yielded a construct with approximately half the activity of the −711/−142 construct (data not shown). These data suggest that the regulatory regions responding to PMA are located between −711 and −142 both within the segment −711 to −441 and −441 to −142. To examine this observation further a plasmid was constructed containing the region from −973 to −711 (pCJ5). This region, as predicted, was not responsive to PMA (Fig. 4b, lanes 5 and 6).

It has been suggested (23) that AP-1 binding sites may play a role in regulating the transcription of c-*jun* and that it is possible that the uAP-1 site contributes to activation within the plasmid −441/−142. To address this possibility, we mutated this uAP-1 site from 5′TTACCTCA3′ to 5′-TATGGAAT3′. Gel shift analysis using the mutated oligomers showed that the mutated sequence was incapable of binding either complex 1 or 2 (data not shown). The mutated (pCJm7) and control plasmids (pCJ7) containing sequences from −441/−142 were transfected separately into U937 cells with or without PMA treatment, and CAT activity was determined. The mutation of this uAP-1 sequence (Fig. 5, pCJm7) had no effect on enhancer activity in response to PMA.

DISCUSSION

Previous work has suggested that the upstream region of the c-*jun* promoter from −132 to +170 contains important regulatory elements which control PMA-induced transcription of the c-*jun* gene (23). DNase I footprinting of this region demonstrated that there are three protected regions, an Sp1-like motif, a CTF binding region, and an AP-1 binding sequence. Because this short sequence contains only three major regions which bind protein factors, it is possible to examine the role of each of these in mediating PMA-induced transcription. As suggested previously, we have found that the dAP-1 sequence is stimulated to enhance transcription by PMA. This is demonstrated by the observation that mutation of this site alone decreases transcription, and mutation of both the CTF and Sp1 binding sequences, leaving the AP-1 site intact, does not fully abrogate stimulation by PMA. Previously, it has been demonstrated that deletion of the sequence containing both the Sp1 and CTF sites from −132 to −80 increases PMA-induced transcription from the remaining AP-1 binding site (23). Here it is demonstrated that mutation of the Sp1 site itself allows a greater response to PMA, suggesting that the protein(s) binding to this site represses transcription. Sp1 binds to GC boxes (GGGCGG) and stimulates transcription in a wide variety of mammalian cell types *in vivo* and large number of promoters *in vitro* (30, 31). However, it appears that there is a family of proteins that binds GC boxes, some of which may be repressors (32). The recently cloned ETF protein binds to GC-rich sequences in the epidermal growth factor receptor, β-actin, and calcium-dependent protease promoter repressing their expression (33). Our data suggest that the GC box in the c-*jun* sequence is occupied by a negative repressor of transcription, since either mutating or deleting this site (data not shown) enhanced PMA-induced c-*jun* expression.

Mutation of the CTF binding site alone did not have any effect on transcription. Mutation of the CTF site in combination with either the AP-1 or Sp1 sites decreased the PMA-induced transcription from this promoter. A family of proteins generated by alternative splicing binds to the CTF/NF-1 sites (34) and initiates transcription of a number of cellular and viral promoters (35) as well as mediating adenovirus replication (36). Gel shift analysis demonstrates that PMA does not increase the amount of DNA binding to this CTF sequence. However, the protein bound to this CTF site appears to enhance transcription stimulated through the AP-1 site.

To examine the role of the CTF and AP-1 sites in the presence of other enhancer elements, we mutated these sites in a construct containing the −1639/+740 region of the c-*jun* promoter. Mutation of these sites, either alone or in combination, had little effect on transcriptional regulation in these long constructs. Similar results have also been reported for a similar construct in HeLa tk^- cells (38) and for the upstream region of the collagenase gene (37). A single AP-1 site in a small region of the collagenase promoter controls PMA-induced transcription. However, in larger constructs this AP-1 sequence is not sufficient to account for PMA-induced transcriptional activation (37). Our data on the c-*jun* promoter suggested that other regions of the c-*jun* promoter may play a role in mediating transcription. To examine these potential sites in more detail, we sequenced the 1.5 kb of the upstream region c-*jun* promoter. This sequence is highly related to that already published for mouse and rat c-*jun* (27, 28), suggesting that the control elements within these three species are also conserved. As predicted, we have found two of

the three retinoic acid response elements which are found in the murine sequence to also be conserved in the human c-*jun*.

Scanning this sequence, we have identified an AP-1 binding site at −183 to −191 (uAP-1) which is related to the AP-1 site previously identified in the SV-40 enhancer. Gel shift analysis demonstrates that this sequence is capable of binding a complex which migrates at the same location as that which is bound by the collagenase AP-1. The binding of this complex is competed by a 50-fold excess of both the dAP-1 sequence (−73/−62) and the col-AP-1 sequence. Also, binding of this complex is abrogated by anti-jun anti-fos (data not shown) antibodies. These data suggest that the uAP-1 is capable of binding the Jun/Fos complex. The uAP-1 also binds a second distinct protein complex in control and PMA-treated cells. This complex runs with a slower mobility than the Jun/Fos complex; it is not bound by the col AP-1 sequence; and binding is inhibited by high concentrations of unlabelled dAP-1 sequence but only minimally inhibited by the col-AP-1 sequence. The formation of this complex is also blocked by anti-jun antibodies. It has been demonstrated that Jun is capable of binding other leucine zipper proteins, including the cyclic AMP response element binding protein (26). Since U937 cells contain endogenous Jun protein, a Jun protein-DNA complex exists in untreated cells which is capable of binding the uAP-1 SV-40-like sequence but not the col AP-1. These data suggest that small changes in the canonical AP-1 sequence may allow for a complex set of additional proteins to bind to these sequences.

By using a set of constructs spanning varying portions of the upstream promoter from −1639/−142 placed in front of a CAT recorder gene, the region from −711 to −142 has been shown to contain DNA sequences which are important for the regulation of PMA-induced c-*jun* transcription. Regulatory regions appear to lie both within the −711/−441 and between −441/−142. Band shift experiments (data not shown) demonstrate that both of these 300 bp sequences are capable of binding transcription factors. Mutation of the uAP-1 in a construct spanning the region from −441 to −142 did not affect transcription, suggesting in the context of this 300 bp fragment that this sequence does not mediate phorbol ester-induced transcription. This region did not contain either an AP-2 or AP-3 (39) consensus sequence, suggesting that another sequence or set of sequences mediates the phorbol ester induction of c-*jun* transcription.

The dAP-1 sequence plays an important role in mediating transcription by phorbol esters in the 300 bp sequence from −132 to +170. As demonstrated, this may occur through activation of Jun homodimers. However, in the context of the larger promoter sequence, both the dAP-1 and the uAP-1 do not appear to play a major role in transcriptional control. This suggests that additional proteins, yet to be identified, play a role in PMA-mediated activation of c-*jun* transcription in human hematopoietic cells.

ACKNOWLEDGEMENTS

We would like to thank Dr M.Karin for supplying the constructs which were necessary to begin this study. Dr Peter Angel was extremely helpful in first pointing out the uAP-1 sequence. This work was support by a PHS grant CA 42533 to A.S.K., and training grant CA09128 to C.C.F. and T.U.

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