Jun family members are controlled by a calcium-regulated, cyclosporin A-sensitive signaling pathway in activated T lymphocytes

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The octamer-binding transcription factor Oct-1 is involved in a wide variety of cellular processes but appears to lack a strong transcriptional activation domain, suggesting that it functions in the context of other proteins. We demonstrated previously that Oct-1, in association with a 40-kD protein, OAP40, contributes to the induction of interleukin-2 (IL-2), an early activation gene and major growth factor for T lymphocytes. Here we report that amino acid sequences obtained from purified OAP40 are identical to regions within JunD and c-Jun. We demonstrate that each of these Jun family members can participate in a complex that includes Oct-1 and a regulatory element in the IL-2 enhancer. In transient transfections, both JunD and c-Jun can contribute to activation-specific transcription mediated by this antigen receptor response element. These studies reveal a role, distinct from AP-1 activity, for Jun family members that is controlled by a calcium-triggered, cyclosporin A-sensitive mechanism.

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Mature T lymphocytes circulate in a quiescent state until they encounter foreign antigen. Presentation of antigen initiates an intracellular signaling cascade that results in a new activated phenotype (Crabtree 1989). Once stimulated, T lymphocytes produce a variety of cytokines that direct the development and proliferation of the T cell as well as other cells participating in the immune response. The production of interleukin-2 (IL-2), one of the first of these cytokines to be synthesized, is thought to reflect the commitment of the T cell to the program of differentiation initiated by antigenic stimulation (Crabtree 1989). Signals initiated by engagement of the antigen receptor are received in the nucleus by the regulatory regions of early activation genes such as the IL-2 gene. The identification and dissection of a minimal enhancer region of ~300 bp located 5' of the IL-2 transcription start site (Durand et al. 1987) led to the discovery of response elements that contribute to activation-specific transcription (Durand et al. 1988). These elements have since been studied to identify the individual transcription factors that are responsible for enhancer function and, in turn, to elucidate the mechanisms that control the activity of these transcription factors.

The homeo domain protein Oct-1, along with a second binding activity, Oct-1-associated protein40 (OAP40), contributes to transcription mediated by an antigen receptor response element (ARRE-1) in the IL-2 enhancer (Ullman et al. 1991). This context of a tissue- and activation-specific enhancer contrasted with previous descriptions of Oct-1 as a regulator of housekeeping genes; however Oct-1 has been shown to contribute to inducible transcription in at least two other cases. The cell-cycle modulation of histone H2b transcription and the trans-activation of herpes simplex virus (HSV) immediate early genes are both dependent on Oct-1 (Fletcher et al. 1987; Gerster and Roeder 1988; LaBella et al. 1988). The phosphorylation state of Oct-1 is thought to regulate inducible activity in the former of these examples (Segil et al. 1991). In the latter scenario, trans-activation depends on a viral protein, VP16, in addition to Oct-1 (Gerster and Roeder 1988; O'Hare and Goding 1988; Preston et al. 1988). VP16, a structural protein found in the viral tegument, is a prototypic acidic transcriptional activator. Oct-1, when interacting with the TAATGARAT DNA recognition motif, aids in tethering VP16 to viral immediate early promoters. Thus, though Oct-1 itself is not a potent transcriptional activator, it contributes to the inducible transcription of HSV immediate early genes by targeting a strong transcriptional activator to a particular context. OAP40 appears to play a role in...
ARRE-1 transcription that is in some ways functionally analogous to the role of VP16 (Ullman et al. 1991).

Here, we report that amino acid sequences obtained from purified OAP40 were found to be identical to sequences from Jun family members. The Jun proteins belong to a class of transcription factors termed bZIP proteins [Kerppola and Curran 1991] owing to the presence of a basic region adjacent to a dimerization motif called a leucine zipper (Landschultz et al. 1988). c-Jun was first identified as a component of AP-1, a TPA-inducible transcription factor [Bohmann et al. 1987, Angel et al. 1988]. Other family members were consequently identified based on sequence homology and/or induction of expression following mitogenic stimulation [Hirai et al. 1989; Ryder et al. 1989]. In addition to forming homodimers, these proteins can form heterodimers with each other as well as with other groups of proteins such as Fos and Fos-related proteins and members of the CREB/ATF family [Nakabeppu et al. 1988; Hai and Curran 1991].

The transcriptional activity of Jun proteins is controlled by complex regulatory mechanisms. For example, the state of phosphorylation at specific residues [Binétruy et al. 1991; Boyle et al. 1991, Pulverer et al. 1991, Hunter and Karin 1992], a cell type-specific inhibitory protein [Baichwal and Tian 1990], and different dimerizing partners [Chiu et al. 1989; Schütte et al. 1989] have been shown to modulate Jun activity. In the studies referred to above the transcriptional function of Jun proteins has been assessed in terms of AP-1 activity. The results presented here demonstrate that in activated T cells, Jun proteins contribute to a transcriptional activity with properties distinct from AP-1. Following T-cell stimulation, the activity of Jun proteins appears to be regulated by two different pathways. One is phorbol ester dependent—indicating a dependence on protein kinase C activation—and results in increased AP-1 activity. The second pathway, described here, is triggered by a flux in intracellular calcium and is sensitive to the immunosuppressant cyclosporin A. While this calcium-mediated signaling pathway does not affect AP-1 activity, it regulates the participation of Jun in OAP activity. The recent demonstrations that Jun and Fos proteins also play a role in the lymphoid-restricted transcription factor NF-AT [Jain et al. 1992; Northrop et al. 1993] may indicate that Jun–Fos family members have a broader role in transcriptional regulation than was first characterized.

Results

Jun family members participate in OAP-binding activity

As described previously, OAP was purified to apparent homogeneity from stimulated Jurkat cells by subjecting crude nuclear extracts to ion exchange chromatography followed by DNA affinity chromatography and, finally, separation on a C4 HPLC reverse-phase column [Ullman et al. 1991]. A protein with a molecular mass of 40 kD, as estimated from SDS-PAGE, was isolated that could reconstitute the Oct-1 + OAP gel mobility shift when mixed with a crude fraction containing Oct-1. To determine the identity of this 40-kD protein, endoproteinase Lys-C was used to generate peptide fragments for amino acid sequencing. The amino acid sequences of seven peptides were determined of which four correspond to JunD, one to c-Jun, and two to a region common to both JunD and c-Jun [Fig. 1].

The observation that ARRE-1 is sensitive to glucocorticoids [Northrop et al. 1992], along with reports on sensitivity of AP-1 to glucocorticoids [Diamond et al. 1990], had previously raised the question of whether Jun family members play a role in OAP activity. A comparison of a consensus AP-1 site and the region of ARRE-1 necessary for OAP activity reveals a 5- of 7-bp alignment between the two sites [TGAGTCA vs. TGtGTaA; Northrop et al. 1992]. Furthermore, Northrop et al. demonstrated that AP-1 and OAP share sequence specificity, yet the exact relationship between the two activities remained unclear. The amino acid sequence data obtained from purified OAP shown in Figure 1 strongly suggested that Jun proteins are directly involved in OAP activity. To confirm that Jun proteins are present in the Oct-1 + OAP gel mobility shift, nuclear extracts from stimulated Jurkat cells were used in a gel mobility-shift assay that was then transferred to nitrocellulose to identify components of the different DNA–protein complexes. Both the upper and lower complexes formed with the ARRE-1 oligonucleotide were detected with Oct-1-specific antisera [Fig. 2; lane 3; Ullman et al. 1991]. When Jun-specific antisera was used for immunodetection, the Oct-1 + OAP complex was not observed.
Figure 2. The Oct-1 + OAP/ARE-1 gel mobility-shift complex is recognized by Jun-specific antisera. Migration of AP-1, Oct-1, and Oct-1 + OAP in a gel mobility-shift assay are shown in lanes 1 and 2. Binding reactions using an excess of unlabeled oligonucleotide were run in parallel and transferred to nitrocellulose. An excess of oligonucleotide was used to maximize DNA-binding activity; however, this may also result in OAP binding to ARE-1 alone, which is possible (Ullman et al. 1991) although not favored when less oligonucleotide is present (lane 1). The lower diffuse band (indicated by *) in lane 5 is probably OAP bound to ARE-1 without Oct-1. Likewise, the intensity of the Oct-1 + OAP complex is relatively stronger when less oligonucleotide is present [lane 1] compared with a binding reaction performed in the presence of a great excess of oligonucleotide [lane 3]. Oct-1-specific antisera [lanes 3,4] or Jun-specific antisera [lanes 5,6] were used to detect the presence of these particular proteins in the DNA-protein complexes.

One prediction that arises from the results described above is that recombinant Jun protein may be able to reconstitute OAP activity in a binding assay. To ascertain if this is the case, we used nuclear extracts prepared from nonstimulated Jurkat cells and performed gel mobility-shift assays in the presence and absence of recombinant forms of Jun and Fos proteins [expressed as fusion proteins with glutathione S-transferase (GST) at their amino termini]. When added to nuclear extract alone, GST-c-Jun or GST-JunD did not affect the formation of DNA-protein complexes; however both GST-c-Jun and GST-JunD, when added to nuclear extract in combination with GST-Fos, formed an upper complex in the gel reminiscent of the Oct-1 + OAP mobility shift [Fig. 3a, lanes 6,7, open arrowhead]. This complex migrated more slowly than an Oct-1 + OAP complex formed in nuclear extracts from stimulated Jurkat cells [Fig. 3a, lane 8]. However, because these recombinant proteins contain an extra 26 kD contributed by GST, the size of the complex is expected to be larger than that composed of endogenous proteins. GST-Fos appears to have a weak reconstitutive activity when added to nuclear extract alone [Fig. 3a, lane 5]; however, this is most likely the...
result of the formation of heterodimers with the small amount of constitutive Jun proteins present in the nuclear extract. To confirm the identity of the proteins involved in each complex we included antisera directed against GST (Fig. 3b, lanes 2,5,8) or antisera specific for Oct-1 (Fig 3b, lanes 3,6,9) in the binding reactions. Although the endogenous Oct-1 + OAP complex in stimulated extracts is unaffected by anti-GST, the upper shift formed in the presence of recombinant proteins is inhibited, indicating that antibody bound to the GST domain specifically interferes with the Oct-1 + OAP-like complex. Both the upper and lower complexes are supershifted by anti-Oct-1 in all cases, confirming that Oct-1 participates along with recombinant Jun–Fos proteins in forming a complex with the ARRE-1 oligonucleotide. Whereas Jun and Fos have some affinity for ARRE-1 in the absence of Oct-1 (data not shown), when Oct-1 is present in the gel mobility-shift assay, the Jun–Fos complex appears to favor binding ARRE-1 in conjunction with Oct-1. The requirement for Fos in addition to Jun may be because a heterodimer is more stable than a Jun–Jun dimer or forms more stable interactions in a DNA-protein complex. Although this does not rule out the possibility that a homodimer has physiological activity, it may mean that a heterodimer will be more easily detected in some assays. An important technical point is that these bacterially produced Jun proteins did not bind to an AP-1 site as homodimers (data not shown). There may be modifications that occur in eukaryotic cells that allow Jun proteins to work efficiently as homodimers. Alternatively, the GST domain may interfere with Jun–Jun but not Jun–Fos interactions. These points are addressed further by the functional studies presented and discussed below. These binding assays, while confirming the ability of c-Jun and JunD to participate in OAP-like binding activity, leave open the question as to whether additional proteins present in the nuclear extract prepared from nonstimulated Jurkat cells also contribute to complex formation.

**OAP and AP-1 are distinct functional activities**

To understand the relationship between OAP and AP-1, we first examined the requirements for their binding activity in Jurkat cells. Both AP-1 and OAP are maximally induced by a combination of phorbol myristate acetate and calcium ionophore and are relatively insensitive to treatment with the immunosuppressive agent cyclosporin A (Fig. 4). These binding studies indicate that in terms of their ability to interact with DNA, OAP and AP-1 are very similar. In contrast, as described by Mattila et al. (1990), the two activities appear functionally distinct. The AP-1 reporter construct used in these functional studies employed the SV40 promoter containing several Sp1 sites, leaving open the possibility that modulation of AP-1 activity might be masked by the strong constitutive activity of Sp1.

To compare the activation requirements of AP-1 and ARRE-1 sites in isolation, multimers of each site were cloned into a reporter construct containing the minimal γ-fibrinogen promoter, which has very low basal activity (Durand et al. 1988). AP-1 activity was found to be inducible with PMA treatment alone, whereas ARRE-1 activity stringently required two signals for activation (Fig. 5). In addition, as opposed to AP-1 function, ARRE-1 function is sensitive to CsA (Fig. 5). Of note is the observation that PMA-stimulated AP-1 activity is greater than activity induced by the dual stimulation of PMA plus ionomycin, whereas the converse is observed with AP-1-binding activity (Fig. 4). Because the transfected cells are stimulated for 6 hr before harvesting cells for the luciferase assay, we also looked at later time points to see whether PMA-induced AP-1-binding activity was induced with slower kinetics than the PMA plus ionomycin activity. The results were qualitatively the same, however, with either 2 or 5 hr of stimulation (data not shown). This lack of correlation between binding activity and functional activity may be the result of post-translational modifications, which increase the transcriptional activity of AP-1 without increasing binding activity. Several examples of post-translational control of Jun proteins (Binétruy et al. 1991; Pulverer et al. 1991) have already been identified that could potentially contribute to AP-1 activity in this manner. AP-1 and OAP clearly share some features, yet there are several distinctions between the two. The simplest explanation of these results is that different intracellular signaling pathways, one involving calcium as a second messenger and
Figure 5. Functional distinctions between AP-1 and Oct-1 + OAP activity. TAg Jurkat cells were transiently transfected with an empty expression vector (PBJ5) and reporter constructs containing the luciferase gene driven by the α-fibrinogen promoter and multiple copies of either the AP-1 site or ARRE-1. Transfections were split in five and stimulated under the conditions indicated. Luciferase values were then quantitated and are expressed relative to the value obtained from the PMA plus ionomycin stimulation in each transfection. The number of independent measurements for each stimulation condition is indicated above the corresponding bar.

one independent of calcium, allow Jun proteins to function in different contexts.

c-Jun and JunD contribute functionally to Oct-1 + OAP activity in a calcium-dependent, cyclosporin A-sensitive manner

To directly assess the contribution of Jun and Fos proteins to Oct-1 + OAP activity, we first transfected junD, fos, or empty expression vectors and analyzed their effect on ARRE-1-directed transcription. Basal activity was consistently increased three- to eightfold in the presence of junD or fos, and, more dramatically, expression of junD or fos was found to replace the requirement for PMA, leaving ARRE-1 responsive to calcium ionophore alone [Fig. 6a]. junB, c-jun, and junD, all under the control of the Rous sarcoma virus (RSV) enhancer, were assessed in parallel for their ability to replace PMA stimulation [Fig. 6b]. These transfections revealed the specificity of this functional property because both a closely related protein [JunB] and an unrelated protein [HNF-1β] had no activity in this context while c-Jun and JunD could each contribute to ARRE-1-driven transcriptional induction.

We then assayed junD-transfected cells in the presence of CsA to further dissect the signaling mechanisms that lead to Jun–Fos function at the ARRE-1 site. As shown in Figure 7, this immunosuppressive agent blocks the calcium-triggered signal needed for JunD to contribute to ARRE-1 activity, reducing ionomycin-stimulated activity from 64% of PMA plus ionomycin-stimulated activity to 3%. Similar results have been observed with c-Jun transfectants [data not shown]. Although JunD stimulates AP-1 activity [compare relative ionomycin value of 20% in the presence of Jun [Fig. 7] to 3.6% [Fig. 5]], CsA has no significant effect on this elevated activity [Fig. 7]. These results parallel the CsA sensitivity of ARRE-1- and AP-1-directed transcription when assayed without exogenous Jun present [Fig. 5] and point toward a physiological mechanism that enables Jun–Fos proteins to participate specifically in ARRE-1 activity.

Discussion

Jun proteins contribute to OAP activity

Our results indicate that both c-Jun and JunD, but not JunB, participate with Oct-1 in forming an active complex with an antigen receptor response element of the IL-2 enhancer ARRE-1. We previously defined this complex-
Intracellular signaling pathways lead to multiple roles of Jun family members during T lymphocyte activation

The requirement for overexpression of Jun proteins and the observation that in some instances overexpression still does not completely replace a PMA signal indicates that these proteins are controlled, at least in part, qualitatively by PMA-triggered signals. When Jun proteins are present at superphysiological levels, an event such as a post-translational modification may occur at a low frequency due to the excess of substrate available rather than due to an increase in activity of the enzyme responsible for post-translational modification. Although only a minor portion of the exogenously expressed proteins would be modified, in terms of absolute levels enough of this form would be present to respond to calcium-mediated signals. Thus, although expression of Jun or Fos proteins alleviates the requirement for costimulation with PMA, this does not necessarily mean that the sole contribution of the PMA-responsive signaling pathway is the increase in expression of Jun–Fos proteins.

Alternatively, the observation that overexpression of JunD and c-jun partially replaces the PMA-induced events controlling Oct-1/OAP-dependent transcription may indicate that a different protein is the physiological mediator of OAP activity. This possibility has been illustrated previously in the case of ACE2 and SW15 (Dohrmann et al. 1992) in which a related protein when overexpressed can complement a function that it does not normally perform. Likewise, a Jun-related protein—antigenically and functionally related—could be the true physiological mediator of OAP activity. This possibility seems unlikely, however, because we know from the purification that the major nuclear proteins in stimulated Jurkat cells found to bind to this site are JunD and c-Jun and from the transient transfection analysis that these proteins are able to function at this site. Therefore, unless JunD and c-Jun are somehow sequestered or otherwise prevented from interacting with Oct-1/ARRE-1, they would be expected to contribute to IL-2 enhancer function following T lymphocyte stimulation.

Jun proteins have been implicated previously in two other transcription factor activities found in activated T lymphocytes. First, “classical” AP-1 activity has been proposed to contribute to the transcription of activation-specific genes (Serfling et al. 1989). Recently another role for Jun and Fos proteins has come to light. In this case, they are thought to contribute to the activity of NF-AT, a lymphoid-specific transcription factor (Jain et al. 1992, Northrop et al. 1993). OAP and NF-AT are known to share several functional features such as the requirements for a calcium-mediated signal and sensitivity to the immunosuppressive agents CsA and FK506, yet an important distinction remains. When assayed in extracts made from Jurkat cells stimulated in the presence of CsA, NF-AT DNA-binding activity is greatly decreased while OAP DNA-binding activity is relatively unaffected (Fig. 4). In the case of NF-AT, this appears to be the result of the failure of a component needed for NF-
AT DNA-binding activity to associate with the nuclear fraction in the presence of CsA or FK506 [Flanagan et al. 1991]. Jun and Fos are thought to comprise the nuclear component of NF-AT [Jain et al. 1992, Northrop et al. 1993]. Upon appearance of the cytoplasmic component, a complex forms with a binding specificity distinct from AP-1. In effect, this cytoplasmic component of NF-AT confers a cell type-specific function on Jun and Fos. The common role of Jun and Fos proteins in the context of ARRE-1 (Oct-1 + OAP) and ARRE-2 (NF-AT) may explain to some extent the relationship between OAP and NF-AT. One possible interpretation of these observations is that the cytoplasmic component of NF-AT, perhaps an enzyme, is necessary for OAP function but not binding. Alternatively, NF-AT and OAP may each be controlled by independent CsA-sensitive mechanisms. A third possibility is that Jun and/or Fos proteins, in addition to the cytoplasmic component of NF-AT, are direct targets of a CsA-sensitive activation mechanism. CsA sensitivity may result from multiple mechanisms which, for any one activity, could be redundant.

The phosphatase calcineurin has been demonstrated to be an essential component of the \( \text{Ca}^{2+} \)-mediated CsA-sensitive pathway leading to the transcriptional activity of both NF-AT and Oct-1 + OAP following T-cell stimulation [Clipstone and Crabtree 1992]. This observation raises the question of whether calcineurin could affect Jun activity directly or indirectly. In terms of direct mechanisms, the simplest possibility is that calcineurin itself dephosphorylates specific residues found in Jun and, in doing so, exposes or creates a surface that allows transcriptional activity in this particular context. Signaling pathways are often controlled by a contingent series of phosphorylations and/or dephosphorylations so the possibility remains that other enzymes lie between calcineurin and Jun or Fos. An indirect mechanism that seems equally possible is that Oct-1 is the target of control. For instance, there may be a CsA-sensitive modification of Oct-1 that confers the ability to function in conjunction with Jun and Fos.

An important question that arises from these studies is whether the sequence of the OAP-binding site itself or the context of this binding site directs Jun to function in response to a set of signals distinct from those controlling AP-1 activity. This question is central to the dissection of the mechanisms controlling Jun function during T-lymphocyte activation. We have made site-directed mutations that change the OAP site into a consensus AP-1 site but leave the octamer motif in the same proximity. This creates a regulatory element that is somewhat inducible by PMA alone but is still further induced, in a CsA-sensitive manner, by the addition of ionomycin in conjunction with PMA [K.S. Ullman, J.P. Northrop and G.R. Crabtree, unpubl.]. This observation suggests that PMA-inducible AP-1 function is restricted by the sequence of the Jun recognition motif and that juxtaposition with Oct-1 confers the ability to be regulated in a calcium-dependent, CsA-sensitive manner. Although the precise mechanism by which Oct-1 and Jun proteins respond to an increase in intracellular calcium remains undefined, our results point to a new context for Jun family members. The role of these ubiquitous transcription factors in ARRE-1 regulation during T-lymphocyte activation emphasizes the importance of combinatorial use of ubiquitous transcription factors as a means of producing biologically specific patterns of transcription.

**Materials and methods**

**Purification, protease digestion, and amino acid sequencing of OAP**

Endoproteinase Lys-C was used to generate peptides from OAP\(^{400}\) activity purified as described previously [Ullman et al. 1991]. This material was separated on a C8 HPLC column (300 Å, Shandon) and collected on precyced, polybrene-coated glass fiber filters [Applied Biosystems]. Automated amino acid sequencing was performed on fractions corresponding to the predominant peaks of absorbance.

**Preparation of nuclear extracts and conditions of binding assays**

Nuclear extracts [Ohlsson and Edlund 1986] were prepared from TAg Jurkat cells {Jurkat cells expressing T antigen from a stably integrated construct as described by Northrop et al. 1993} that had been stimulated for 2 hr under different conditions as indicated [NS, nonstimulated; P, 20 ng/ml PMA (Sigma); I, 2 \( \mu \text{M} \) ionomycin (Calbiochem); P + I, PMA plus ionomycin, CsA, 100 \( \mu \text{g/ml} \) of cyclosporin A (Sandoc)]. Six micrograms of each extract was used in gel mobility-shift reactions containing an oligonucleotide probe corresponding to a binding site for AP-1 (TGCGTACTCAGCGGTCG, Lee et al. 1987); Oct-1 + OAP (ARRE-1, TTGAATAATGCTAAATATATGTA-AAAACAT, Durand et al. 1988); or NF-AT (ARRE-2, GATCTAAAACGCTTACTCAT, Durand et al. 1988). Binding assays in which bacterially produced c-Jun, JunD, and Fos were used to reconstitute OAP-binding activity were performed similarly. Approximately 0.5 \( \mu \text{g} \) of bacterial protein [of which only a portion was full-length and contained the DNA-binding domain] was incubated with 5 \( \mu \text{g} \) of nonstimulated Jurkat nuclear extract. When antisera were used, 1 \( \mu \text{l} \) of antisera was added last to the binding reaction. The Oct-1-specific antisera has been described previously [Ullman et al. 1991] and the anti-GST rabbit antisera was obtained by injection of a GST fusion protein.

The Western on the gel mobility-shift assay was performed as described previously [Ullman et al. 1991]. Binding reactions using heparin–Sepharose-fractionated Jurkat nuclear extract were scaled up, \( ^{32}\text{P} \)-labeled oligonucleotide was added to an aliquot containing one-tenth the reaction, and 44 ng of unlabeled oligonucleotide was added to the remainder of the reaction. Following electrophoresis, the lanes containing the larger reactions were transferred to nitrocellulose following standard protocols. The nitrocellulose was blocked with 5% nonfat dried milk in PBS containing 0.05% Tween 20. Primary antibody was added in the same buffer with 0.5% milk. The anti-Jun rabbit antisera [a kind gift from V. Baichwal] was raised against full-length c-Jun expressed in bacteria. A secondary antibody conjugated to horseradish peroxidase was used for detection with a chemiluminescent system [ECL, Amersham].

**Bacterial expression constructs and production and purification of recombinant protein**

The entire coding region of mouse c-jun and junD and the re-
gion starting from amino acid 22 in rat fos were cloned into the pGEX-2T vector (Pharmacia). These constructs were transformed in *Escherichia coli* strain BL21. Fusion protein expression was induced with 4 mM isopropyl-β-D-thiogalactoside. Bacteria were sonicated in extraction buffer [EB: 50 mM Tris (pH 8), 200 mM NaCl, 10 mM EDTA; 10% glycerol; 1 mM DTT; 0.2 mM PMSF] plus 1% NP-40. Soluble fusion proteins were then purified using glutathione-Sepharose (Pharmacia).

Plasmids and transient transfection of Jurkat cells

TAG Jurkat cells grown in RPMI supplemented with 5% enriched calf serum and 5% fetal calf serum were transiently transfected with constructs containing the luciferase gene being driven by the γ-fibrinogen minimal promoter under the control of either ARRE-1 or AP-1 sites (four copies of oligonucleotides listed above). For each experimental point, 1 × 10⁶ Jurkat cells were transiently transfected by electroporation (1 × 10⁷ to 2 × 10⁷ cells in 300 µl; 960 mF; 250 V) with 3 lag of reporter plasmid and 2 µg of an expression vector where indicated. Cells transfected under the same conditions were split into separate pools for stimulation (see above). Cells were lysed in 25 mM Tris buffer, and luciferase activity was quantitated using a luciferometer (Analytical Luminescence Laboratory). Experimental values are expressed relative to the activity found in extracts from the PMA plus ionomycin stimulation in each transfection.

The expression constructs SV2flos, RSVjunB, RSVc-Jun, RSVjunD (all of mouse origin), and RSVHNF-B were a kind gift from M. Yaniv. 6XHISjunD was constructed by linking a 6-his-tidine tail to the amino terminus of mouse JunD under the control of the SRα promoter in the PBJ5 expression vector (see Northrop et al. 1993).

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