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T-Cell Activation Signals and Human T-Cell Leukemia Virus Type I-Encoded p40^x Protein Activate the Mouse Granulocyte-Macrophage Colony-Stimulating Factor Gene through a Common DNA Element

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Activation of T cells by an antigen, a mitogen, or a combination of a phorbol ester (12-*O*-tetradecanoylphorbol-13-acetate [TPA]) and a calcium ionophore (A23187) leads to induction of a set of lymphokine genes. Treatment of human T-cell leukemia line Jurkat by a mitogen or p40^x, a transactivator protein encoded by human T-cell leukemia virus type I, activates many transfected lymphokine genes in a transient transfection assay. To study the mechanism of lymphokine gene induction, we examined the effects of mitogen stimulation and p40^x on the gene for the mouse granulocyte-macrophage colony-stimulating factor (GM-CSF) in Jurkat cells. Deletion and mutation analyses showed that the 5′-flanking region of the gene for the GM-CSF is composed of two types of regulatory elements. One sequence, located at positions −95 to −73, determines response to stimulation by either TPA-A23187 or p40^x. This region contains conserved lymphokine element 2, which appears in the gene for interleukin 3 (IL-3) and is followed by a GC-rich stretch. This GC-rich stretch alone specifies inducible response to p40^x but not to TPA-A23187. Another sequence, located at positions −113 to −96 upstream of a TATA-like sequence, mediates inducible response to p40^x but not to TPA-A23187. This sequence includes conserved lymphokine element 1, which appears in several lymphokine-cytokine genes, such as those for IL-3, G-CSF, and IL-2. We previously showed that the simian virus 40 early region promoter was also induced by a mitogen or p40^x in Jurkat cells. Deletion analysis showed that the minimum regions required for stimulation by both signals are identical. These results, which indicate that p40^x stimulates transcription of the gene for the GM-CSF or the simian virus 40 early region promoter through the same DNA element or an overlapping DNA element required for induction by a mitogen, lend further support to the notion that p40^x can exert its function by activating a component(s) of the T-cell signal transduction pathway which is activated by an antigen or a mitogen.

Expression of many eucaryotic genes is regulated by hormones, growth factors, or other stimuli that interact with specific receptors to induce various intracellular processes. Activation of protein kinase C and increase of Ca^{2+} mobilization are used as signal transduction processes in many different types of cells (2, 26). When T cells are exposed to antigens, the T-cell antigen receptor-CD3 complex transduces the extracellular stimulus across the plasma membrane, generating intracellular signals. These signals activate phosphoinositide turnover, leading to production of diacylglycerol, which activates protein kinase C, and inositol trisphosphate, which results in increased Ca^{2+} mobilization or influx or both (42). Signal transduction events further downstream trigger a series of biochemical reactions in the nucleus which result in production of a battery of lymphokines. These can then mediate numerous effector functions to help coordinate the immune and inflammatory responses (20). Nuclear oncogenes, such as c-*fos* and c-*myc*, and the gene that encodes a component of the interleukin 2 (IL-2) receptor system (p55) are also induced in this process. Activation of T cells by an antigen can be mimicked by lectins, such as concanavalin A and phytohemagglutinin (PHA), anti-T-cell receptor antibodies, anti-CD3 antibodies, Ca^{2+} ionophores, or phorbol esters, such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA), which directly activate protein kinase C. Among four major colony-stimulating factors (CSF), IL-3 (multi-CSF) and granulocyte-macrophage CSF (GM-CSF) are produced by activated T cells and stimulate early progenitor cells to produce hemopoietic cells of multiple lineages (20). IL-4, IL-5, and IL-6 (B-cell-stimulatory factor 2 [BSF-2]) are pleiotropic lymphokines that stimulate B cells, T cells, and hemopoietic cells (44).

Human T-cell leukemia virus type I (HTLV-I) is an etiologic agent of adult T-cell leukemia, a malignancy of $CD4^+$ T cells (45). As with antigen-activated T cells, HTLV-I-transformed T cells express the IL-2 receptor (p55) gene and various lymphokine genes, such as those for the GM-CSF, IL-5, IL-6, and gamma interferon. The pX region located near the 3′ end of the HTLV-I genome encodes three different proteins, $p21^{x\text{-}III}$, $p27^{x\text{-}III}$, and p40^x. The function of $p21^{x\text{-}III}$ is unknown. $p27^{x\text{-}III}$ modulates the synthesis of viral RNA at a posttranscriptional level (12). p40^x is a nuclear protein that activates transcription from the promoter in the viral long terminal repeat. Activation of various genes in HTLV-I-transformed T cells may be ascribed to p40^x, since p40^x alone or in combination with lectin activates genes for the IL-2 receptor (p55) (5, 11, 17), IL-2 (11, 17, 34), the GM-CSF, and IL-3 (22). The mouse gene for the GM-CSF is activated in Jurkat cells also by PHA-TPA or TPA-calcium ionophore A23187 in transient transfection assays.

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For comparison, we transfected the simian virus 40 (SV40) early region promoter, which responds in the same way as the GM-CSF promoter in Jurkat cells. The SV40 promoter does not work efficiently in uninduced Jurkat cells and is strongly activated by $p40^x$ or PHA-TPA treatment (22). The SV40 enhancer sequence is composed of an array of short sequence motifs that may contribute cell type specificity or inducible response to signals such as cyclic AMP or phorbol ester TPA in certain cell types (1, 4, 10, 14, 19). Recently, various DNA-binding proteins that specifically recognize these motifs have been identified (13). We showed that the *cis*-acting element required for $p40^x$ or PHA-TPA stimulation is functionally equivalent to the enhancer sequence in the SV40 early region promoter since a plasmid carrying an enhancer-deleted SV40 early region promoter failed to respond to either $p40^x$ or PHA-TPA (22).

In this paper, we extend these observations and describe *cis*-acting DNA elements within the 5′-flanking region of the gene for the GM-CSF and the SV40 enhancer sequence that mediate the response to mitogen stimulation and $p40^x$ *trans*-activation in Jurkat cells. Our results indicate that $p40^x$ activates the gene for the GM-CSF through two distinct DNA elements, one of which is also required for activation by a mitogen and the other of which is conserved in many lymphokine genes and is not stimulated by a mitogen. In the SV40 enhancer, the DNA sequence motifs that mediate the responses to $p40^x$ and mitogens are identical; these motifs are compared with those of the gene for the GM-CSF. Our results, which demonstrate that stimulation by mitogens and $p40^x$ is mediated by a common DNA element in the gene for the GM-CSF or in the SV40 enhancer motif, strongly suggest that $p40^x$ exerts its function by interacting with the T-cell signal transduction pathway.

Mouse and human genes for the GM-CSF, which exist as a single-copy gene in the haploid genome, are composed of four exons and three introns and are organized similarly (21). Generally, 5′-flanking regions are highly conserved between mouse and human lymphokine genes. There is about 85% homology in the 5′-flanking regions from the TATA boxes to around 330 base pairs (bp) further upstream of the human and mouse genes for the GM-CSF (21). Although there is no obvious homology among different lymphokine genes, comparison of the 5′-flanking sequences of the mouse genes for the GM-CSF and IL-3 revealed two homologous DNA motifs at positions −108 to −99 and −94 to −88 in the mouse gene for the GM-CSF (21). These two motifs are completely conserved in the human gene for the GM-CSF and in mouse and human genes for IL-3 and are referred to in this paper as conserved lymphokine element 1 (CLE1) and CLE2, respectively (see Fig. 3 and 4). Downstream of CLE2 is a sequence motif similar to the recognition site of transcription factor SP1, which will be referred to as the GC box (6). A TATA box is located at positions −29 to −25. The importance of the 5′-flanking regions of the gene for the GM-CSF for lectin-induced expression was confirmed by transfection experiments with the chloramphenicol acetyltransferase (CAT) gene (*cat*) as a reporter gene. The human T-cell leukemia line Jurkat, supplied by R. D. Malefijt, Immunological Laboratory, UNISET, Dardilly, France, was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and 0.05 mM 2-mercaptoethanol. A DEAE-dextran method was used for transfection (9, 38). For cells in suspension, 5×10^6 cells washed with Tris-buffered saline were suspended in 1 ml of Tris-buffered saline containing 0.5 mg of DEAE-dextran per ml and 15 μg of DNA (10 μg of a *cat* plasmid and 5 μg of either pcDSRα-pX or puc18) and incubated for 25 min at room temperature. The cells were suspended in 10 ml of tissue culture medium containing 0.1 mM chloroquine diphosphate (Sigma Chemical Co.) and incubated for 1 h at 37°C. Chloroquine-containing medium was then replaced with normal medium and incubated for an additional 40 h. For mitogen activation, cells were incubated with 50 μg of TPA (Sigma) per ml and 0.5 μM A23187 (Calbiochem-Behring) in medium containing 1% fetal bovine serum for 8 h and harvested for the CAT assay. Cell extracts were prepared by three cycles of freezing and thawing, and CAT assays were performed as previously described (22). Briefly, cell extracts containing 20 to 100 μg of protein were incubated with [^{14}C]chloramphenicol at 37°C for 8 to 10 h. The acetylated forms of chloramphenicol were separated by thin-layer chromatography, and the radioactivity of the spots was determined. The activities of various *cat* constructions were compared with that of pSV0CAT in uninduced cells. Each construct was transfected at least three times, and an average is shown. After transfection of Jurkat cells with pmoGM-CAT-226, which carries 226 bp of the 5′-flanking region of the mouse gene for the GM-CSF, there was about

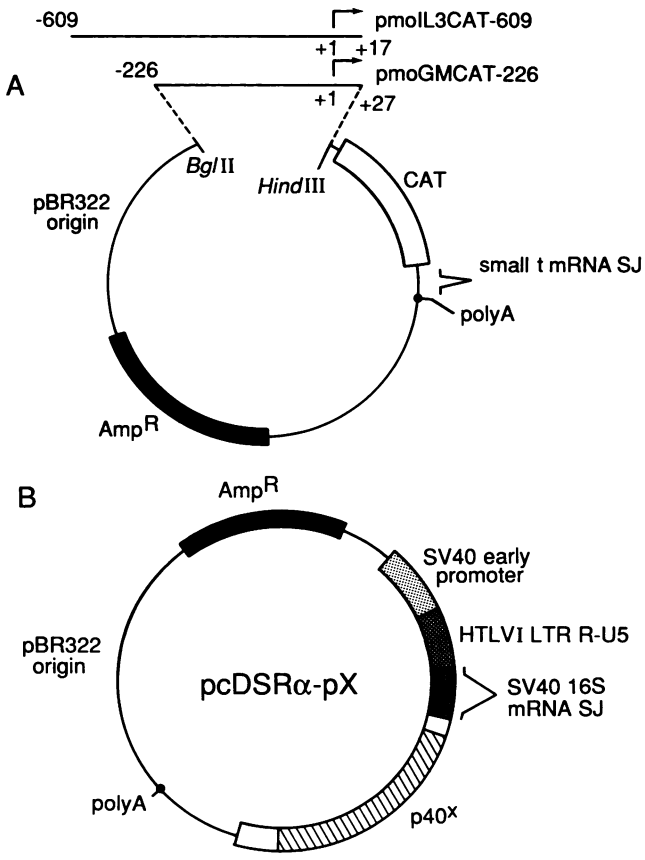

FIG. 1. (A) Structure of plasmids carrying the 5′-flanking region of the mouse gene for the GM-CSF or IL-3 linked to the *cat* gene. Symbols: ■, β-lactamase; ▭, *cat* gene. Numbers show positions with respect to the transcription initiation site, and arrows show the direction of transcription. Abbreviations: SJ, splice junction; polyA, SV40 early mRNA polyadenylation site. (B) Structure of plasmid pcDSRα-pX. Hatched bar, Coding region of $p40^x$, $p27^{x\text{-}III}$, and $p21^{x\text{-}III}$; polyA, SV40 late mRNA polyadenylation site. To achieve high-level expression of $p40^x$, the *Bam*HI-*Nde*I fragment of pMTPX (31), which contains the HTLV-I pX coding region, was placed downstream of a strong promoter, SRα (40), to yield pcDSRα-pX.

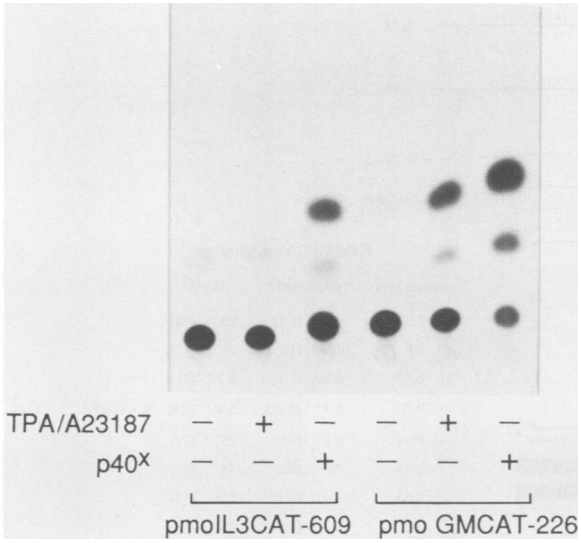

FIG. 2. Expression of CAT activity of the mouse GM-CSF–*cat* or IL-3–*cat* fusion plasmid in Jurkat cells. Plasmids pmoGMCAT-226 and pmoIL3CAT-609 were transfected with or without pcDSRα-pX. At 40 h posttransfection, half of the transfected cells were stimulated with TPA (50 ng/ml) and A23187 (0.5 μM) for 8 h. The cell extracts containing 20 μg of protein were incubated with [^{14}C]chloramphenicol at 37°C for 10 h. The substrate converted to the acetylated form was separated by thin-layer chromatography.

a 20-fold increase in CAT activity after treatment with TPA (50 ng/ml) and A23187 (0.5 μM) and about a 60-fold increase when the cells were cotransfected with plasmid pcDSRα-pX (Fig. 1). Similarly, p40^x stimulated plasmid pmoIL3CAT-609 about 10-fold, whereas TPA-A23187 did not appreciably affect the IL-3 promoter (Fig. 2).

To map the region required for activation by TPA-A23187 or p40^x, a series of deletions was generated by using *Bal* 31 nuclease and synthetic oligonucleotides. Deletions that encompass positions −1100 and −226 increased not only the induced level of expression by TPA-A23187 or p40^x but also the basal level of expression (Fig. 3). Deletions extending to position −113, which still maintain all three DNA motifs (CLE1, CLE2, and the GC box), responded fully to both p40^x and TPA-A23187 stimulation. Deletions extending to position −96, which remove CLE1, decreased the level of induction by p40^x without appreciable effect on TPA-A23187 induction. In contrast, a deletion extending to −84, which leaves only the GC box, completely abolished TPA-A23187 induction and most of the p40^x stimulation. Removal of the GC box (pmoGMCAT-72) did not abolish the residual stimulatory effect of p40^x, but removal of a region farther downstream (positions −72 to −61) reduced the response to p40^x about threefold. Plasmid pSV0CAT, which carries no promoter sequence upstream of the *cat* gene, still showed two- to threefold enhancement by p40^x, although we failed to detect a unique transcription initiation site (data not shown). Therefore, this level of induction by p40^x could be nonspecific activation.

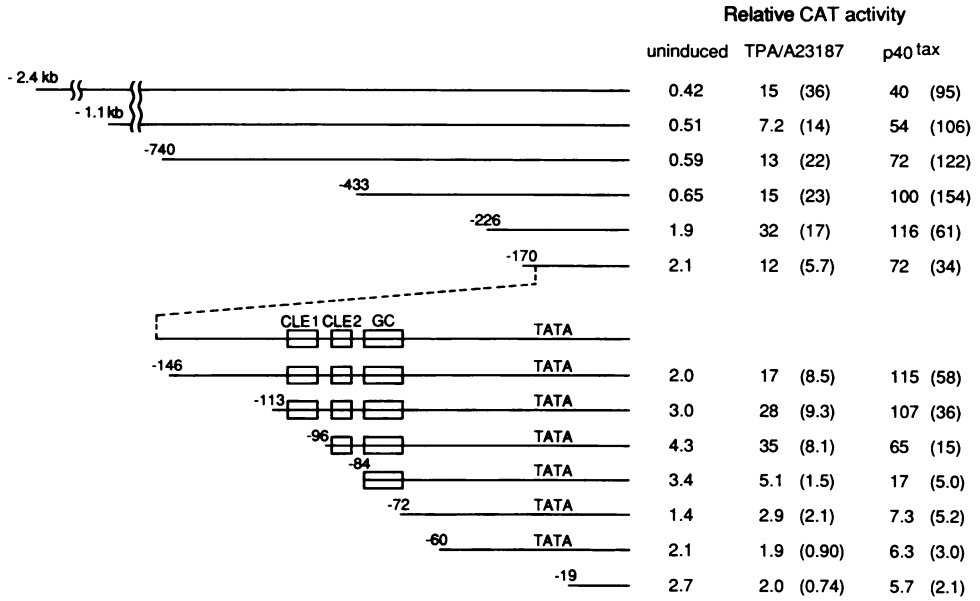

FIG. 3. 5′ Deletion analysis of the mouse GM-CSF promoter in Jurkat cells. CLE1, CLE2, and GC box motifs described in the text are diagrammed. CAT activity is normalized relative to the activity directed by plasmid pSV0CAT in unstimulated cells. Fold induction is shown in parentheses. pmoGMCAT-1.1 contains the mouse gene for the GM-CSF (21), from the *Xmn*I site (at about −1.1 kilobases with respect to the transcription initiation site) to the *Mst*II site (at +27), inserted at the *Hin*dIII site of pSV0CAT (8) in the sense orientation with respect to the *cat* gene. For convenience in making deletions from the 5′ end, the *Hin*dIII site distal to the *cat* gene of pmoGMCAT-1.1 was converted to a unique *Bgl*II site. To construct pmoGMCAT-2.4, the *Eco*RI site at a position of about −2.4 kilobases of the mouse gene for the GM-CSF was converted to a *Bgl*II site and the *Bgl*II-*Sac*I (at −19) fragment was replaced with the *Bgl*II-*Sac*I fragment of pmoGMCAT-1.1. pmoGMCAT-226, -60, and -19 were generated by deleting the *Bgl*II-*Bgl*II fragment after converting a *Stu*I (at −226), *Bst*EII (at −60), or *Sac*I (at −19) site of pmoGMCAT-2.4 to a *Bgl*II site. pmoGMCAT-740, -433, -170, -146, -113, and -96 were constructed by substituting the *Bgl*II-*Hin*dIII fragment of pmoGMCAT-2.4 with fragments generated by *Bal* 31 deletion at the *Bgl*II site of pmoGMCAT-2.4. pmoGMCAT-84 or -72 was constructed by inserting a synthetic oligonucleotide covering from either −84 or −72, respectively, to the *Bst*EII site at −60 into the *Bgl*II-*Bst*EII site of pmoGMCAT-96.

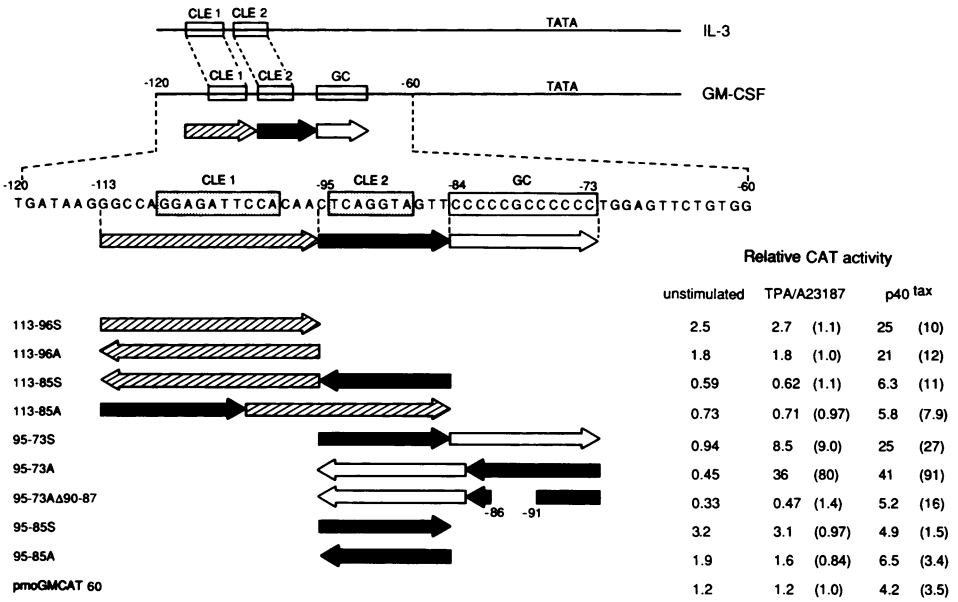

FIG. 4. Relative CAT activities of plasmids carrying various segments of the 5′-flanking region of the mouse gene for the GM-CSF inserted into the *Bgl*II site of pmoGMCAT-60. Hatched, solid, and open arrows represent fragments containing CLE1 (−108 to −99), CLE2 (−94 to −88), and GC box (−84 to −73) motifs, respectively. The activities of various *cat* constructions were compared with that of pSV0CAT in uninduced cells. pmoGMCAT-60, which is not activated by signals, was used to identify DNA motifs required for induction. Values in parentheses represent fold induction by stimulation with TPA-A23187 or p40^x. p113-96, p113-85, p95-85, or p95-73 was constructed by introducing a synthetic oligonucleotide covering −113 to −95, −113 to −85, −95 to −85, or −95 to −73, respectively, into a unique *Bgl*II site of pmoGMCAT-60 in either the sense (S) or the antisense (A) orientation. p95-73AΔ90-87 contains a 4-bp deletion (−90 to −87) of p95-73A.

To identify the minimum region that mediates response to TPA-A23187 or p40^x, the region spanning positions −113 and −73 was divided into subfragments by chemical synthesis (Fig. 4). Each oligonucleotide fragment was inserted at the *Bgl*II site of pmoGMCAT-60, since this plasmid responds to neither TPA-A23187 nor p40^x. The fragment covering positions −95 and −73 that contains both the CLE2 motif and the GC box, when cloned into plasmid pmoGMCAT-60 in either orientation (p95-73S or p95-73A) restored the inducible response to TPA-A23187 and p40^x (Fig. 4). However, the fragment carrying only CLE2 (p95-85S or p95-85A) did not respond to this stimulation. Therefore the CLE2 motif alone is insufficient to mediate the response to TPA-A23187 stimulation. This may account for the lack of response of the IL-3 promoter-*cat* fusion gene to TPA-A23187 stimulation (Fig. 2) (22), since the CLE2 motif found in the 5′-flanking region of the IL-3 gene (21) is not followed by a GC box. The fragment which covers positions −95 and −73 but has a 4-bp deletion within CLE2 (p95-73AΔ90-87) failed to respond to TPA-A23187 and reduced the response to p40^x. These results suggest that both the CLE2 motif and the GC box are required for efficient activation by TPA-A23187 or p40^x. Plasmids pmoGMCAT-84 and p95-73AΔ90-87, which contain only the GC box, showed diminished response to p40^x and no response to TPA-A23187. These results indicate that the GC box alone confers an inducible response to p40^x but not an inducible response to TPA-A23187. p40^x can activate plasmids (113-96S or 113-96A) containing the sequence (−113 and −95) that includes the CLE1 motif in either orientation, although less strongly than can those plasmids that contain the 95-73 construct that includes CLE2 and the GC box. Therefore, the CLE1 motif alone appears to determine the inducible response to p40^x but not the response to TPA-A23187.

The CLE1 motif is conserved in the 5′-flanking region of various lymphokine-cytokine genes, such as those for IL-2, IL-3, and the G-CSF. This sequence also specifies a response to p40^x stimulation. This may account for the activation of many lymphokine promoters by p40^x in Jurkat cells in transient transfection experiments (22). However, since a mitogen failed to activate transcription through CLE1, this element, as well as the CLE2-GC box, which does not exist in 5′-flanking regions of many lymphokine genes, cannot fully account for the coordinate induction of various lymphokine genes by an antigen or a mitogen during T-cell activation. Both CLE1 and CLE2-GC box motifs function

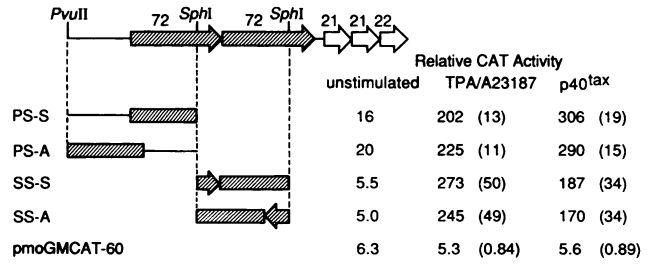

FIG. 5. Relative CAT activities of plasmids carrying the SV40 enhancer region linked to the GM-CSF promoter. Top diagram, SV40 promoter region; hatched and open arrows, 72-bp repeat of the SV40 enhancer and 21-bp repeat recognized by transcription factor SP1, respectively. The activities of various *cat* constructions were compared with that of pSV0CAT in uninduced cells. Values in parentheses indicate fold induction by TPA-A23187 or p40^x stimulation. PS-S, PS-A, SS-S, or SS-A was constructed by inserting the *Pvu*II-*Sph*I (PS) or *Sph*I-*Sph*I fragment (SS) of pSV2CAT (8), which contains the SV40 enhancer, into a unique *Bgl*II site of pmoGMCAT-60 in either the sense (S) or the antisense (A) orientation.

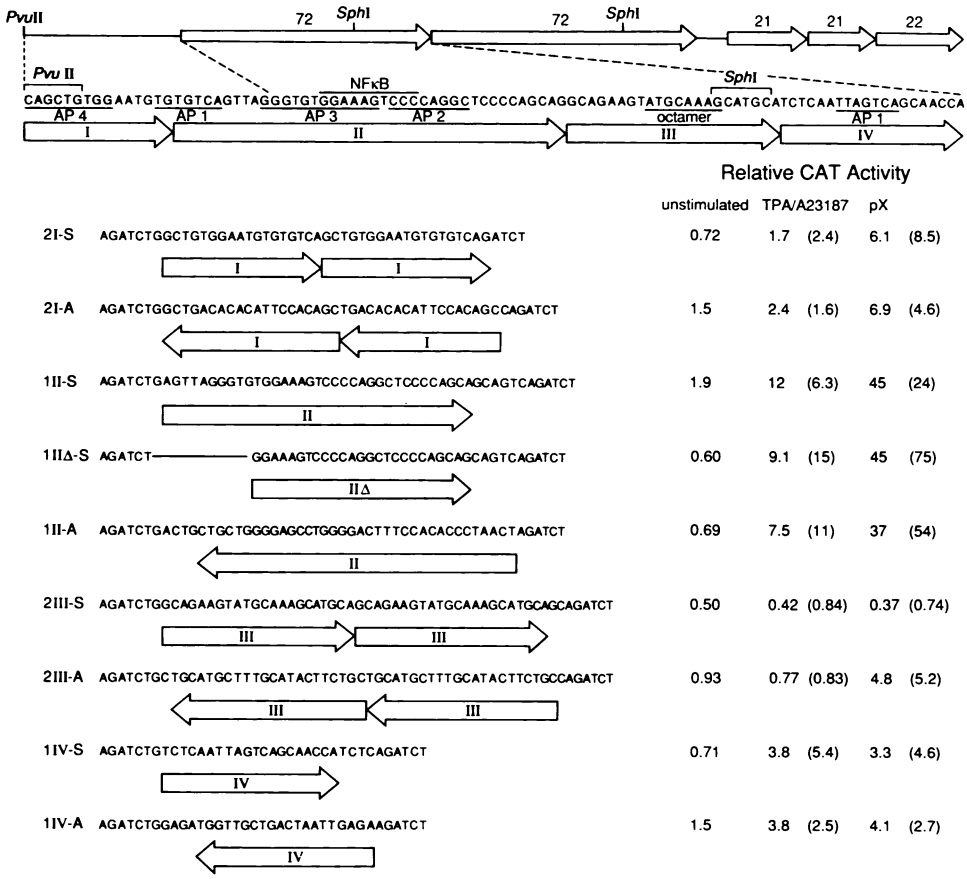

FIG. 6. Relative CAT activities of plasmids containing synthetic oligonucleotides corresponding to various regions of the SV40 enhancer. Top diagram, SV40 promoter and enhancer regions. The SV40 enhancer sequence is dissected into segments represented by open arrows with roman numerals. Either one (II and IV) or two (I and III) copies of each segment were inserted into the *Bgl*II site of pmoGMCAT-60 in either orientation. IIΔ indicates segment II with a deletion at the 5′ end. The activities of various *cat* constructions were compared with that of pSV0CAT in uninduced cells. Values in parentheses indicate fold induction by TPA-A23187 or $p40^x$ stimulation.

regardless of orientation. Shannon et al. (33) identified nuclear proteins that could form a complex with a DNA fragment carrying CLE1 and CLE2, although the significance of these binding data remains to be determined.

Removal of the region that encompasses positions at −72 and −61, which is located downstream of the GC box motif, reduced the level of induction by $p40^x$ from fivefold to threefold. Furthermore, the linker insertion at positions −72 to −62 of plasmid pmoGMCAT-96 was induced by TPA-A23187 and $p40^x$, but the level of induction was about half that of the wild-type construct, suggesting that the region from −72 to −62 also contributes to the promoter function (data not shown). When linker insertion mutations were introduced at positions −54 to −43 or −43 to −32, induction by TPA-A23187 or $p40^x$ was severely impaired (T. Heike and N. Arai, unpublished data). This result may be consistent with the results of Nimer et al. (25), who showed a DNase I footprint over the region from −65 to −31 which corresponds to −57 to −24 in the human gene for the GM-CSF.

We previously showed that the SV40 early promoter, which was as inefficient as the GM-CSF promoter in the uninduced state in Jurkat cells, was strongly activated by stimulation with TPA-PHA, TPA-A23187, or $p40^x$, similar to the gene for the GM-CSF (22). Furthermore, activation of the SV40 promoter by these stimuli seems to be attributable to the enhancer sequence upstream of the *Sph*I site. To compare the regulatory elements of gene for the GM-CSF with those of the SV40 promoter, we further defined the DNA element(s) of the SV40 enhancer that is responsible for TPA-A23187 activation or $p40^x$ activation. Various segments of the SV40 enhancer, either isolated following restriction endonuclease digestion or chemically synthesized, were introduced into the *Bgl*II site of plasmid pmoGMCAT-60. First, an *Sph*I fragment harboring all of the motifs within the 72-bp enhancer (SS-S or SS-A) or a *Pvu*II-*Sph*I fragment harboring the immediate upstream region of the 72-bp repeat in addition but lacking the region downstream of the *Sph*I site (PS-S or PS-A) was introduced into plasmid pmoGMCAT-60 in both orientations (Fig. 5). All of these constructs directed expression of CAT activity 10- to 50-fold over the basal level in the presence of TPA-A23187 or by cotransfection with plasmid pcDSRα-pX. These results indicate that the DNA segment encompassing the 5′ end and the *Sph*I site of the 72-bp repeat is required for activation.

To further localize the DNA segment responsible for TPA-A23187 or $p40^x$ stimulation, the SV40 enhancer region was dissected into four segments. These segments alone functioned as an enhancer in certain cell types when multiple tandem copies were used (27, 30). Each segment of the SV40 enhancer used in this study is recognized by specific DNA-binding proteins. Segment I contains a recognition site for DNA-binding proteins such as AP4 (18). Segment II is

recognized by DNA-binding proteins such as NF-κB (32), AP2, and AP3 (10, 19). Segment III contains the octamer sequence found in immunoglobulin enhancers or promoters (7, 28). This motif has been shown to be required for lymphocyte-specific expression of the immunoglobulin gene (39, 43) and cell cycle-specific expression of the histone H2b gene (36, 37). Segment IV carries the binding site for transcription factor AP1 (1, 14), which was reported to be involved in TPA-dependent signal transduction in HeLa and HepG2 cells. Dimers of segments I and III and monomers of segments II and IV were introduced into the *Bgl*II site of the recipient plasmid pmoGMCAT-60 (Fig. 6). By coincidence, a deletion mutation of segment II that lacks the AP3-binding site (1IIΔ-S) was also generated and used in transfection experiments. The plasmid carrying segment II in either orientation (1II-S or 1II-A) restored the inducible response to both TPA-A23187 and $p40^x$, although the level of induction was lower than that of plasmids carrying a larger segment of the SV40 enhancer element. The AP3-binding site in segment II is not required for induction, since the 1IIΔ-S construct was fully active with both stimuli.

These results indicate that a single copy of the segment carrying the DNA motifs for NF-κB binding and AP2 binding is sufficient for $p40^x$ or TPA-A23187 stimulation. Recently several groups showed that NF-κB-binding sites of the human immunodeficiency virus promoter and the IL-2 receptor (p55) promoter were required for activation of these promoters by a mitogen or $p40^x$ in T cells (3, 15, 16, 24, 29, 35, 41). Therefore, induction of the SV40 enhancer sequence by TPA-A23187 or $p40^x$ in Jurkat cells may also involve binding of NF-κB protein. Although there is little sequence homology between the CLE2-GC box motif and the NF-κB binding site, it is important to determine whether the same DNA-binding protein is involved in activation of the CLE2-GC box motif of the GM-CSF gene and the SV40 enhancer sequence. Other constructs carrying segment I (2I-S and 2I-A), III (2III-S and 2III-A), or IV (1IV-S and 1IV-A) did not appreciably affect the level of CAT activity in response to TPA-A23187 or $p40^x$ stimulation.

Although $p40^x$ is localized to the nucleus, it remains to be determined whether this protein is able to bind to DNA. Our results, which indicate that the TPA-A23187-responsive element and the $p40^x$-responsive element overlap in the gene for the GM-CSF or in the SV40 enhancer, suggest that $p40^x$ exerts its activation through a *cis*-acting regulatory element for the antigen-dependent signal transduction pathway of T cells. It is likely that $p40^x$ mediates its function by interacting with a protein component(s) in the signal transduction pathway.

Bovine papillomavirus-encoded protein E2 also activates the mouse gene for the GM-CSF in Jurkat cells or fibroblasts (22). Stimulation by protein E2 in Jurkat cells, as with $p40^x$, is enhanced by TPA-A23187 and requires CLE1 or the CLE2-GC box in the gene for the GM-CSF and the NF-κB- or AP2-binding site in the SV40 enhancer (T. Heike et al., manuscript in preparation). These results suggest that protein E2 activates the gene for the GM-CSF or the SV40 promoter by a mechanism similar to that of $p40^x$. It is possible that $p40^x$ or protein E2, in both T cells and fibroblasts, activates some cellular component(s) in the signal transduction pathway which results in activation of lymphokine genes in the absence of extracellular stimuli. Analysis of proteins that recognize the regulatory elements in the GM-CSF promoter is required to study the interaction of $p40^x$ and the signal transduction pathway in T cells. The DNA element defined in the present study will help to identify the protein component(s) that interacts with these regulatory sequences and shed more light on the mechanism of T-cell activation, a critical step of the immune response.

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