Regulation of expression of the c-sis proto-oncogene

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ABSTRACT
Regulation of expression of platelet derived growth factor polypeptide B encoded by the c-sis proto-oncogene is important in a number of physiological and pathological conditions. Sequences in the 1028 nucleotide long 5' untranslated region of the c-sis mRNA were found to inhibit protein synthesis. The inhibition is relieved by deletion of nucleotides 154–378 or 398–475. Sequences within 375 nucleotides upstream of the RNA initiation site are important for transcriptional activity. Sequences in two portions of this region, between –375 and –235 nucleotides and between –235 and –99 nucleotides relative to the RNA CAP site are important for full activity. A transcriptional enhancer activity is demonstrated by its ability to increase the activity of the human T lymphotropic virus type (HTLV) I promoter at a distance and in an orientation-independent manner. Furthermore, sequences upstream of the c-sis RNA CAP site respond to the HTLV I transactivator protein to increase RNA synthesis from either the c-sis or HTLV I promoter.

INTRODUCTION
Platelet-derived growth factor (PDGF) is a cationic glycoprotein dimer composed of A and B polypeptides (1,2). These two polypeptides have a similar size, identical array of cysteine residues, and 40% amino acid similarity (3). PDGF can be synthesized in three distinct and active forms, as an AB heterodimer, an AA homodimer, or a BB homodimer (4–6). The A and B polypeptides are encoded by genes located on human chromosomes 7 and 22, respectively (3,7–9). The B chain gene is the c-sis proto-oncogene (10–12).

Expression of the c-sis gene is highly restricted. It is expressed in a) developing placenta (13), b) vascular smooth muscle cells (14–18), c) activated monocytes (19–24), d) endothelial cells undergoing differentiation or in atherosclerotic plaques (25–32), e) astrocytes in the developing central nervous system (33), and f) certain carcinomas (34), melanomas (35), sarcomas (36–39), brain tumor-derived cell lines (15,36,39–42), and human T-lymphotropic (HTLV) type I and II transformed lymphocytes (43–46). The finding of expression of PDGF receptors and c-sis mRNA in mesenchymal tumors has suggested an autocrine role for this growth factor in transformation (47–51). The role of c-sis mRNA expression in leukemogenesis mediated by HTLV-I or II remains uncertain.

The entire c-sis gene has been cloned and the structure of the exons determined by nucleotide sequencing (8,11,44,45,52–55). The gene is composed of seven exons spanning 22 kilobases (kb). Preliminary functional characterization of sequences in the 1028 nucleotide 5' untranslated region has demonstrated an element that regulates translation (54,56). Functional studies utilizing sequences upstream of the RNA CAP site demonstrated the
presence of a transcriptional promoter (54). The current study provides a more detailed characterization of elements in the c-sis gene which regulate transcription and translation, and their response to the trans-activating factors of HTLV-I and II.

MATERIALS AND METHODS

**DNA Clones**

Lambda SIS-A is a 16 kb clone obtained from a normal human genomic DNA library (Fig. 1 and ref. 54). It includes sequences upstream of the c-sis RNA CAP site through the fourth exon. Clone pSIS-1 was obtained from lambda SIS-A by subcloning the 5' most 2.0 kb Bam HI fragment into the Bam HI site of SP65 (Promega Scientific). SIS CAT -1373/486 and SISCAT 486/-1373 include the Sal I – Xho I fragment of pSIS-1 cloned into the Sal I site of SP65-CAT3, a plasmid which includes the CAT gene but no eukaryotic promoter or enhancer (57). SIS CAT -1373/486 was treated with Avr II and Hind III, E. coli DNA polymerase Klenow fragment, and T4 DNA ligase to produce SIS CAT -1373/94. SIS CAT -1373/486 was treated with Bss HII or Sma I and T4 DNA ligase to produce SIS CAT -1373/486 d 154/378 or SIS CAT -1373/486 d 398/475, respectively. SIS CAT -1373/486 was digested with Hpa I and the 2.9 kb fragment cloned into the Sma I site of SP65-CAT3 to produce SIS CAT -743/486. SIS CAT -375/38 was previously designated SIS CAT 1. It was treated with Bst EII, Bal 31, Sac I, T4 DNA polymerase, and T4 DNA ligase to produce a set of deletion mutants. The structure of each of these clones was determined by dideoxy nucleotide sequencing (58). Plasmid SP65 was treated with Hinc II, Pvu II, and T4 DNA ligase to produce SP65HP. Clones pSV2 CAT (59), RSV CAT (60), MPMV CAT3 (57), NE-CAT1 (57), CMV CAT (57), HTLV I CAT (61), CRTAdSL3NEO (62), 91023 NpX 15S (63), pXB-RS (BLV-TAT) (64), and pBLH2CAT (BLV-LTR-CAT) (64) have been described previously. Nucleotide positions within the c-sis gene are indicated relative to the RNA CAP site (54). All nucleic acid manipulations were performed as previously described (65).

**Transfection Assays**

Jurkat and K562 cells were grown in RPMI-1640 medium supplemented with 10% fetal calf serum, 4 mM glutamine, 50 micromolar 2-mercaptoethanol, 50 units/ml penicillin, and 50 micrograms/ml streptomycin. The cells were transfected with ten micrograms of plasmid by the DEAE dextran (57) or electroporation methods (66). HOS, HeLa, A172, and AKR-2B cells were grown in DMEM medium supplemented with 10% fetal calf serum, 110 micrograms/ml pyruvate, 50 units/ml penicillin, and 50 micrograms/ml streptomycin. These cells were transfected with ten micrograms of plasmid by the calcium phosphate precipitation method (57). Cells were harvested 48–72 hrs. later and CAT assays performed as previously described. Variation in replicate CAT assays was less an 6%. Similar transfection efficiencies were confirmed by hybridization of Hirt supernatant DNA from transfected cells (67).

**RNA Assays**

Fifty micrograms of plasmid DNA was transfected by the calcium phosphate precipitation technique onto five 150 mm plates of HeLa cells which were 50% confluent. After three days, RNA was isolated by the method of Chirgwin (68). RNA samples were then added to 100 pmoles of an oligonucleotide (GTGAATTAAGGCCGAGTAAACTTG) complementary to nucleotides 4804–4828 in pSV2CAT, 200 units Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories), and buffer according
RESULTS

Structure of the c-sis Gene

Clones lambda SIS-A (Fig. 1) and L33 span the entire c-sis proto-oncogene (11,54). Comparison of the restriction enzyme maps and nucleotide sequences of these clones with cDNA clones (44,70) demonstrate the presence of 7 exons spanning 22 kb. The 5' and 3' untranslated sequences are 1028 and 1658 bp in length, respectively. The 5' untranslated sequences have a 70% GC content and include 3 minicistrons at nucleotides 588–602, 757–786, and 874–909, upstream of the open reading frame for PDGF B polypeptide which begins at nucleotide 1029. Sequences upstream of the RNA initiation site include decanucleotide sequences identical to the consensus SP1 binding site on the plus and minus strands, respectively, at nucleotides -220 to -211 and -128 to -117 (54,71).
Fig. 2. Sequences Downstream of the c-sis RNA Initiation Site Inhibit CAT Activity. Each of the indicated plasmids was transfected into HOS cells. The transcriptional orientation of c-sis sequences is opposite to that of CAT in SIS CAT 486/−373 which is indicated by an arrow. Sequences from the c-sis gene present in each plasmid are indicated to the left. Autoradiograms of CAT assays are indicated with the unacetylated forms to the left of the 1- and 3-mono acetylated forms. The percent of chloramphenicol which is acetylated is shown to the right.

Sequences in the 5' Untranslated Region of the c-sis mRNA Inhibit Translation
Studies with an in vitro translation system demonstrated that 5' untranslated sequences inhibit PDGF B polypeptide synthesis (54). To examine the activity of these sequences in vivo, a set of plasmids were constructed in which nucleotides −1373 to 486 were fused to the chloramphenicol acetyl transferase (CAT) gene (Fig. 2). These sequences gave rise to very low levels of CAT activity (0.9% acetylation) when placed in the correct orientation, compared to background levels (0.3% acetylation) when placed in the incorrect orientation. Similar results were obtained with a plasmid with nucleotides −743 to 486 placed upstream of the CAT gene (0.7% acetylation). In contrast, deletion of nucleotides 95486 from plasmid SIS CAT −1373/486 gave rise to 5-fold more CAT activity. No difference was detected in the level of CAT mRNAs in these transfected cells compared to that in cells transfected with plasmid SIS CAT −1373/486 (Fig. 3). Furthermore, deletions of nucleotides 154−378 (Fig. 2) or 398−475 (not shown) almost completely relieved the inhibition. These deletions did not affect the minicistrons upstream of the PDGF B polypeptide open reading frame.

Cell Type Dependence of the c-sis Transcriptional Enhancer
Prior studies had suggested that sequences from −375 to 38 expressed significantly higher levels of CAT activity in endothelial cells than fibroblasts (54). To assess possible tissue specific c-sis transcriptional activity in other cells, plasmids with sequences −375 to 38 or −1373 to 94 fused to CAT were transfected into cell lines expressing (HOS, A172) or not expressing endogenous c-sis sequences (HeLa, Jurkat) (36,46). The activity of these plasmids was compared to one lacking a eukaryotic promoter, SP65-CAT3, and those with strong promoter and enhancer activities derived from cytomegalovirus (CMV) immediate early gene, Mason-Pfizer monkey virus (MPMV) or Rous sarcoma virus (RSV) long terminal repeat sequences (LTR), or the early region of simian virus (SV) 40. Low, but significant levels of CAT activity were obtained with SIS CAT −375/38 and SIS CAT −1373/94, compared to SP65-CAT3 and SIS CAT 38/−373. No consistent differences
Fig. 3. Sequences in the 5' Untranslated Region Do Not Affect the Level of c-sis RNA. One (lane 1), 0.5 (lane 2), 0.25 (lane 3), 0.125 (lane 4) or 0.063 (lane 5) micrograms of RNA from HeLa cells transfected with SIS CAT -1373/94 or SIS CAT -1373/486 was reverse transcribed and amplified by polymerase chain amplification reactions. The amplified products were analyzed by dot blot hybridization with a CAT DNA probe. In CAT activity were obtained with plasmid SIS CAT -375/38 compared to SIS CAT -1373/94. Furthermore, no correlation was obtained with the level of endogenous c-sis mRNA. Studies were also carried out with a) unactivated and concanavalin A treated human peripheral blood monocytes, b) untreated and transforming growth factor (TGF) beta treated AKR 2B cells, and c) untreated and phorbol myristic acid treated HeLa or K562 cells. Though each of these agents has previously been shown to increase endogenous c-sis mRNA transcription (20,24,29,72—75), no significant increase in CAT activity could be elicited (not shown).

Deletion Analysis of the c-sis Transcriptional Enhancer

The previous experiments demonstrated that sequences from -1373 to -375 had no significant positive or negative regulatory effects on c-sis transcriptional activity. To further localize the minimal sequences for this activity, a Bal 31 deletion library was constructed, and individual clones transfected into HOS or A172 cells (Fig. 4) or HeLa or AKR 2B cells which gave similar results (not shown). This experiment demonstrated a significant diminution of CAT activity after deletion of nucleotides -375 to -235. Further decrease

Fig. 4. Deletion Analysis of the c-sis Transcriptional Enhancer. A set of clones derived from Bal 31 treatment of SIS CAT -375/38 (See Materials and Methods) are shown at the left side of the figure with the corresponding c-sis sequences indicated. Ten micrograms of each clone was transfected into HOS or A172 cells and a representative experiment is shown. The number of cpm of acetylated product in each cell type is shown and the autoradiogram of CAT assays performed in A172 cells is shown at the right side of the figure.
Fig. 5. Plasmids Used to Test the Activity of the c-sis Enhancer with the HTLV-I Promoter. HTLV-I-CAT is a plasmid with the U3 and R regions of the HTLV-I LTR with enhancer (E) and promoter (P) elements fused to the CAT gene. It was treated with Nde I (N) and T4 DNA ligase to excise the HTLV-I enhancer, generating plasmid HTLV-I P-CAT. The 2.0 kb Bam Hi insert from clone pSIS-1 was cloned into the Bam Hi (B) site of HTLV-I P-CAT in either orientation, generating clones HTLV-I P SIS-CAT 1 and HTLV-I P SIS-CAT 2. The transcriptional orientation of the c-sis sequences in each plasmid is shown by an arrow. HTLV-I P SIS-CAT 1 was treated with Avr II (A), Bal 31, T4 DNA polymerase, Nru I (Nt), and T4 DNA ligase, excising nucleotides -23 to 734, as determined by dideoxy sequencing, and generating clone HTLV-I P SIS-CAT 1 AN in activity was obtained with deletions of nucleotides -235 to -99. A construct with nucleotides -99 to 38 had only a low level of activity in A172 cells, but no detectable activity in HeLa or HOS cells. Two other deletion clones of SIS CAT -375/38 were examined. A clone with a deletion of nucleotides -292 to -173 showed very little CAT activity whereas the CAT activity derived from a clone with a deletion of nucleotides -278 to -161 was 5-10-fold greater. In light of the results above, this would suggest that nucleotides -292 to -278 are important for c-sis transcriptional activity.

The c-sis Transcriptional Enhancer Functions in an Orientation and Position-Independent Manner with a Heterologous Promoter

To characterize the activity of the c-sis enhancer sequences, clones were constructed utilizing the HTLV-I promoter. A plasmid with the HTLV-I LTR upstream of the CAT gene, HTLV-I CAT was used for the plasmid constructions (Fig. 5). The HTLV-I enhancer and trans-activating response elements localized between nucleotides -350 and -55 (62) were excised, providing a plasmid with the HTLV-I promoter only, HTLV-I P CAT. Sequences from the c-sis gene, including nucleotides -1373 to 779 were subcloned into a Bam HI site of HTLV-I P CAT, at a site 1.7 kb downstream of the HTLV-I promoter. Clones with the c-sis sequences in either orientation were obtained and designated HTLV-I P SIS
CAT 1 and HTLV-I P SIS CAT 2. Bal 31 deletion clones of these plasmids were obtained which lacked the c-sis promoter, RNA initiation site, and most of the downstream sequences; these clones were designated HTLV-I P SIS CAT 1 AN (Fig. 5) and HTLV-I P SIS CAT 2 AN (not shown).

Each of these clones was transfected into HOS or HeLa cells and CAT assays performed (Fig. 6). HTLV-I P CAT gave rise to significantly less CAT activity than did HTLV-I CAT. CAT activities similar to those obtained with HTLV-I P CAT were obtained with cells transfected with HTLV-I P CAT SIS CAT 1 or HTLV-I P SIS CAT 2. In contrast, cells transfected with HTLV-I P SIS CAT 1 AN (Fig. 6) or HTLV-I P SIS CAT 2 AN (not shown) demonstrated CAT activities comparable to or greater than that of HTLV-I CAT. This shows the ability of the c-sis enhancer to increase the activity of the HTLV-I promoter. In these cells, pSV2 CAT and RSV CAT showed significant activity whereas SP65-CAT3 showed only background levels of CAT activity.

The HTLV-I and II Trans-Activators Increase c-sis Transcriptional Enhancer Activity

HTLV-I and II encode proteins designated tat 1 and tat 2, respectively, which enhance the transcriptional activity of the HTLV-I LTR, the interleukin 2 (IL2) receptor gene, or the interleukin 2 gene in concert with mitogens or phorbol ester (61,76). The finding of c-sis mRNA expression in most HTLV-I and II transformed cell lines (46,77 and our unpublished findings) suggested that the tat gene products might also trans-activate the c-sis gene at a transcriptional level. Thus, either HTLV-I CAT or SIS CAT -1373/94 was co-transfected with a control plasmid or HTLV-I or II tat expression plasmids (Fig. 7). These data demonstrate enhancement of both the HTLV-I CAT and SIS CAT -1373/94 activities by both TAT genes. Co-transfection experiments performed with the bovine
leukemia virus (BLV) tat gene showed no increase in c-sis CAT activity despite significant increase of the BLV LTR CAT activity.

Similar studies were performed with the HTLV-I P CAT plasmids with the c-sis enhancer transfected into HeLa (not shown) or AKR 2B cells (Fig. 8). Whereas co-transfection of MPMV CAT3 with a control plasmid gave rise to low levels of CAT activity, no significant activity was seen with the other plasmids under assay conditions chosen for their lack of

Fig. 7. C-sis Sequences Respond to the HTLV I and II Tat Products. Ten micrograms of each plasmid was transfected into HeLa cells with ten micrograms of a control plasmid, SP65HP, a TAT I expressing plasmid (CRTAdSL3NEO), or a TAT II expressing plasmid (91023 NpX).

Fig. 8. C-sis Sequences Respond to the HTLV I Tat Protein in Enhancing Transcription from the HTLV I Promoter. Ten micrograms of each plasmid was transfected into AKR 2B cells a) without or b) with 10 micrograms of plasmid CRTAdSL3NEO which expresses the HTLV I tat gene product.
sensitivity. However, co-transfection of the tat 1 gene led to a significant increase in HTLV-I CAT or HTLV-I P SIS CAT 1 AN activity, but only minimal or no increases in the activity of the other plasmids.

DISCUSSION

Structure of the c-sis Gene
The c-sis gene, which encodes the PDGF B polypeptide is composed of 7 exons over 22 kb (Fig. 1 an ref. 53,54). Striking similarity with the PDGF A gene is seen, which also includes 7 exons over approximately 24 kb (78). Both genes include a large 5' untranslated sequence with a high GC content in exon 1 and a long 3' untranslated region in exon 7. An AT-rich sequence which may mediate RNA degradation is present in the 3'untranslated region of the PDGF B mRNA (45,79) but not the PDGF A mRNA (78). This is consistent with the long half of the A-chain mRNA (>3 hrs.) compared to the B-chain mRNA (35-40 min) and would suggest that alteration in mRNA stability could play a role in gene regulation (80,81). Splice sites between coding exons occur in similar locations in each gene (78). Alternative splicing of the PDGF A chain mRNA may skip exon 6 (82,83); this has not been detected in PDGF B mRNA expression. Structural alterations involving a loss of part or all of the c-sis gene are found frequently in meningiomas (84 and unpublished observations with M. Smidt).

Regulation of Translation by 5' Untranslated c-sis Sequences
The 5' untranslated sequences of the c-sis gene which include 1028 bp are longer than those of most other genes (85). The presence of upstream minicistrons is similar to that of a number of other genes including proto-oncogenes erb A, abl, fgr, N-myc, bcl-2, int-1, neu, syn, and N-ras (86—89). ATG codons upstream of the authentic initiator codon are also found in the PDGF A chain gene (79). Our previous work has demonstrated that the 5'untranslated sequences profoundly inhibit PDGF B-chain polypeptide synthesis in wheat germ extracts (54). Data provided here also demonstrates inhibition of translation by these sequences in vivo (Fig. 2). Elements mediating this inhibition were localized to either of two sequences between nucleotides 154 and 378 or between nucleotides 398 and 485. Neither of these sequences affect the minicistrons suggesting that inhibition of translation is not due to protein synthesis from the upstream open reading frames. This is to be distinguished from the cases of yeast GCN4 and CPA1 mRNAs in which upstream AUG codons affect translational efficiency (90,91). These findings suggest that secondary structure is most likely involved in translational inhibition in light of the considerable secondary structure present in this region of the mRNA which has a GC content of 70%, and the fact that the inhibition can be relieved by deletion of either of two distinct sequences. Similar results have been obtained by Rao and coworkers (56). Thus, it is likely that although ribosome movement on the mRNA may unfold RNA secondary structure in most cases, there may be situations in which the energy requirements are too high for this to proceed efficiently. There are several other examples in which a stable hairpin loop has been shown to down- regulate translation (92—94). The finding of low levels of PDGF B polypeptide synthesis in vivo despite significant levels of c-sis mRNA would suggest that this form of translational inhibition is operative in vivo (95). This inhibition may be at least partially relieved by the presence of strong transcriptional activity. Utilizing an SV40 enhancer and promoter, we have found no significant differences in the transforming activity of c-sis cDNA for NIH 3T3 cells in the presence or absence of the 5'untranslated sequences (my unpublished observations). Translational inhibition may also be relieved in vivo by trans-
acting factors which enhance protein synthesis as have been demonstrated in other systems (96,97) or by alternative splicing; evidence for these mechanisms functioning in c-sis expression is not yet available.

The c-sis Transcriptional Enhancer

The data presented here maps the c-sis transcriptional enhancer to a region within 375 bp of the RNA CAP site. Sequences between nucleotides −1373 and −375 have no positive or negative regulatory role in this activity (Table 1). Sequences downstream of the RNA CAP site which alter transcriptional activity have not been found, but only sequences within exons 1, 6, and 7, and intron 6 have been examined for enhancer function.

Within the region 375 bp upstream of the RNA CAP site, two regions have been identified which confer positive transcriptional regulatory activity (Fig. 3). Sequences between nucleotides −375 and −235 have an important role in transcriptional activity; sequences between −278 and −292 bp have been shown to play a critical role in this regard. A silencer region between nucleotides −352 and −323, recently described in studies with K562 cells (98), does not affect c-sis transcriptional activity in HOS or A172 cells.

Sequences between −235 and −99 bp also influence the level of transcriptional activity, but no one element within this domain could be identified. Though, two consensus SP1 binding sites are found within this region (54), their role with regards to transcriptional activity is unclear. Additional positive regulator elements downstream of position −99 may be present as suggested by the transcriptional activity of nucleotides −99 to +38 in A172 cells, and to a lesser extent in HeLa and HOS cells. The presence of such a positive regulatory element has been described in a recent study (98).

Experiments were performed to demonstrate that the c-sis enhancer can complement a heterologous promoter derived from the HTLV-I LTR. The c-sis sequences are as active as the HTLV-I enhancer sequences in this regard. They can act at a distance of 1.7 kb, and in either orientation. It was interesting to note, however, that if the c-sis promoter and RNA CAP site was included in these plasmids, no enhancer activity for the HTLV-I promoter could be detected. This likely represented competition between the two promoters for binding RNA polymerase II or other factors critical for RNA synthesis. This phenomenon has been observed in other cases in which two promoters are situated close to one another (99).

Table 1. Activity of the c-sis Transcriptional Promoter in Different Tissue Types

<table>
<thead>
<tr>
<th>Plasmid Transfected</th>
<th>Jurkat</th>
<th>Relative CAT Activity</th>
<th>HeLa</th>
<th>A172</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIS CAT −375/ 38</td>
<td>0.032</td>
<td>0.051</td>
<td>0.071</td>
<td>0.078</td>
</tr>
<tr>
<td>SIS CAT 38/−373</td>
<td>0.017</td>
<td>0.020</td>
<td>0.007</td>
<td>0.011</td>
</tr>
<tr>
<td>SIS CAT −1373/ 94</td>
<td>0.032</td>
<td>0.063</td>
<td>0.063</td>
<td>0.026</td>
</tr>
<tr>
<td>SP65 CAT3</td>
<td>ND</td>
<td>0.018</td>
<td>0.010</td>
<td>0.020</td>
</tr>
<tr>
<td>NE-CAT1</td>
<td>ND</td>
<td>0.080</td>
<td>0.330</td>
<td>0.041</td>
</tr>
<tr>
<td>MPMV-CAT3</td>
<td>3.270</td>
<td>2.550</td>
<td>1.037</td>
<td>1.537</td>
</tr>
<tr>
<td>CMV-CAT</td>
<td>ND</td>
<td>2.579</td>
<td>1.161</td>
<td>1.130</td>
</tr>
<tr>
<td>RSV-CAT</td>
<td>ND</td>
<td>0.320</td>
<td>0.122</td>
<td>0.252</td>
</tr>
<tr>
<td>pSV2-CAT</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
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</tr>
</tbody>
</table>

CAT measurements were performed at 4–5 different time points in 2–4 replicate transfection experiments in each cell type. CAT activity, measured by the slope of acetylation of chloramphenicol versus time of incubation, is divided in each case by the value obtained in the same cell type transfected with pSV2-CAT. ND, not determined.
Regulation of c-sis Transcription

Our previous data suggested that there were high levels of c-sis transcription in endothelial cells compared to fibroblasts (54), which reflected the level of endogenous c-sis mRNAs. Regulation of c-sis transcription in endothelial cells may be critical to normal development of vessels and to the development of atherosclerosis (26–28,30–32,70). However, the work shown here with cell lines which express or do not express endogenous c-sis mRNA failed to demonstrate differences in the level of activity of CAT expressed from upstream c-sis sequences. Furthermore, alterations in c-sis driven CAT activity could not be demonstrated with treatment of monocytes with concanavalin A, K562 cells with phorbol esters, or AKR 2B cells with TGF beta. In each case, published data had suggested that regulation occurred at the level of transcription (20,24,72–75). The failure of the SIS CAT plasmids to reflect these transcriptional alterations may be due to a) regulation by these factors at least partially by other mechanisms, e.g. RNA processing or degradation, or b) by the activity of a cis-acting element which has been omitted from our plasmid, e.g. an enhancer downstream or more than 1.3 kb upstream of the RNA CAP site. Regulation of c-sis gene expression under these circumstances may be important for responses to wounds and other inflammatory stimuli, the development of fibrotic conditions e.g. idiopathic pulmonary fibrosis in which PDGF expression has been implicated etiologically (100–103), and in the genesis of certain mesenchymal tumors including sarcomas and brain tumors (36).

In contrast, transcriptional regulation of the c-sis gene was demonstrated with the HTLV-I and II tat gene products. The activity on the upstream c-sis sequences was demonstrated either with the c-sis promoter or the HTLV-I promoter. The cis acting element conferring tat responsiveness remains to be further localized within the 1.4 kb sequences used in these analyses. No sequence similarities of the c-sis gene to the 21 bp repeated elements of the HTLV-I or II LTRs (62,104,105) or the trans-activator response region of the IL2 receptor (106) were found. In at least the case of the HTLV-I and II LTRs, it appears that the tat product is not directly mediating this activity, but rather secondary factors are responsible for the interactions with the 21 bp elements (62). Thus, distinct secondary factors may be operative in activating each cellular gene and viral genes.

Though PDGF A and B mRNAs and protein products have been demonstrated in most HTLV-I and II transformed cell lines (43–46, 77 and my unpublished findings), their role in viral-mediated leukemogenesis is unclear. Though extracellular membrane receptors for PDGF have not been identified on normal lymphocytes or HTLV-I or II infected lymphocytes (personal communications with T. Deuel and L.T. Williams), these experiments are inconclusive. PDGF receptors may be present on a small subset of lymphocytes only transiently during transformation, and then become down-regulated. Alternatively, expression of PDGF receptors may occur exclusively intracellularly within a subset of lymphocytes. Assays of PDGF receptor mRNA in HTLV infected cells should be helpful in assessing this possibility. Lastly, it remains possible that a distinct PDGF receptor is mediating responsiveness to PDGF products expressed in HTLV infected cells. Evidence for a second class of PDGF receptor has recently been provided (107110). Examination of these questions may better define biochemical mechanisms of transformation by HTLV-I and II. Recently published work has also shown activation of the GM-CSF and c-fos genes by HTLV TAT I (111,112). The element in c-fos responsive to TAT overlaps that which is responsive to v-sis conditioned medium. Thus, TAT activation of sis may play a role in fos activation. The role of these genes in transformation by HTLV-I or II remain to be determined.
ADDENDUM
The HTLV TAT proteins have now been designated TAX (113).

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