trans-Activation of the Simian Virus 40 Enhancer by a pX Product of Human T-Cell Leukemia Virus Type I

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A trans-acting factor, p40, of human T-cell leukemia virus type I profoundly potentiated the function of the enhancer from simian virus 40 but not polyomavirus and Roux sarcoma and murine sarcoma viruses. This trans-activation was seen in a limited repertoire of cells, in contrast to trans-activation of the human T-cell leukemia virus type 1 enhancer by p40.

Enhancers are short stretches of DNA that potentiate the transcription of their own and heterologous genes in a cis-acting fashion. A number of viral (2, 4, 11, 19, 27, 32) and cellular (1, 3, 8, 10, 23, 33, 34) genes have been found to be associated with enhancer sequences. The activity of enhancers is thought to be regulated by their interaction with protein factors, termed trans-acting transcriptional activators. In retroviruses, including oncogenic viruses, the trans-activation mechanism is required for not only expression of viral genes but also viral replication. Human T-cell leukemia virus type I (HTLV-I), which appears to be the etiological agent of adult T-cell leukemia and is a member of the family of retroviruses (14, 17, 35, 36), displays a unique feature of trans-activation of its own viral gene expression. The genome of HTLV-I contains long terminal repeats (LTRs) and the three standard retroviral genes gag, pol, and env. In addition, HTLV-I has a novel open reading frame region, designated as pX, between env and the 3' LTR (25). One of its products, p40, trans-activates gene expression from the promoter unit in the U3 region of the HTLV-I LTR (9, 22, 26, 29). This function was demonstrated to be mediated by a 21-base-pair (bp) motif sequence within the U3 region which functions as an enhancer and is indispensable for the replication of HTLV-I (28). On the other hand, HTLV-I has no typical oncogene and is randomly integrated into the cellular genome (24). The mechanism of cellular transformation by HTLV-I could, therefore, be different from that by other retroviruses, but clear information on the transformation mechanism has not yet been demonstrated. However, products of pX are suggested to play a crucial role in transformation. Hence, it is of importance to elucidate the entire function of p40 with respect to leukemogenesis. During our studies on the trans-acting function of p40, we noted that p40 modulates the function of the simian virus 40 (SV40) early promoter. In the present study, we report that p40 trans-activates the SV40 enhancer in some cell lines.

To assess the trans-acting effects of p40 on cognate and noncognate viral promoters, we used a set of plasmids containing the bacterial chloramphenicol acetyltransferase (CAT) gene under the control of various viral promoter units (Fig. 1). The promoter units used were the HTLV-I LTR, the Roux sarcoma virus (RSV) LTR, and the SV40 early promoter. They were independently linked to the CAT gene, generating pCHL4 (22), pRSVcat (11), and pSV2cat (12), respectively. Other CAT constructs, pMEHL4 and pWEN-CAT, which carry hybrid promoters consisting of the murine sarcoma virus (MSV) enhancer with the enhancerless HTLV-I LTR (pHL4) or the polyomavirus enhancer with the SV40 core promoter, respectively, were also used. These plasmids were cotransfected into 17 different human and mouse cell lines of hematopoietic origin by the DEAE-dextran method (22, 23) along with pMAXneo (22), which can provide the trans-acting p40 function. The cell lines used included those of T and B lymphocyte, monocyte, natural killer cell, and erythrocyte lineages. CAT enzyme activity in cultures of cotransfected cells was compared with that in cells cotransfected with pMAXneo/M, which is a frameshift mutant of pMAXneo that is incapable of producing the functional trans-activator (22).

Each plasmid (10 μg) was transfected with 10 μg of either pMAXneo or pMAXneo/M into cells (8 × 104). DEAE-dextran was used at a concentration of 500 μg/ml for all cell lines except LCL-Kan and HSB2, for which 250 μg/ml was used. The transfected cells were cultured in RPMI 1640 medium supplemented with 20% fetal calf serum, 2 mM l-glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml and were harvested for the CAT assay 48 h after transfection. CAT activity was determined as described previously (12, 22); usually, 20 to 60 μg of total protein from the extract was incubated with 0.1 μCi of [14C]chloramphenicol (54 mCi/mmol; Amersham International, Buckinghamshire, United Kingdom) and 0.5 mM acetyl coenzyme A (Sigma Chemical Co., St. Louis, Mo.) in 500 mM Tris hydrochloride (pH 7.8) for 2 h at 37°C. The products of the reaction were extracted with ethyl acetate, chromatographed on silica gel thin-layer plates (E. Merck AG, Darmstadt, Federal Republic of Germany) in 95% chloroform–5% methanol, and autoradiographed.

In all cell lines tested, cotransfection of pCHL4 with pMAXneo resulted in a progressive increase in the level of CAT activity as compared with that in cells cotransfected with pCHL4 and pMAXneo/M (Table 1). These results are consistent with previous observations in lymphoid and nonlymphoid cells (9, 22, 26, 28, 29). In contrast, in all cells tested, HTLV-I p40 appeared to have no significant effect on CAT expression directed by the promoter unit of RSV (pRSVcat) or the hybrid promoters containing the MSV (pMEHL4) or polyomavirus (pWEN-CAT) enhancer. However, the CAT construct bearing the SV40 early promoter unit (pSV2cat) had patterns different from those with...
either the HTLV-I LTR or other distinct viral promoter units (Table 1). Cotransfection of pSV2cat with pMAXneo resulted in significantly increased (5- to 20-fold) CAT activity in the human T-cell lines Jurkat, HSB2, and Karpas T and in an erythroleukemic cell line, K562. The level of CAT activity varied in different cell lines, but we considered that p40 had a significant effect when CAT expression with pMAXneo was more than three times that with pMAXneo/M, as indicated by the boldface type in Table 1, except with pCHL4.

p40 has been shown to act directly or indirectly on the 21-bp enhancer sequence in the U3 region of the HTLV-I LTR (28). The SV40 promoter unit used contains an enhancer consisting of a 72-bp direct repeat, GC boxes, and the core promoter. Accordingly, we next examined which element of the SV40 promoter unit is responsible for transactivation by p40. For this, we used pSV1Ccat as an enhancerless mutant in which a sequence covering most of the 72-bp direct repeat in pSV2cat is deleted but the GC boxes are still present (Fig. 1). This plasmid was introduced with either pMAXneo or pMAXneo/M into the cells in which p40 trans-activated CAT expression from pSV2cat, and CAT activity was assayed. The results showed that p40 had no effect on the enhancerless SV40 promoter in pSV1Ccat and that the conversion of [14C]chloramphenicol was as low as it was with pSV2cat and pMAXneo/M in all the cell lines tested except for K562, in which the level of CAT activity after cotransfection with pSV1Ccat and pMAXneo was 20-fold lower than it was after cotransfection with pSV2cat and pMAXneo/M (Fig. 2). These results strongly suggested that the SV40 72-bp direct repeat enhancer region is required for trans-activation by p40 of HTLV-I.

This probability was further tested by examination of the CAT activity from CAT-expressing vectors in which the
TABLE 1. Effect of HTLV-I p40 on the activities of viral promoters in various hematopoietic cells

<table>
<thead>
<tr>
<th>Cells*</th>
<th>pCHL4</th>
<th>pMEHL4</th>
<th>pRSVcat</th>
<th>pWEN·CAT</th>
<th>pSV2cat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+p40</td>
<td>−p40</td>
<td>+p40</td>
<td>−p40</td>
<td>+p40</td>
</tr>
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<td>Jurkat</td>
<td>5.9</td>
<td>0.7</td>
<td>3.5</td>
<td>2.8</td>
<td>0.8</td>
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<tr>
<td>HSB2</td>
<td>7.3</td>
<td>0.3</td>
<td>1.3</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>TALL-1</td>
<td>5.4</td>
<td>1.1</td>
<td>2.9</td>
<td>1.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Molt-4</td>
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<td>0.9</td>
<td>1.4</td>
<td>1.6</td>
<td>17.3</td>
</tr>
<tr>
<td>Karpas T</td>
<td>29.4</td>
<td>0.9</td>
<td>7.1</td>
<td>11.9</td>
<td>13.5</td>
</tr>
<tr>
<td>CCRF-CEM</td>
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<td>3.7</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
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<td>8.1</td>
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<td></td>
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<td>Daudi</td>
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<td>0.9</td>
<td>2.4</td>
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<td>0.5</td>
<td>0.4</td>
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<td>P3HR1</td>
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<td>LCL-Kan</td>
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<td>0.1</td>
<td>0.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Natural killer</td>
<td></td>
<td></td>
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<td></td>
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<td>NK-7</td>
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<td>2.3</td>
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<td>K562</td>
<td></td>
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<td></td>
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</tr>
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</table>

* All cells were maintained in RPMI 1640 medium with 10% fetal calf serum, 2 mM l-glutamine, and antibiotics. All the cell lines were of human origin, except for EL-4 and NK-7, which were of murine origin.
* CAT expression is expressed as the percentage of [14C]chloramphenicol converted into acetylated forms. +p40 and −p40 indicate cotransfection with pMAXneo and pMAXneo/M, respectively. Boldface indicates a significant effect of p40 (see text). ND, Not determined.

FIG. 2. p40-dependent trans-activation of the SV40 enhancer. Transfection was done at least in triplicate for each combination of constructs and cells, and typical results are shown. Conditions for experiments and presentation of data were as described in the text and in Table 1, footnotes a and b (nd = ND).
CAT genes are driven by heterologous promoters containing the SV40 enhancer. For construction of these vectors, a 233-bp NcoI-PvuII fragment from the SV40 enhancer region was introduced into pdHL4, which has the HTLV-I LTR lacking the U3 enhancer, at the Clal site in the sense and antisense orientations, yielding pSEHL4 and pSERHL4, respectively (Fig. 1). These hybrid plasmids were similarly cotransfected along with pMAXneo into Jurkat, HS62, Karpas T, and K562 cells, and the CAT activities were compared with those with pMAXneo/M. In all four cell lines, p40 induced a 4- to 10-fold increase in activity (Fig. 2). This enhancing effect was independent of the orientation of the inserted enhancer fragment of SV40. Moreover, enhancement by p40 was demonstrated with other hybrid plasmids (pSEBH14 and pSEBRHL4) carrying the SV40 enhancer fragment at the BamHI site located downstream from the CAT-coding region (Fig. 1), although the magnitudes of the enhancement were lower than with pSEHL4 or pSERHL4 (Fig. 2). These results clearly showed that the p40 enhancing effect is also independent of the location of the cis-acting element. With these plasmids, however, no increase by p40 was seen in U937 and Raji cell lines, as with pSV2cat (Fig. 2). Although the SV40 fragment used as an enhancer contains the GC boxes as well as the 72-bp enhancer sequence, the 72-bp enhancer sequence seems to be responsible for facilitating gene expression mediated by p40 in a trans-acting manner, because the enhancement by p40 was not dependent on the orientation or location of the SV40 element, whereas the GC box function is reported to be dependent on its location (31).

The present study is an attempt to gain insight into the mechanism of trans-activation by HTLV-I p40, which is a nuclear molecule that is probably crucial for leukemogenesis. Examination of the activities of several distinct viral promoter units showed that the SV40 enhancer as well as the HTLV-I LTR was responsive to trans-activation mediated by p40. These results suggested that the activation could occur at a transcriptional level. In addition, we found that trans-activation of the SV40 enhancer was restricted to certain cell lines.

It is unlikely that the high level of CAT activity from the plasmids bearing the SV40 element in the presence of p40 in some cells was due to an increase in the copy number of the plasmids, because the activation by p40 was observed with hybrid plasmids carrying the SV40 fragment, which is inadequate for replication, and because these cells did not express the SV40 large T antigen, which is absolutely required for the replication of SV40.

The repertoire of cell lines showing a p40 enhancing effect on the SV40 enhancer is much smaller than that showing enhancement of the HTLV-I LTR, which is trans-activated in all the cells tested so far. This restricted pattern is distinct from that of polyomavirus, MSV, and RSV enhancers. This difference indicates that the SV40 enhancer does not share a common mode of action of p40 with the HTLV-I enhancer. These observations suggest that each enhancer, including the enhancers of polyomavirus, MSV and RSV, interacts with a respective trans-activator. Therefore, although it is still uncertain whether p40 itself or a cellular factor induced or modified by p40 interacts directly with the 21-bp sequence in the HTLV-I enhancer, our results clearly show that the HTLV-I trans-activator p40 does not directly interact with the SV40 enhancer but is competent in activating at least a cellular factor that modulates the function of the SV40 enhancer. p40 may transcriationally induce the expression of some cellular genes, including the gene for a trans-activator for the SV40 enhancer in certain cells. Recently, transcription of the cellular genes for interleukin 2 and its receptor in Jurkat cells was reported to be induced by introduction of a p40-expressing vector (16, 21). Alternatively, p40 may function as a modulator to activate some preexisting cellular factors in a limited number of cell lines. The latter possibility is supported by a recent report that a tumor promoter, 12-O-tetradecanoylphorbol-13-acetate, activates the SV40 enhancer function by modification of a cellular factor(s) that could interact with the enhancer (15).

A computer search has identified a pentanucleotide, CT-GAC, that is present in enhancers of SV40 and HTLV-I (our unpublished data). This sequence is also found in the 5' regulatory regions of the interleukin 2, interleukin 2 receptor, and adenovirus E3 genes, which are all trans-activated by HTLV-I p40 or the equivalent product of the closely related HTLV-II; the adenovirus E3 gene is also trans-activated by its own viral E1A product via a cellular factor (5). In the SV40 enhancer, this sequence is located at the end of an element designated as P (7) and is also present in the element designated as A by Herr and Clarke (13); both of these elements are thought to participate in SV40 enhancer function, but the sequence is distinct from the known core sequence of the SV40 enhancer. This sequence might be commonly involved in the function of the enhancers rather than in the determination of their respective specificities. Apart from this sequence, we could not find any significant homology between the enhancers of HTLV-I and SV40. It may be significant that K562 cells have an abnormality in the expression of the c-abl proto-oncogene, which encodes a tyrosine-specific protein kinase (6), and that Jurkat cells are reported to express the lsk/high gene product, which is also a tyrosine-specific protein kinase (20, 30). These tyrosine kinases may lead to the expression of some cellular genes, including the gene for a possible factor that, after modification by HTLV-I p40, interacts with the SV40 enhancer. This mechanism could explain why only a few cell lines show trans-activation of the SV40 enhancer by p40.

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LITERATURE CITED


