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CELL-LINE SPECIFIC ACTIVATION OF SV40 TRANSCRIPTIONAL ENHANCER BY p40^{tax} OF HTLV-1

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A transcriptional *trans*-activator p40^{tax} of HTLV-1 was reported to activate HTLV-1 enhancer, but not SV40 or Rous sarcoma virus enhancer. However, in certain cell lines, we found that SV40 enhancer was activated by p40^{tax}. These cell lines were mostly T cells, where the SV40 enhancer showed only low activity without p40^{tax}. Since p40^{tax}-mediated activation of the LTR is not cell line-specific, the activation of enhancers by p40^{tax} depends on the combination of enhancer and cell type used for the test. Thus, apparent activation by p40^{tax} depends on variable cellular components involved in transcriptional regulation.

Key words: HTLV-1 *trans*-activator — SV40 enhancer — pX protein — Cell specific enhancer

Human T-cell leukemia virus type 1 (HTLV-1)[1,2] contains an extra sequence "pX" in its genome[3] and the *tax* gene in this region codes for a *trans*-acting factor, p40^{tax}. p40^{tax} activates transcription from HTLV-1 long terminal repeat (LTR)[4–9] responding to an enhancer element in the LTR.[10–12] In addition to the LTR, cellular genes of interleukin-2 (IL-2) and its receptor (IL-2R) are also activated by p40^{tax}.[13–15] This function of p40^{tax} may account for the abnormal, polyclonal proliferation of HTLV-1-infectd T-cells at an early stage of adult T-cell leukemia development.[16] However, other viral promoters such as SV40 early promoter or Rous sarcoma virus (RSV) LTR, which show high activities in a variety of cells, were not further activated by p40^{tax} in several cell lines.[5] Thus, p40^{tax} was concluded to have some specificities for activation of promoters.

In contrast to the previous findings that SV40 promoter was not activated by p40^{tax},[5] we found here that the SV40 enhancer is *trans*-activated by p40^{tax} in certain cell lines, most of which were T cells. The SV40 promoter is known to be a strong promoter in a variety of cell types, but it does not work efficiently in T cells. This unexpected, cell line-specific activation of SV40 promoter by p40^{tax} would provide a useful system for analyzing the mechanism of the transcriptional activation by p40^{tax}, because the SV40 promoter is well studied and several sequence motifs and their binding proteins have been identified.[17,18] In addition, the system might also be useful for studying the regulation of gene expression in T cells, since the effect was mostly observed in T cells.

pSV2CAT construct was transfected with or without *tax* expression plasmid pMTPX[9] by the DEAE-dextran method, and the CAT activity was assayed as described previously.[5] pSV2CAT[19] expressed CAT activity at only low levels in T cell lines, but the expression was strongly activated upon cotransfection of *tax* expression plasmid. These results were obtained in T cell lines, such as HSB-2, CEM, Molt 3, and Jurkat and also in non-T cell lines K562 (pre-erythroid), HL60 (pre-myeloid) and Ball-1 (B cell). Some typical results are presented in Fig. 1. In contrast to these cell lines, most B cell lines so far tested (fifteen cell lines) showed high CAT activity without *tax* plasmid and the activity was not enhanced further by *tax* (Fig. 1). The results with B cell lines are similar to those previously reported with fibroblasts and epithelial cells.[5] pRSVCAT containing the RSV LTR tested in control experiments was not activated significantly by *tax* even in T cell lines. Therefore, it was concluded that activation of SV40 promoter by p40^{tax} is a specific event, not a

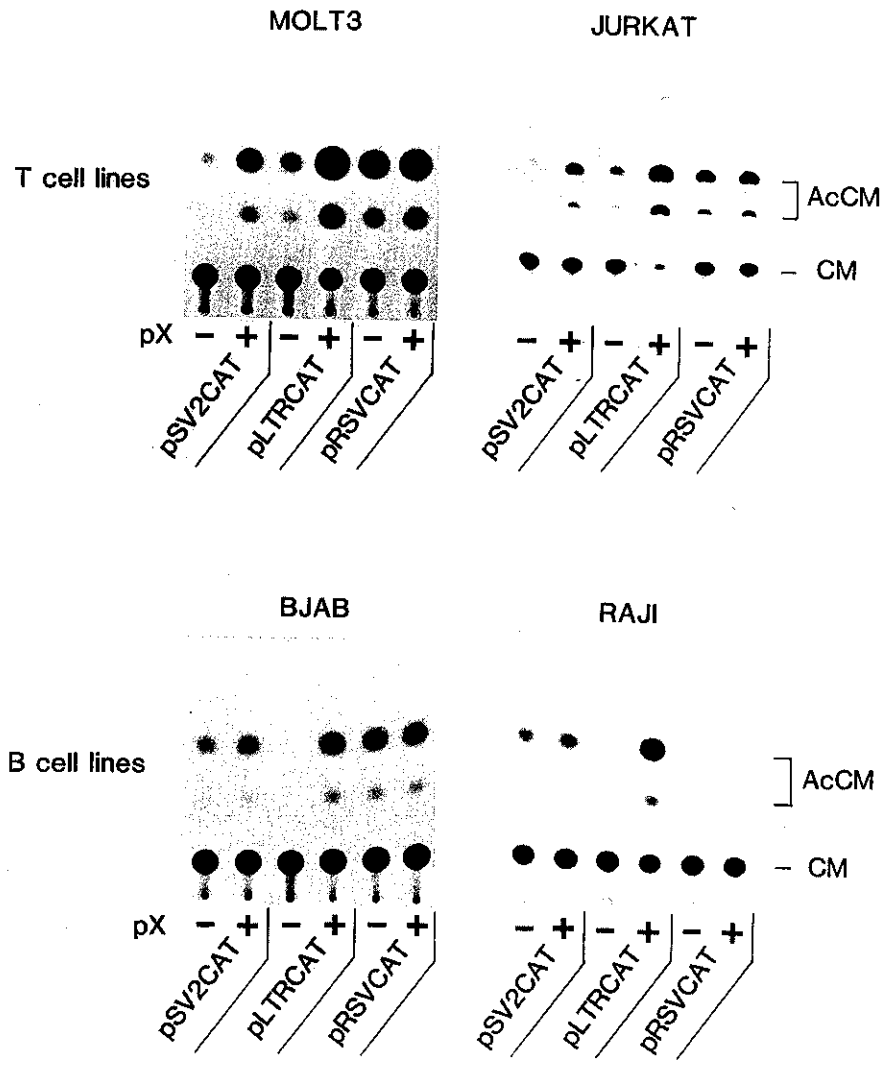

Fig. 1. Activation of CAT expression by $p40^{tax}$ in various cell lines. A CAT construct (4 μg) was transfected into each cell line with (+) or without (−) *tax* expression plasmid pMTPX (1 μg), and CAT activity was assayed 2 days after transfection as described previously.[5] AcCM represents the acetylated form of chloramphenicol (CM).

simple reflection of a general activation of cellular functions. This cell-line specificity is in contrast to *trans*-activation of the HTLV-1 LTR, which did not show any cell-line specificity.[4, 5] According to Cross *et al.*,[15] however, pSV2CAT was not activated by $p40^{tax}$ in Jurkat cells. The reason for this contradiction between their and our results is not known. Subclones of Jurkat cells might explain these contradictory findings.

To determine the sequence responsible for *tax*-mediated *trans*-activation, a 200 bp fragment containing the SV40 enhancer was inserted into an enhancer-less promoter pdN55 of the HTLV-1 LTR to construct pSVdN55 (Table I). In the absence of $p40^{tax}$, pSVdN55 expressed a low level of CAT activity similar to that with pdN55 in T cell lines such as HSB-2, CEM (Table I), Molt 3, and Jurkat (data not shown). These results revealed that

Table I. Relative CAT Expression with Various Enhancers

Plasmid construction		Relative CAT activities in HSB-2		CEM		BJAB		K562	
	pX	−	+	−	+	−	+	−	+
pdN55	R U5 CAT	1	3	1	3	1	1	1	21
pLTRCAT	U3 R U5 CAT	1	60	1	140	1	13	18	2800
pSVdN55	SV R U5 CAT	1	39	1	150	37	45	13	790
pRSVdN55	RSV R U5 CAT	NT	NT	360	410	NT	NT	690	760

The enhancer-less CAT construct pdN55 was derived by deletion of the *Hin*d III-*Nde* I fragment from the pLTRCAT. Into the *Nde* I site of pdN55, a fragment (200 bp) containing SV40 enhancer (nucleotide positions 95–294 of the SV40 map) was inserted to construct pSVdN55, and RSV enhancer (428 bp *Pvu* II-*Eco* RI fragment) to construct pRSVdN55. CAT enzymes formed in the presence (+) or absence (−) of *tax* plasmid were assayed as in Fig. 1 and the activities are presented in ratios to that of pdN55 without *tax* expression in each cell line. This corresponds to 2.7% acetylation of CM per hour per 100 μg of proteins in HSB-2 cells, 0.6% in CEM, 0.2% in BJAB, and 0.8% in K562 cells. NT, "not tested."

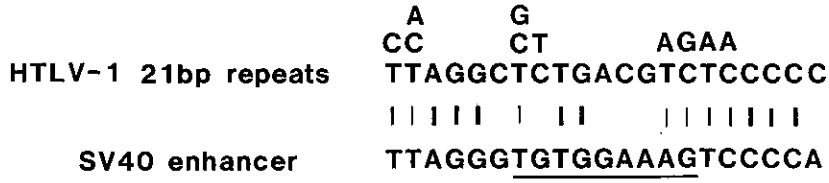

Fig. 2. Nucleotide sequence homology between SV40 early enhancer and HTLV-1 enhancer. HTLV-1 LTR has three direct repeats of 21 bp which differ from each other in a few bases and only the different bases are shown above the 21 bp sequence. The underlined sequence is the "core" sequence of the SV40 enhancer.

SV40 enhancer was virtually inactive in T cell lines. Cotransfection of *tax* expression plasmid, however, enhanced the CAT expression about 40 to 150 fold. On the other hand, the enhancer-less construct pdN55 was not activated (Table I). These observations clearly indicate that the SV40 enhancer sequence is responsible for the cell-line-specific *trans*-activation by $p40^{tax}$.

When other enhancers including those of the RSV LTR (Table I), E1A promoter of adenovirus type 2, LTR of Moloney murine leukemia virus and early promoter of polyoma virus (data not shown), were tested, none of them was activated by $p40^{tax}$. Thus, *trans*-activation by $p40^{tax}$ is apparently restricted to certain types of enhancer. In this respect, a homology of the SV40 enhancer with HTLV-1 enhancer[10] may be significant although it is very limited (Fig. 2).

It might be also noteworthy that enhancer-less promoter pdN55 could be augmented significantly by $p40^{tax}$ only in a few cell lines such as K562 (Table I) and Ball-1 (data not shown). These activations of enhancer-less promoter do not seem to be attributed to

enhancer specific factors, and therefore other factors were also proposed to be involved in such activation.

These observations clearly show that *tax* activation of a certain enhancer or promoter depends on the combination of the enhancer and cell type in which the activity is tested. That is, the factor(s) involved in activation of the SV40 enhancer may be less active or limited in T cell lines when compared with those in other cell lines, but $p40^{tax}$ can complement some of these defects. Thus, it is possible that a cellular factor involved in *tax*-mediated activation of SV40 enhancer could be different from that for LTR activation, because the LTR activation did not show any cell line specificity. Supporting this idea, a cell-type specific protein that binds to the SV40 enhancer was reported by Davidson *et al.*[20] $p40^{tax}$, therefore, might function similarly to the E1A protein of adenoviruses, which does not bind DNA directly, but modifies the activity of DNA-binding proteins.[21,22] The E1A protein can also activate an enhancer-less promoter similarly to $p40^{tax}$.[23] In fact, Chen *et al.*[24] have reported functional similarity between $p40^{tax}$ and the E1A protein; namely they cross-activate mutual target promoters.

Characterization of factor(s) involved in the *trans*-activation by $p40^{tax}$ would provide insights into the mechanism of cellular gene activation by exogenous viral factors, which might be associated with tumorigenesis. In addition, a noteworthy observation is the very low activity of SV40 early promoter in T cell lines, although this promoter is widely used as an expression vector or a positive standard for high expression.

While the manuscript was in preparation, a similar result has been reported by Saito *et al.*[25]

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