Effect of Human T-Cell Leukemia Virus Type I Tax Protein on Activation of the Human Vimentin Gene

ALAIN LILIENBAUM,¹ MADELEINE DUC DODON,² CYRILLE ALEXANDRE,² LOUIS GAZZOLO,²
AND DENISE PAULIN^{1*}

Université Paris 7, Institut Pasteur, 25 Rue du Docteur Roux, 75015 Paris,¹ and UMR 30 Centre National de la Recherche Scientifique-UCBL, ImmunoVirologie Moléculaire et Cellulaire, Faculté de Médecine A. Carrel, 69372 Lyon,² France

Received 2 February 1989/Accepted 27 September 1989

We report that the expression of the vimentin gene, a cytoskeletal growth-regulated gene, is activated in *trans* by the Tax (p40x) transactivator protein encoded by the human T-cell leukemia virus type I. Expression of the Tax protein activates a number of cellular genes, such as those coding for the α chain of the high-affinity interleukin-2 receptor and interleukin-2. These findings indicate that the Tax protein is involved in the unregulated T-cell growth associated with human T-cell leukemia virus type I infection. Higher levels of vimentin mRNA were expressed in two human T-cell leukemia virus type I-transformed T cell lines, C91/PL and C81-66/45, when compared with that in Jurkat T cells. We demonstrate that this activation is conferred by the vimentin upstream flanking sequences. Indeed, enhanced activity was detected when constructs with the vimentin promoter linked to the chloramphenicol acetyltransferase gene were transfected in HeLa cells and in two cell lines of hematopoietic origin (Jurkat T lymphoblastoid cells and U937 promonocytic cells) together with a Tax expression plasmid. By introducing a series of deletions in the vimentin promoter, we further restrict these sequences to 30 base pairs, located between 241 and 210 base pairs upstream of the mRNA cap site. A 40-base-pair oligonucleotide containing this regulatory region proved sufficient to confer Tax inducibility upon a heterologous promoter linked to chloramphenicol acetyltransferase. Importantly, this segment includes an 11-base-pair promoter segment that has homology with the binding site for the NF- κ B transactivating factor. Our findings indicate that constitutive expression of the vimentin gene under the control of the Tax protein may be relevant in understanding the progression of the lymphoproliferative process associated with human T-cell leukemia virus type I infection.

Epidemiological studies have shown that human T-cell leukemia virus type I (HTLV-I) is associated with adult T-cell leukemia; however, the role of HTLV-I in the leukemogenesis process has not yet been established. Only a very low percentage of seropositive individuals ever develop the leukemia after a latent period that may be as long as 30 years after primary infection. Furthermore, the leukemic cells do not express viral antigens (38, 39). These observations suggest that HTLV-I is mainly involved in the initiation of the lymphoproliferative process but is not required for the maintenance of the malignant phenotype. Recent data have shown that HTLV-I gene products are involved in the activation of T cells. Viral envelope glycoproteins of HTLV-I can promote polyclonal T-cell expansion, suggesting that the *env* products play a major role in the early phase of T-cell activation (6, 9). After proviral integration, the Tax protein, one of the three proteins encoded by the pX region, is able to trigger the expression of interleukin-2 (IL-2) receptors (IL-2Rs) (4, 12, 18) and the synthesis of IL-2 (12, 18, 35), probably by inducing cellular factors acting on the transcription of these genes involved in T-cell proliferation (2). These observations support a role for the Tax protein in sustaining a constitutive cell activation. Furthermore, the abundant transcription of cellular genes in T lymphocytes infected with HTLV-I favors such a hypothesis (17).

In this report, we show that HTLV-I-transformed T lymphocytes express high levels of vimentin mRNA. This observation, which was made in the course of a comparative study involving B and T lymphoblastoid cells, led us to postulate that the vimentin gene may be transactivated by the HTLV-I Tax protein. Vimentin, a member of the intermediate filament family, behaves like a growth-regulated gene in many cells, including fibroblasts and human peripheral blood mononuclear cells (13). Stimulation of quiescent fibroblasts (BALBc/3T3) by serum or by a growth factor such as the platelet-derived growth factor or B and T lymphocytes by lectins such as phytohemagglutinin results in an increase of the vimentin mRNA levels (7).

To evaluate a possible effect of the Tax protein on the expression of the human vimentin gene, we isolated DNA sequences within the upstream region of the human vimentin gene that were important for gene transcription. Transient transfection assays together with a Tax expression plasmid indicated that this protein is able to activate the human vimentin promoter in human cells of nonhematopoietic or hematopoietic origin. Furthermore, experiments with deletion mutants of the human vimentin promoter region linked to a chloramphenicol acetyltransferase (CAT) gene indicated that a region at -241 to -140 base pairs (bp) relative to the transcription initiation site is sufficient for Tax-inducible CAT expression. Further experiments revealed that a 30-bp promoter segment of this region, homologous with the binding site of NF- κ B, was required for Tax-induced activity of the human vimentin promoter.

* Corresponding author.

MATERIALS AND METHODS

Plasmids. We have previously cloned and identified part of the human vimentin gene (24). This cDNA started at exon 2 and did not include the 5'-flanking sequence. To obtain a clone containing the 5'-regulatory sequences, we screened a human genomic library with human vimentin cDNA. The clone lambda-V54 was then selected for further studies.

To investigate the functional importance of the regulatory regions necessary for the activation by the HTLV-I Tax protein, a series of deletion mutants from the 5' end was constructed by taking advantage of unique restriction sites. These clones, all ending at nucleotide +93 from the cap site, were placed upstream of the bacterial CAT reporter gene. The nine clones generated started at positions -1710, -957, -830, -529, -424, -338, -241, -140, and -78, respectively, and are shown in Fig. 2. Two additional deletion mutants starting at positions -210 and -170, respectively (designated as the -210 and -170 mutants), were generated by digestion of the mutant starting at position -241 with exonuclease III and mung bean nuclease. Sequencing of these mutants was carried out by the dideoxy-chain termination method (31) with a Sequanase sequencing kit (U.S. Biochemical Corp.).

The pMTPX plasmid, described by Seiki et al. (33), expresses the Tax (p40), Rex (p27), and p21 proteins of HTLV-I under the control of the metallothionein promoter.

The pBS plasmid was generated by digestion of pMTPX by *Bam*HI (position 5095) and *Sph*I (position 5126) (32). The 5' and 3' overhangs were filled in with Klenow fragment and T4 polymerase, and blunt ends were ligated. This double digestion removes a fragment of 31 bp that contains the ATG of Rex protein (position 5125). This plasmid expresses only the Tax and p21 proteins.

The pMTΔ plasmid was obtained by digestion of pMTPX by *Bam*HI (position 5095) and *Spa*I (position 8473). The 5' and 3' overhangs were filled in, and blunt ends were ligated. This control plasmid does not express any protein of HTLV-I encoded for by the pX open reading frame.

The NFtk-CAT plasmid was obtained by cloning a double-strand oligonucleotide, 5' ACTATCATCCGAAAGCCCC CAAAAGTCCCAGCCCAGCGC 3', synthesized after the nucleotide sequence of the vimentin promoter between nucleotides -197 and -237, with a *Hind*III site added at the 5' end and an *Sph*I site at the 3' end, into a pBLCAT2 vector, which contains the herpes simplex virus thymidine kinase (tk) promoter controlling the transcription of a CAT gene (16).

Cell lines. The HTLV-I-transformed T-cell lines C91/PL (25) and C81-66/45 (30) (kindly provided by R. C. Gallo, Bethesda, Md.) were grown in RPMI 1640 supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, and antibiotics. These cell lines were obtained after transformation of cord blood cells cocultivated with HTLV-I-producing cells. Both cell lines express the Tax protein, but the C81-66/45 cell line is nonproductively infected with HTLV-I, since these cells do not express *gag* antigens or reverse transcriptase activity and viral particles are not detected in the culture medium.

The Jurkat cells, a human T-cell leukemia line (supplied by R. DeWaal Malefijt, Unicet, Lyon), and the U937 promonocytic cell line were grown in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, and antibiotics. HeLa cells, a human epithelioid carcinoma line, were grown in minimum essential medium supplemented with 10% FCS, 2 mM L-glutamine, and antibiotics.

Transfection and CAT assays. A DEAE-dextran method was used for transfection of cells growing in suspension (adapted from Ohtani et al. [23] with minor modifications). Briefly, 5×10^6 cells washed with Hanks solution were suspended in 1 ml of RPMI 1640 containing 0.3 mg of DEAE-dextran per ml and 10 µg of DNA (4 µg of CAT plasmid and 2 µg of pMTPX supplemented with 4 µg of carrier pUC18) and incubated for 30 min (C81-66/45 and U937) or 2 h (Jurkat) at 37°C. The cells were then washed and suspended in medium containing 10% FCS, incubated at 37°C for 48 h, and then harvested for the CAT assay. Cell extracts were prepared by lysis in 250 mM Tris (pH 8) containing 0.05% sodium dodecyl sulfate, and CAT assays were performed as previously described (11). Briefly, half of the cell extract was incubated with 0.1 µCi of [¹⁴C]chloramphenicol at 37°C for 90 min. The acetylated forms of chloramphenicol were separated by thin-layer chromatography, and the radioactive spots were cut from the chromatography plate and counted in a liquid scintillation counter to quantitate the CAT activity.

The calcium phosphate coprecipitation technique was carried out for the transfection of HeLa cells by the method of Chen and Okayama (3). Briefly, HeLa cells were seeded at 1.5×10^6 cells per 100-mm plate in minimal essential medium supplemented with either 10 or 2% FCS. Four micrograms of the indicated CAT construct DNA was transfected along with 2 µg of pMTPX and pUC18 up to a final DNA concentration of 20 µg. After 12 h of incubation, cells were washed and incubated in minimal essential medium supplemented with the appropriate serum concentration for another 24 h. CAT activity in cell extracts was determined as described above.

Northern blots. Total RNA was prepared for adding 20 ml of 3 M LiCl-6 M urea to each g of cells (previously washed three times with phosphate-buffered saline [PBS]), and the mixture was homogenized in a Teflon-glass Potter homogenizer. The RNA was then prepared as described by Auffray and Rougeon (1). RNA electrophoresis under denaturing conditions in the presence of formamide, transfer to nitrocellulose, and hybridization were carried out as described by Goldberg (10). Hybridization of 10^6 cpm of ³²P-labeled nick-translated probe cHuVim1 (24) per ml to filter-bound RNA was performed at 42°C for 15 h. As a control, the same blots were hybridized with glyceraldehyde-3-phosphate dehydrogenase cDNA. After hybridization, the filters were washed three times at room temperature with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-0.1% sodium dodecyl sulfate for 15 min each, twice at 15°C with 1× SSC-0.1% sodium dodecyl sulfate and then twice with 0.1× SSC-0.1% sodium dodecyl sulfate at 65°C for 30 min.

RESULTS

Transcriptional expression of the vimentin gene in HTLV-I-infected T-cell lines. We previously reported that normal human T lymphocytes isolated from peripheral blood express vimentin at all stages of maturation (5). We further demonstrated that the amount of this protein was regulated at the transcriptional level (15). Because HTLV-I deregulates T-cell growth, we hypothesized that viral infection might alter the transcription of the vimentin gene in T cells. To investigate this hypothesis, total cellular RNA was extracted from C91/PL, C81-66/45, and Jurkat cells (T-cell lines) and examined by Northern analysis. The steady-state levels of vimentin mRNA were three- and fivefold higher in the HTLV-I-transformed T-cell lines C91/PL and C81-66/45,

FIG. 1. Transcriptional expression of the vimentin gene in Jurkat cells (lane 1) and in two HTLV-I-infected T-cell lines, C91/PL (lane 2) and C81-66/45 (lane 3). Total cellular RNA was extracted from 10⁷ cells and subjected to Northern blot analysis as described in Materials and Methods. Filter-bound RNA was then hybridized to ³²P-labeled human vimentin cDNA (cHuVim 1) (10⁸ cpm/μg) and then to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

respectively, than in Jurkat cells (Fig. 1). In these three cell lines, the amounts of glyceraldehyde-3-phosphate dehydrogenase mRNA were identical.

These results support the view that the increase in vimentin gene expression might be related to the infection of T cells by HTLV-I. Furthermore, the sole expression of the Tax protein by C81-66/45 cells (data not shown) raised the possibility that Tax is responsible for the transcriptional activation of the human vimentin gene in HTLV-I-infected T cells. Before directly assessing the effect of Tax on vimentin gene expression, we performed a functional analysis of the human vimentin promoter in human cells.

Expression of the human vimentin gene in mammalian cell lines: deletion analysis of the vimentin promoter. As described in Materials and Methods, a series of truncated promoter constructs linked to the CAT reporter gene was generated. The different plasmids were then transfected into exponentially growing HeLa cells cultured in medium supplemented with 10% FCS. Transfected cells were cultured for 48 h, washed, lysed, and assayed for CAT activity.

The full-length promoter gave a significantly high CAT activity, which was unaffected by deletion as far as position -957 (Fig. 2). A higher activity was detected when the promoter was deleted to position -830. The CAT activity was reduced 10-fold with the vimentin promoter deleted to position -529. The same low activity was maintained in position -338. The CAT activity reached the same level observed with the full-length promoter with deletion to position -241. CAT activity dropped approximately sevenfold when the promoter was further deleted to position -140. Removal of the CAAT box by deletion to position -78 abolished the activity.

These observations indicate that the human vimentin promoter is composed of at least two positive elements (the first between positions -1710 and -529 and the second between positions -241 and -140) and of one negative element (from positions -529 to -241). These results obtained with human cells confirm those reported by Rittling and Baserga (26), who analyzed the human vimentin promoter in transiently or stably transfected murine 3T3 cells.

The above data were obtained with HeLa cells growing in medium supplemented with 10% FCS. Since the regulation of vimentin is cell growth dependent, the functional analysis of the human vimentin promoter was then performed in HeLa cells maintained in medium supplemented with 2% FCS. As expected, lowering the serum concentration resulted in a significant decrease in the promoter activity (Fig. 2). The higher CAT activity observed with plasmid -830 confirmed the presence of a serum-responsive element in this region. Indeed, an AP1/c-jun binding site recently identified at nucleotide -707 mediated serum and phorbol ester inducibility of the human vimentin promoter (27). Nevertheless, our results show an increased activity of the -830 mutant compared with that of the -957 mutant in HeLa cells when cultured in 2% FCS but not when cultured in 10% FCS. Furthermore, a marked decrease in activity was ob-

FIG. 2. Functional analysis of the activity of the vimentin deletion mutants. The 5' deletions of the 5'-flanking region were generated and cloned upstream of a promoterless CAT gene. Constructs were then used for transfection in HeLa cells cultivated in medium supplemented with either 10 or 2% FCS or in Jurkat and U937 promonocytic cells cultivated in medium supplemented with 10% FCS. After 48 h, the cells were harvested and assayed for CAT activity as described in Materials and Methods. Conversion of [¹⁴C]chloramphenicol to 1- or 3-acetylated chloramphenicol was measured, and the results are expressed as percent acetylated chloramphenicol. Symbols: —, vimentin 5'-flanking sequences (the numbers on the left represent the nucleotide lengths of the inserts from the transcription initiation sites); □, CAT coding sequence. ND, Not done.

TABLE 1. Effect of the Tax protein on the activity of the human vimentin promoter-CAT fusion plasmids transfected in human T and non-T cells^a

Transfected cells	Addition of pMTPX	% Acetylated chloramphenicol in transfected cells					
		957 ^b	529	338	241	140	78
C81-66/45	—	2.9	0.9	8.6	31.1	3.4	2.7
Jurkat	+	12.4	6.1	8.2	61.0	12.6	1.1
U937	+	48.6	17.6	73.4	94.2	13.0	0.7
HeLa	+	9.4	35.9	44.7	80.1	15.6	1.0

^a Human vimentin promoter-CAT fusion plasmids having various 5' deletion endpoints were transfected with the pMTPX plasmid into Jurkat T cells and U937 promonocytic cells growing in medium supplemented with 10% FCS and into HeLa cells maintained in medium supplemented with 2% FCS. C81-66/45, a Tax-expressing cell line, was not transfected with the pMTPX plasmid. Results are expressed as percent acetylated [¹⁴C]chloramphenicol. CAT activity values of each promoter deletion mutant transfected in Jurkat, U937, and HeLa cells without the pMTPX plasmid are those reported in Fig. 2.

^b Numbers in column subheadings represent the nucleotide lengths of the inserts from the transcription initiation sites.

tained upon deletion to position -529 in HeLa cells grown at both serum concentrations. These observations rather suggest that a serum response element is located upstream of nucleotide -830 and that a positive element that functions at both serum concentrations lies between nucleotides -830 and -529.

The functional analysis of the human vimentin promoter was next examined in two human cell lines of hematopoietic origin: Jurkat T cells and U937 human promonocytic cells. These cells were cultured in medium supplemented with 10% FCS. In contrast with HeLa cells cultured at the same FCS concentration, a low basal CAT activity was observed in U937 and Jurkat cells transfected with the different constructs. These results indicated that the two serum-responsive regions identified in HeLa cells are not active in these two hematopoietic cell lines. This comparative analysis indicates that specific serum-inducible factors acting on the transcription of the vimentin gene in epithelial adherent cells are either inactive or absent in the hematopoietic cellular environment.

Effect of the Tax protein on human vimentin promoter activity. In conjunction with the above analysis it was of interest to study the promoter activity of the different deletion mutants in C81-66/45 cells. Elevated CAT activity was obtained after transfection of the promoter deleted to position -241 (Table 1). As emphasized above, C81-66/45 cells expressed only the Tax protein together with high levels of vimentin. These results indicate that the Tax protein may be involved in the active transcription of this cytoskeletal protein gene. The ninefold decrease in CAT activity observed with the -140 truncated promoter compared to the -241 deletion mutant indicated that the region between positions -241 and -140 should confer Tax inducibility to the human vimentin promoter.

To further confirm the effect of the Tax protein on the regulation of the human vimentin promoter, transient transfection studies were then performed in Jurkat T cells and in U937 promonocytic cells (growing in medium supplemented with 10% FCS) and in HeLa cells (cultured in medium with 2% FCS). In all of these cell lines, the HTLV-I long terminal repeat promoter was strongly activated (data not shown) after transfection of a long terminal repeat-CAT construct with the pMTPX plasmid, which expressed the three pX proteins: Tax (p40x), Rex (p27x), and p21x.

FIG. 3. Activity of the promoter CAT fusion plasmid deleted to position -241 transfected with different pX gene expression plasmids in human U937 cells. Cells were transfected with this together with the pMTPX plasmid (expressing Tax, Rex, and p21 proteins) or with the pBS plasmid (expressing Tax and p21 proteins) or with the control pMtΔ plasmid. Results are expressed as the percent acetylation of [¹⁴C]chloramphenicol to 1- or 3-acetylated chloramphenicol. Shown is the autoradiogram of the thin-layer chromatography plate.

Jurkat, U937, and HeLa cells were transfected with each of six CAT fusion plasmids encompassing different elements of the vimentin promoter, namely, constructs -957, -529, -338, -241, -140, and -78, together with the pMTPX plasmid. Forty-eight hours later, CAT activity was determined in extracts of the transfected cells. Tax-dependent CAT activity increased as the 5' sequence was shortened to reach a maximum with the human vimentin promoter mutant deleted to position -241 (Table 1). Removal of nucleotides between positions -241 and -140 caused decreases in CAT activity of five-, seven-, and fivefold in Jurkat, U937, and HeLa cells, respectively. The Tax-inducible promoter activity is not retained with the construct containing only 78 nucleotides upstream of the transcription initiation site. Finally, in HeLa cells, a region from positions -529 to -338, previously defined as negative in HeLa cells maintained in medium supplemented with 10% FCS, did not interfere with inducibility conferred by Tax to the promoter.

Finally, the activation of the human vimentin promoter in the different cell lines was dependent of the expression of the Tax protein, since a mutant pX plasmid that expressed only the Tax protein could fully support activation of the human vimentin gene. Conversely, a mutant plasmid that did not express any pX-related proteins failed to activate the vimentin promoter (Fig. 3).

These results indicated that the 5'-flanking region of the human vimentin gene contains nucleotides that can be activated by the Tax protein. Furthermore, the 5' boundary of the *cis*-acting element required mainly for this activation is localized between 241 and 140 bp upstream from the transcription initiation site.

Analysis of the -241- to -140-bp region: localization of an

FIG. 4. Analysis of the Tax-inducible regulatory domain of the vimentin gene. The upper part of the figure shows a schematic representation of the positions of the NF- κ B and of the related CLE2 motifs. In the lower part of the figure are presented the activities of the human vimentin promoter-CAT fusion plasmids deleted to positions -241, -210, -173, and -140 and transfected in Jurkat cells with (■) or without (□) the pMTPX plasmid. Results are expressed as percent acetylation of [14 C]chloramphenicol or 1- or 3-acetylated chloramphenicol.

NF- κ B consensus sequence. Sequence analysis of the region between positions -241 and -140 revealed the presence of three elements which are potential targets of Tax activation. Thus, an element having homology with the NF- κ B binding site was localized at nucleotides -227 to -217. This element has been involved in the transactivation by Tax of the IL-2R α -chain gene (14). The CLE2 element, found twice, between nucleotides -197 and -191 and between nucleotides -160 and -154, was implicated in the Tax activation of the granulocyte-macrophage colony-stimulating factor gene (21).

To identify which of these elements is required to confer Tax inducibility to the vimentin promoter, we generated two new deletion plasmids by exonuclease III and mung bean nuclease digestion of the promoter construct starting at position -241. These new constructs retained only 210 and 173 nucleotides upstream from the transcription initiation site. A 10-fold decrease in the Tax-induced promoter activity was observed in Jurkat cells transfected with these constructs, when compared with the promoter activity in cells transfected with the -240 construct (Fig. 4). The same results were obtained after transfection of these constructs in U937 and HeLa cells (data not shown).

These data demonstrate Tax-inducible promoter activity in the 5'-flanking region of the human vimentin promoter located between positions -241 and -210. They further suggest that the NF- κ B present in this region may be responsible for Tax activation. To confirm this possibility, we constructed the NF κ -CAT transcription unit. Synthetic oligonucleotide duplexes encompassing nucleotides -197 to -237 of the human vimentin promoter were cloned immediately upstream from the herpes simplex virus tk-CAT construct. NF κ -CAT and tk-CAT plasmids were then transfected alone in C81-66/45 cells or together with the pMTPX plasmid in U937 and Jurkat cells. The NF κ -CAT construct was activated in C81-66/45 cells, in U937 cells, and in Jurkat cells (but only in the presence of the Tax expression plas-

mid), whereas tk-CAT was not Tax inducible in these cell lines (Fig. 5). Collectively, these results demonstrate that the 40-bp segment spanning the NF- κ B site proved sufficient to confer Tax inducibility to a heterologous promoter.

DISCUSSION

Infection of human T cells in vitro by HTLV-I leads to the autonomous growth of these cells in the absence of IL-2. This immortalization event has been ascribed to the Tax protein, which was first characterized, as a protein transactivating the viral gene transcription, through the enhancer element located in the HTLV-I long terminal repeat (22). Furthermore, Tax was shown to alter, either alone or in combination with lectins, the expression of cellular genes involved in T-cell activation and growth, such as those

FIG. 5. Activity of tk-CAT plasmids containing a vimentin promoter segment spanning the NF- κ B element. tk-CAT or NF κ -CAT plasmids were transfected either together with the pMTPX plasmid expressing the Tax protein in U937 and Jurkat cells or alone in C81-66/45 cells. Results are expressed as percent acetylation of [14 C]chloramphenicol to 1- or 3-acetylated chloramphenicol.

coding for the IL-2R α chain (4, 12, 18), IL-2 (12, 18, 35), granulocyte-macrophage colony-stimulating factor (20, 21), and IL-3 (20). Recently, this nuclear transactivator protein was found to activate the *c-fos* promoter (8).

In the present study, we have demonstrated that the Tax protein is involved in the transcriptional activation of the vimentin gene. Vimentin is of interest not only for its structural role in the cytoskeleton but also as a growth-regulated gene. In HTLV-I-transformed T cells, the vimentin gene expression, as assessed by the levels of vimentin mRNA, was found to be three to five times higher than that in HTLV-I-free lymphoblastoid T cells. The observation that one of the HTLV-I-infected cells studied (C-8166/45) expressed only the Tax protein further implicated this retroviral gene product in the increased expression of the vimentin gene. We then showed that Tax protein activates in transiently transfected cells the human vimentin promoter in Jurkat T cells, HeLa epithelial cells, and U937 promonocytic cells. Indeed, the functional analysis performed with deletion plasmids of the 5'-flanking region of the vimentin gene showed that the *cis*-acting element required for Tax activation of the human vimentin gene is localized within 241 bp upstream from the transcription initiation site. Moreover, a plasmid carrying only 140 bp upstream from the transcription initiation site failed to respond to Tax. Therefore, the 5' boundary of the *cis*-acting element required for Tax activation is localized between 241 and 140 bp upstream of the transcription initiation site. In this region, two types of sequences were identified. The first one, between positions -227 and -217, is homologous to a sequence present in the IL-2R (α chain) gene, which has homology with the binding site for NF- κ B. This sequence has been involved in the transactivation of the IL-2R gene by the Tax protein (2, 29). The second type is represented twice and is closely related to a sequence called CLE2 (common lymphokine element) present in the promoter of the granulocyte-macrophage colony-stimulating factor gene (19), the IL-2 gene, and the IL-2R gene (21). This element has been involved in the Tax activation of the mouse granulocyte-macrophage colony-stimulating factor.

Results obtained with constructs retaining only 210 and 173 bp upstream from the transcription initiation site clearly indicate that the two CLE2-related sequences are not implicated in the Tax response of the vimentin promoter. In contrast, results obtained with constructs 241 and 210 bp confirm that the NF- κ B sequence confers Tax inducibility to the vimentin promoter. Furthermore, a 40-bp oligonucleotide containing this regulatory region proved sufficient to confer Tax inducibility upon a heterologous promoter. However, the possibility cannot be excluded that other transcription factors may also interact with the 40-bp region that was inserted into the tk promoter.

Our results show that Tax can activate the human vimentin gene transiently transfected into T cells or non-T cells, such as monocytes and epithelial cells. We therefore speculate that Tax can cooperate with preexisting cellular transcriptional factors and that the synergistic effect of cellular and viral proteins can lead to the activation of the vimentin gene. Alternatively, Tax could induce a common transcription activation pathway in different cellular environments. The induction by Tax of cellular proteins that activate the κ B elements in the IL-2R α gene favors the latter possibility (2).

We were unable to demonstrate by Northern analysis a difference in the transcriptional activation of the vimentin gene between a Jurkat T-cell clone stably expressing Tax cDNA and the parental T-cell line. This contrasts with the

induction of CAT activity in the same cells transiently transfected by the pMTPX plasmid. Consequently, the low production of Tax in stably transfected Jurkat clones may not allow the transcriptional activation of genes involved in T-cell growth. Indeed, Tax expression in these cells was lower than that in C81-66/45 cells (results not shown). Experiments are in progress to determine whether treatment with phorbol myristate acetate and forskolin, which increases the level of the Tax production, will result in the transcriptional activation of the vimentin gene, as it has been recently shown for genes coding for IL-2R, IL-2, and granulocyte-macrophage colony-stimulating factor (37). Preliminary results indicate that phorbol myristate acetate plus forskolin treatment of one stably Tax-expressing Jurkat clone did confer Tax inducibility to a transfected NF κ -CAT plasmid, whereas no CAT activity could be detected in the untreated Tax-expressing clone (A. Salvetti, personal communication). It is possible that a second event, in addition to Tax-mediated transcriptional activation, may be required for an increased level of vimentin mRNA.

Vimentin behaves like a growth-regulated gene. Indeed, steady-state levels of vimentin mRNA are low in G_0 cells, peak in mid- G_1 , and decrease to the prestimulation state at the onset of the S phase (13). It has been established that progression from G_0 resting T lymphocytes to the G_1 period of the cell cycle is characterized by the appearance of *c-fos* and early *c-myc*. Furthermore, the induction of IL-2 production triggers a late series of cell cycle progression events culminating in proliferation (34). It thus appears that vimentin expression is involved in the progression phase toward cellular proliferation. Therefore, the present study emphasizes that Tax is involved in the induction of a set of genes that are essential for T-cell proliferation.

The present report defines a new role of the Tax protein in including high levels of a structural protein. Vimentin is an essential gene belonging to the family of the intermediate filaments. Whether vimentin expression is related to the leukemogenic process remains to be elucidated. Electron microscopy studies and indirect immunofluorescence observations with anti-intermediate filament antibodies by light microscopy have shown that arrays of intermediate filaments form a complex network connecting the plasma membrane to the nuclear surface throughout the cytoplasm (for a review, see reference 36). This dynamic network may therefore be implicated not only in cytoskeletal organization but also in signal transduction, particularly in cells endowed with a high proliferative ability. One could therefore speculate that in HTLV-I-transformed T cells, the presence of an efficient intermediate filament network is required to convey signals in and out of the nucleus. Indeed, one property that distinguishes normal T lymphocytes from HTLV-I-transformed cells is that the latter are most often independent of IL-2 for their growth. They are characterized by the presence of IL-2R on their membrane, express the nuclear *c-fos* proto-oncogene, and display a cytoplasmic vimentin network. The constitutive expression of these proteins could therefore account for the autonomous growth of these HTLV-I-transformed cells. Another provocative hypothesis is supported by the recent observation (28) showing homology of vimentin with the *dbl* proto-oncogene at the level of the amino acid sequence, indicating that constitutive expression of the cytoskeletal protein may be involved in the transforming event alone or in cooperation with the *c-fos* proto-oncogene.

ACKNOWLEDGMENTS

We thank Fabian Wild, Khash Khazaie, and Marc Vasseur for critically reading the manuscript and for valuable discussions. We are grateful to Zhenlin Li for expert nucleotide sequence analysis and to Patrick Vicart for oligonucleotide cloning.

This work was supported in part by grants from Association de Recherches sur le Cancer, Association Française contre les Myopathies, Association Nationale de Recherche contre le SIDA, Fédération Nationale des Centres de Lutte Contre le Cancer, and Fondation de France.

LITERATURE CITED

- Auffray, C., and F. Rougeon. 1980. Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumor RNA. *Eur. J. Biochem.* **107**:303-314.
- Ballard, D. W., E. Böhnlein, J. W. Lowenthal, Y. Wano, B. R. Franza, and W. C. Greene. 1988. HTLV-I Tax induces cellular proteins that activate the kB element in the IL-2 receptor α gene. *Science* **241**:1652-1655.
- Chen, C., and H. Okayama. 1987. high-efficiency transformation of mammalian cells by plasmid DNA. *Mol. Cell. Biol.* **7**:2745-2752.
- Cross, S. L., M. B. Feinberg, J. B. Wolf, N. J. Holbrook, F. Wong-Staal, and W. J. Leonard. 1987. Regulation of the human interleukin-2 receptor α chain promoter: activation of a non functional promoter by the transactivator gene of HTLV-I. *Cell* **49**:47-56.
- Dellagi, K., W. Vainchenker, G. Vinci, D. Paulin, and J. C. Brouet. 1983. Alteration of vimentin intermediate filament expression during differentiation of human hematopoietic cells. *EMBO J.* **2**:1509-1514.
- Duc Dodon, M., and L. Gazzolo. 1987. Loss of interleukin-2 requirement for the generation of T colonies defines an early event of human T-lymphotropic virus type I infection. *Blood* **69**:12-17.
- Ferrari, S., R. Battini, L. Kaczmarek, S. Rittling, B. Calabretta, J. Kim de Riel, V. Philiponis, J. F. Wei, and R. Baserga. 1986. Coding sequence and growth regulation of the human vimentin gene. *Mol. Cell. Biol.* **6**:3614-3620.
- Fuji, M., P. Sassone-Corsi, and I. M. Verma. 1988. *c-fos* promoter trans-activation by the tax₁ protein of human T-cell leukemia virus type I. *Proc. Natl. Acad. Sci. USA* **85**:8526-8530.
- Gazzolo, L., and M. Duc Dodon. 1987. Direct activation of resting T lymphocytes by human T-lymphotropic virus type I. *Nature (London)* **326**:714-717.
- Goldberg, D. A. 1980. Isolation and partial characterization of the *Drosophila* alcohol dehydrogenase gene. *Proc. Natl. Acad. Sci. USA* **77**:5794-5798.
- Gorman, C. M., L. F. Moffat, and B. H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* **2**:1044-1051.
- Inoue, J., M. Seiki, T. Taniguchi, S. Tsuru, and M. Yoshida. 1986. Induction of interleukin 2 receptor gene expression by p40^x encoded by human T-cell leukemia virus type 1. *EMBO J.* **5**:2883-2888.
- Kaczmarek, L. 1986. Biology of disease: protooncogene expression during the cell cycle. *Lab. Invest.* **54**:365-376.
- Leung, K., and G. J. Nabel. 1988. HTLV-1 transactivator induces interleukin-2 receptor expression through an NF-kB-like factor. *Nature (London)* **333**:776-778.
- Lilienbaum, A., V. Legagneux, M. M. Portier, K. Dellagi, and D. Paulin. 1986. Vimentin gene: expression in human lymphocytes and in Burkitt's lymphoma cells. *EMBO J.* **5**:2809-2814.
- Luckow, B., and G. Schütz. 1987. CAT constructions with multiple unique restriction sites for the functional analysis of eucaryotic promoters and regulatory elements. *Nucleic Acids Res.* **15**:5490.
- Manzari, V., R. C. Gallo, G. Franchini, E. Westin, N. L. Cecherini, M. Popovic, and F. Wong-Staal. 1983. Abundant transcription of a cellular gene in T cells infected with human T-cell leukemia-lymphoma virus. *Proc. Natl. Acad. Sci. USA* **80**:11-15.
- Maruyama, M., H. Shibuya, H. Harada, M. Hatakeyama, M. Seiki, T. Fujita, J. Inoue, M. Yoshida, and T. Taniguchi. 1987. Evidence for aberrant activation of the interleukin-2 autocrine loop by HTLV-I-encoded p40^x and T3/Ti complex triggering. *Cell* **48**:343-350.
- Miyatake, S., T. Otsuka, T. Yokota, F. Lee, and K. Arai. 1985. Structure of the chromosomal gene for granulocyte-macrophage colony stimulating factor: comparison of the mouse and human genes. *EMBO J.* **4**:2561-2568.
- Miyatake, S., M. Seiki, R. D. Malefijt, T. Heike, J. Fujisawa, Y. Takebe, J. Nishida, J. Shlomai, T. Yokota, M. Yoshida, K. Arai, and N. Arai. 1988. Activation of T cell derived lymphokine gene in T cells and fibroblasts: effects of human T cell leukemia virus type I p40^x protein and bovine papilloma virus encoded E2 protein. *Nucleic Acids Res.* **16**:6547-6566.
- Miyatake, S., M. Seiki, M. Yoshida, and K. Arai. 1988. 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. *Mol. Cell. Biol.* **8**:5581-5587.
- Nyborg, J. K., W. S. Dynan, I. S. Y. Chen, and W. Wachsman. 1988. Binding of host-cell factors to DNA sequences in the long terminal repeat of human T-cell leukemia virus type 1: implication for viral gene expression. *Proc. Natl. Acad. Sci. USA* **85**:1457-1461.
- Ohtani, K., M. Nakamura, S. Saito, T. Noda, Y. Ito, K. Sugamura, and Y. Hinuma. 1987. Identification of two distinct elements in the long terminal repeat of HTLV-I responsible for the maximum gene expression. *EMBO J.* **6**:389-395.
- Perreau, J., A. Lilienbaum, M. Vasseur, and D. Paulin. 1988. Nucleotide sequence of the human vimentin gene and regulation of its transcription in tissues and cultured cells. *Gene* **62**:7-16.
- Popovic, M., G. Lange-Wantzin, P. S. Sarin, D. Mann, and R. C. Gallo. 1983. Transformation of human umbilical cord blood T-cells by human T-cell leukemia/lymphoma virus. *Proc. Natl. Acad. Sci. USA* **80**:5402-5406.
- Rittling, S. R., and R. Baserga. 1987. Functional analysis and growth factor regulation of the human vimentin promoter. *Mol. Cell. Biol.* **7**:3908-3915.
- Rittling, S. R., L. Coutinho, T. Amram, and M. Kolbe. 1989. AP1/jun binding sites mediate serum inducibility of the human vimentin promoter. *Nucleic Acids Res.* **17**:1619-1633.
- Ron, D., S. R. Tronick, S. A. Aaronson, and A. Eva. 1988. Molecular cloning and characterization of the human *dbl* proto-oncogene: evidence that its overexpression is sufficient to transform NIH/3T3 cells. *EMBO J.* **7**:2465-2472.
- Ruben, S., H. Poteat, T. H. Tan, K. Kawakami, R. Roeder, W. Hasteline, and C. A. Rosen. 1988. Cellular transcription factors and regulation of IL-2 receptor gene expression by HTLV-I Tax gene product. *Science* **241**:89-92.
- Salahuddin, S. Z., P. D. Markham, F. Wong-Staal, G. Franchini, V. S. Kalyanaraman, and R. C. Gallo. 1983. Restricted expression of human T-cell leukemia-lymphoma virus (HTLV) in transformed human umbilical cord blood lymphocytes. *Virology* **129**:51-64.
- Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463-5467.
- Seiki, M., S. Hattori, Y. Hirayama, and M. Yoshida. 1983. Human adult T-cell leukemia virus: complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. *Proc. Natl. Acad. Sci. USA* **80**:3618-3622.
- Seiki, M., J. Inoue, T. Takeda, A. Hikikoshi, M. Sato, and M. Yoshida. 1985. The p40^x of human T-cell leukemia virus type I is a *trans*-acting activator of viral gene transcription. *Gann* **76**:1127-1131.
- Shipp, M., and E. L. Reinherz. 1987. Differential expression of nuclear proto-oncogenes in T cells triggered with mitogenic and nonmitogenic T3 and T11 activation signals. *J. Immunol.* **139**:2143-2148.
- Siekevitz, M., S. F. Josephs, M. Dukovich, N. Pfeffer, F. Wong-Staal, and W. G. Greene. 1987. Activation of the HIV-1 LTR by T cell mitogens and the *trans*-activator protein of HTLV-I.

- Science **238**:1575–1578.
36. **Steinert, P. M., and D. R. Roop.** 1988. Molecular and cellular biology of intermediate filaments. *Annu. Rev. Biochem.* **57**: 593–625.
37. **Wano, Y., M. Feinberg, J. B. Hosking, H. Bogerd, and W. C. Greene.** 1988. Stable expression of the Tax gene of type I human T-cell leukemia virus in human T cell activates specific cellular genes involved in growth. *Proc. Natl. Acad. Sci. USA* **85**: 9733–9737.
38. **Wong-Staal, F., and R. C. Gallo.** 1985. Human T-lymphotropic retroviruses. *Nature (London)* **317**:395–403.
39. **Yoshida, M., and M. Seiki.** 1987. Recent advances in the molecular biology of HTLV-1: *trans*-activation of viral and cellular genes. *Annu. Rev. Immunol.* **5**:541–559.