Expression and Characterization of the *trans*-Activating Protein Tax of Human T-Cell Leukemia Virus Type I in *Saccharomyces cerevisiae*

LING-CHUAN C. WU,^{1*} TSE-HUA TAN,^{2†} AND S. I. SHAHIED¹

Public Health and Environmental Laboratories, New Jersey State Department of Health, Trenton, New Jersey 08625-0360,¹ and Biological Carcinogenesis and Development Program, Program Resources, Inc./DynCorp., National Cancer Institute-Frederick Cancer Research and Development Center, P.O. Box B, Frederick, Maryland 21702-1201²

Received 4 May 1992/Accepted 21 August 1992

The *trans*-activator protein Tax of human T-cell leukemia virus type I (HTLV-I) stimulates transcription of the viral genome from the long terminal repeat. With a reporter *HIS4TATA::lacZ* fusion gene, the transcriptional activity of the Tax-responsive element in the long terminal repeat was tested in *Saccharomyces cerevisiae*. We found that fragments containing the 21-bp repeat of the HTLV-I enhancer stimulate synthesis of β -galactosidase activity 15- to 20-fold. To test the ability of the Tax protein to *trans* activate the HTLV-I enhancer in yeast cells, the *pX* region of HTLV-I, encoding the Tax protein, was cloned under the control of the yeast *GALI* promoter. The expressed Tax protein is localized in the nucleus and associated with the yeast nuclear matrix fraction. In yeast cells that contained the integrated *tax* gene, two- to sixfold stimulation of expression from the HTLV-I enhancer was detected at the early stages of *tax* induction. This *in vivo* reconstitution system provides a new approach for examining the host factor(s), the signal transduction mechanism(s), and the role of nuclear architecture involved in Tax-mediated *trans* activation.

Human T-cell leukemia virus type I (HTLV-I) is etiologically associated with adult T-cell leukemia/lymphoma (64). In addition to the *gag*, *pol*, and *env* genomic regions common to human retroviruses, this virus possesses at least two regulatory genes, *tax* and *rex*, for autoregulation of its gene expression (58, 64). The 40-kDa Tax protein encoded by the *pX* region of the HTLV-I genome (32) is essential for HTLV-I replication and functions as a *trans* activator to stimulate transcription from the U3 region of the HTLV-I long terminal repeat (LTR) (9, 13, 46, 53, 54). The HTLV-I Tax-responsive element (TRE) consists of three copies of 21-bp imperfect repeats located between positions -252 and -54, with a core pentanucleotide sequence, TGACG, in each of the 21-bp motifs (15, 39, 44, 48, 56). This pentanucleotide sequence is also present in the cyclic AMP (cAMP)-responsive element (CRE) (26, 40, 56) and in the activating transcription factor (ATF) binding site (22, 56), raising the possibility that Tax works via normal cellular signal transduction pathways. The TRE confers responsiveness to cAMP and adenovirus E1A protein, and the effects of cAMP and Tax are additive (26, 40, 56).

Molecular studies have shown that Tax can also activate other viral and cellular enhancer sequences, including human immunodeficiency virus type 1 (HIV-1) (50), simian virus 40, cytomegalovirus, immunoglobulin kappa gene, interleukin-2 receptor α chain, interleukin-2, class I major histocompatibility complex antigen, and tumor necrosis factor beta (reviewed in reference 4). Tax indeed behaves like a T-cell mitogen by inducing the expression of interleukin-2 and the interleukin-2 receptor α chain (49, 59). The oncogenic potential of Tax protein was demonstrated in both the

transgenic mouse (23) and rat fibroblast (57) models. Cotransfection of *tax* with the *ras* oncogene induced the formation of foci that were highly tumorigenic in nude mice (41). Tax also activated the human vimentin gene, a component of the cytoskeletal network implicated in signal transduction (33). Apart from its positive transcriptional effects, Tax negatively regulates the expression of human β -polymerase, a cellular enzyme involved in DNA repair, suggesting a possible pathway for the genome damage observed in adult T-cell leukemia (28).

The precise molecular mechanism by which Tax mediates these diverse transcriptional effects remains unclear. Tax does not bind to DNA specifically (5, 16); rather, it functions by inducing or modifying the activity of selective host transcription factors. Several potential candidates for this host factor have been described (5, 35, 37, 55). Protein kinase C-like activity has been implicated in Tax-mediated *trans* activation (56), whereas inhibitors of *de novo* protein synthesis have no effect (17).

Recent evidence suggests that the RNA polymerase II transcriptional system is highly conserved throughout evolution from yeasts to mammals (20, 34). In order to develop a simple model system with which to study Tax-mediated *trans* activation and to define the cellular components involved, we expressed the *tax* gene in the yeast *Saccharomyces cerevisiae* and examined the regulation of the reporter β -galactosidase gene, in which the upstream activating sequence (UAS) elements required for transcription were replaced by HTLV-I enhancer elements. In this study, we demonstrated that a sequence of the HTLV-I enhancer could substitute for the yeast UAS and direct an activated level of β -galactosidase synthesis in yeast cells and that expression of Tax yielded *trans* activation of the HTLV-I enhancer. A two- to sixfold Tax-mediated *trans* activation of the HTLV-I

* Corresponding author.

† Present address: Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030.

enhancer was detected transiently in the early stages of Tax induction.

MATERIALS AND METHODS

Yeast strains and media. The *S. cerevisiae* strains used in this study were YPH102 (α *ura3-52 lys2-801 ade2-101 his3-200 leu2*) (gift from James R. Broach) and DBY745 (α *ade1-100 leu2-3 leu2-112 ura3-52*). DNA transformations in yeast cells were performed by the lithium acetate method of Ito et al. (24). Rich medium (YEPD) and minimal medium (SC or SD) for yeast cultures were prepared as described by Sherman et al. (47). For galactose induction, cells were grown in SD plus 2% (wt/vol) glucose, a medium in which the *GAL1* promoter is repressed. At late log phase, the culture was diluted 20-fold into SD with 2% (wt/vol) raffinose. After 4 h at 30°C, galactose was added to a final concentration of 2% (wt/vol). This culture was kept at 30°C for 18 h, after which the cells were harvested. Synthetic 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal) plates were prepared as described by Rose and Botstein (42).

Plasmid construction. The reporter plasmid pML87 (gift from Marc Lamphier) (Fig. 1) is a shuttle vector that carries a 5'-truncated *Escherichia coli lacZ* gene fused in phase to the yeast *HIS4* promoter, a fragment of the yeast 2 μ m plasmid required for autonomous replication, and the *URA3* gene, for selection in yeast cells. To eliminate the *HIS4* UAS region, we digested pML87 with *Sma*I, added *Xho*I linkers, digested it with *Xho*I, and self-ligated it to yield plasmid p Δ UAS (Fig. 1). The DNA fragment containing the HTLV-I enhancer was derived from pHTLV-ICAT (53); the region from -321 to -54 (*Sma*I to *Nde*I sites of the HTLV-I LTR) was excised and cloned into the *Xho*I site of p Δ UAS with proper linkers. This plasmid was designated pHTLV-I (Fig. 1).

Plasmid YLHTLV-I was constructed by digesting YCP50 (43) with *Eco*RI and *Hind*III and inserting a 4.6-kb *Eco*RI-*Hind*III genomic fragment of *LYS2* (11). The resulting plasmid was digested with *Hind*III and *Nsi*I, and the large fragment containing *ARS1*, *CEN4*, and *LYS2* was purified and ligated to an *Nsi*I-*Tth*111I reporter DNA fragment derived from pHTLV-I (Fig. 1).

For inducible overexpression of the HTLV-I Tax protein in yeast cells, we restricted plasmid pHTax1 (44) with *Tth*111I and *Hinc*II. This digestion released a 1,205-bp fragment which encodes most of the Tax protein except for 8 amino acids at the N terminus. DNA sequences encoding the 8 amino acids at the 5' end were cloned in with a corresponding synthetic oligonucleotide. This construction gave rise to a full-length *tax* gene not interrupted by the intron sequence. The full-length *tax* gene was inserted into multicopy plasmid YEPTOP2-pGAL1 (61), with the yeast DNA topoisomerase II gene previously deleted from the *GAL1* promoter. The resulting plasmid, YEPTAX-pGAL1, carries the 2 μ m circle origin of replication, the *URA3* gene adjacent to the 3' end of the *tax* gene, and the *tax* gene under control of the yeast *GAL1* promoter. To facilitate the integration of the *GAL1* promoter and the *tax* gene (*pGAL1::TAX*) into the yeast chromosome, the 2,241-bp 2 μ m circle-containing fragment was excised by digesting plasmid YEPTAX-pGAL1 with *Eco*RI. The remaining DNA was religated and linearized by digestion with *Nsi*I, which has a unique cutting site within the *URA3* gene. The DNA was used to transform YPH102, a *Ura*⁻ host strain, and the *Ura*⁺ transformants (strain YLW1) were selected for Western immunoblot analyses. For a negative control, a similar

procedure was used to construct *tax*-minus transformants (strain YLW2). In this case, we integrated an *Nsi*I-digested DNA fragment derived from YEPTOP2-pGAL1 with the 2 μ m circle and the DNA topoisomerase II gene previously deleted.

RNase protection assay. Cells transformed with pML87, p Δ UAS, and pHTLV-I were grown to mid-log phase, and total RNA was isolated (10). Ten micrograms of total RNA was hybridized to a ³²P-labeled β -galactosidase probe generated by inserting the 581-bp *Xho*I-*Hpa*I fragment of pML87 (Fig. 1A) into pSPT18 (Boehringer Mannheim) and in vitro transcription. For quantitation of *tax* mRNA in different sugars, YLW1 and YLW2 cells were induced for total RNA isolation. Twenty micrograms of total RNA was hybridized to the *tax* probe, an antisense *tax* transcript spanning nucleotides 1 to 200 at the 5' end. As an internal control, a yeast actin antisense probe was transcribed by using the pYST122 plasmid (gift from Tapan Som) and hybridized to 5 μ g of total RNA. RNA hybridization and RNase protection assays were done as recommended by the manufacturer (Ambion). Protected RNA fragments were separated by electrophoresis on a 4% acrylamide-8 M urea gel and visualized by autoradiography.

β -Galactosidase assays. Strains were grown in SD to a density of 1×10^7 to 2×10^7 cells per ml. Aliquots (50 ml) of the cells were harvested by centrifugation, washed once in cold TE (10 mM Tris-HCl [pH 8.0], 1 mM EDTA), and frozen at -20°C in 0.3 ml of breaking buffer (42). The cells were thawed, and the suspension was disrupted by vortexing with an equal volume of glass beads (Sigma; 0.45 to 0.50 mm diameter) at 4°C. β -Galactosidase assays were performed on an aliquot of unclarified lysate with the substrate chlorophenyl red β -D-galactopyranoside (Boehringer Mannheim) at a final concentration of 3 mg/ml in 100 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.2)-154 mM NaCl-2 mM MgCl₂-1% bovine serum albumin-0.05% Tween 20. After 20 min at 30°C, reactions were terminated by the addition of ZnCl₂ to a final concentration of 1 mM. Cell debris was removed by centrifugation at 12,000 rpm for 15 min at room temperature before spectrophotometric measurement. The remaining lysate were centrifuged at 12,000 rpm for 10 min at 4°C. Aliquots of each supernatant were removed and assayed for total protein (Bio-Rad).

Western immunoblot analysis. Whole-cell extracts for Western blot analysis were prepared by gentle digestion of the yeast cell wall with Zymolyase (1 mg of Zymolyase 100T per ml of wet yeast cell volume, 30°C for 20 min). The spheroplasted cells were resuspended and boiled immediately in 20% sodium dodecyl sulfate (SDS)-40 mM dithiothreitol. Aliquots of the cell extract were diluted threefold with water, and the protein was precipitated with 10% trichloroacetic acid. The protein pellet was resuspended in sample loading buffer, and samples were fractionated by SDS-polyacrylamide gel electrophoresis (PAGE) (31). The protein was electroblotted onto nitrocellulose filters as suggested by Bio-Rad. Immunological detection of the Tax protein and yeast DNA topoisomerase II was done by incubating the nitrocellulose filters with anti-Tax antibody (gift from S. Oroszlan) or anti-yeast DNA topoisomerase II antibody (gift from James C. Wang), respectively, and then with goat anti-rabbit immunoglobulin G (IgG) conjugated with calf alkaline phosphatase (Cappel). After several washes of the filters, immobilized alkaline phosphatase was visualized with a histochemical stain (5' to 3').

Immunofluorescence microscopy. DBY745 cells trans-

formed with YEPTAX-pGAL1 were induced to detect the cellular localization of Tax protein. Cells were digested with Zymolyase as described above and then fixed in 3.7% formaldehyde. The fixed cells were made permeable to the antibody by sequential incubation with 0.2% Triton X-100 for 10 min (room temperature), methanol for 6 min (-20°C), and acetone for 30 s (-20°C). The anti-Tax antibody was preabsorbed with DBY745 whole cells and diluted 1,000-fold. The first antibody incubation was carried out at 30°C overnight on microwell slides. After several washes, goat anti-rabbit IgG conjugated with fluorescein (Cappel) was applied to the cells at a 1:5,000 dilution. To localize cellular DNA, cells were washed and stained with $1\text{ }\mu\text{g}$ of 4,6-diamidino-2-phenylindole (DAPI) per ml for 5 min.

Preparation of the yeast nuclear matrix fraction. The protocol for fractionation of yeast nuclei and the preparation of a nuclear matrix fraction was essentially that described before (63). DBY745 cells transformed with YEPTAX-pGAL1 were induced, harvested, and digested with Zymolyase as described above. Spheroplasts were resuspended in 4.75 volumes of extraction buffer (50 mM Tris-HCl [pH 7.5], 50 mM NaCl, 5 mM MgCl_2 , 1 mM phenylmethylsulfonyl fluoride, $2\text{ }\mu\text{g}$ of pepstatin A per ml). Spheroplasts were lysed by addition of 0.25 volume of 20% Triton X-100 followed by 10 strokes with a tight-pestle Dounce homogenizer to yield a crude homogenate. The crude homogenate was centrifuged to yield a postnuclear supernatant and a pellet, which was washed twice with extraction buffer to generate two wash supernatants (W1 and W2) and a nuclear pellet. The nuclear pellet was resuspended in 1 volume of 20 mM Tris-HCl (pH 7.5)–5 mM MgCl_2 containing $10\text{ }\mu\text{g}$ of DNase I and $8\text{ }\mu\text{g}$ of RNase per ml and incubated at 37°C for 15 min. The digested nuclei were collected by centrifugation, and the nuclease supernatant was retained. The pellet was resuspended in 0.9 volume of 290 mM sucrose–10 mM Tris-HCl (pH 7.5)–0.1 mM MgCl_2 , to which 0.1 volume of 20% Triton X-100 was added. After incubation at 0°C for 10 min, the suspension was centrifuged, and the supernatant was removed. The pellet was resuspended in 0.5 volume of 100 mM Tris-HCl (pH 7.5)–290 mM sucrose–0.1 mM MgCl_2 , and 0.5 volume of 2 M NaCl was added. After 10 min at 0°C , the suspension was centrifuged, and the supernatant (S1) was removed. The salt extraction was repeated to yield a second salt wash supernatant (S2), and a final pellet, designated the nuclear matrix fraction.

RESULTS

Transcriptional regulatory element from HTLV-I functions as an enhancer in yeast cells. To test whether the transcriptional regulatory sequence from HTLV-I could stimulate gene expression in yeast cells, we constructed the tester plasmid pHTLV-I (Fig. 1). This plasmid is a derivative of a *URA3*-containing $2\text{ }\mu\text{m}$ circle yeast shuttle vector that carries a *HIS4::lacZ* fusion under the control of the yeast *HIS4* promoter (pML87 [Fig. 1]). We deleted 1,855 bp from the upstream portion of this promoter, removing the *HIS4* UAS, and replaced it with a unique *XhoI* linker. The resulting plasmid (pΔUAS, Fig. 1), which retains the downstream *HIS4* TATA region, produced very low levels of β -galactosidase (Fig. 2A). Strains containing this plasmid fail to turn blue on plates containing the chromogenic β -galactosidase substrate X-gal. By contrast, the parental plasmid pML87 produced approximately 100 U of β -galactosidase activity (Fig. 2A), and the strains containing pML87 are blue on X-gal plates (data not shown). A 267-bp DNA fragment

FIG. 1. (A) Schematic diagrams of plasmid pML87. Region 2u represents the 2,241-bp *EcoRI* fragment of the B form of the yeast $2\text{ }\mu\text{m}$ plasmid. Transcription of *lacZ* is under the control of the yeast *HIS4* promoter. (B) Organization of the regulatory regions. pML87 plasmid was constructed with a yeast *HIS4* promoter fused to an *E. coli lacZ* gene (B-gal, hatched box). Two regions of the *HIS4* promoter, the TATA box at -60 and the UAS at -120 to -210 , were identified as essential for promoter function (3). The *lacZ* gene was transcribed under the control of the yeast *HIS4* promoter, with the transcriptional start at 0. To construct a UAS-less *HIS4::lacZ* promoter, an *XhoI* linker was inserted to separate the *HIS4* UAS from the TATA box. pΔUAS was then constructed by deletion of the DNA sequence from the *BamHI* to the *XhoI* site. To construct plasmid pHTLV-I, a 267-bp fragment spanning the HTLV-I enhancer (positions -321 to -54 , *SmaI* to *NdeI*, of the HTLV-I LTR) was inserted in pΔUAS upstream from the *XhoI* linker. The 267-bp HTLV-I enhancer sequence contains three copies of a 21-bp repeat which is also characterized as a TRE (indicated by arrows).

spanning the HTLV-I transcriptional regulatory element was inserted into the *XhoI* site of pΔUAS to generate pHTLV-I (Fig. 1). The 267-bp fragment was derived from positions -321 to -54 of the HTLV-I LTR (*SmaI* to *NdeI*), which includes all essential *cis* elements for Tax-mediated *trans* activation. Insertion of the HTLV-I sequence elicited β -galactosidase activity levels ranging from 25 to 30 U (Fig. 2A). Colonies with this level of β -galactosidase cleaved sufficient X-gal to turn blue on X-gal plates (data not shown).

To determine whether the stimulation of β -galactosidase

FIG. 2. (A) β -Galactosidase activity produced after transformation of yeast cells by plasmids carrying different regulatory elements. Strain YPH102 was transformed with plasmid pML87 (left), p Δ UAS (center), or pHTLV-I (right). The units of β -galactosidase activity produced by each of the clones are shown above the bars. β -Galactosidase assays were performed as described in Materials and Methods. (B) RNase protection analysis of RNAs in YPH102 cells transformed by pML87, p Δ UAS, and pHTLV-I. RNase protection assays were performed as described in Materials and Methods. RNAs were hybridized to antisense RNA probes for yeast actin (B1) or the *lacZ* gene (B2). With the actin probe (B1), a major RNA of 258 nucleotides was protected in cells transformed by pML87 (lane 2), p Δ UAS (lane 3), and pHTLV-I (lane 4). The 331-nucleotide transcript is the undigested actin probe (lane 1). With the *lacZ* probe (B2), a fragment of 506 nucleotides was protected by the *lacZ* antisense probe in pML87 (lane 2) and pHTLV-I (lane 4) but not in p Δ UAS (lane 3). The 581-nucleotide transcript (lane 1) is the undigested *lacZ* probe. The autoradiographs were developed after 30 min and 1 day of exposure for actin probe and *lacZ* probe, respectively.

activity from the HTLV-I enhancer is a consequence of increased transcription, we isolated RNA from YPH102 cells transformed by pML87, p Δ UAS, and pHTLV-I and performed RNase protection assays. Each RNA sample was hybridized, in separate experiments, either to a probe containing yeast actin antisense RNA or to one with *lacZ* antisense RNA. Hybridization to the actin probe (Fig. 2B1) showed that approximately the same amount of total mRNA was present in all samples, evident as equivalent amounts of hybridization to the endogenous actin transcripts. By contrast, when the RNAs were hybridized to the *lacZ* probe (Fig. 2B2), no RNA could be detected in cells transformed by p Δ UAS, but intermediate amounts were produced by pHTLV-I, and large amounts were produced by pML87. The

FIG. 3. Western blot analysis of Tax protein synthesized in yeast cells. (A and B) Cultures of DBY745 (A) and DBY745 transformed with YEPTAX-pGAL1 (B) were grown and induced with 2% galactose as described in the text. Aliquots of the culture were collected at the time points indicated (in hours postinduction), cell lysates were prepared and fractionated by SDS-12.5% PAGE, and the proteins were transferred to a nitrocellulose filter. The blot was incubated with polyclonal rabbit antibodies directed against Tax. (C and D) YPH102 (Ura⁻) host cells were integrated with DNA fragments containing either the *GAL1* promoter (strain YLW2) (C) or *pGAL1::TAX* (strain YLW1) (D). The integration occurred at the *URA3* gene, and Ura⁺ transformants were selected. Cell extracts were prepared from either a glucose-grown culture (Gl) or after 18 h of galactose induction (Ga). The proteins were fractionated for Western blotting.

RNA levels were roughly consistent with the amounts of β -galactosidase activity produced by these constructs (Fig. 2A). Moreover, the initiation site of β -galactosidase mRNA in pML87 and pHTLV-I is the same, as the same sizes of RNAs were protected from RNase digestion in pML87 and pHTLV-I (Fig. 2B2).

Tax-mediated *trans* activation of HTLV-I enhancer in yeast cells. Tax protein can be expressed in yeast cells. However, some observations indicate that selection of the proper yeast promoter for expression of Tax protein is critical. First, we found that overexpression of Tax with *pGAL1* on a high-copy-number plasmid is deleterious to the cells (see below). Second, leaky expression of Tax protein upon transformation may induce negative selection. This is supported by the results with two plasmid constructions described below. A high-copy-number plasmid bearing the *tax* gene under the control of the yeast alcohol dehydrogenase 1 promoter (2) transformed yeast cells but failed to show the 40-kDa Tax protein upon induction. We also cloned the *tax* gene under a downregulation mutant of *pGAL1*, *pGAL1 Δ 24* (60), which expressed 2% of the wild-type *pGAL1* level of protein upon induction but is leaky in glucose repression (62). The plasmid with the *tax* gene cloned under *pGAL1 Δ 24* failed to transform yeast cells (data not shown).

To circumvent these problems, we integrated a piece of DNA containing the *URA3* gene and *pGAL1::TAX* into the yeast chromosome (strain YLW1; Fig. 3D). Ura⁺ transformants were selected, and cell extracts were prepared for Western blot analyses. As shown in Fig. 3D, the 40-kDa Tax protein was detected in cell extracts prepared from YLW1 upon galactose induction, whereas in extracts of YLW1 grown on glucose or of strain YLW2, which carries the vector lacking the *tax* gene insertion, the 40-kDa Tax protein band was absent (Fig. 3C). We did not observe a growth-inhibitory effect of Tax protein in YLW1 cells upon galactose induction (data not shown). Although Tax protein in raffinose-grown cells is not detectable by Western blot analyses (Fig. 3B, lane 0), a low level of leaky expression of *pGAL1* under this nonrepressing condition is suspected.

The expression level of *tax* mRNA in YLW1 and YLW2 cells was quantitated by RNase protection assays. As shown in Fig. 4, total RNA generated from YLW1 and YLW2 cells

FIG. 4. RNase protection analyses of RNAs in YLW1 and YLW2 cells. Cells were grown and induced as described in Materials and Methods. RNAs were hybridized to antisense RNA probes for yeast actin (A1 and A2) or the *tax* gene (T1 and T2). With the actin probe, a major RNA of 258 nucleotides was protected in all samples (A1 and A2, lanes 2 to 5). The 331-nucleotide transcript is the undigested probe (lane 1). With the *tax* probe, a fragment of 174 nucleotides was protected in YLW1 cells 4 h after being shifted into raffinose-containing medium (T1, lane 3) and 3 h (T1, lane 4) and 18 h (T1, lane 5) after supplementation with galactose. This fragment is absent in YLW2 (T2) and in YLW1 cells grown in glucose (T1, lane 2). The 200-nucleotide transcript is the undigested probe (lane 1).

grown with different sugars was hybridized to either the yeast actin probe (Fig. 4, A1 and A2) or the *tax* antisense RNA probe (Fig. 4, T1 and T2). Approximately the same amount of actin mRNA was present in all samples (Fig. 4, A1 and A2). In YLW2 cells, *tax* mRNA was not detected under any growth conditions (Fig. 4, T2), while in YLW1 cells (Fig. 4, T1), protected 174-nucleotide transcripts were found 4 h after the cells were shifted to raffinose-containing medium (lane 3) and 3 h (lane 4) and 18 h (lane 5) after being shifted to growth in galactose, but no protected fragments were present in cells grown with glucose (18 h, lane 2).

To test whether induction of Tax could *trans* activate the HTLV-I enhancer in *S. cerevisiae*, we transformed reporter plasmid YLHTLV-I into both strains YLW1 and YLW2. YLHTLV-I contained a *lacZ* gene linked to a promoter consisting of the HTLV-I enhancer and *HIS4* TATA box, so that expression of β -galactosidase was driven by the HTLV-I enhancer. As shown in Fig. 5, there was a transient induction of β -galactosidase activity that correlated with the expression of the Tax protein. An average induction of two- to sixfold was detected in the early stages of induction. Several aspects of this pattern are worth noting. First, a trace amount of Tax protein is enough to *trans* activate, as noted by the sixfold activation after a shift to raffinose for 4 h. Second, the *trans* activation is transient; it peaked at 4 h after the shift to raffinose and gradually decreased within 13 h after withdrawal from glucose repression. Third, a prolonged (or high-level) exposure to Tax protein may suppress *trans* activation, as no obvious *trans* activation could be detected after 18 h of induction in galactose (data not shown). This result is consistent with the observation that Tax protein produced in *E. coli* can stimulate *trans* activation within 2 h after protoplast fusion to mouse L cells integrated with a reporter gene. The efficiency of *trans*

FIG. 5. Tax-mediated *trans* activation of the HTLV-I enhancer in yeast cells. YLHTLV-I was transformed into either YLW2 (cross-hatched bars) or YLW1 (open bars). Cells were induced as described in the text. Aliquots of the culture were withdrawn after 18 h of growth in glucose (Glu), 4 h after a shift to raffinose [Raf(4)/Glu(0)], and 3, 6, and 9 h after addition of 2% galactose (Gal). Cell extracts were prepared, and units of β -galactosidase activity were determined.

activation reached a maximum 8 h after fusion, followed by a sharp decrease 16 h after fusion (16).

Tax protein overexpressed in yeast cells is transported into the nucleus. High-level synthesis of Tax protein could be achieved by conditional expression from a *GAL1* promoter. We constructed plasmid YEPTAX-pGAL1, which has the entire *tax* coding sequence inserted into a high-copy-number yeast expression vector and allowed inducible expression of Tax protein under the control of the yeast *GAL1* promoter. As shown in Fig. 3A, no 40-kDa tax protein was observed in the host strain DBY745. In transformed cells, supplementation with 2% galactose resulted in the induction of a 40-kDa protein which was recognized by anti-Tax antiserum (Fig. 3B). Peak Tax expression was detected after 18 h of induction (Fig. 3B). Overexpression of Tax protein from this plasmid inhibited colony formation by the transformed cells, but the inhibition was reversible, and colony formation could be restored by placing galactose-induced cells on glucose-containing plates. As shown in Fig. 6, cultures of DBY745 or DBY745 transformed with YEPTAX-pGAL1 were diluted and plated onto either galactose- or glucose-containing SC plates after 18 h of galactose induction. No colonies from the transformed cell culture appeared on galactose plates, while the same inoculum gave rise to numerous colonies on glucose-containing plates (Fig. 6B). This growth-inhibitory effect was not observed on DBY745 host cells (Fig. 6A).

Tax protein is localized predominantly in the nucleus in mammalian systems (19, 30). This subcellular localization of Tax protein in yeast cells was confirmed by indirect immunofluorescent staining of fixed cells. As shown in Fig. 7A, intense fluorescence was detected in the nuclei of DBY745 cells transformed with YEPTAX-pGAL1 upon induction. No fluorescence was observed when the cells were grown under glucose repression (Fig. 7C) or if preimmune serum was used (data not shown). In the experiment shown, the cells were also stained with DAPI to verify the position of the nucleus (Fig. 7B and D). The *tax* gene product was

FIG. 6. Overexpression of Tax protein inhibited yeast colony formation. Cultures of DBY745 host cells (A) and DBY745 cells transformed with YEPTAX-pGAL1 (B) were induced with galactose for 18 h as described in the text. Aliquots of the culture were diluted and plated on SC supplemented with galactose (Ga) or glucose (Gl).

transported into the nucleus, as was evident when the patterns of DAPI and anti-Tax antibody staining were compared (Fig. 7). Moreover, the Tax protein did not appear to be randomly distributed within the nucleus but was concentrated and granulated (Fig. 7A). The reason for the weak DAPI staining under induced conditions (Fig. 7B) is unclear.

Tax protein overexpressed in yeast cells cofractionates with the yeast nuclear matrix. In preliminary experiments designed to purify Tax protein from yeast cells, we observed that Tax protein was highly resistant to salt extraction. This observation, in conjunction with the finding that Tax protein in yeast nuclei exhibited a unique punctate staining pattern (Fig. 7A), led us to suspect that Tax may associate with the yeast nuclear matrix. In order to address this question, the subnuclear fractionation of yeast cells was performed as follows. A culture of YEPTAX-pGAL1-transformed cells was induced as described above. The cells were converted to spheroplasts and mechanically sheared in hypotonic buffer containing Triton X-100. This resulted in cell lysis, disruption of the cytoskeleton, lysis of membrane-bound internal organelles, and removal of the inner and outer nuclear membranes. Nuclear matrix components are resistant to extraction with nonionic detergents such as Triton X-100 under low-salt conditions. The residual pelletable material obtained after spheroplast lysis consisted primarily of nuclei with some cell wall debris, which was largely nonproteinaceous. The nuclear pellet was treated with DNase and RNase and subsequently fractionated by sequential extraction with 2% Triton X-100 and 1 M NaCl. The salt-insoluble fraction was pelleted to yield the final nuclear matrix fraction. The proteins isolated from each fraction were examined by SDS-12.5% PAGE and stained with Coomassie blue (data not shown). The partitioning of Tax protein during subnuclear fractionation of yeast cells was examined by immunoblot analysis on SDS-PAGE-fractionated samples of each of

FIG. 7. Subcellular localization of Tax protein produced in yeast cells. YEPTAX-pGAL1-transformed DBY745 cells were processed for indirect immunofluorescence assays. Cells grown in either induced (Gal) or repressed (Glu) conditions were harvested, digested with Zymolyase, and immobilized on polylysine-coated slides. The slides were fixed with formaldehyde and double stained with anti-Tax antibody (A and C) and DAPI (B and D).

the fractions in the preparation. The results of such an analysis are shown in Fig. 8A. Tax protein was present in the crude homogenate (CH) and partitioned mostly to the nuclear pellet (PN). It was not liberated by treatment with nuclease (NS) or nonionic detergent (TS). The salt extraction resulted in a nearly equal partitioning of Tax protein between the first salt supernatant (S1) and the nuclear matrix fraction (NM) (Fig. 8A).

DNA topoisomerase II is a major polypeptide component of the nuclear matrix prepared from *Drosophila melanogaster* (6) and calf thymus (21). Yeast DNA topoisomerase II fractionates in a similar manner, except that it is more sensitive to salt extraction (63). As a control, a Western blot of the various fractions obtained during the preparation of the yeast nuclear matrix was probed with antibody directed against yeast DNA topoisomerase II. As shown in Fig. 8B, this antibody cross-reacts with a 170-kDa protein that fractionates predominantly to the nucleus and can be extracted from the nucleus with 1 M NaCl (Fig. 8B, S1). Lower-molecular-weight species visible in the crude homogenate (CH) and postnuclear supernatant (PS) result from nonspecific interactions.

DISCUSSION

We have shown that a transcriptional regulatory element from HTLV-I can substitute for a UAS element in *S. cerevisiae* to stimulate expression of a linked β -galactosidase gene. The insertion of sequence from -321 to -54 of the HTLV-I LTR transcriptional regulatory region stimulated β -galactosidase activity 15- to 20-fold. This region of the HTLV-I LTR, which is also responsible for Tax-mediated *trans* activation of HTLV-I, consists of three copies of 21-bp imperfect repeats. With the *tax* gene integrated so that expression of the gene was tightly controlled by the yeast *GAL1* promoter, we detected an additional two- to sixfold activation of β -galactosidase expression. This indicates that Tax protein is able to *trans* activate the HTLV-I enhancer in yeast cells. The *trans* activation is transient and requires a

FIG. 8. (A) Distribution of Tax protein following subcellular fractionation of yeast cells. YEPTAX-pGAL1-transformed DBY745 cells were grown in SD lacking uracil and supplemented with 2% galactose. Cells were lysed and fractionated as described in the text. Equivalent samples from each fraction were electrophoresed on SDS-10% PAGE, and a Western blot analysis with anti-Tax antibody was carried out. Samples tested were the crude homogenate (CH), postnuclear supernatant (PS), two washes (W1 and W2), nuclear pellet (PN), nuclease-digested pellet (DN), nuclease-released supernatant (NS), Triton X-100-extractable supernatant (TS), two salt-extractable supernatants (S1 and S2), and the nuclear matrix (NM). (B) Distribution of yeast DNA topoisomerase II (Topo II) during subcellular fractionation. Cell extracts prepared for analyzing Tax protein distribution were electrophoresed on SDS-8% PAGE. The Western blot was probed with anti-yeast DNA topoisomerase II antibody. Sample designations are the same as in panel A.

low level of *tax* expression. A growth-inhibitory effect was observed with high levels of *tax* expression. It is conceivable that the long-term, higher expression of *tax* leads to the repression of some host factors which indirectly suppress Tax-mediated *trans* activation. The same phenomenon was observed in Jurkat T lymphocytes constitutively expressing *tax*; the ability of mitogens to activate NF- κ B was blocked and suppressed, as opposed to activation in B lymphoid cells (45).

We do not know whether the mechanism of transcriptional regulation and Tax-mediated *trans* activation of HTLV-I in yeast cells is similar to that in mammalian cells, although several aspects of Tax *trans* activation are consistent with this interpretation. First, Tax-mediated *trans* activation of the HTLV-I enhancer was observed not only in lymphoid cells (54) but also in nonlymphoid cells (13, 15, 56) and an insect cell line (27), indicating that T-cell-specific host factors are not required. Second, the core pentanucleotide sequence TGACG in each of the 21-bp TRE repeats is also a consensus sequence for CREB/ATF binding. An ATF-like activity has been identified in *S. cerevisiae*, and a mammalian ATF site can function as UAS in yeast cells (34). The yeast ATF-like activity may recognize the ATF binding site in the TRE and mediate *trans* activation. Third, the pathway

for Tax *trans* activation of the HTLV-I LTR in mammalian cells may be regulated by a cAMP/protein kinase A-mediated or a protein kinase C-mediated signal transduction pathway (29, 56). Similar signal transduction pathways exist in *S. cerevisiae* (7, 36). We are currently in the process of testing the effect of cAMP on Tax-mediated *trans* activation of the HTLV-I enhancer in yeast cells. In addition, we have been able to isolate several host mutants which showed enhanced or diminished HTLV-I enhancer activity in yeast cells (data not shown). We are currently characterizing these mutants.

On the basis of both subcellular fractionation techniques and immunocytochemical analysis at the light-microscopic level, we have shown that Tax protein is quantitatively found in the nucleus despite significant overproduction in yeast cells. This represents one piece of evidence that Tax protein behaves in a biologically relevant manner in yeast cells. Within the nucleus, Tax was distributed almost equally between the high-salt extractable fraction and the nuclear matrix. These cell biology properties resemble those observed in mammalian cells (30, 38, 51) and indicate a function similar to that in mammalian cells, since the nuclear localization and the *trans*-activation ability of Tax are coupled (18, 52). Although the role of the nuclear matrix in transcriptional regulation is so far unclear, several studies indicate that it may play an important role. The transcriptional complex, i.e., nascent RNA transcripts, active RNA polymerase II, and transcribed DNA sequence, is associated with the nuclear matrix (1, 25). Several gene products whose ability to promote oncogenic transformation stems from their role as transcriptional regulators are also associated with the nuclear matrix. Among them are the large T antigen of polyomavirus (8), *myc* gene products (12), and the E1A protein of adenovirus (14). The colocalization of Tax protein, the enhancer element, a host factor(s), and the RNA polymerase II transcriptional machinery on the nuclear skeletal structure may underlie the ability of Tax protein to *trans* activate.

In conclusion, we have reconstituted a viral *trans*-activation system in yeast cells. This system should provide a new approach by which to study the host factors involved in Tax-mediated *trans* activation, the upstream and downstream effectors that interact with Tax, and the molecular mechanism governing the involvement of the nuclear matrix in Tax function.

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REFERENCES

1. Abulafia, R., A. BenZe'ev, N. Hay, and Y. Aloni. 1984. Control of late simian virus 40 transcription by the attenuation mechanism and transcriptionally active ternary complexes are associated with the nuclear matrix. *J. Mol. Biol.* 5:467-487.
2. Ammerer, G. 1983. Expression of genes in yeast using the *ADC1* promoter. *Methods Enzymol.* 101:192-201.
3. Arndt, K., C. Styles, and G. R. Fink. 1987. Multiple global regulators control *HIS4* transcription in yeast. *Science* 237:874-880.
4. Baeuerle, P. A. 1991. The inducible transcription activator NF- κ B: regulation by distinct protein subunits. *Biochim.*

- Biophys. Acta 1072:63–80.
5. Beimling, P., and K. Moelling. 1989. Isolation and characterization of the tax protein of HTLV-I. *Oncogene* 4:511–516.
 6. Berrios, M., N. Osheroff, and P. A. Fisher. 1985. *In situ* localization of DNA topoisomerase II, a major polypeptide component of the *Drosophila* nuclear matrix fraction. *Proc. Natl. Acad. Sci. USA* 82:4142–4146.
 7. Bourne, H. R., D. A. Sanders, and F. McCormick. 1990. The GTPase superfamily: a conserved switch for diverse cell functions. *Nature (London)* 348:125–132.
 8. Buckler-White, A. J., G. W. Humphrey, and V. Pigiet. 1980. Association of polyoma T antigen and DNA with the nuclear matrix from lytically infected 3T6 cells. *Cell* 22:37–46.
 9. Cann, A. J., J. D. Rosenblatt, W. Wachsmann, N. P. Shah, and I. S. Y. Chen. 1985. Identification of the gene responsible for human T-cell leukaemia virus transcriptional regulation. *Nature (London)* 318:571–574.
 10. Carlson, M., and D. Botstein. 1982. Two differentially regulated mRNAs with different 5' ends encode secreted and intracellular forms of yeast invertase. *Cell* 28:145–154.
 11. Eibel, H., and P. Philippsen. 1983. Identification of the cloned *Saccharomyces cerevisiae* *LYS2* gene by an integrative transformation approach. *Mol. Gen. Genet.* 191:66–73.
 12. Eisenman, R. N., C. Y. Tachibana, H. D. Abrams, and S. R. Hann. 1985. *v-myc*- and *c-myc*-encoded proteins are associated with the nuclear matrix. *Mol. Cell. Biol.* 5:114–126.
 13. Felber, B. K., H. Paskalis, C. Fleinman-Ewing, F. Wong-Staal, and G. N. Pavlakis. 1985. The pX protein of HTLV-I is a transcriptional activator of its long terminal repeats. *Science* 229:675–679.
 14. Feldman, L. T., and J. R. Nevins. 1983. Localization of the adenovirus E1Aa protein, a positive-acting transcriptional factor, in infected cells. *Mol. Cell. Biol.* 3:829–838.
 15. Fujisawa, F., M. Seiki, M. Sato, and M. Yoshida. 1985. A transcriptional enhancer sequence of HTLV-I is responsible for *trans*-activator mediated by p40x of HTLV-I. *EMBO J.* 5:713–718.
 16. Giam, C.-Z., M. Nerenberg, G. Khoury, and G. Jay. 1986. Expression of the complete human T-cell leukemia virus type I *pX* coding sequence as a functional protein in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 83:7192–7196.
 17. Giam, C.-Z., and Y.-L. Xu. 1989. HTLV-I *tax* gene product activates transcription via pre-existing cellular factors and cAMP responsive element. *J. Biol. Chem.* 264:15236–15241.
 18. Gitlin, S. D., P. F. Lindholm, S. J. Marriott, and J. N. Brady. 1991. Transdominant human T-cell lymphotropic virus type I *TAX*, mutant that fails to localize to the nucleus. *J. Virol.* 65:2612–2621.
 19. Goh, W. C., J. G. Sodroski, C. A. Rosen, M. Essex, and W. A. Haseltine. 1985. Subcellular localization of the product of the long open reading frame of human T-cell leukemia virus type I. *Science* 227:1227–1228.
 20. Guarente, L. 1988. UASs and enhancers: common mechanism of transcriptional activation in yeast and mammals. *Cell* 52:303–305.
 21. Halligan, B. D., K. A. Edwards, and L. F. Liu. 1985. Purification and characterization of a type II DNA topoisomerase from bovine calf thymus. *J. Biol. Chem.* 260:2475–2482.
 22. Hardy, S., and T. Shenk. 1988. Adenoviral control regions activated by E1A and cAMP response element bind to the same factor. *Proc. Natl. Acad. Sci. USA* 85:4171–4175.
 23. Hinrichs, S. H., M. Nerenberg, R. K. Reynolds, G. Khoury, and G. Jay. 1987. A transgenic mouse model for human neurofibromatosis. *Science* 237:1341–1343.
 24. Ito, H., Y. Fukuda, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* 153:163–168.
 25. Jackson, D. A., and P. R. Cook. 1985. Transcription occurs at a nucleoskeleton. *EMBO J.* 4:919–925.
 26. Jeang, K.-T., I. Boros, J. Brady, M. Radonovich, and G. Khoury. 1988. Characterization of cellular factors that interact with the human T-cell leukemia virus type I p40^x-responsive 21-base-pair sequence. *J. Virol.* 62:4499–4509.
 27. Jeang, K.-T., C.-Z. Giam, M. Nerenberg, and G. Khoury. 1987. Abundant synthesis of functional human T-cell leukemia virus type I p40^x protein in eukaryotic cells by using a baculovirus expression vector. *J. Virol.* 61:708–713.
 28. Jeang, K.-T., S. G. Widen, O. J. Semmes IV, and S. H. Wilson. 1990. HTLV-I *trans*-activator protein, tax, is a *trans*-repressor of the human β -polymerase gene. *Science* 247:1082–1084.
 29. Kadison, P., H. T. Poteat, K. M. Klein, and D. V. Faller. 1990. Role of protein kinase A in tax *trans*-activation of the human T-cell leukemia virus type I long terminal repeat. *J. Virol.* 64:2141–2148.
 30. Kiyokawa, T., T. Kawaguchi, M. Seiki, and M. Yoshida. 1985. Association of the *pX* gene product of human T-cell leukemia virus type-I with nucleus. *Virology* 147:462–465.
 31. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680–685.
 32. Lee, T.-H., J. E. Colligan, J. G. Sodroski, W. A. Haseltine, S. Z. Salahuddin, F. Wong-Staal, R. C. Gallo, and M. Essex. 1984. Antigens encoded by the 3' terminal region of human T-cell leukemia virus: evidence for a functional gene. *Science* 226:57–61.
 33. Lilenbaum, A., M. Duc Dodon, C. Alexandre, L. Gazzolo, and D. Paulin. 1990. Effect of human T-cell leukemia virus type I Tax protein on activation of the human vimentin gene. *J. Virol.* 64:256–263.
 34. Lin, Y.-S., and M. R. Green. 1989. Identification and purification of a *Saccharomyces cerevisiae* protein with the DNA binding specificity of mammalian activating transcription factor. *Proc. Natl. Acad. Sci. USA* 86:109–113.
 35. Marriott, S. J., P. F. Lindholm, K. M. Brown, S. D. Gitlin, J. F. Duvall, M. F. Radonovich, and J. N. Brady. 1990. A 36-kilodalton cellular transcription factor mediates an indirect interaction of human T-cell leukemia/lymphoma virus type I Tax1 with a responsive element in the viral long terminal repeat. *Mol. Cell. Biol.* 10:4192–4201.
 36. Matsumoto, K., I. Uno, Y. Oshima, and T. Ishikawa. 1982. Isolation and characterization of yeast mutants deficient in adenylate cyclase and cAMP-dependent protein kinase. *Proc. Natl. Acad. Sci. USA* 79:2355–2359.
 37. Nyborg, J. K., and W. S. Dynan. 1990. Interaction of cellular proteins with the human T-cell leukemia virus type I transcriptional control region. Purification of cellular proteins that bind the 21-base-pair repeat elements. *J. Biol. Chem.* 265:8230–8236.
 38. Oda, T., S. Watanabe, H. Sumii, T. Nakamura, Y. Arakaki, and K. Shimotohno. 1987. Immunoelectron microscopic localization of the *pX* gene products in mammalian T-cell leukemia virus type 1-producing cells. *Cancer Res.* 47:2077–2082.
 39. Paskalis, H., B. K. Felber, and G. N. Pavlakis. 1986. *cis*-acting sequences responsible for the transcriptional activation of human T-cell leukemia virus type I constitute a conditional enhancer. *Proc. Natl. Acad. Sci. USA* 83:6558–6562.
 40. Poteat, H. T., P. Kadison, K. McGuire, L. Park, R. E. Park, J. G. Sodroski, and W. A. Haseltine. 1989. Response of the human T-cell leukemia virus type I long terminal repeat to cyclic AMP. *J. Virol.* 63:1604–1611.
 41. Pozzatti, R., J. Vogel, and G. Jay. 1990. The human T-lymphotropic virus type I *tax* gene can cooperate with the *ras* oncogene to induce neoplastic transformation of cells. *Mol. Cell. Biol.* 10:413–417.
 42. Rose, M., and D. Botstein. 1983. Construction and use of gene fusions to *lacZ* (β -galactosidase) that are expressed in yeast. *Methods Enzymol.* 101:167–180.
 43. Rose, M. D., P. Novik, J. H. Thomas, D. Botstein, and G. R. Fink. 1987. A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. *Gene* 60:237–243.
 44. Rosen, C. A., R. Park, J. G. Sodroski, and W. A. Haseltine. 1987. Multiple sequence elements are required for regulation of human T-cell leukemia virus gene expression. *Proc. Natl. Acad. Sci. USA* 84:4919–4923.
 45. Ruben, S. M., and C. A. Rosen. 1990. Suppression of signals required for activation of transcription factor NF- κ B in cells

- constitutively expressing the HTLV-I tax protein. *New Biol.* 2:894-902.
46. Seiki, M., J. Inoue, T. Takeda, and M. Yoshida. 1986. Direct evidence that p40x of human T-cell leukemia virus type I is a *trans*-acting transcriptional activator. *EMBO J.* 5:561-565.
 47. Sherman, F., G. R. Fink, and J. B. Hicks. 1983. *Methods in yeast genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 48. Shimotohno, K., M. Takanao, T. Teruuchi, and M. Miwa. 1986. Requirement of multiple copies of a 21-nucleotide sequence in the U3 regions of human T-cell leukemia virus type I and type II long terminal repeats for *trans*-acting activation of transcription. *Proc. Natl. Acad. Sci. USA* 83:8112-8116.
 49. Siekevitz, M., M. B. Feinberg, N. Holbrook, F. Wong-Staal, and W. C. Greene. 1987. Activation of interleukin-2 and interleukin-2 receptor (tac) promote expression by the *trans*-activator (tax) gene product of human T-cell leukemia virus type I. *Proc. Natl. Acad. Sci. USA* 84:5389-5393.
 50. Siekevitz, M., S. F. Josephs, M. Dukovich, N. Pfeffer, F. Wong-Staal, and W. C. Greene. 1987. Activation of the HIV-1 LTR by T-cell mitogens and the *trans*-activator protein of HTLV-I. *Science* 238:1575-1578.
 51. Slamon, D. J., W. J. Boyle, D. E. Keith, M. F. Press, D. W. Golde, and L. M. Souza. 1988. Subnuclear localization of the *trans*-activating protein of human T-cell leukemia virus type I. *J. Virol.* 62:680-686.
 52. Smith, M. R., and W. C. Greene. 1990. Identification of HTLV-I tax *trans*-activator mutants exhibiting novel transcriptional phenotype. *Genes Dev.* 4:1875-1885.
 53. Sodroski, J., C. Rosen, W. C. Goh, and W. Haseltine. 1985. A transcriptional activator protein encoded by the *x-lor* region of the human T-cell leukemia virus type I. *Virology* 144:59-65.
 54. Sodroski, J. G., C. A. Rosen, and W. A. Haseltine. 1984. *trans*-Acting transcriptional activation of the long terminal repeat of human T lymphotropic viruses in infected cells. *Science* 225:381-385.
 55. Tan, T.-H., M. Horikoshi, and R. G. Roeder. 1989. Purification and characterization of multiple nuclear factors that bind to the Tax-inducible enhancer within the human T-cell leukemia virus type 1 long terminal repeat. *Mol. Cell. Biol.* 9:1733-1745.
 56. Tan, T.-H., R. Jia, and R. G. Roeder. 1989. Utilization of signal transduction pathway by the human T-cell leukemia virus type I transcriptional activator *tax*. *J. Virol.* 63:3761-3768.
 57. Tanaka, A., C. Takahashi, S. Yamaoka, T. Nasoka, M. Maki, and M. Hatanaka. 1990. Oncogenic transformation by the *tax* gene of human T-cell leukemia virus type I *in vitro*. *Proc. Natl. Acad. Sci. USA* 87:1071-1075.
 58. Varmus, H. 1988. Regulation of HIV and HTLV gene expression. *Genes Dev.* 2:1055-1062.
 59. Wano, C. M., N. Feinberg, J. B. Hosking, H. Bogerd, and C. Greene. 1988. Stable expression of the *tax* gene of type I human T-cell leukemia virus in human T cells activates specific cellular genes involved in growth. *Proc. Natl. Acad. Sci. USA* 85:9733-9737.
 60. West, R. W., Jr., R. R. Yocum, and M. Ptashne. 1984. *Saccharomyces cerevisiae* GAL1-GAL10 divergent promoter region: location and function of the upstream activating sequence UAS_G. *Mol. Cell. Biol.* 4:2467-2478.
 61. Worland, S. T., and J. C. Wang. 1989. Inducible overexpression, purification, and active site mapping of DNA topoisomerase II from the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* 264:4412-4416.
 62. Wu, L.-C. C. Unpublished results.
 63. Wu, L.-C. C., P. A. Fisher, and J. R. Broach. 1987. A yeast plasmid partitioning protein is a karyoskeletal component. *J. Biol. Chem.* 262:883-891.
 64. Yoshida, M., and M. Seiki. 1987. Recent advances in the molecular biology of HTLV-I: *trans*-activation of viral and cellular genes. *Annu. Rev. Immunol.* 5:541-559.