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16. All of these lines expressed the indicated viral gene products, as determined by immunoprecipitation with specific antibodies, and all but the v-*myc*–infected cells formed foci in soft agar at frequencies of at least 30 per 10^4 cells plated.
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HTLV-I Trans-Activator Protein, Tax, Is a Trans-Repressor of the Human β-Polymerase Gene

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Human T cell leukemia virus type I (HTLV-I) is the etiological agent for adult T cell leukemia (ATL). The HTLV-I trans-activator protein Tax can activate the expression of its own long terminal repeat (LTR) and many cellular and viral genes. Tax down-regulated the expression of human β-polymerase (huβ-pol), a cellular enzyme involved in host cell DNA repair. This finding suggests a possible correlation between HTLV-I infection and host chromosomal damage, which is often seen in ATL cells.

HTLV-I IS ETIOLOGICALLY ASSOCIATED with one particular type of ATL (*1*). This human retrovirus contains genes coding for the Gag, Pol, and Env structural proteins and is also replication competent. It does not have a typical oncogene, and the mechanism, or mechanisms, for HTLV-I–induced leukemogenesis remains unclear.

A salient feature of HTLV-I is the presence at the 3′ end of its genome of overlapping open reading frames encoding three proteins (40, 27, and 21 kD) (*2*). It is known that the 40-kD (Tax) protein is a potent trans-activator of viral LTR-directed transcription (*3*). The 27-kD (Rex) protein functions in some aspect of post-transcriptional processing of viral RNAs (*4*). The role of the 21-kD polypeptide is currently unknown.

Molecular studies have shown that HTLV-I Tax can activate some cellular genes such as interleukin-2 (IL-2), IL-2 receptor α (IL-2Rα), c-*fos*, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (*5*). Through this process, it has been suggested that Tax can manifest an oncogenic potential (*6*) similar to that observed in a transgenic mouse model (*7*). A plausible pathway by which Tax can influence T cell proliferation is through the induction of both IL-2 and IL-2Rα chain. However, these properties may not be sufficient for cellular transformation, since in ATL cells elevated expression of IL-2Rα is not always accompanied by the production of IL-2 (*8*).

From an alternative perspective, HTLV-I may alter host cell metabolism through the repression of selected cellular genes. The ubiquitous finding that ATL cells are karyotypically abberrant (*9*) has prompted some investigators to suggest that host genome damage may be an additional event needed for HTLV-I to induce malignancies (*6*). We explored the possibility that a viral trans-acting function could be associated with host DNA abnormalities.

The human β-polymerase (huβ-pol) is a 39-kD DNA-polymerizing enzyme that functions not in the replication but in the repair of DNA (*10*). The promoter-regulatory sequences of the huβ-pol gene have been characterized (*11*). Upstream of the huβ-pol transcriptional initiation site is a TGACGTCA motif (*11*). In other systems, this sequence confers cyclic adenosine monophosphate (cAMP) responsiveness to linked genes (*12*), and it also constitutes one portion of the Tax-responsive element in the HTLV-I LTR (*13*). We reason that the biological behavior of the huβ-pol promoter could likely be modulated by Tax.

Trans-regulation of the huβ-pol promoter by Tax was tested in a transient co-transfection assay. The homology between the TGACGTCA motifs of huβ-pol and the HTLV-I LTR predicted a positive trans-acting effect by Tax. When a Tax-producing plasmid (pCMVPx), containing a cDNA coding for the 40-kD protein (*7*) driven by the simian cytomegalovirus (CMV) immediate-early promoter (*14*), was introduced into human epithelial cells (HeLa) with either of two huβ-pol–CAT hybrid genes pβP2 or pβP8 [containing a −1152 to +62 or a −113 to +62 fragment of the huβ-pol placed upstream of the CAT gene, respectively (*11*)], the results obtained were unexpected. We found that increasing the amount of pCMVPx substantially reduced the activity of the huβ-pol promoter (Fig. 1A, lanes 4 to 6 and lanes 7 to 9). In contrast, the same amounts of pCMVPx DNA markedly activated the expression of the HTLV-I LTR (pU3RCAT; Fig. 1A, lanes 1 to 3). When CAT mRNA levels were quantitated instead of CAT enzyme activity, similar results were seen (Fig. 1B). These findings suggest that the repression by Tax of the huβ-pol promoter occurred at the level of transcription. Similar results were obtained with a plasmid in which Tax production was driven by HTLV-I LTR (*7*), whereas no significant repression was observed in co-transfections with Rex- or p21-producing plasmids (*15*).

If the above observations are indeed valid for HTLV-I biology, one would expect similar findings in ATL cells. We analyzed two independently derived cell lines of HTLV-I–transformed T lymphocytes (C8166-45 and MT-4) (*16*). Both were found to express HTLV-I Tax constitutively (Fig. 2A, lanes 2 and 3). However, when compared to Jurkat cells (Fig. 2B, lane 1), each had greatly reduced synthesis of the huβ-pol mRNA (Fig. 2B, lanes 2 and 3). There are "normal" variations between cell lines in the amount of steady-state huβ-pol mRNA. For example, we found that human erythroleukemia K562 cells contain 50% as much steady-state huβ-pol mRNA relative to Jurkat cells (*15*). However, this level is still four to six times higher than that seen for C8166-45 or MT-4 cells. These findings in permanently propagating cells corroborate in part the parallel observations from

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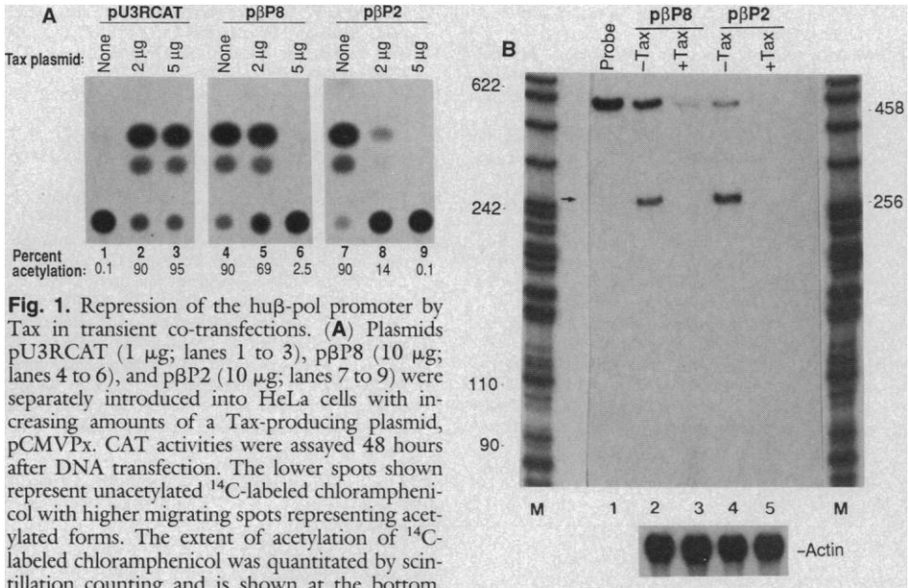

Fig. 1. Repression of the huβ-pol promoter by Tax in transient co-transfections. (**A**) Plasmids pU3RCAT (1 μg; lanes 1 to 3), pβP8 (10 μg; lanes 4 to 6), and pβP2 (10 μg; lanes 7 to 9) were separately introduced into HeLa cells with increasing amounts of a Tax-producing plasmid, pCMVPx. CAT activities were assayed 48 hours after DNA transfection. The lower spots shown represent unacetylated ^{14}C-labeled chloramphenicol with higher migrating spots representing acetylated forms. The extent of acetylation of ^{14}C-labeled chloramphenicol was quantitated by scintillation counting and is shown at the bottom. The numbers shown represent the average from three experiments. Absolute values of acetylation varied by less than 20% from experiment to experiment. Analogous results were also seen in transfections of CV-1 and Jurkat cells. (**B**) Reduced transcription from the huβ-pol promoter in the presence of Tax. Total cellular RNA was isolated from HeLa cells transfected with pβP8 and pβP2 without (lanes 2 and 4) or with (lanes 3 and 5) pCMVPx, respectively. The amount of CAT-specific mRNA was assayed by S1 nuclease protection with a 458-bp single-stranded DNA probe (*20*). Lane 1 contains ^{32}P-labeled single-stranded probe alone. The arrow points to a 256-bp band, which is the expected size protected from S1 nuclease digestion. Molecular size markers (lane M) are pBR322-Hpa II–digested fragments. For normalization, equal amounts of RNAs used in the S1 assays (lanes 2 to 5) were also hybridized to a human β-actin probe in a Northern analysis (shown at bottom).

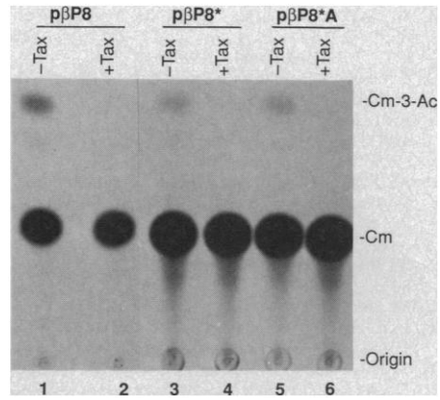

Fig. 3. The TGACGTCA motif is not necessary for downregulation by Tax. The pβP8* and pβP8*A constructions are identical to the pβP8 plasmid, except for the following (underlined) changes in the TGACGTCA sequence: pβP8* TGGGTGCACCCA and pβP8*A TGGGCCCA. The pβP8, pβP8*, or pβP8*A were co-transfected with or without pCMVPx (compare lane 1 to 2, lane 3 to 4, and lane 5 to 6). Reactions shown in lanes 1 and 2 were performed with one-fifth the amount of extract as those shown in lanes 3 to 6, and were exposed for autoradiography for one-seventh the amount of time. Cm, chloramphenicol; Cm-3-Ac, acetylated chloramphenicol.

the transient transfection assays.

The mechanism of action of Tax is yet to be fully elucidated. In several studies, it is clear that a conserved TGACGTCA motif is a crucial component of the DNA target within the HTLV-I LTR, which is transactivated by Tax (*13*). We investigated whether this consensus found in the huβ-pol promoter could also be the target for Tax-mediated repression. Starting with the pβP8 construction (*11*), we engineered two site-directed mutations that altered the TGACGTCA sequence (pβP8* and pβP8*A; Fig. 3). When pβP8* and pβP8*A were assayed in transient co-transfections with pCMVPx, both were found to be reduced in basal expression but each was still downregulated by Tax (Fig. 3). Thus, the TGACGTCA octanucleotide is an important basal component of the huβ-pol promoter. However, in this context, it is not required for Tax-mediated repression.

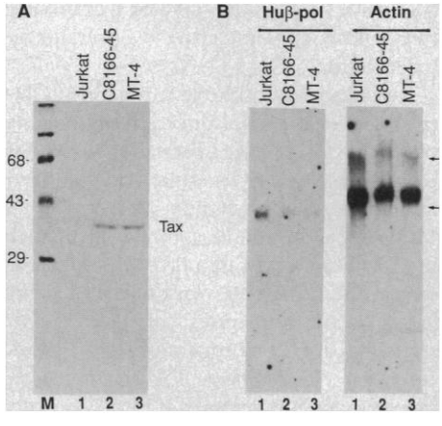

Fig. 2. Expression of the huβ-pol gene is reduced in HTLV-I–transformed cells. (**A**) Immunoblotting of Jurkat, C8166-45, and MT-4 cells with a Tax-specific antiserum. Visualization of antibody-antigen reactions was performed with ^{125}I-labeled protein A. Lane M contains ^{14}C-labeled molecular size markers. (**B**) Expression of huβ-pol transcript in Jurkat, C8166-45, and MT-4 cells. Total cellular RNAs (25 μg) were hybridized in two identical filters to either a ^{32}P-labeled huβ-pol cDNA (left) or a human β-actin probe (right). The relative positions of the 28*S* (top arrow) and 18*S* (bottom arrow) ribosomal RNAs are indicated.

In this study, we report on the negative regulation of one cellular gene by the HTLV-I Tax protein. Previous analyses have found that Tax can activate the expression of many genes that share no apparent homology to each other in their regulatory sequences (*5*). Tax is not a sequence-specific DNA-binding protein, and it likely uses a multitude of cellular factors to mediate diverse functions (*13, 17*). We do not currently know whether the observed repression of huβ-pol is a direct or an indirect effect engendered by Tax. At very high levels of expression Tax can be toxic to the cell (*18*). Our transient transfection studies controlled for this possibility (see pU3RCAT; Fig. 1A) and were conducted with amounts of a Tax-producing plasmid that previously exhibited no detectable toxicity (*18*). The finding that huβ-pol expression is diminished in ATL cells (Fig. 2) also argues against toxicity from the introduction of exogenous DNA. We believe that huβ-pol may represent only one of many cellular genes that can be down regulated by HTLV-I. Another candidate is the gene for the CD3 receptor, which is severely reduced in expression in ATL cells (*19*).

Less than 1% of individuals infected with HTLV-I eventually progress to leukemia (*6*). One interpretation of this natural progression of ATL is that a second event must be involved in viral transformation. The suggestion that this additional event is preexisting host DNA damage (*6*) is compatible with our present findings. In such a model, one can hypothesize that in the rare infection of a chromosomally damaged lymphocyte, the abrogation of the cellular DNA-repair system contributes towards leukemogenesis.

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Enhancement of SIV Infection with Soluble Receptor Molecules

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The CD4 receptor on human T cells has been shown to play an integral part in the human immunodeficiency virus type 1 (HIV-1) infection process. Recombinant soluble human CD4 (rCD4) was tested for its ability to inhibit SIVagm, an HIV-like virus that naturally infects African green monkeys, in order to define T cell surface receptors critical for SIVagm infection. The rCD4 was found to enhance SIVagm infection of a human T cell line by as much as 18-fold, whereas HIV-1 infection was blocked by rCD4. Induction of syncytium formation and de novo protein synthesis were observed within the first 24 hours after SIVagm infection, whereas this process took 4 to 6 days in the absence of rCD4. This enhancing effect could be inhibited by monoclonal antibodies directed to rCD4. The enhancing effect could be abrogated with antibodies from naturally infected African green monkeys with inhibitory titers of from 1:2,000 to 1:10,000; these antibodies did not neutralize SIVagm infection in the absence of rCD4. Viral enhancement of SIVagm infection by rCD4 may result from the modulation of the viral membrane through gp120-CD4 binding, thus facilitating secondary events involved in viral fusion and penetration.

RECEPTOR-MEDIATED INFECTION of T cells by HIV-1 has been studied extensively, and reports have shown that the interaction of HIV-1 gp120 with CD4 is the primary event required for infection of susceptible human T cells (*1, 2*). Subsequent events responsible for penetration are theorized to include direct fusion processes by way of a region within the transmembrane protein (TMP) (*3*). The exact sequence of events leading to penetration, uncoating, and replication of HIV is unknown. Members of the immunodeficiency virus family that were originally isolated from nonhuman primates also replicate efficiently in human CD4-positive T cells (*4–10*), although some restriction of infection has been reported for SIVagm and SIVmac (*8–11*). We began these studies to define the nature of receptor-mediated infection by SIVagm viruses and to evaluate the biological relation of SIVagm with HIV-1 in their requirements for gaining cell entry and replication.

Monoclonal antibodies (MAbs) to CD4 (including anti-Leu3a and OKT4A) define major epitopes on CD4 that overlap those regions responsible for gp120 binding and therefore efficiently block HIV-1 infection of CD4-bearing T cell lines (*2, 12*). To determine if SIVagm(tyo-1), an isolate previously characterized and shown to share approximately 50% nucleic acid sequence homology with HIV-1 (*8*), also uses similar epitopes on CD4 for gp120 binding, a panel of mouse MAbs to CD4 (anti-CD4) were tested for their ability to block infection of a CD4-positive T cell line (MOLT-4 cl.8) by SIVagm(tyo-1) and HIV-1. This cell line is highly permissive for SIVagm infection as assessed by syncytium formation, unlike most other human CD4-bearing T cell lines (*6*). Those antibodies capable of blocking HIV-1 were also able to block SIVagm(tyo-1) infection, as measured by a total reduction in syncytium formation (Table 1). Monoclonals that bind to regions other than the NH_2-terminal V1 domain of CD4 did not block either HIV-1 or SIVagm(tyo-1) infection. These data suggest that SIVagm(tyo-1) uses essentially the same CD4 epitopes as HIV-1 for binding and that binding through the CD4 molecule is a prerequisite for infection.

Recombinant soluble human CD4 (rCD4) blocks HIV-1 infection of human T cell lines (*13, 14*). Incubation of rCD4 (25 μg/ml) with HIV-1 resulted in complete inhibition of syncytium formation in MOLT-4 cl.8 cells. In contrast, incubation of rCD4 with SIVagm(tyo-1) resulted in syncytium formation within the first 24 hours of infection rather than 4 to 6 days after infection as normally observed in the absence of rCD4 (Fig. 1). The enhanced rate of syncytium formation in the presence of rCD4 makes it unlikely that the lack of inhibition is due to a lower affinity of human-derived CD4 for SIVagm. The effects of rCD4 on SIVagm were concentration dependent; concentrations as low as 250 ng/ml enhanced syncytium formation by SIVagm at 1 to 2 days after infection. At concentrations below 250 ng/ml, there was no enhancement, and syncytium formation was observed at 6 days. The effect of rCD4 on HIV-1 was also concentration depen-

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