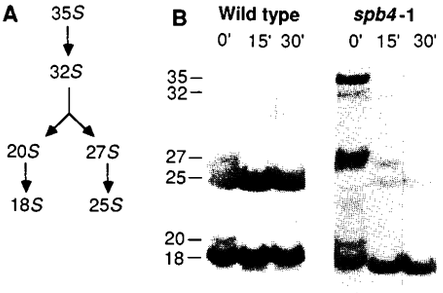

Fig. 3. *SPB4* is involved in the maturation of 25*S* rRNA. (**A**) Schematic representation of the processing of pre-rRNA in *S. cerevisiae*. (**B**) Pulse-chase analysis of pre-rRNA processing in wild-type cells (YAS43, MAT**a** *his3 leu2 trp1 ura3*) and the *spb4-1* mutant (YAS165). The location of the different rRNAs on the gel are shown, and the length of the chase period in minutes is indicated. Approximately 10^9 exponentially growing cells in 45 ml of minimal medium supplemented with the auxotrophic requirements (*20*) were concentrated to 1 ml, and the rRNA was pulse-labeled for 6 min with 50 μCi of [methyl-^{3}H]methionine (Amersham, 75 Ci/mmol) at 30°C (rRNA is selectively labeled in this procedure by methylation through the methyl donor *S*-adenosylmethionine) (*8*). The chase period was initiated by dilution of cells into 45 ml of minimal medium plus 1 mg/ml unlabeled methionine. Samples (10 ml) were withdrawn at the indicated times, and total RNA was prepared as previously described (*18*). RNA quantities yielding equivalent 18*S* rRNA band intensities (~20,000 cpm) were denatured with glyoxal and fractionated on a 1% agarose gel in 10 m*M* sodium phosphate, *p*H 7.0 (*19*), fixed in sodium salicylate (*21*), and visualized by fluorography for 5 days at −70°C. Some mature rRNA appears in the 0′ lanes because of the length of the pulse-labeling. The slightly altered 18*S* rRNA migration seen in the *spb4-1* chase lanes is not reproducible between experiments.

bypasses the cellular requirement for PAB. These results suggest that the *spb2* mutants previously isolated (*2*) are deficient in a functional *RPL46* gene product.

The discovery of a putative RNA helicase that is involved in the maturation of rRNA defines a new substrate for this family of proteins. The availability of the *spb4* conditional mutations, together with the potential of developing an in vitro assay for the protein's putative ATPase and helicase activities, should allow the identification of the essential residues for these functions. The srmB protein of *Escherichia coli* is also a member of this family, and its overexpression allows the assembly of the 50*S* ribosomal subunit in the presence of a temperature-sensitive *RPL24* mutation (*11*). Whether this protein is utilized for unwinding rRNA in a wild-type background, and whether this potential activity is similar to that of SPB4, remains to be determined.

The experiments presented here suggest that the formation of the 60*S* ribosomal subunit involves specific, enzymatic unwinding of the rRNA by SPB4. It is unlikely that SPB4 is part of the mature ribosome since ribosomal proteins of this molecular weight have not been reported (*12*). SPB4 could be part of the preribosomal particle in the nucleus that is involved in ribosomal biogenesis. *Spb4-1* might exert its suppressor effect by changing the regions of rRNA that are unwound during subunit assembly, thereby altering the structure of the subunit and possibly its protein components. For example, RPL46 and other proteins may not associate with this modified subunit, and this could allow a basal level of PAB-independent translation initiation. We suspect that equivalent mutations in helicases involved in the assembly of other ribonucleoproteins, like the splicesome, could have analogous effect on their composition and specificity.

If PAB-independent translation is made possible by altering the native 60*S* subunit structure, then a detailed analysis of the *spb* mutants will yield much information about the multiple gene products that play a role in the assembly of this particle. The ability to positively select for the *spb* mutations (*2*) will greatly simplify these studies. Future work will focus on determining if the 60*S* ribosomal subunits in *spb1–spb7* mutants have distinct compositions of ribosomal proteins, and how the requirements of PAB for initiation is mediated by this subunit.

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Tyrosine Kinase Activity and Transformation Potency of *bcr-abl* Oncogene Products

TRACY G. LUGO, ANN-MARIE PENDERGAST, ALEXANDER J. MULLER, OWEN N. WITTE

Oncogenic activation of the proto-oncogene c-*abl* in human leukemias occurs as a result of the addition of exons from the gene *bcr* and truncation of the first *abl* exon. Analysis of tyrosine kinase activity and quantitative measurement of transformation potency in a single-step assay indicate that variation in *bcr* exon contribution results in a functional difference between p210$^{bcr\text{-}abl}$ and p185$^{bcr\text{-}abl}$ proteins. Thus, foreign upstream sequences are important in the deregulation of the kinase activity of the *abl* product, and the extent of deregulation correlates with the pathological effects of the *bcr-abl* proteins.

AMINO-TERMINAL DELETIONS FREquently activate tyrosine kinase oncogene products [for example, *abl* (*1*), *src* (*2*), and *erb*B (*3*)]. The activation of c-*abl* in human leukemias occurs after a chromosomal translocation that replaces the first exon of c-*abl* with sequences from *bcr*, which results in the generation of a fused *bcr-abl* transcript (*4*). Alternative forms of the *bcr-abl* oncogene product, p210$^{bcr\text{-}abl}$ and p185$^{bcr\text{-}abl}$, are characteristic of chronic myelogenous leukemia (CML) and Philadelphia chromosome–positive acute lymphocytic leukemia (ALL), respectively. In ALL the breakpoint on chromosome 22 occurs within the first intron of *bcr*, whereas in

Department of Microbiology and Molecular Biology Institute and the Howard Hughes Medical Institute, University of California, Los Angeles, CA 90024.

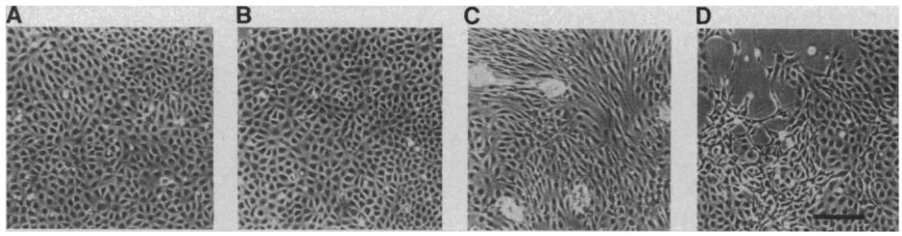

Fig. 1. Morphology of Rat 1 cells mock-infected (**A**) or infected with p210$^{bcr\text{-}abl}$ (**B**), p185$^{bcr\text{-}abl}$ (**C**), or p160$^{v\text{-}abl}$ (**D**). Phase-contrast micrographs were taken 18 days after infection with viruses (*8*). Optical magnification was ×100.

Table 1. Focus formation in soft agar by Rat 1 fibroblasts after acute infection with *bcr-abl* viruses. Values reported are from individual experiments. Assays for anchorage-independent growth in 20% fetal bovine serum were performed as described (*8*), except that cells were plated in soft agar 48 hours after infection.

Virus	Foci per 10^4 cells plated
None	<0.5, <0.5, <0.5
p210$^{bcr\text{-}abl}$	<0.5, <0.5, 1.4, 18
p185$^{bcr\text{-}abl}$	275, 380, 240, 500
p160$^{v\text{-}abl}$	1000, >1000

CML it lies within a 5-kb region further downstream (*5, 6*). Both gene products include identical sequences derived from c-*abl*, including the tyrosine kinase domain; they differ by the deletion of 501 internal *bcr*-derived residues in p185$^{bcr\text{-}abl}$. A study of c-*abl* deletion mutants has shown that removal of NH_2-terminal sequences is necessary for c-*abl* oncogenic activation (*1*). If NH_2-terminal deletion is the critical event in c-*abl* protein activation, the two *bcr-abl* proteins should have similar transforming activity, because they have identical NH_2-termini.

Both p185$^{bcr\text{-}abl}$ and p210$^{bcr\text{-}abl}$ can transform primary bone marrow cells, and p185$^{bcr\text{-}abl}$ is a more potent carcinogen in hematopoietic cells than p210$^{bcr\text{-}abl}$ (*7*). The transformed cells in these cultures represent clonal or oligoclonal outgrowths from the mass population of infected cells and may have accumulated secondary oncogenic events. We have used recombinant retroviruses to express *bcr-abl* proteins in Rat 1 fibroblasts in order to compare their oncogenicity directly and quantitatively in a single step with an assay for growth in soft agar (*8*). We found that p185$^{bcr\text{-}abl}$ is more effective than p210$^{bcr\text{-}abl}$ at eliciting transformation of Rat 1 cells after acute infection (Table 1). The frequency of macroscopic foci formed in soft agar by Rat 1 cells infected with p185$^{bcr\text{-}abl}$ virus was 100-fold higher than that seen after infection with p210$^{bcr\text{-}abl}$ virus in the same experiment, in which virus stocks matched for *abl* RNA content by slot blotting were used (*9*). Abelson murine leukemia virus expressing v-*abl* was included as a positive control. Patches of morphologically transformed cells were evident in the monolayer of p185-virus–infected Rat 1 cells; these did not appear in the p210-virus–infected cells (Fig. 1). We found similar extents of viral infection [an average of one integration per cell as determined by Southern (DNA) blotting] in all the infected populations (*9*).

To make a quantitative comparison of the relative kinase activities of the *bcr-abl* proteins, we measured the autophosphorylation activity of samples containing approximately equimolar amounts of p185$^{bcr\text{-}abl}$ and p210$^{bcr\text{-}abl}$, which were prepared by in vitro translation in a reticulocyte lysate (Fig. 2, A and B). The amount of ^{32}P incorporated into the p185$^{bcr\text{-}abl}$ sample was five to ten times as large as that incorporated into the comparable p210$^{bcr\text{-}abl}$ sample. We observed a similar result when we compared the autokinase activities of p185$^{bcr\text{-}abl}$ and p210$^{bcr\text{-}abl}$ proteins that had been immunoprecipitated from rodent fibroblasts after acute infection with *bcr-abl* retroviruses (Fig. 2, C and D). Tryptic phosphopeptide maps have shown that p185$^{bcr\text{-}abl}$ has fewer autophosphorylation sites than p210$^{bcr\text{-}abl}$, and the extent of phosphate incorporation into the peptides that are phosphorylated in p185$^{bcr\text{-}abl}$ is similar to the incorporation in those in p210$^{bcr\text{-}abl}$ (*10*). Therefore the

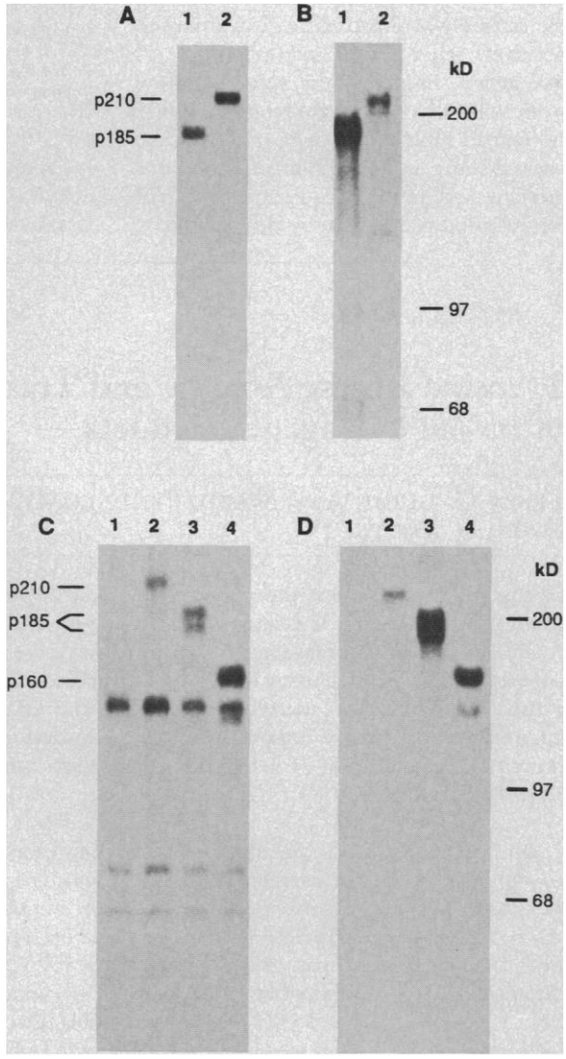

Fig. 2. Autokinase activity of *bcr-abl* proteins synthesized in vitro or immunoprecipitated from *bcr-abl* virus–infected Rat 1 cells. (**A** and **B**) ^{35}S-labeled *bcr-abl* proteins were prepared by in vitro transcription and translation as described (*12*). Each reaction mixture was immunoprecipitated with the αpEX5 antibody to the COOH-terminus of the *abl* protein (*13*). Samples were divided and one set was subjected to autophosphorylation in the presence of [γ-^{32}P]ATP (adenosine triphosphate) (*14*). Incorporated ^{35}S was determined by subjecting equal volumes of ^{35}S-labeled material to SDS–polyacrylamide gel electrophoresis (SDS-PAGE) on a 7% acrylamide gel and by determining the radioactivity present in the *bcr-abl* protein bands by scintillation counting. ^{35}S-labeled and ^{35}S- and ^{32}P-labeled p185$^{bcr\text{-}abl}$ (lane 1) and p210$^{bcr\text{-}abl}$ (lane 2) proteins, normalized for incorporation of ^{35}S, were subjected to SDS-PAGE on 7% acrylamide gels. ^{35}S-labeled *bcr-abl* proteins were detected by fluorography (A), and the ^{32}P-labeled *bcr-abl* proteins by autoradiography through four sheets of aluminum foil, which reduced the ^{35}S signal to negligible levels (B). (**C** and **D**) Rat 1 cells (lane 1) were infected with retroviruses containing p210$^{bcr\text{-}abl}$ (lane 2), p185$^{bcr\text{-}abl}$ (lane 3), or p160$^{v\text{-}abl}$ (lane 4) oncogenes. Forty-eight hours after infection, equal numbers of cells were either labeled with [^{35}S]methionine (C) for 3 hours and then lysed, and the *abl* proteins immunoprecipitated with αpEX5 (C), or were immediately lysed, and the *abl* proteins immunoprecipitated and autophosphorylated in vitro in the presence of [γ-^{32}P]ATP (*14*) (D).

amount of ^{32}P incorporated into equimolar samples of p185$^{bcr\text{-}abl}$ and p210$^{bcr\text{-}abl}$ must correspond to the number of molecules undergoing autophosphorylation during the course of these assays and thus to the kinase activity of the proteins. Also, the specific activity of p185$^{bcr\text{-}abl}$ for phosphorylation of angiotensin, an exogenous substrate, was five times as high as that of p210$^{bcr\text{-}abl}$ (Fig. 3). Thus the loss of *bcr* sequences leads to a more profound activation of the *abl* tyrosine kinase in p185$^{bcr\text{-}abl}$.

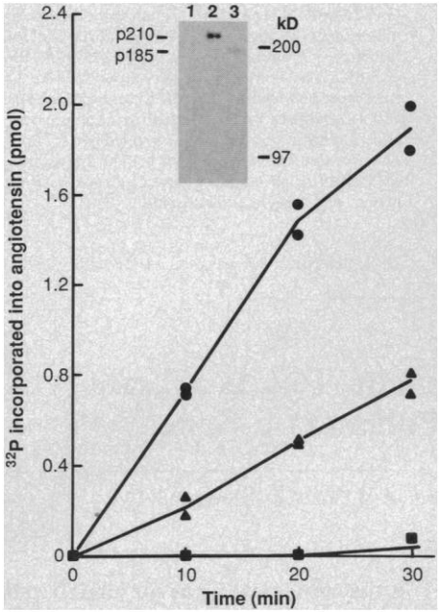

Fig. 3. Relative specific activities of p185$^{bcr\text{-}abl}$ and p210$^{bcr\text{-}abl}$ for phosphorylation of angiotensin. Control and infected cells were labeled for 6 hours with [^{35}S]methionine, cell lysates were normalized for ^{35}S incorporation into protein by scintillation counting of trichloroacetic acid precipitates, and the normalized lysates were immunoprecipitated with αpEX5. Phosphorylation of angiotensin II was assayed in the presence of 20 μ*M* [γ-^{32}P]ATP (*15*). The time course of ^{32}P incorporation into angiotensin catalyzed by immunoprecipitates of uninfected Rat 1 cells (■), p210-expressing cells (▲), and p185-expressing cells (●) was followed. The mean number of picomoles of ^{32}P incorporated into angiotensin per minute (calculated from values observed at the 10- and 20-min time points) was 0.023 for the p210$^{bcr\text{-}abl}$ sample and 0.074 for the p185$^{bcr\text{-}abl}$ sample. To determine the relative amounts of *bcr-abl* proteins in the reactions, we subjected one-fifth of each immunoprecipitate to SDS-PAGE, and incorporated ^{35}S was visualized by fluorography (**Inset**): lane 1, control Rat 1 cells; lane 2, p210-expressing cells; and lane 3, p185-expressing cells. Densitometric scanning of the fluorogram indicated that the p210$^{bcr\text{-}abl}$ sample contained 1.5 times as many *bcr-abl* molecules as the p185$^{bcr\text{-}abl}$ sample, after the values were adjusted for the number of methionine residues in each protein. Specific activities were compared by dividing the number of picomoles of ^{32}P incorporated into angiotensin per minute by the p210$^{bcr\text{-}abl}$ sample by 1.5; the specific activity of the p185$^{bcr\text{-}abl}$ sample was thus 4.8 times as high as that of the p210$^{bcr\text{-}abl}$ sample.

We used protein immunoblotting with an antibody to phosphotyrosine to compare the profiles of phosphorylated proteins in p185- and p210-virus–infected fibroblasts (Fig. 4). The *bcr-abl* proteins themselves were the major phosphorylated species in all the *bcr-abl*–infected cell populations. A distinct band of proteins with apparent molecular masses of ~60 to 62 kD was visible in lysates of *bcr-abl*–infected Rat 1 cells, but not in those of control Rat 1 cells. The faster migrating 60-kD species was detectable in lysates of p210-virus–infected cells but was more prominent in p185-virus–infected cells. The slower migrating 62-kD components (pp62) appeared exclusively in p185-virus–infected cells. This slower species, which may be a more highly phosphorylated derivative of the faster migrating form, also appears in Rat 1 cells expressing p160$^{v\text{-}abl}$ (*9*). We have not yet identified this protein (or proteins), but in a series of Rat 1 cell lines expressing other oncogenes, including other protein tyrosine and serine kinases, only v-*src* expression was accompanied by the appearance of a similar ~60-kD protein reactive with antibody to phosphotyrosine (Fig. 4). It is possible that this species may be part of a pathway common to the two cytoplasmic tyrosine kinases.

The p185$^{bcr\text{-}abl}$ fusion protein has consistently been associated with an aggressive form of leukemia that is distinct from CML (*6, 11*). Our results indicate that the form of *bcr-abl* more frequently associated with acute diseases encodes a more active tyrosine kinase and has much greater transforming activity in both hematopoietic cells and fibroblasts. This complexity of oncogene activation suggests that the *bcr* gene segments function as important determinants of pathogenesis for the generation of Philadelphia chromosome–positive human leukemias.

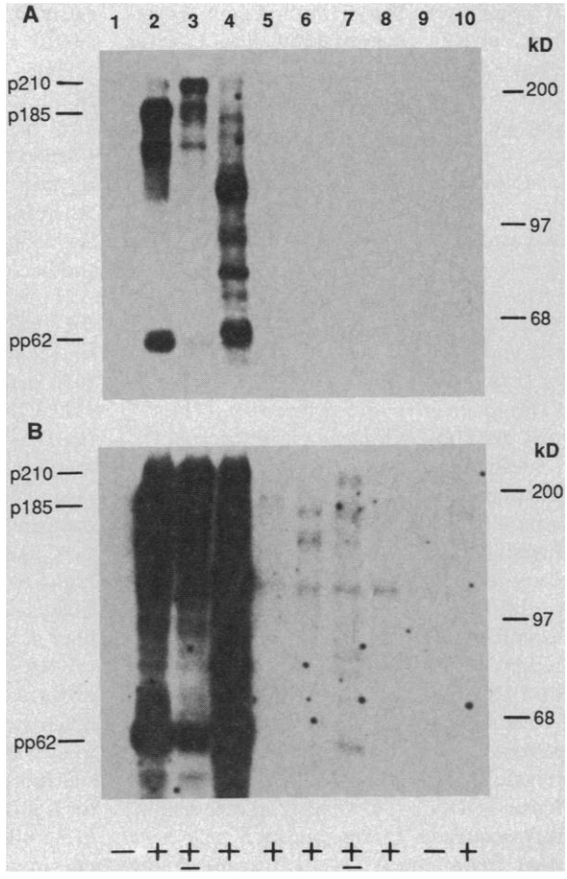

Fig. 4. Proteins phosphorylated on tyrosine in Rat 1 cells expressing activated oncogenes. Rat 1 cells were infected with retroviral vectors expressing oncogenes: lane 1, control vector not containing oncogene sequences; lane 2, p185$^{bcr\text{-}abl}$; lane 3, p210$^{bcr\text{-}abl}$; lane 4, v-*src*; lane 5, v-*mos*; lane 6, v-*fms*; lane 7, v-*fes*; lane 8, v-H-*ras*; lane 9, v-*myc*; lane 10, v-H-*ras* and v-*myc* coexpressed (*16*). Equivalent amounts of cellular protein (27 μg) from each of the cell lines were separated by electrophoresis on a SDS–8% polyacrylamide gel, transferred electrophoretically to a nitrocellulose filter, and subjected to immunoblotting with antibodies to phosphotyrosine (*17*). After antibody treatment, the filters were washed and then incubated with ^{125}I-labeled protein A for 1 hour at room temperature (*18*). The filters were subjected to autoradiography for 24 hours (**A**) and 168 hours (**B**) at −70°C with an intensifying screen. The positions of p210$^{bcr\text{-}abl}$, p185$^{bcr\text{-}abl}$, and the pp62 protein are indicated. The cells were examined for morphological transformation; + indicates transformation, ± partial transformation (*8*) and − no transformation.

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HTLV-I Trans-Activator Protein, Tax, Is a Trans-Repressor of the Human β-Polymerase Gene

KUAN-TEH JEANG,* STEVEN G. WIDEN, OLIVER J. SEMMES IV, SAMUEL H. WILSON

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

K.-T. Jeang and O. J. Semmes IV, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.
S. G. Widen and S. H. Wilson, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

*To whom correspondence should be addressed.