brucei (*14*). This protein belongs to a novel class of glycan-anchored membrane proteins (*15*) that are thought to function in signal transduction (*16*) and intracellular targeting (*17*). The presence of this acidic repeat domain suggests that the VHL protein may be localized on the cell membrane and may be involved in signal transduction or cell adhesion. Further clues to the function of the VHL protein may emerge as more sequence information is obtained.

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8. Polyadenylated RNA from all tissue types tested (heart, skeletal muscle, brain, placenta, lung, liver, kidney, pancreas, prostate) showed a strong hybridization signal at 6 to 6.5 kb. In addition, expression was observed by RT-PCR analysis of total RNA from VHL lymphoblastoid cell lines. RNA and corresponding cDNA were prepared and amplified (*5*) with primers representing the g7 cDNA sequence (Fig. 4).
9. FISH was performed as in K. Tory *et al.* [*Genomics* **13**, 275 (1992)]. Phage p191, containing an 80-kb insert, was isolated by D. A. Smoller from a three-hit p1 phage genomic library (Genome Systems, Inc., St. Louis, MO).
10. The Southern blots contained DNA samples (purchased from BIOS Laboratories, New Haven, CT) from the following species: human, chimpanzee, macaque, cow, rat, mouse, chicken, frog, fruit fly, sea urchin, and yeast (*Saccharomyces cerevisiae*). Blots were (pre)hybridized in Church buffer [G. M. Church and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1991 (1984)] at 65°C for 18 hours and washed in 0.1× Church buffer at 60°C for 60 min.
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18. Previously we did not observe crossovers between the D3S601 locus and VHL in >300 informative meioses (*4*). However, we recently detected one such crossover, which allowed us to place the VHL locus distal to D3S601 (E. R. Maher *et al.*, unpublished results).
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20. This work is the result of a 6-year effort and represents a truly communal enterprise. We appreciate the cooperation of the VHL families in the United States, Canada, and Europe. B. Zbar, M. Lerman, and the NCI–FCRDC group are grateful to A. Rabson for his unwavering support. We thank M. F. Singer for the λgt11 teratocarcinoma cDNA library, H. B. Grossman for RCC cell lines UMRC5 and UMRC6, E. Brundage for help in screening YAC libraries, and J. Klabansky and D. Shores for managing the manuscript. Supported by grants from Action Research and the Cancer Research Campaign (to E.R.M.), the Japanese Overseas Cancer Fellowship of the Foundation for Promotion of Cancer Research (to M.Y.), and the William K. Warren Foundation (to D.S.R.D.).

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Repression of MHC Class I Gene Promoter Activity by Two-Exon Tat of HIV

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Major histocompatibility complex (MHC) class I molecules are the major receptors for viral peptides and serve as targets for specific cytotoxic T lymphocytes. Human immunodeficiency virus–type 1 (HIV-1) specifically decreased activity of an MHC class I gene promoter up to 12-fold. Repression was effected by the HIV-1 Tat protein derived from a spliced viral transcript (two-exon Tat). These studies define an activity for two-exon Tat distinct from that of one-exon Tat and suggest a mechanism whereby HIV-1–infected cells might be able to avoid immune surveillance, allowing the virus to persist in the infected host.

Major histocompatibility complex molecules play a pivotal role in the initiation and propagation of immune responses. Immune surveillance for viral infections is provided primarily by MHC class I antigens, which bind intracellularly generated viral peptides and act as targets for antiviral cellular immune responses (*1*). Many viruses are known to repress MHC class I expression, among them a number of retroviruses such as the Moloney leukemia virus (*2*). Decreases in MHC class I levels provide a mechanism for the virus to evade the host immune response. HIV-1 is a complex retrovirus that primarily infects $CD4^+$ T cells and monocytes, ultimately causing a depletion in the $CD4^+$ T cell population and a profound immunodeficiency (*3*). Because HIV-1 is known to establish persistent infections, suggesting a viral mechanism to avoid immune surveillance, we have examined its effect on MHC class I expression.

To assess the effect of HIV-1 on MHC class I expression, we transiently transfected human HeLa cells with an HIV-1_{LAI}–derived construct, pNL-A1, which expresses all viral gene products except Gag and Pol (*4*), and measured cell surface class I expression by staining with an antibody to human leukocyte antigen, W6/32, 48 hours after transfection. To distinguish pNL-A1–transfected from nontransfected HeLa cells, we also stained cells with an antibody to gp120 (pNL-A1 encodes gp120). Two-color flow cytometry was performed, and the mean cell surface level of class I on $gp120^+$ cells was compared with that on $gp120^-$ cells in the same population (*5*). Whereas $gp120^-$ cells stained with a mean fluorescence intensity (MFI) of 100 ± 8, $gp120^+$ cells expressed significantly lower levels of MHC class I, with an MFI of 49.2 ± 2.1 ($P < 0.0004$, five independent experiments). Thus, one or more HIV-1 gene products (but not Gag or Pol) are capable of markedly decreasing endogenous MHC class I expression. The extent of reduction observed in the present studies reflects only the difference that occurs during the transient 48-hour assay and could be an underestimate of the actual reduction of class I expression occurring in transfected HeLa cells. These results are consistent with a previous report describing a transient decrease in class I expression in HIV-1–infected T cell lines (*6*). Changes in class I expression of the magnitude observed here have been shown to alter susceptibility of cells to cytolysis by cytotoxic T cells (*7*).

To determine whether the HIV-1–mediated reduction of class I expression was transcriptional, we examined the effect of

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HIV-1 gene products on an MHC class I promoter ligated to the reporter gene chloramphenicol acetyltransferase (CAT). A class I promoter construct containing 313 base pairs (bp) of 5′ flanking sequences (*8*) was cotransfected into HeLa cells with varying amounts of either pNL-A1 or a control construct, pAR, which expresses only a subset of the pNL-A1–encoded viral proteins (Tat, Vpu, an aberrant Env/Nef fusion protein, and a truncated Gag protein) (*4*). Cotransfection of the class I promoter with pNL-A1 markedly reduced promoter-directed CAT activity to 11.7 ± 1.5% of controls cotransfected with carrier DNA alone. In contrast, cotransfection with the pAR construct did not reduce class I promoter activity (*9*).

The repression mediated by pNL-A1 was not due to nonspecific effects of HIV-1–encoded products on the class I promoter, because expression of CAT under the control of SV40, Rous sarcoma virus (RSV), or murine leukemia virus (MuLV) promoters was not significantly affected when the promoter was cotransfected with pNL-A1 into HeLa cells (Table 1). Because the constructs examined were identical except for the upstream promoter region, the failure of pNL-A1 to decrease CAT activity other than in the class I promoter construct also eliminates possible posttranscriptional effects. Furthermore, pNL-A1 inhibited the expression of a second reporter gene, luciferase, directed by the class I promoter [(*10*); see also Table 2], indicating that the pNL-A1 effect did not specifically target the CAT reporter. These experiments demonstrate that HIV-1–mediated repression of MHC class I expression occurs transcriptionally.

To map the target site of the HIV-1–mediated repression, we tested deletion constructs of the class I promoter for their sensitivity to pNL-A1. A variety of regulatory elements have been identified between −209 and −68 bp in the 5′ flanking region of the MHC class I gene (*11*), including a constitutive enhancer (enhancer A), an interferon response element, a cAMP (adenosine 3′,5′-monophosphate) response element (CRE), and a weak enhancer (enhancer B). Enhancer A has been shown previously to bind a homodimer of the p50 NFκB; alternative processing of the NFκB precursor after HIV-1 infection has been reported (*12*). Deletion to within 68 bp of the initiation of transcription did not abrogate repression by pNL-A1 (Fig. 1). Thus, the effect was not mediated by enhancer A-NFκB, the CRE, or the enhancer B regions, all of which are deleted from this construct. Rather, the HIV-1–responsive element maps to within the 68-bp region of the minimal promoter, a region that is only known to contain the CAT and TATA boxes.

Because pNL-A1 but not pAR reduced class I promoter activity, HIV-1 sequences responsible for class I promoter repression map to a region of the proviral genome 3′ of the *pol* gene (Fig. 2). Contained within this region are seven genes: *vif*, *vpu*, *vpr*, *rev*, *tat*, *env*, and *nef*. Mutations affecting six of these genes were introduced into the pNL-A1 construct and cotransfected into HeLa cells with the class I promoter constructs, −135CAT and −313CAT. Each of these mutant derivatives of pNL-A1 efficiently repressed the class I promoter (Fig. 2) (*10, 13*). Indeed, deletion of *nef* significantly augmented the repression of the class I promoter. Thus, the six HIV-1 gene products—Nef, Rev, Vif, Vpr, Vpu, or Env—were not responsible for repressing the promoter. The effects of the Nef and Rev proteins were also tested directly; expression vectors containing *rev* or *nef* under the control of a cytomegalovirus (CMV) promoter did not reduce class I promoter activity over a range of concentrations (1 to 6 μg of plasmid DNA) tested (*10*). Of the seven gene products encoded by pNL-A1, the only one not ruled out in this experiment is Tat (*14*).

Tat is a viral-encoded regulatory protein that transactivates HIV long terminal repeat (LTR)–directed gene expression. Tat is synthesized as either an 86 (or somewhat longer)–amino acid protein derived from a spliced mRNA (two-exon Tat) or a 72–amino acid product containing only the first coding exon (one-exon Tat), the latter appearing after the onset of Rev expression. Whereas pNL-A1 directs the synthesis of both forms of Tat, only the single-exon form is expressed by pAR. Although both Tat species transactivate the HIV LTR in tissue culture systems (*15*), the functional significance of the two forms of Tat in vivo is not presently understood. Because pNL-A1 but not pAR inhibited the class I promoter, the results obtained in the pNL-A1 mutagenesis experiment (Fig. 2) suggested that the observed repression was due to the two-exon species of Tat.

To examine this possibility further, we cotransfected expression vectors encoding HIV-1_{LAI}–derived two-exon Tat (86 amino acids in length) and one-exon Tat (72 amino acids in length) into HeLa cells with a class I promoter construct (−135Luciferase) (*16*). Two-exon Tat, in the absence of any other HIV-1 transcripts, markedly decreased class I promoter activity, whereas one-exon Tat had only a minor effect (Table 2). This suggests that the

Table 1. pNLA1 selectively reduces class I promoter activity. HeLa cells were cotransfected (*9*) with the indicated promoter CAT construct (5 μg) and with the HIV-1 proviral constructs pAR or pNL-A1 (5 μg). Values (an average of four duplicate samples) represent CAT activity normalized to control transfections without an HIV-1 proviral construct. All assays were normalized to β-galactosidase activity.

Promoter	pAR/control	pNL-A1/control
Class I	1.35 ± 0.095	0.46 ± 0.02
SV40	1.29 ± 0.13	1.51 ± 0.26
RSV	0.99 ± 0.003	1.00 ± 0.003
MuLV	3.16 ± 0.04	1.90 ± 0.135

Table 2. Repression of class I promoter activity by two-exon Tat in the absence of any other viral gene products. HeLa cells were cotransfected (*9*) with a class I promoter construct containing 135 bp of promoter proximal sequence ligated to the luciferase reporter gene (−135 luciferase; 5 μg) and the indicated Tat expression vectors (5 μg) or with a control expression vector consisting of the SV40 early promoter with a nonfunctional insert (*16*). The effect of Tat on MHC class I promoter activity is expressed relative to that of the control expression vector. Luciferase activity was determined on a Monolight 2010 luminometer (Analytical Luminescence Laboratory) and was normalized to β-galactosidase activity.

Tat	Form	Origin	Relative promoter activity (Tat/control)
Tat 86	Two-exon	HIV-1_{LAI}	0.172 ± 0.009
Tat 72	One-exon	HIV-1_{LAI}	0.767 ± 0.088
Tat 49	Truncation	HIV-1_{LAI}	0.867 ± 0.089
Tat 101	Two-exon	HIV-1_{SF2}	0.412 ± 0.024

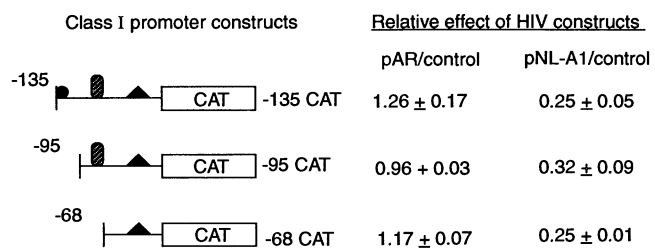
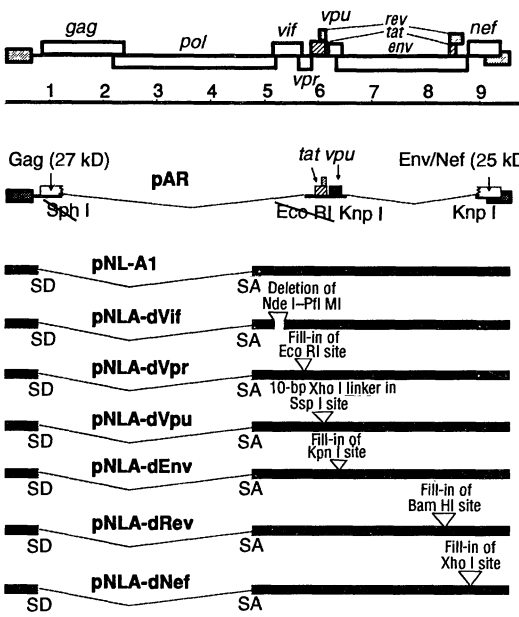


Fig. 1. HIV-1–mediated negative regulation of the class I promoter requires only 68 bp of promoter-proximal sequences. Truncated MHC class I promoter CAT constructs (5 μg) were cotransfected with pAR or pNL-A1 (5 μg) into HeLa cells, and CAT activity was determined (*9*). Results are expressed relative to control values. Figure summarizes data obtained from two independent experiments performed in duplicate.



Genes not expressed	Class I promoter constructs -135 (relative to control)	-313 (relative to control)
(gag), pol, vif, vpr, rev, env, (nef)	1.030 ± 0.140	1.100 ± 0.026
gag,pol	0.097 ± 0.003	0.295 ± 0.005
gag, pol, vif	0.060 ± 0.005	0.084 ± 0.010
gag, pol, vpr	0.108 ± 0.010	0.515 ± 0.038
gag, pol, vpu	0.084 ± 0.024	0.490 ± 0.087
gag, pol, env	0.086 ± 0.002	0.395 ± 0.015
gag, pol, env, vif, vpr?, vpu, rev	0.164 ± 0.040	ND
gag, pol, nef	0.012 ± 0.001	ND

Fig. 2. Repression of class I promoter activity not eliminated by HIV-1 mutations that eliminate the expression of Gag, Pol, Vif, Vpr, Vpu, Rev, Nef, or Env. Derivative pNL-A1 constructs (5 μg) containing the indicated mutations to eliminate expression of specific HIV-1 genes were generated and cotransfected along with −135CAT or −313CAT (5 μg) into HeLa cells, and CAT activity was determined (*9*). Results are expressed relative to control values.

repression of the class I promoter by pNL-A1 but not by its derivative pAR resulted from its capacity to express two-exon Tat and indicates that the COOH-terminal domain of two-exon Tat is essential for repression. These experiments define a function of two-exon HIV-1 Tat distinct from that of one-exon Tat and indicate that the HIV-1 Tat protein can act both as a transcriptional repressor as well as a transcriptional activator.

A two-exon Tat (101 amino acids in length) derived from the HIV-1_{SF2} isolate also repressed class I promoter activity, but to a lesser extent than the HIV-1_{LAI}–derived two-exon Tat (Table 2). This may reflect differences in the amino acid sequence between positions 73 and 86 or in the 15 additional residues in the HIV-1_{SF2} Tat. The present studies raise the possibility that differences in the strength of Tat-mediated repression of class I expression may influence the persistence and pathogenicity associated with different viral strains.

The mechanism by which two-exon Tat represses class I promoter activity is not known. Tat may act directly by regulating class I promoter activity or function indirectly by transactivating a gene that regulates class I promoter activity. Tat has been reported to interact with cellular factors such as Tat-binding protein (TBP)–1 and mammalian suppressor of sgv1 (MSS1) during the transactivation of the HIV LTR (*17*). Whether these factors are involved in regulating class I gene expression, either alone or in concert with Tat, is not known.

Because MHC class I molecules are the primary presenters of viral peptides to T cells, reduction in cell surface levels of class I might lead to a reduced ability of an infected cell to be recognized by HIV-1–specific cytotoxic T lymphocytes. Down-regulation of class I molecules in an HIV-1–infected cell could provide a mechanism for that cell to avoid normal immune surveillance.

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9. HeLa cells (1 × 10^6 cells in Dulbecco's minimum essential medium supplemented with 2 mM L-glutamine, Hepes, gentamicin sulfate, and 10% fetal bovine serum seeded 24 hours before transfection in a 100-mm^2 tissue culture dish) were cotransfected by standard calcium phosphate precipitation (*8*) with 10 μg of −313CAT, 100 ng of CMV–β-galactosidase (as an internal control), and 10 μg of the HIV-1 proviral constructs, pAR or pNL-A1. (In other experiments, where less test DNA was used, carrier salmon sperm or pUC 19 DNA was added to each sample to achieve a constant total amount of 20 μg of transfected DNA) Cells were harvested after 48 hours, and extracts were prepared. CAT assays were performed after normalization to β-galactosidase Data were analyzed with an AMBIS 4000 radioanalytic imaging detector. Results are expressed relative to the control and summarize data obtained from six independent transfections, each of which was performed in triplicate.
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13. The −135CAT construct is generally more efficiently repressed by pNL-A1 than is −313CAT. The molecular basis for this is not currently understood. The possibility that DNA sequence elements located between −135 and −313 bp may modulate repression is being investigated
14. Other possibilities have been eliminated as follows. (i) Inhibition of class I promoter activity might occur as a result of direct competition for transcription factors by HIV-1 proviral DNA sequences This possibility is unlikely because neither the HIV-1 LTR alone nor pNL-A1 DNA sequences lacking the HIV-1 LTR repress class I promoter activity (*10*). (ii) Because pAR does not produce either infectious virus or viral particles, simple sequestration of transcription factors within a viral particle cannot account for the lack of repression
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