Characterization of Murine Monoclonal Antibodies to the *tat* Protein from Human Immunodeficiency Virus Type 1

DAVID A. BRAKE,¹ JAAP GOUDSMIT,² WILLY J. A. KRONE,² PAT SCHAMMEL,³
NANCY APPLEBY,³ ROB H. MELOEN,⁴ AND CHRISTINE DEBOUCK^{1*}

Department of Molecular Genetics, Smith Kline and French Laboratories, King of Prussia, Pennsylvania 19406-0939¹;
Human Retrovirus Laboratory, Department of Medical Microbiology, Academic Medical Center, Amsterdam,
The Netherlands²; Monoclonal Antibody Facility, Cell Biology Research Laboratory,
Beckman Instruments, Irvine, California 92714³; and Central Veterinary Institute, Lelystad, The Netherlands⁴

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A panel of murine monoclonal antibodies (MAbs) to the human immunodeficiency virus type 1 *trans*-activator *tat* protein were characterized. The anti-*tat* MAbs were mapped to the different domains of the *tat* protein by Western blot (immunoblot) and Pepsican analyses. One-half of the MAbs tested mapped to the amino-terminal proline-rich region, and one-third of the MAbs tested mapped to the lysine-arginine-rich region of *tat*. The individual MAbs were tested for inhibition of *tat*-mediated *trans* activation, using a cell-based in vitro assay system. MAbs which mapped to the amino-terminal region of the *tat* protein demonstrated the highest degree of inhibition, whereas MAbs reactive to other portions of the molecule exhibited a less pronounced effect on *tat* function.

The etiological agent of the acquired immunodeficiency syndrome is believed to be a retrovirus, designated human immunodeficiency virus type 1 (HIV-1) (4, 13). HIV-1 exhibits the same *gag-pol-env* genomic organization as all retroviruses, and it also encodes a handful of additional proteins, most of which have been shown to play a regulatory function (7, 27). One of these regulatory proteins, termed *tat* or *trans* activator, is absolutely required for viral gene expression and replication (8, 11) and is thus a potential target for therapeutic intervention in the treatment of HIV-1 infection. This therapeutic approach is not trivial, since the precise mechanism by which *tat* regulates HIV-1 gene expression and viral growth is still unknown. *trans* activation by transcriptional and posttranscriptional mechanisms have been proposed (6). Site-directed mutagenesis of the *trans*-activation response element located within the HIV-1 long terminal repeat (LTR) has been used to define critical sequences necessary for *tat*-induced viral gene expression (10, 15). A similar approach has been utilized to identify functional domains of the *tat* protein apparently responsible for *trans* activation and subcellular localization (14, 25). In addition, a number of *cis*-acting regulatory elements located within the HIV-1 LTR mediate responses to a wide range of constitutive and inducible cellular transcription factors which may act synergistically with *tat* to further amplify viral gene expression (7). In an effort to gain additional information on the structure and function of *tat*, we developed murine monoclonal antibodies (MAbs) against a bacterially produced *tat* protein. We used these anti-*tat* MAbs to identify immunodominant epitopes on *tat* and to try to define domains of *tat* involved in *trans* activation of the HIV-1 LTR.

BALB/c mice were immunized subcutaneously with 25 µg of full-length *tat* protein purified from induced pOTS-TATIII (1; T. Smith, S. Franklin, and C. Deboeck, unpublished data) in Freund complete adjuvant and then given two boosts in incomplete Freund adjuvant 4 and 6 weeks later. Spleens from immunized mice were removed, and hybridomas were

produced by using standard techniques (19). Wells were screened by enzyme immunoassay, using a direct antigen-coated plate method; positive wells were cloned by limiting dilution. Cells retesting positive were expanded and injected into pristane-primed BALB/c mice, and ascites were harvested 1 to 2 weeks later. Total antibody titers were obtained by enzyme immunoassay, and immunoglobulin G levels were measured, using a Beckman ICS rate nephelometer. Antibodies were purified by protein A chromatography and concentrated, and protein content was determined by bicinchoninic acid.

A total of twelve murine MAbs that were reactive to the recombinant *tat* protein by enzyme immunoassay were obtained. The reactivity of each MAb against the various domains of *tat* was first determined by Western blot (immunoblot) analysis, using a panel of *tat* deletion mutants. The recombinant *tat* proteins analyzed included the full-length *tat* molecule (residues 1 to 86) and *tat* deletion mutants lacking either the proline-rich (residues 5 to 22), the cysteine-rich (residues 23 to 42), or the lysine-arginine-rich (residues 44 to 63) region (Fig. 1) (Table 1). All MAbs, with the exception of MAb 6, recognized the full-length *tat* protein by Western blot. One-half of the antibodies tested mapped to the proline-rich region, indicating that in mice, epitopes within the first 22 amino acids of the *tat* protein are immunodominant. This result is similar to data obtained with human sera, in which a major immunodominant epitope located at the amino terminus of *tat* was identified (20). One-third of the antibodies localized to the lysine-arginine-rich domain of *tat* and one antibody, MAb 9, reacted with all three deletion mutants and was thus mapped to the carboxyl-terminal domain of *tat*. None of the MAbs tested mapped to the cysteine-rich central domain of *tat* by Western blot analysis. This result is not totally unexpected, given that this region has been proposed to bind heavy metal ions such as zinc and to be involved in a metal-linked dimerization of the *tat* protein (12). Such a structural configuration could preclude the cysteine-rich domain from being efficiently recognized by the immune system.

* Corresponding author.

¹MET GLU PRO VAL ASP PRO ARG LEU GLU PRO TRP LYS¹²
¹³HIS PRO GLY SER GLN PRO LYS THR ALA CYS THR ASN²⁴
²⁵CYS TYR CYS LYS LYS CYS CYS PHE HIS CYS GLN VAL²⁶
²⁷CYS PHE ILE THR LYS ALA LEU GLY ILE SER TYR GLY²⁸
²⁹ARG LYS LYS ARG ARG GLN ARG ARG ARG PRO PRO GLN³⁰
³¹GLY SER GLN THR HIS GLN VAL SER LEU SER LYS GLN³²
³³PRO THR SER GLN SER ARG GLY ASP PRO THR GLY PRO³⁴
³⁵LYS GLU⁸⁶

FIG. 1. Predicted amino acid sequence of HIV-1 *tat* protein. The full-length 86-amino-acid sequence of the *tat* protein deduced from the HTLV-IIIIB isolate (3) is shown. Bars below the amino acid sequence illustrate the proline-rich (solid bar), cysteine-rich (stippled bar), and lysine-arginine-rich (hatched bar) regions of *tat*. The expression vectors pOTS-TATIII-DP, pOTS-TATIII-DC, and pOTS-TATIII-DLA encode mutant *tat* proteins with deletions that approximate these regions (Table 1).

In order to more precisely define the epitopes recognized by each MAb on the *tat* protein, the reactivity of all MAbs to overlapping nonapeptides (1 to 9, 2 to 10, etc.) spanning the entire *tat* sequence was determined by Pepsan essentially as described elsewhere (16) (Table 2). Although no linear domain of nine amino acids was found to bind the proline region-specific MAbs 5, 8, and 11, the other seven MAbs reacted with one or more overlapping nonapeptides that were in agreement with the mapping by Western blot analysis.

TABLE 1. Epitope mapping of *tat* MAbs by Western blot analysis

MAb	Reaction of MAb to <i>tat</i> construct ^a :				Epitope recognized
	Full length	DP	DC	DLA	
1	+	—	+	+	PRO
2	+	+	+	—	L/A
3	+	—	+	+	PRO
4	+	+	+	—	L/A
5	+	—	+	+	PRO
6	—	—	—	—	None
7	+	—	+	+	PRO
8	+	—	+	+	PRO
9	+	+	+	+	Carboxyl terminal ^b
10	+	+	+	—	L/A
11	+	—	+	+	PRO
12	+	+	+	—	L/A

^a Construction of the full-length HIV-1 *tat* bacterial expression plasmid pOTS-TATIII was previously described (1) and was used to isolate all fragments described below. Plasmids pOTS-TATIII-DP, pOTS-TATIII-DC, and pOTS-TATIII-DLA contain a deletion of the proline-, cysteine-, or lysine-arginine-rich region of *tat*, respectively. For pOTS-TATIII-DP, missing amino acids 5 to 22, pOTS-TATIII was linearized with *Bam*HI, treated with mung bean nuclease, and then digested with *Xba*I. Gel-purified vector was then ligated to a 548-base-pair *Rsa*I-*Xba*I fragment. Plasmid pOTS-TATIII-DC, lacking amino acids 23 to 42, was constructed by digestion of pOTS-TATIII with *Mst*II and treatment with Klenow, followed by digestion with *Bgl*II. Gel-purified vector was then ligated to a 732-base-pair *Bgl*II-*Rsa*I fragment. Similarly, pOTS-TATIII-DLA, lacking amino acids 44 to 63, was generated by *Mst*II digestion and Klenow treatment, followed by *Sph*I digestion. Gel-purified vector was then ligated to a 288-base-pair *Hin*III-filled *Sph*I fragment. Protein samples were obtained 5 h after nalidixic acid induction in *Escherichia coli* AR120 and were subjected to 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1). After electrophoretic transfer to nitrocellulose, blots were treated with the appropriate MAb. +, MAb reactive with *tat* protein; —, MAb nonreactive with *tat* protein. PRO, Proline rich; L/A, lysine-arginine rich.

^b Epitope recognized is located downstream of amino acid 63.

MAbs 1 and 3 recognized the amino-terminal peptide VDPRLPEWK within the proline-rich domain and did not react with any of the other 77 peptides, indicating the importance of the terminal Val-4 and Lys-12 residues for the binding specificity of these MAbs. Although MAb 7 also recognized this peptide sequence, it also reacted with two peptides lacking the carboxyl-terminal Lys or Trp, indicating that amino-terminal Val-4 and Pro-10 are the residues crucial for binding to MAb 7. In addition to their binding to the nonapeptide VDPRLPEWK derived from the sequence of the LAV-1-HTLV-IIIIB HIV-1 isolate, the reactivity of MAb 1 and MAb 3 to the corresponding peptides from the SF2 (26) and MAL/ELI (2) HIV-1 isolates was also tested. MAb 1 but not MAb 3 bound to the SF2 VDPNLEPWK peptide, whereas the MAL/ELI VDPNLEPWK peptide was not recognized by either MAb, raising the possibility that anti-*tat* MAbs could be used to discriminate between *tat* variants.

Three MAbs (2, 4, and 12) bound similarly to seven overlapping nonapeptides composing residues from the basic lysine-arginine-rich region. Since none of the other 71 peptides bound these three MAbs, residues Arg-56 and Pro-58 appear to be crucial for antibody binding. The best binding was found to nonapeptides with these two amino acid residues positioned in the middle of the peptide, i.e., RQR RRRPPQG/QRRRPPQGS for MAb 2, RQRRRPPQG for MAb 4, and KRRQRRRPP/RQRRRPPQ/RQRRRPPQG/QRRRPPQGS for MAb 12. One MAb (MAb 9) was found to react with five peptides covering sequences at the carboxyl-terminal end of *tat*. Residues critical for binding were Gln-76 and Asp-80 spaced by three amino acids, as indicated by the absence of reactivity to any of the bordering 73 nonapeptides.

All MAbs which recognized the full-length *tat* protein by Western blot (Table 1) were found to react to *tat* in transiently transfected COS cells by indirect immunofluorescence (data not shown). In this assay, the *tat* protein was predominantly localized within the nucleoli of the transfected cells as reported by others (9, 18, 25), but some nuclear and cytoplasmic staining was also observed and appeared to correlate in most experiments with higher levels of protein expression.

The observation that the anti-*tat* MAbs recognized native *tat* protein in transfected cells raised the possibility that some of these antibodies could inhibit *tat* function within cells. Because a cell-free biochemical assay for *tat* function has not yet been developed, the purified MAbs were tested for inhibition of *tat* function within cells, using a novel quantitative assay for *tat*-mediated *trans* activation. This system consists of a HeLa cell line, HTNT9.2, which constitutively expresses *tat* which in turn *trans* activates an integrated copy of the HIV-1 LTR adjacent to the human tissue plasminogen activator (tPA) gene (Fig. 2) (manuscript in preparation). A control HeLa cell line, TNT2.1, which also expresses *tat* constitutively but contains an integrated copy of the *tat* nonresponsive Rous sarcoma virus promoter upstream of the tPA gene, was similarly derived. Transient transfection of HTNT9.2 and TNT2.1 with a plasmid containing the HIV-1 LTR fused to the chloramphenicol acetyltransferase (CAT) gene resulted in a high level of CAT activity, confirming the presence of active *tat* in these cell lines. The tPA produced is secreted and can be quantitated by a simple chromogenic assay (5), thus rendering this assay much more convenient than the conventional CAT assay. A modification of a protocol for hypotonic lysis of pinocytotic vesicles (22) was used to introduce the individual MAbs (200 µg) into cultured HTNT9.2 cells. After this procedure, cells

TABLE 2. Epitope mapping of *tat* MABs by Pepscan analysis

MAB	Nonapeptide	Reactivity ^a	MAB	Nonapeptide	Reactivity ^a
1	VDPRLEPWK ^b	+	7	EPVDPRLEP ^d	++
2	KRRRQRRRP ^c	++		PVDPRLEPW	+
	KRRQRRRPP	++		VDPRLEPWK	++
	RRQRRRPPQ	++	8	None	-
	RQRRRPPQG	+++	9	QPTSQSRGD ^e	+++
	QRRRPPQGS	+++		PTSQSRGDP	++
	RRRPPQGSQ	+		TSQSRGDPT	++
	RRPPQGSQT	++		SQSRGDPTG	+++
3	VDPRLEPWK ^b	++		QSRGDPTGP	++
4	KRRRQRRRP ^c	+	11	None	-
	KRRQRRRPP	++	12	KRRRQRRRP ^c	+
	RRQRRRPPQ	++		KRRQRRRPP	+++
	RQRRRPPQG	+++		RRQRRRPPQ	+++
	QRRRPPQGS	++		RQRRRPPQG	+++
	RRRPPQGSQ	-		QRRRPPQGS	+++
	RRPPQGSQT	+		RRRPPQGSQ	-
5	None	-		RRPPQGSQT	++

^a Since Pepscan is a qualitative assay, reactivity of a nonapeptide with a MAB is estimated. MAB 6 and MAB 10 were not available at time of assay. Strong reactivity (+++), moderate reactivity (++), weak reactivity (+), and no reactivity (-) are indicated.

^b Valine 4 is first residue.

^c Lysine 50 is first residue in first nonapeptide.

^d Glutamic acid 2 is first residue in first nonapeptide.

^e Glutamine 72 is first residue in first nonapeptide.

were plated in microtiter wells, and culture supernatants were removed 90 min later and assayed for levels of tPA expression. Results of a typical experiment are presented in Fig. 3. A commercially obtained murine immunoglobulin G showed only a 9% level of inhibition of tPA expression in the HTNT9.2 cell line. Six of the eleven anti-*tat* MABs tested exhibited significant inhibition of tPA expression when supernatants from HTNT9.2 cells were assayed 90 min after exposure to the antibody. The inhibition seen in the *tat*-responsive HTNT9.2 cell line was due to MAB specificity and not MAB toxicity, since none of the anti-*tat* MABs tested possessed inhibitory activity when introduced into the *tat* nonresponsive control TNT2.1 cell line (data not shown). The degree of inhibition in HTNT9.2 ranged from 17% (MAB 4) to 41% (MAB 3) and appeared to correlate with the epitope recognized. The differences in the ability of the MABs to inhibit *tat trans* activation were not caused by a differential uptake of MABs by cells, since equivalent concentrations of intracellular MABs were detected by Western blot 90 min postincubation (data not shown). Two of the four MABs

which mapped to the basic domain of *tat* had a moderate inhibitory effect on *trans* activation, whereas MAB 9, which maps to the carboxyl-terminal region of *tat*, lacked any significant inhibitory effect, a result consistent with the fact that this region of the protein is dispensable for *trans*

FIG. 2. Schematic representation of the HTNT expression cassettes. The eucaryotic HTNT vector contains two expression cassettes as shown. In one cassette (right box), the HIV-1 LTR promoter directs the expression of the reporter gene tPA followed by the neomycin resistance gene (Neo) in a polycistronic arrangement and the bovine growth hormone (BgH) polyadenylation site (23). The second cassette (left box) contains the simian virus 40 early promoter, the full-length *tat* gene (3), and the bovine growth hormone (BgH) polyadenylation site. Stable HTNT transfectants were selected in the presence of G418. The arrows above the diagram indicate the direction of transcription, and the arrow below the diagram represents the *tat*-mediated *trans* activation of the HIV-1 LTR.

FIG. 3. MAB-mediated inhibition of *tat* function in HTNT9.2 cells. Cells (4×10^6) were treated with 200 μ g of MAB and plated in each of nine microtiter wells. After 90 min, triplicate wells were pooled and samples (three samples per MAB) were assayed in duplicate to determine levels of tPA expression. Shown are the mean inhibition values (standard deviation, <10% in all experiments) of each MAB from a single, representative experiment. The proline region-specific MABs (solid bars), basic region-specific MABs (hatched bars), carboxyl terminus-specific MAB (vertically striped bar), and a nonspecific immunoglobulin G control (open bar) are shown. To control for differential uptake and stability of MABs, the HTNT9.2 intracellular levels of representative MABs 7 (high inhibition level), 4 (moderate inhibition level), and 10 (no inhibition) were measured and determined to be equivalent 90 min postincubation (data not shown). To control for MAB toxicity, these same MABs (4, 7, and 10) were assayed for tPA inhibition in the *tat* nonresponsive TNT2.1 cell line and found to possess no significant inhibitory activity (data not shown).

activation. The three MAbs which demonstrated the highest degree of *tat* inhibition recognize the same linear epitope, VDPRLPEWK, in the amino-terminal proline-rich region of *tat*, suggesting that this region is important for *tat* function. These results are in agreement with our observations that a *tat* deletion mutant missing the proline-rich region fails to *trans* activate the HIV-1 LTR (data not shown). These data are also concordant with published results from several laboratories (14, 21, 25), including recent data which demonstrated that substitution of the acidic residues within the amino-terminal portion of the molecule had a profound effect on *tat* function (24). In contrast, a recent report describing the use of *tat* deletion peptides (17) has suggested that the first 36 amino acids of *tat* are unnecessary for *trans* activation, but these conflictual results have not been resolved.

Finally, it is interesting that the inhibition of *tat* activity seen in these experiments was partial and temporary. The maximal inhibition was observed at 90 min and did not exceed 41% (Mab 3); when assayed 3 h after their introduction into the indicator cells, all MAbs failed to effectively block *tat*-induced *trans* activation of tPA expression (unpublished observations). This short-lived inhibitory effect may be explained by the constitutive production of *tat* protein in the stable transfected HTNT9.2 cell line. It may be difficult to identify compounds which effect complete and sustained inhibition of *tat* function since very low levels of *tat* are apparently sufficient to induce full *trans* activation of HIV-1 LTR-directed gene expression.

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LITERATURE CITED

1. Aldovini, A., C. Debouck, M. B. Feinberg, M. Rosenberg, S. K. Arya, and F. Wong-Staal. 1986. Synthesis of the complete *trans*-activation gene product of human T-lymphotropic virus type III in *Escherichia coli*: demonstration of immunogenicity *in vivo* and expression *in vitro*. *Proc. Natl. Acad. Sci. USA* 83:6672-6676.
2. Alizon, M., S. Wain-Hobson, L. Montagnier, and P. Sonigo. 1986. Genetic variability of the AIDS virus: nucleotide sequence analysis of two isolates from African patients. *Cell* 46:63-74.
3. Arya, S. K., C. Guo, S. F. Josephs, and F. Wong-Staal. 1985. *trans*-Activator gene of human T-lymphotropic virus type III (HTLV-III). *Science* 229:69-73.
4. Barre-Sinoussi, F., J. C. Chermann, R. Rey, M. T. Nugeyre, S. Chanaret, J. Gruet, C. Dauguet, C. Axler-Blin, F. Brun-Vezinet, C. Rouzioux, W. Rozenbaum, and L. Montagnier. 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immunodeficiency syndrome (AIDS). *Science* 220:868-871.
5. Connors, R. W., R. W. Sweet, J. P. Noveral, D. S. Pfarr, J. J. Trill, R. J. Shebuski, B. A. Berkowitz, D. Williams, S. Franklin, and M. E. Reff. 1988. DHFR coamplification of tPA in DHFR⁺ bovine endothelial cells: *in vitro* characterization of the purified serine protease. *DNA* 7:651-661.
6. Cullen, B. R. 1986. *trans* activation of human immunodeficiency virus occurs via a bimodal mechanism. *Cell* 46:973-982.
7. Cullen, B. R., and W. C. Greene. 1989. Regulatory pathways governing HIV-1 replication. *Cell* 58:423-426.
8. Dayton, A. I., J. G. Sodroski, C. A. Rosen, W. C. Goh, and W. A. Haseltine. 1986. The *trans*-activator gene of the human T cell lymphotropic virus type III is required for replication. *Cell* 44:941-947.
9. Falkner, F. G., T. R. Fuerst, and B. Moss. 1988. Use of vaccinia virus vectors to study the synthesis, intracellular localization, and action of the human immunodeficiency virus *trans*-activator protein. *Virology* 164:450-457.
10. Feng, S., and E. C. Holland. 1988. HIV-1 *tat trans*-activation requires the loop sequence within *tar*. *Nature (London)* 334:165-167.
11. Fisher, A. G., A. Aldovini, C. Debouck, R. C. Gallo, and F. Wong-Staal. 1986. The *trans*-activator gene of HTLV-III is essential for virus replication. *Nature (London)* 320:367-371.
12. Frankel, A. D., D. S. Bredt, and C. O. Pabo. 1988. Tat protein from human immunodeficiency virus forms a metal-linked dimer. *Science* 240:70-73.
13. Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleska, B. Safai, G. White, P. Foster, and P. D. Markham. 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* 224:500-503.
14. Garcia, J. A., D. Harrich, L. Pearson, R. Mitsuyasu, and R. B. Gaynor. 1988. Functional domains required for *tat*-induced transcriptional activation of the HIV-1 long terminal repeat. *EMBO J.* 7:3143-3147.
15. Garcia, J. A., D. Harrich, E. Soultanakis, F. Wu, R. Mitsuyasu, and R. B. Gaynor. 1989. Human immunodeficiency virus type 1 LTR TATA and TAR region sequences required for transcriptional regulation. *EMBO J.* 8:765-778.
16. Geysen, H. M., R. W. Meloen, and S. J. Barteling. 1984. Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. *Proc. Natl. Acad. Sci. USA* 81:3998-4002.
17. Green, M., and P. M. Lowenstein. 1988. Autonomous functional domains of chemically synthesized human immunodeficiency *tat trans*-activator protein. *Cell* 55:1179-1188.
18. Hauber, J., A. Perkins, E. P. Heimer, and B. R. Cullen. 1987. *trans*-Activation of human immunodeficiency virus gene expression is mediated by nuclear events. *Proc. Natl. Acad. Sci. USA* 84:6364-6368.
19. Kohler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature (London)* 256:495-497.
20. Krone, W. J. A., C. Debouck, L. G. Epstein, P. Heutink, R. Meloen, and J. Goudsmit. 1988. Natural antibodies to HIV-tat epitopes and expression of HIV-1 genes *in vivo*. *J. Med. Virol.* 26:261-270.
21. Kuppuswamy, M., T. Subramanian, A. Srinivasan, and G. Chinnadurai. 1989. Multiple functional domains of *tat*, the *trans*-activator of HIV-1, defined by mutational analysis. *Nucleic Acids Res.* 17:3551-3561.
22. Okada, C. Y., and M. Rechsteiner. 1982. Introduction of macromolecules into cultured mammalian cells by osmotic lysis of pinocytotic vesicles. *Cell* 29:33-41.
23. Pfarr, D. S., L. A. Rieser, R. P. Woychik, F. M. Rottman, M. Rosenberg, and M. E. Reff. 1986. Differential effects of polyadenylation regions on gene expression in mammalian cells. *DNA* 5:115-122.
24. Rappoport, J., S.-J. Lee, K. Khalili, and F. Wong-Staal. 1989. The acidic amino-terminal region of the HIV-1 *tat* protein constitutes an essential activating domain. *New Biol.* 1:101-110.
25. Ruben, S., A. Perkins, R. Purcell, K. Joung, R. Sia, R. Burghoff, W. A. Haseltine, and C. A. Rosen. 1989. Structural and functional characterization of human immunodeficiency virus *tat* protein. *J. Virol.* 63:1-8.
26. Sanchez-Pescador, R., M. D. Power, P. J. Barr, K. S. Steimer, M. M. Stempien, S. L. Brown-Shimer, W. W. Gee, A. Renard, A. Randolph, J. A. Levy, D. Dina, and P. A. Luciw. 1985. Nucleotide sequence and expression of an AIDS-associated retrovirus (ARV-2). *Science* 227:484-492.
27. Varmus, H. 1988. Regulation of HIV and HTLV gene expression. *Genes Dev.* 2:1055-1062.