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Evidence that a Sequence Similar to TAR Is Important for Induction of the JC Virus Late Promoter by Human Immunodeficiency Virus Type 1 Tat

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A specific RNA sequence located in the leader of all human immunodeficiency virus type 1 (HIV-1) mRNAs termed the transactivation response element, or TAR, is a primary target for induction of HIV-1 long terminal repeat activity by the HIV-1-derived *trans*-regulatory protein, Tat. Human neurotropic virus, JC virus (JCV), a causative agent of the degenerative demyelinating disease progressive multifocal leukoencephalopathy, contains sequences in the 5′ end of the late RNA species with an extensive homology to HIV-1 TAR. In this study, we examined the possible role of the JCV-derived TAR-homologous sequence in Tat-mediated activation of the JCV late promoter (Tada et al., Proc. Natl. Acad. Sci. USA 87:3479–3483, 1990). Results from site-directed mutagenesis revealed that critical G residues required for the function of HIV-1 TAR that are conserved in the JCV TAR homolog play an important role in Tat activation of the JCV promoter. In addition, in vivo competition studies suggest that shared regulatory components mediate Tat activation of the JCV late and HIV-1 long terminal repeat promoters. Furthermore, we showed that the JCV-derived TAR sequence behaves in the same way as HIV-1 TAR in response to two distinct Tat mutants, one of which that has no ability to bind to HIV-1 TAR and another that lacks transcriptional activity on a responsive promoter. These results suggest that the TAR homolog of the JCV late promoter is responsive to HIV-1 Tat induction and thus may participate in the overall activation of the JCV late promoter mediated by this transactivation.

Progressive multifocal leukoencephalopathy (PML) is a rare human disease characterized by progressive demyelinating lesions in the brain secondary to viral infection and impaired cellular immunity (37, 38). JC virus (JCV), a ubiquitous human polyomavirus which replicates exclusively in glial cells, is the causative agent of this disease (3). Although 70 to 80% of the adult population are seropositive for JCV, the incidence of PML is very low (26, 27). Serological surveys have demonstrated that primary JCV infection usually occurs during childhood, although no acute illness associated with primary infection has been recognized (26, 37). The cofactors responsible for activation of latent JCV and induction of PML are not known, but this disease occurs almost exclusively in a background of chronic immunosuppression.

Neurologic complications are a dominant feature of AIDS. At least 60% of AIDS patients develop neurologic disorders, and 90% demonstrate neurologic abnormalities at autopsy (21, 29). The incidence of PML among AIDS patients is approximately 4%, which is significantly higher than the incidence among individuals immunosuppressed by other causes (1). In addition to the diagnoses of classical PML which are confirmed by detection of JCV, a large number of AIDS patients diagnosed with AIDS dementia complex demonstrate features that are strikingly similar to this disease, such as the appearance of bizarre astrocytes, gliosis, enlargement of oligodendrocytes, and progressive demyelination (9, 24). The strong correlation between human immunodeficiency virus type 1 (HIV-1) expression in the brain and the severity of neurologic symptoms strongly suggests a role for this virus in central nervous system pathology. Recently, we have demonstrated that the HIV-1-encoded *trans*-regulatory protein Tat is a potent activator of JCV gene expression (36). Tat greatly increases the rate of transcription from the JCV late (JCV_L) promoter by a mechanism that is independent from and synergistic with the transactivation caused by the JCV regulatory protein T antigen (4). Tat is a transcriptional activator protein that is the essential component for the establishment of a productive HIV-1 infection (5). The target sequence for Tat induction on the HIV-1 promoter is located in the leader of the viral transcripts between nucleotides +19 and +42 in the R region of the long terminal repeat (LTR) (5, 14, 16). This *trans*-acting responsive region (TAR) has the capacity to form a stem-loop structure within the RNA molecule. It seems that this secondary structure plays an important role for Tat-induced activation of the viral promoter (10, 34, 36). Results from in vitro RNA binding studies have demonstrated that Tat has the capacity to bind the TAR RNA molecule and that this interaction may facilitate and/or stabilize the association of RNA polymerase II for initiating viral RNA synthesis (2, 3). In addition to Tat, several cellular proteins also bind to the TAR element in a sequence-specific manner (11, 23, 39).

The regulatory region of JCV contains two 98-bp tandem repeats that control cell-specific expression of viral early and late genes in glial cells (18, 35). Of particular interest is the presence of a region with a substantial sequence homology with the critical region of the HIV-1 TAR in the leader of the JCV_L transcripts. In this study, we asked whether the TAR

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homolog in the JCV genome responds to HIV-1 Tat induction. For this purpose, we replaced the HIV-1 TAR element with its homolog in JCV and found that Tat was able to induce expression from this chimeric promoter. Results from site-directed mutagenesis indicated that the Tat-binding sequence as well as the region where a cellular factor(s) interacts is important for Tat responsiveness of the TAR homolog. In addition, we found that Tat mutants with no binding activity to TAR RNA are unable to enhance activity of the chimeric promoter harboring the TAR homolog.

MATERIALS AND METHODS

Plasmid constructs. pJCV$_L$-CAT was constructed by cloning the 256-bp *Pvu*II-*Hin*dIII fragment (map units 0.67 to 0.72) of JCV containing the late gene control region into the *Bgl*II site of pCAT$_{3M}$ (20). pJCT was made by placing the JCV early DNA fragment that expresses the viral early region under the control of the ICP4 promoter (kindly provided by J. Remenick, National Cancer Institute). pCD$_{16}$ contains upstream HIV-1 LTR DNA sequence from nucleotides −176 to +80 cloned in front of the reporter chloramphenicol acetyltransferase (CAT) gene (25). The ΔTAR deletion in the LTR-CAT construct was created by restriction digestion with *Bgl*II-*Hin*dIII and then blunting with Klenow enzyme and religation. The JCV-TAR construct was made by inserting synthetic double-stranded DNA fragments representing JCV TAR-homologous sequence (5′-GATCT AGCTCATACCTAGGGAGCCACCAGCTAA-3′) between the *Bgl*II-*Hin*dIII sites in the ΔTAR-LTR plasmid (HIV-1 ΔTAR). The p5TAR chimera contains five copies of the entire TAR sequence (+1 to +63) of HIV-1 cloned in front of the HIV-1 LTR (22). The previously described K9 Tat mutant (3) was kindly provided by A. Frankel (Whitehead Institute, Cambridge, Mass.). The pgly$_{2,5,9}$ chimera was made by site-directed mutagenesis, using a 42-base oligonucleotide containing the triple missense mutant (32). Plasmid pA-Tat contains the Tat gene of HIV-1 under the control of the β-actin promoter.

Site-directed mutagenesis was done by cloning a 1,950-bp fragment containing sequences derived from the JCV control sequence of pJCV$_L$-CAT into replicative-form M13mp18. Seventeen-mer synthetic oligonucleotides (5′-CTAGTTA GCCAACCAGC-3′) were used to induce a mutation at a target region by standard procedures (40). The integrity of all constructs was verified by DNA sequencing.

RNA analysis. RNA was extracted from transfected cells by the hot acid-phenol procedure as described previously (31). DNA was removed by treatment of RNAs with 50 μg of DNase I per ml. Labeled single-stranded M13-derived probe was mixed with 20 to 50 μg of purified RNAs in 20 μl of hybridization buffer containing 0.4 M NaCl, 0.04 M PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); pH 6.4], 1.25 mM EDTA, and 80% formamide and heat denatured at 65°C for 15 min and then gradually cooled to 37°C. After incubation in 37°C for 16 h, hybridizations were terminated by the addition of 180 μl of 0.25 M NaCl–0.03 M Na acetate (pH 4.6)–1 mM $ZnSO_4$–800 U of S1 nuclease per ml. The reaction mixtures were incubated for 60 min at 37°C. The S1-protected fragments were extracted with phenol-chloroform, ethanol precipitated, and resolved by a denaturing acrylamide-urea gel.

For primer extension after hybridizations, hybrids were recovered by precipitation with ethanol and primer extensions were done with reverse transcriptase as described previously (15). The single-stranded S1 and primer extension probes were obtained by uniform labeling of the M13-CAT construct (19) with [^{32}P]dCTP (800 Ci/mmol) during strand synthesis with universal M13 primer. The M13-CAT recombinant contained a *Bgl*II-*Eco*RI fragment from pA$_{10}$CAT$_2$ (19) spanning 256 bp of CAT-coding sequences in the M13mp9 background. For generating S1 probe, after synthesis of the ^{32}P-labeled probe, a 458-nucleotide single-stranded fragment was cleaved with *Hin*cII endonuclease. Only 256 nucleotides of the probe spanning the CAT gene have sequence homology to JCV-derived CAT RNA. For primer extension, a 265-nucleotide single-stranded DNA containing the 5′ portion of the CAT-coding sequence and the M13 primer was generated by digesting the ^{32}P-labeled M13-CAT with *Hin*dIII.

Transfection. Calcium phosphate precipitation transfection was done by the method described previously (13), except that glycerol shock was included. Briefly, a total of 15 μg of DNA per 60-mm dish was used in each experiment. The calcium phosphate precipitate remained on the cells for 2 to 4 h before glycerol shock. Forty-eight hours after transfection, the cells were collected, and a crude protein extract was prepared by freeze-thawing. Equal amounts of protein (75 to 100 μg) were incubated in a 2-h reaction to assay for CAT activity by the procedure described previously (12). Fold induction was measured by scintillation counting of spots corresponding to acetylated and nonacetylated forms from the thin-layer chromatography plate. Each experiment was repeated multiple times to ensure transfection efficiency and reproducibility of the results.

RESULTS

Analysis of JCV$_L$ RNAs in the presence of HIV-1 Tat. JCV has multiple late transcriptional units that are transcribed from regions scattered throughout the viral regulatory regions (38). There are six major RNA species starting from the nucleotides in the vicinity of the origin of DNA replication (L$_1$), the A+T-rich boxes within each 98-bp repeat (L$_2$, L$_4$), the positions 3′ of each repeat (L$_3$, L$_5$), and the sequences spanning outside of the 98-bp repeat at the 3′ site (L$_6$) (Fig. 1A). There is a stretch of 15 nucleotides in the center of each 98-bp repeat, between nucleotides 79 to 94 and 181 to 196, that has a substantial sequence similarity (86%) with HIV-1 TAR. As a first step to identify the target element for the induction of the JCV$_L$ promoter by Tat, we examined the amount and identified the 5′ termini of JCV$_L$ RNAs by S1 and primer extension analyses, respectively. To this end, a human glioblastoma cell line (U87-MG) was transfected with pJCV$_L$-CAT alone or together with a plasmid expressing Tat, and after 48 h, total RNAs were harvested and hybridized to the single-stranded 458-bp S1 and 265-bp primer extension probes, separately. The homology of these probes with chimeric CAT transcripts rests only in the CAT sequences (Fig. 1B). Thus, the S1-protected fragment represents the steady-state levels of the late RNAs and not their 5′ termini, whereas elongation of the 265-bp probe to late RNA start sites in the primer extension assay determines the 5′ termini of the Tat-induced transcripts. As shown in Fig. 1C, a 256-nucleotide S1-protected fragment is detected in the presence of the Tat-producing plasmid, suggesting that Tat enhances the level of CAT RNA in these cells. Results from primer extension analysis revealed accumulation of a major cDNA fragment, corresponding to the late transcripts, initiating from nucleotides 95 to 100 (L$_3$).

Role of TAR homolog in JCV$_L$ induction. The major JCV$_L$ RNA which is induced by HIV-1 Tat possesses a TAR-

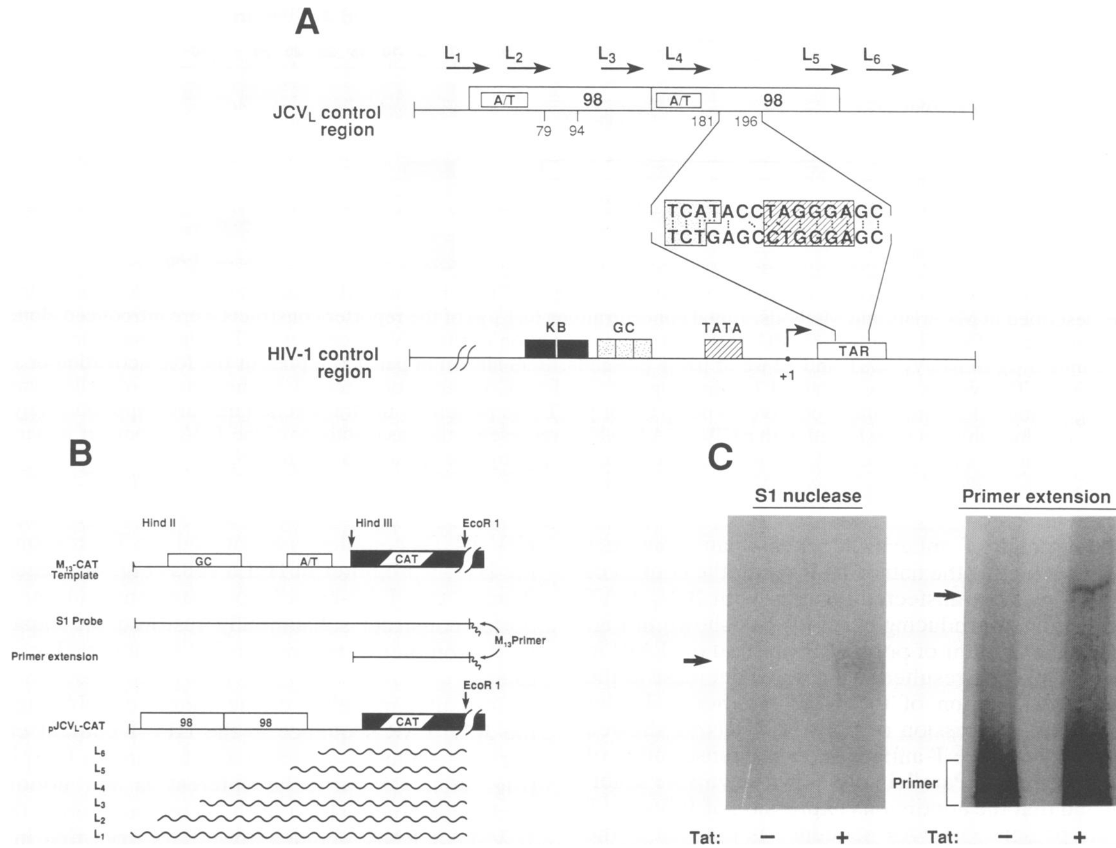

FIG. 1. Analysis of JCV_L RNAs in cells cotransfected with HIV-1 Tat. (A) Structural organization of the JCV and HIV-1 regulatory regions and location of the TAR homolog in the JCV_L promoter sequence. Arrows illustrate positions of JCV_L RNA start sites (38). (B) Diagram of M13-CAT construct used to prepare single-stranded probe for S1 nuclease and primer extension analyses of CAT transcripts. A 458-bp S1 probe was generated by digesting the [^{32}P]dCTP-labeled DNA with *Hin*dIII. Only the 256-bp fragment of the S1 probe has homology to CAT in RNA. The primer extension probe was made by releasing a 265-bp cDNA to CAT sequence with the *Hin*dIII restriction enzyme. L_1 to L_6 represent chimeric JCV_L-CAT RNAs. (C) S1 nuclease and primer extension analyses of JCV-derived CAT RNA from glial cells transfected with pJCV_L-CAT alone or together with Tat expressor plasmid. At 48 h after transfection, total RNA was prepared from transfected cells, hybridized with the respective probes separately, and, after 16 h, treated with S1 nuclease or reverse transcriptase. The products were analyzed by electrophoresis in a denaturing acrylamide-urea gel. Arrows show positions of the 256-bp S1-protected fragment (left) and the 410-bp primer extension product (right). The molecular weight marker used in this study was pBR322 cleaved with the MSPI restriction enzyme (not shown).

homologous sequence in the leader region. Therefore, we used deletion and site-directed mutagenesis approaches to examine the importance of the TAR homolog in conferring Tat responsiveness to the JCV_L promoter. The deletion was generated by removing the distal copy of the 98-bp repeat that encompasses the leader of the affected L_3 RNA (Fig. 2A). By site-directed mutagenesis, we introduced two base substitutions in the most conserved and critical nucleotides of the TAR homolog in the distal 98-bp sequence, altering CTAGGGAGC to CTAGTTAGC (Fig. 2A). This sequence in the HIV-1 TAR forms a loop in the top of the TAR hairpin RNA (7). Conversion of this sequence from CTG<u>GG</u>AGC to CTG<u>TT</u>AGC impairs Tat-mediated HIV-1 gene transcription (7). As shown in Fig. 2B, conversion of the G residues to Ts or removal of the distal copy of the 98-bp repeat significantly decreased the extent of the late promoter induction by Tat in glial cells. In a parallel transfection experiment, we examined the activity of these mutants in the presence of the JCV early protein T antigen. Similar results were obtained when experiments were done in the presence of cytosine arabinoside (10 μg/μl), which blocks viral DNA replication (data not shown). Results showed no drastic reduction in the T-induced transcriptional activity of the base-substituted mutant (Fig. 2B). Deletion of the entire 98-bp sequence, however, reduced (approximately 50%) late promoter activity induced by T antigen. These results suggest that the AGGGA sequence of the TAR homolog spanning the JCV_L RNA leader plays an important role in activation of JCV_L gene expression by Tat. The low, yet persistent, level of JCV_L promoter activity in these mutants suggests the involvement of a secondary target for Tat induction in the viral genome (4a).

Effect of poly-TAR RNAs on JCV_L activation by Tat. Recently Lisziewicz et al. (22), by overexpressing multimerized HIV TAR sequences, showed a block in Tat response of the viral LTR in transfection experiments. They concluded that overproduction of TAR RNA down-regulates Tat-mediated activation by sequestering Tat and some cellular components participating in this induction. We examined whether overexpression of the HIV-1 TAR sequence affects the extent of Tat induction of the JCV_L promoter in glial cells. In a parallel experiment, we tested the effect of poly-TAR sequences on induction of the JCV_L promoter by

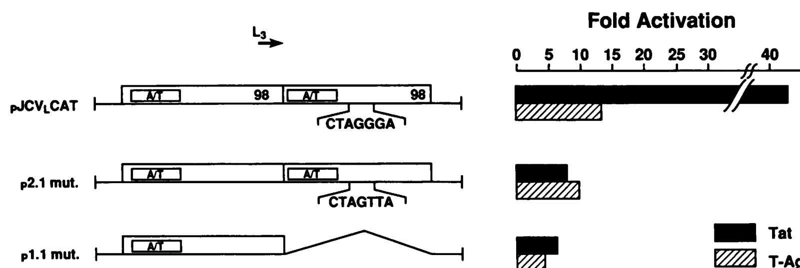

FIG. 2. Site-directed mutagenesis of the conserved G residues in the JCV TAR homolog. Base substitution and deletion mutagenesis were performed as described in Materials and Methods. Equal concentrations (0.1 μg) of the reporter constructs were introduced alone or together with plasmids producing Tat (pTat) into U87-MG glial cells. Transactivation of the JCV_L promoter by T antigen was done in a transfection mixture containing 3 μg of $pJCV_L$-CAT and 10 μg of pJCT plasmids. Bars shown in panel B represent the fold activation of JCV_L and its mutant derivatives. The values were obtained by dividing the level of CAT activity detected in the extract of cells transfected with CAT-containing plasmid alone from those observed when pTat or pJCT was present in the transfection mixture. Approximately 1% of CAT conversion was obtained in cells transfected with $pJCV_L$-CAT, which represents the basal activity of the JCV_L promoter in our assay. In all cases, the final amount of the transfected DNA was kept constant (15 μg) by adding salmon sperm DNA.

the viral early protein T antigen. A recombinant construct containing five copies of the native TAR under the control of the HIV-1 LTR was cotransfected together with JCV_L-CAT and Tat- or T-antigen-producing plasmids. As shown in Fig. 3A, increasing the amount of poly-TAR plasmid (p5TAR) in the transfection mixture resulted in a gradual decrease in the Tat-mediated transcription of the JCV_L promoter. Under similar conditions, expression of poly-TAR RNAs showed no inhibitory effect on the T-antigen-induced transcription of the viral late promoter. Parallel studies with cytosine arabinoside revealed that the continuous expression of JCV_L by T antigen in the presence of TAR sequence is not due to the amplification of template DNA in transfected cells (3a). Moreover, in the control experiments, p5TAR effectively down-regulated the increased levels of HIV-1 LTR and JCV_L promoters induced by Tat in glial cells (data not shown). These results suggest that similar, if not identical, regulatory components are involved in the Tat-derived induction of the HIV-1 LTR and JCV_L promoter through the TAR sequence. Furthermore, these observations corroborate our earlier studies (4) indicating that JCV T antigen and Tat operate through different biological pathways to stimulate JCV_L promoter activity.

Replacement of HIV-1 TAR with its homolog derived from JCV promoter. In a different approach, we examined the inducibility of the JCV-derived TAR sequence by Tat in a chimeric LTR-CAT plasmid in which the native HIV-1 TAR was replaced with the JCV TAR-homologous sequence (Fig. 4A). As expected, deletion of the TAR sequence from the

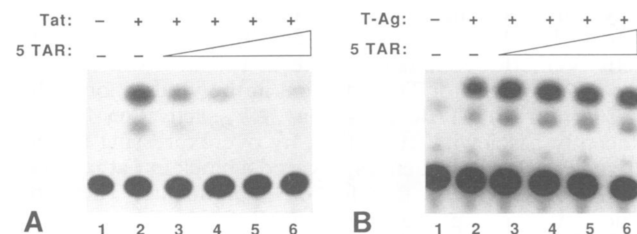

FIG. 3. Inhibition of Tat-mediated JCV_L promoter activity by multiple HIV-1 TAR elements. U87-MG cells were transfected with $pJCV_L$-CAT (0.5 μg) alone or together with 5 μg of pTAR (A)- or pT-Ag (B)-producing plasmids and increasing amounts of p5TAR. Lanes 3 to 6 contain 1.1, 2.2, 4.4, and 8.8 μg of p5TAR in the cotransfection mixture.

HIV-1 LTR completely abrogated the Tat responsiveness of the HIV-1 promoter in HeLa cells (Fig. 4B, lanes 10 to 12). Placing the TAR-homologous sequence in the ΔTAR reporter construct substantially restored the capacity of the LTR promoter to respond to Tat induction (Fig. 4B, lanes 4 to 6).

Computer modeling of the chimeric RNA suggested that the JCV TAR sequence in the HIV-1 context can perform intramolecular base pairing and fold into a hairpin structure (Fig. 4C). Of particular interest is the notion that the nucleotide composition of the stem and loop in the chimeric JCV TAR RNA significantly differs from that in HIV TAR (Fig. 4C). The sequences residing in the loop of the native TAR are positioned in the stem of the chimeric hairpin RNA in the vicinity of the UCAU loop. Note that UCU forms a bulge in the HIV TAR (Fig. 4C) and is believed to be the target for Tat binding (2, 3, 7). The secondary structure of RNA predicted by computer may not represent its structural configuration in vivo.

To examine the importance of the CAGGGAG and the neighboring UCAU sequences in activation of the chimeric promoter by Tat, two G residues of the CUAG<u>GG</u>AG sequence and C and U residues of the nearby loop UCAU were converted to Ts and As, respectively (Fig. 4C). When the response of the mutated chimeric TAR to Tat was examined by CAT assay, results indicated that mutations in the primary sequence of the chimeric HIV-JCV TAR abrogated inducibility of the viral promoter by Tat (Fig. 4B, lanes 7 to 9). Therefore, we considered the possibility that these two regions are important for Tat induction by serving as targets for binding of cellular proteins and perhaps HIV-1 Tat protein. Experiments are in progress to examine interactions of Tat and cellular proteins with the TAR-homologous sequence. From these observations, one could envision that the existence of the two critical domains, in close proximity to each other, and the formation of a stem-loop structure are more critical than their positions on the stem or loop of the hairpin structure in conferring Tat responsiveness. Studies utilizing compensating mutants in the stem region which restore the stem-loop structure of the JCV-TAR mutant will determine the importance of the secondary structure in activation of the JCV TAR by Tat (4a).

Effect of Tat mutants on JCV TAR homolog. A concept that has been emerging from several studies on HIV-1 Tat structure and function is that Tat, like many eukaryotic

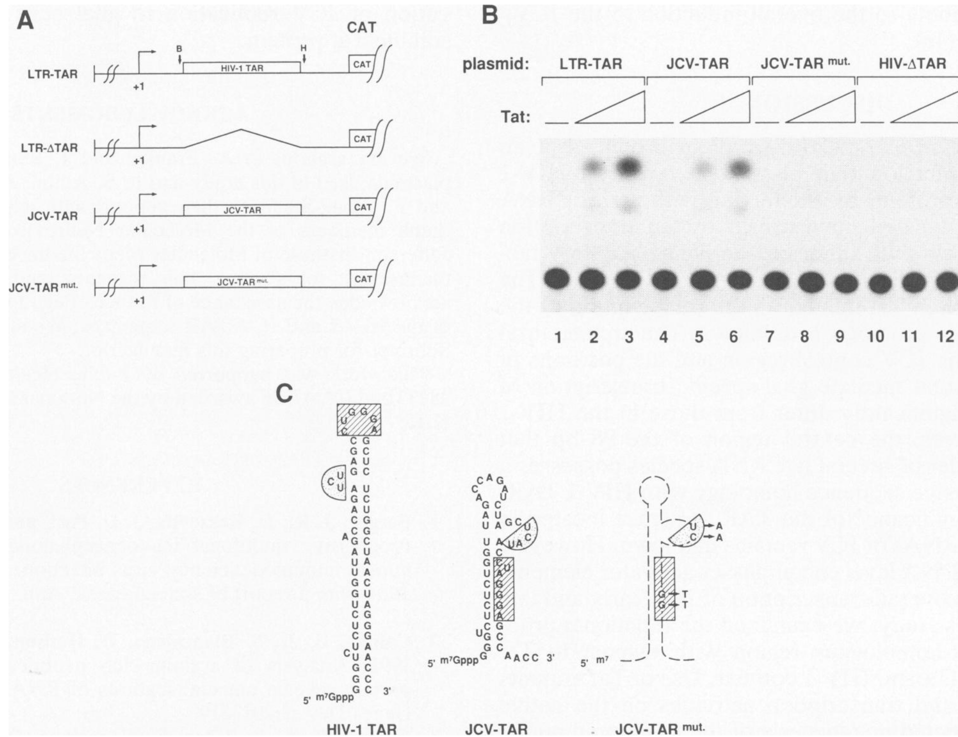

FIG. 4. Inducibility of JCV-derived TAR sequence by Tat in the HIV-1 promoter context. (A) Structure of LTR TAR-containing reporter construct and its mutant derivatives with no TAR (LTR ΔTAR), with JCV TAR, or with the JCV TAR mutant. (B) Cotransfection of HeLa cells with LTR-CAT constructs (0.5 μg) and pTat (2.5 and 5.0 μg). (C) Computer modeling of HIV-1 TAR, JCV TAR, and the JCV TAR mutant. Positions of the conserved regions providing targets for binding of Tat and a cellular factor(s) in HIV-1 TAR and their homologs in JCV TAR are shown.

transactivators, contains distinct binding and activating domains (30). Evidently, the arginine-rich region of Tat spanning residues 49 to 57 is critical for binding of Tat to its intended RNA target, TAR (2, 3), whereas other regions such as the acidic domain at the amino terminal (32) and a cysteine-rich region located between amino acids 22 and 37 are essential for transcriptional activity of the protein. To investigate the importance of the previously identified activator and binding domains of Tat on the induction of the JCV TAR homolog-containing promoter, we chose to use two classes of Tat mutants, one containing substitutions in the acidic residues ($gly_{2,5,9}$) that exerts no transcriptional activity on the HIV-1 LTR (32), and another with substitutions in the arginine-rich region (K9) that eliminates the TAR-binding ability of Tat (Fig. 5A). These mutants were cotransfected with the HIV-1 LTR or chimeric LTR with the TAR homolog in HeLa cells. The results in Fig. 5B show that mutations in the acidic amino-terminal region of Tat or in the arginine-rich domain abolished the ability of Tat to enhance transcription from the LTR-JCV chimeric promoter. These results suggest that similar regions of Tat are responsible for induction of transcription through the TAR homolog derived from the JCV_L promoter. Analysis of CAT activity after cotransfection of these mutants with the JCV_L-CAT reporter demonstrates that both the amino-terminal and arginine-rich regions are important for full induction of the JCV_L promoter by Tat in glial cells (data not shown). Of particular interest was the notion that, unlike $gly_{2,5,9}$, the K9 mutant showed a reduced but consistent level of activity on the JCV_L promoter, suggesting that a TAR-independent mech-

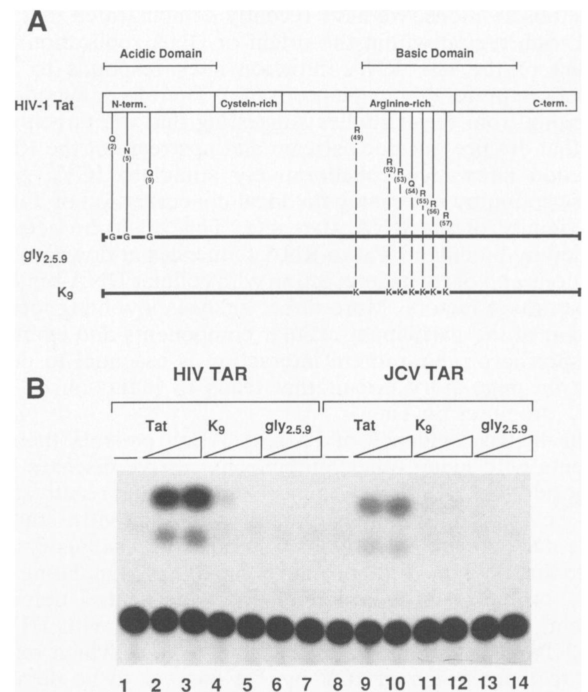

FIG. 5. *trans* activation of JCV-derived TAR-containing LTR by Tat and its mutants. (A) Schematic representation of prototype Tat protein, the positions of various regions, and the two mutants $gly_{2,5,9}$ and K9. (B) Activity of the HIV-1 promoter containing JCV TAR sequence in the absence and presence of 2.5 and 5.0 μg of Tat and its derivative mutants in HeLa cells.

anism may contribute to the overall induction of the JCV_L promoter by Tat (4a).

DISCUSSION

Tat is a potent *trans*-regulatory protein produced at an early phase of infection that plays a key role in HIV-1 replication. This protein, by interacting with a short RNA sequence (TAR) located downstream of the transcription start site, and likely with other cell-derived regulatory factors, induces the rate of HIV-1 RNA transcription. The mechanism by which Tat influences the transcription of the heterologous JCV_L promoter is unknown. The architectural organization of the JCV control region and the positions of regulatory areas that mediate glial-specific transcription of the JCV_L gene significantly differ from those in the HIV-1 promoter. However, the central region of the 98 bp that resides in the leader of several late RNA species possesses a region with extensive sequence homology with HIV-1 TAR. The biological significance of the TAR sequence located in the leader of late RNAs of JCV remains unknown. However, this region at the DNA level encompasses activator elements that contribute to overall transcription of JCV early and late promoters. In this study, we examined the functional property of the TAR-homologous region with respect to Tat induction in the JCV and HIV-1 context. Use of Tat mutants with no binding and transcription activities on the native TAR and site-directed mutagenesis of the conserved nucleotides within the TAR homolog revealed that common elements are important for full activation of the JCV and HIV-1 promoters by Tat. The low-level induction of the JCV_L promoter-harboring mutations in the TAR-homologous sequence by Tat suggests that a secondary target for Tat induction may be operative in glial cells. In support of these observations, we have recently demonstrated that the G+C-rich region within the origin of DNA replication upstream of the late RNA initiation sites responds to Tat induction, preferably in glial cells (4a). Therefore, a model is emerging from these studies suggesting that the targets for Tat that are present downstream and upstream of the RNA initiation sites may collaboratively stimulate JCV_L gene transcription by increasing the local concentration of Tat in the vicinity of the RNA start site. This could be accomplished by binding of Tat to RNA sequences at downstream positions and/or by its interaction with cellular DNA-binding transcription factors. More direct analysis involving identification of the participant cellular components and examining specific protein-protein interaction is essential to decipher the regulatory circuit that leads to induction of the JCV_L promoter by Tat.

The higher incidence of PML in AIDS patients than in patients with many other immunosuppressive diseases has suggested that HIV-1 plays a more direct role in reactivating JCV beyond just immunosuppression. Our in vitro studies presented here and in previous communications (36) demonstrate that the HIV-1 Tat protein is capable of stimulating the JCV_L promoter in cells derived from the central nervous system. Although superinfection of glial cells with HIV-1 and JCV in the brains of patients with AIDS which might lead to reactivation of JCV by Tat has yet to be demonstrated, an alternative mechanism which involves internalization of Tat protein by cells may provide an independent route for accessibility of the JCV_L promoter by Tat. The Tat protein of HIV-1 has been detected in the media of infected cells and shown to be taken up by neighboring uninfected cells (6, 8). Experiments are in progress to examine reactivation of JCV replication in glial cells after exposure to soluble Tat protein.

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