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Activation of the JC virus Tat-responsive transcriptional control element by association of the Tat protein of human immunodeficiency virus 1 with cellular protein Purα

(AIDS/progressive multifocal leukoencephalopathy/glial cells/papovavirusPurA)

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ABSTRACT **JC virus is activated to replicate in glial cells of many AIDS patients with neurological disorders. In human glial cells, the human immunodeficiency virus 1 (HIV-1) Tat protein activates the major late promoter of JC virus through a Tat-responsive DNA element, termed *upTAR*, which is a recognition site for cellular Purα, a sequence-specific single-stranded DNA binding protein implicated in cell cycle control of DNA replication and transcription. Tat interacts with two leucine-rich repeats in Purα to form a complex that can be immunoprecipitated from cell extracts. Tat enhances the ability of purified glutathione *S*-transferase–Purα (GST–Purα) to bind the *upTAR* element. Tat acts synergistically with Purα, in a cell-cycle-dependent manner, to activate transcription at an *upTAR* element placed upstream of a heterologous promoter. Since Purα is ubiquitously expressed in human cells and since *PUR* elements are located near many promoters and origins of replication, the Tat–Purα interaction may be implicated in effects of HIV-1 throughout the full range of HIV-1-infected cells.**

JC virus (JCV) is the etiologic agent of progressive multifocal leukoencephalopathy, a demyelinating neurodegenerative disease. While JCV is normally latent in humans, it is activated in the central nervous systems of certain immunocompromised individuals. A high preponderance of JCV infection in brains of AIDS patients has suggested that the human immunodeficiency virus 1 (HIV-1) itself, independently of its effects on the immune system, may play a role in activation (1, 2). JCV has a highly specific tropism for glial cells. HIV-1 also infects glial cells in the brain, and aside from the as yet undemonstrated possibility of coinfection, JCV-infected cells would be accessible to the HIV-1 Tat protein (3–5).

The Tat protein encoded by HIV-1 is an RNA-binding protein that stimulates transcription at the viral long terminal repeat promoter through specific interaction with an element (transactivation region, or TAR) present within the 5′ untranslated leader of HIV-1 transcripts (6–12). While the precise mechanism of Tat activation is unknown, evidence suggests that the protein acts on transcription by introducing associated cellular factors for either initiation, elongation or antitermination into the vicinity of the transcriptional apparatus (8, 13–15). While cellular proteins have been identified that bind to Tat (16–20), the mechanism by which these might activate transcription of any given gene is not known. The 5′ leaders of certain of the late JCV transcripts contain elements homologous to TAR (21), and transcription of these mRNAs is strongly stimulated by Tat (22). Furthermore, JCV transcriptional activation by Tat requires interaction with a cellular protein and the Tat-responsive *upTAR* element located in the JCV late region promoter upstream of the transcriptional start sites. Tat itself does not bind this element (23). Since the *upTAR* element contains consensus binding sites for Purα, an evolutionarily conserved nuclear protein (24–26), we sought to determine whether this protein would interact with both Tat and *upTAR*.

Purα is a nuclear protein of 322 aa (25, 26) that has been implicated in control of both gene transcription (27–29) and DNA replication (24, 26, 30). In avian cells, Purα mediates effects of Rous sarcoma virus on cellular gene transcription (28). Purα contains a region with limited homology to the simian virus 40 large tumor antigen (26), and this region is implicated in the binding of each of these proteins to Rb, the retinoblastoma tumor suppressor gene product (31). Purα is a single-stranded DNA binding protein with specific affinity for repeats of the sequence GGN (25). The JCV *upTAR* element, located just distal to the viral origin of DNA replication relative to the late mRNA start sites, contains the sequence GGAGGCGGAGGC, which is essential for Tat responsiveness (23). Herein we show that human Purα has a specific affinity for the Tat protein in glial cells and that Tat and Purα act synergistically *in vivo* to enhance transcription at the JCV Tat-responsive *upTAR* element.

MATERIALS AND METHODS

Plasmids and Vectors. Plasmids used for transfection were constructed as follows. Plasmid UP-2 is vector pBLCat-2, containing the *Escherichia coli* chloramphenicol acetyltransferase (CAT) gene linked to the herpes simplex virus thymidine kinase (TK) gene promoter and, upstream of the promoter, the *upTAR* element, synthesized as follows with 4-bp restriction site ends: 5′-AGCTTGGAGGCGGAGGCG-3′ and 3′-ACCTCCGCCTCCGCAGCT-5′. Plasmid UP-3 is pBLCat-2 containing the *upTAR* element mutated as follows: 5′-AGCTTGGAAAGTTAGGCG-3′ and 3′-ACCTTTCAATCCGCAGCT-5′. Plasmid pActin-Tat has been described (22). Plasmid pEBV-Pur contains the 1.1-kb Purα cDNA of pPUR6 (25) under control of the Epstein–Barr virus promoter.

Immunoprecipitation. Glioblastoma cell line 5–10, constitutively synthesizing Tat expressed under control of the simian virus 40 late promoter, was cultured in Dulbecco's modified

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Abbreviations: JCV, polyoma virus JC; GST, glutathione *S*-transferase; HIV-1, human immunodeficiency virus 1; CAT, chloramphenicol acetyltransferase; LRR, leucine-rich repeat; TK, thymidine kinase.

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Eagle's medium (DMEM) containing 10% fetal bovine serum in 75-cm^2 plates to 5×10^5 cells per plate. Extracts of 10^7 cells were prepared by lysis at 0°C in 4.5 ml of Lysis 150 buffer (31) supplemented to 250 mM NaCl. After 2 min in lysis buffer, extracts were clarified by centrifugation at 14,000 × *g* for 4 min in an Eppendorf centrifuge. After addition of 1.5 ml of Lysis 150 buffer with no NaCl, monoclonal antibody 9C12 (2.0 μg) was added to 1.0-ml aliquots of extract. After incubation at 0°C for 30 min, magnetic beads coupled to sheep anti-mouse antibody (Dynal, Great Neck, NY; 500 μg of beads coupled to approximately 5 μg of antibody) were added to each 1-ml aliquot. After 2 hr at 4°C with gentle shaking, beads were collected by magnetism and washed five times with 1 ml of 0.15 M NaCl/0.015 M sodium citrate (pH 7.0) containing 1 mM EDTA. Proteins attached to sheep anti-mouse magnetic beads were eluted in 40 μl of SDS sample buffer and subjected to SDS/polyacrylamide gel electrophoresis on 10% gels. After blotting to an Immobilon membrane (Millipore), the membrane was probed with rabbit anti-Tat antibody 705 (National Institute of Allergy and Infectious Diseases) followed by peroxidase-conjugated goat anti-rabbit antibody using the Dupont Renaissance system.

Electrophoresis of Protein–Protein Complexes in the Presence or Absence of a Purα DNA Recognition Element. This method for measuring protein–protein interaction is based on the ability of one protein—e.g., Purα—to bind its recognition element while attached to a nitrocellulose filter. Samples of purified glutathione *S*-transferase (GST)–Purα (Pur) and GST–Tat (Tat) or GST–Tat72 (Tat72), each at 7.5 pmol, were incubated, either singly or in combination in buffer described previously (31) for 1 hr at 30°C. To assay effects of DNA binding by Purα upon the GST–Purα interaction with GST–Tat or GST–Tat72, unlabeled JCV+, the single-stranded DNA purine-rich upTAR 24-mer oligonucleotide was either omitted or included at 10 ng/ml in the incubation. Samples were loaded onto a nondenaturing 3% gel as described (31, 32) and subjected to electrophoresis at 40 V for 36 hr. Proteins thus separated were electroblotted to a nitrocellulose filter. The filter was cut into sections. Sections were probed either with anti-Tat antibody, to detect Tat, or with labeled *upTAR* oligonucleotide, to detect Purα DNA binding. Standard gel shifts, to measure Purα ability to bind its single-stranded DNA recognition element, were performed as described using labeled 24-mer oligonucleotide MFO677, the purine-rich strand of a c-*myc* locus *PUR* element (24).

Transfections and CAT Assays. Cells were plated at 5×10^5 per plate in DMEM without fetal bovine serum. After 60 hr cells were released from G_0 phase by transferring to medium containing 10% fetal bovine serum. Transfections were performed, using a standard calcium phosphate protocol as described (22), at the following times as indicated in each panel: 4 hr (G_0/G_1 phase), 12 hr (G_1/S phase), or 16 hr (S phase). Cells were transfected, either singly or in combination as indicated in the figure legends, with CAT reporter plasmid UP-2 (3 μg), containing the *upTAR* element linked to a heterologous TK promoter and CAT gene, plasmid pActin-Tat (2.5 μg), containing the full-length 86-codon Tat gene under control of a human β-actin promoter, and plasmid pEBV-Pur (0.01, 0.05, or 0.10 μg), expressing the human Purα cDNA. Nonsynchronous cells were transfected with either plasmid UP-2 or UP-3, containing a mutated *upTAR* element, and plasmid pEBV-Pur as indicated. CAT assays were performed as described (22). Each assay point utilized 100 μg of cell extract protein. Quantitation was by liquid scintillation spectrometry.

RESULTS

Tat and Purα Coimmunoprecipitate from Extracts of Glial Cells Constitutively Expressing Tat. We examined the ability of Tat and Purα to form a complex in cells where both proteins are present. The human glioblastoma cell line 5–10 has been derived from line U-87MG transfected with a vector for expression of the *tat* gene linked to a simian virus 40 promoter and synthesizes Tat constitutively (33). These cells also contain Purα, as does every mammalian tissue thus far tested. Fig. 1*A*, a Western blot with anti-Tat antibody, shows that Tat and Purα can be immunoextracted as a complex from lysates of 5–10 cells using a monoclonal antibody to Purα. Lysates of 5–10 cells possess a protein migrating at approximately 16 kDa that reacts with the anti-Tat antibody, as well as a larger aggregated Tat form (left lane). The 5–10 cells possess a large amount of this aggregated Tat form, which may be an artifact of overexpression. Control extracts of U-87MG glial cells, which do not synthesize Tat, do not show any Tat bands on a Western blot, confirming that the bands in lane 1 do represent Tat. The 16-kDa protein is immunoprecipitated by the addition of anti-Purα antibody 5B11 (right lane). It has previously been observed that monomeric Tat protein migrates at a position of 17 kDa on SDS gels (20), a position higher than expected based on amino acid sequence. The 16-kDa band we observe migrates at the position of purified Tat protein obtained from J. Karn (MRC Laboratory of Molecular Biology, Cambridge, U.K.) (data not shown). Fig. 1*B* shows that Tat in a cell lysate specifically reacts with Purα fused to GST and coupled to glutathione-agarose beads. Only the beads containing Purα, and not those containing control GST, bind the purified Tat. Under the conditions employed, approximately 40% of the purified Tat is retained on the beads. In the far right lane, a 5–10 cell lysate has been passed over the Pur–GST affinity column, showing that Tat is retained.

Purα Association with Tat Enhances Purα Binding to its Single-Stranded Recognition Element. The gel shift experiments in Fig. 2*A* show that presence of Tat, at a 1:1 molar ratio to Purα, enhances Purα binding nearly 10-fold. Compare JCV+, the purine-rich strand of the *upTAR* element, in the

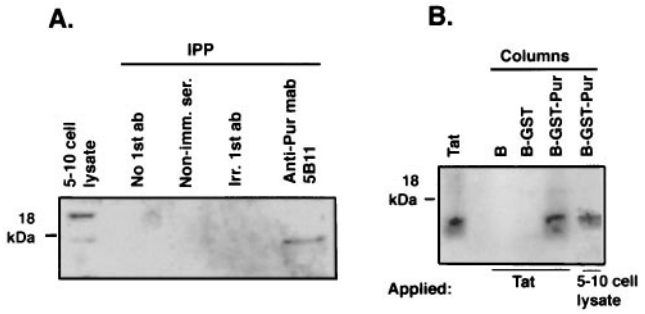


FIG. 1. Detection of a Tat–Purα complex in human glioblastoma cells. (*A*) Immunoprecipitation of a Tat–Purα complex from extracts of human glioblastoma 5–10 cells, which constitutively express the HIV-1 *tat* gene, with an anti-Purα monoclonal antibody. The left lane represents 25 μl of untreated lysate. For the lanes labeled IPP, immunoprecipitation was carried out using magnetic beads alone (no first antibody), mouse nonimmune serum (Non-imm. ser.), an irrelevant first antibody, anti-influenza virus hemaggltuinin epitope monoclonal antibody 12CA5 (Irr. 1st ab), or anti-Purα antibody 5B11 (Anti-Pur mab 5B11). (*B*) Complex formation between Tat and GST–Purα in glioblastoma cell lysates. Lanes labeled Columns represent protein eluted from columns consisting of glutathione-agarose beads alone (lane B), beads coupled to GST (lane B-GST), or beads coupled to GST–Purα (lane B-GST-Pur). The left lane represents purified Tat protein (400 ng). For the lanes labeled Tat, 400 ng of purified Tat protein was passed over the column. For the lane labeled 5–10 cell lysate, 20 μl of clarified lysate was passed over the column. The gel bands were transferred to an Immobilon membrane and the membrane was probed with anti-Tat antibody. The four lanes at left were exposed to film for equal time, and the lane at far right was exposed 20 times longer. The position 18 kDa represents migration of a prestained lysozyme marker.

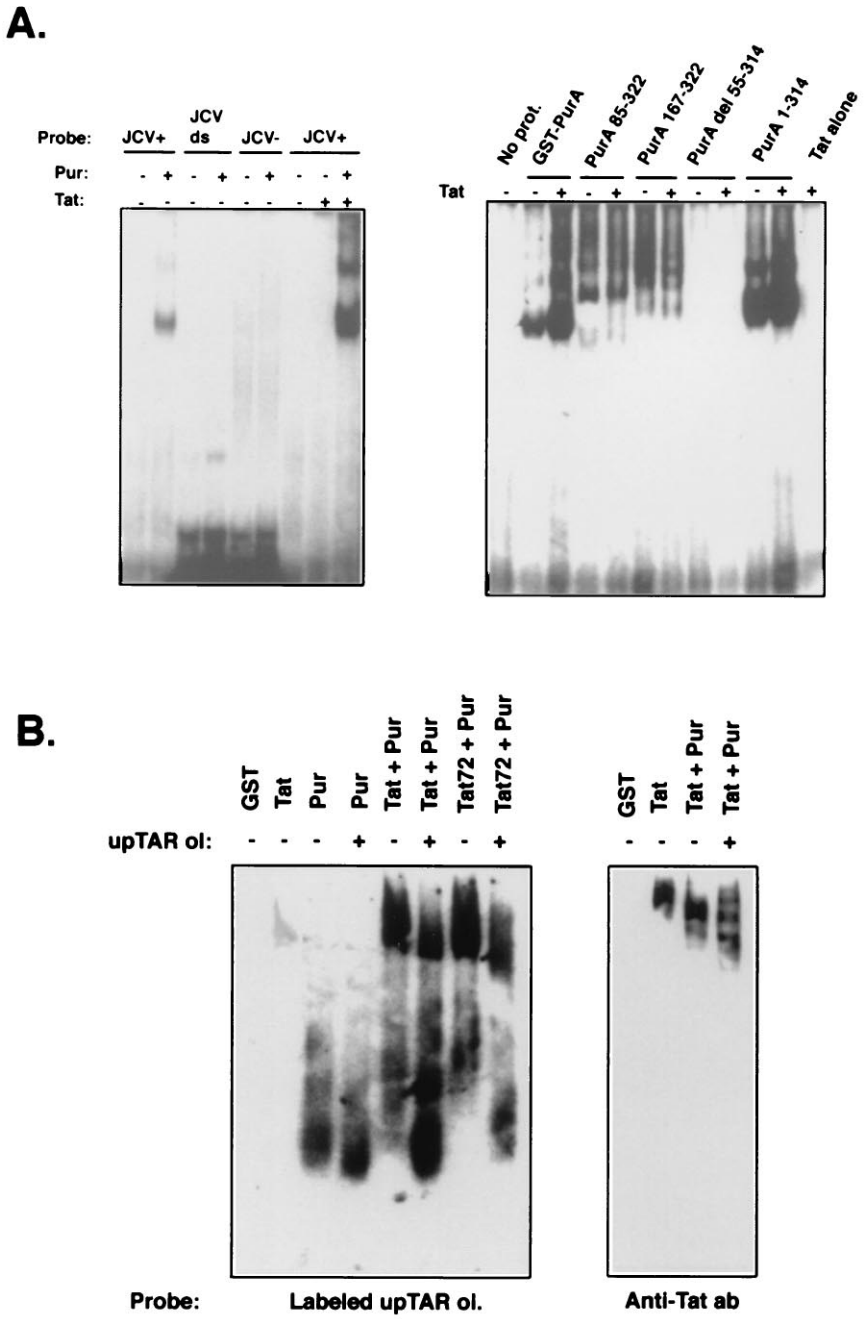


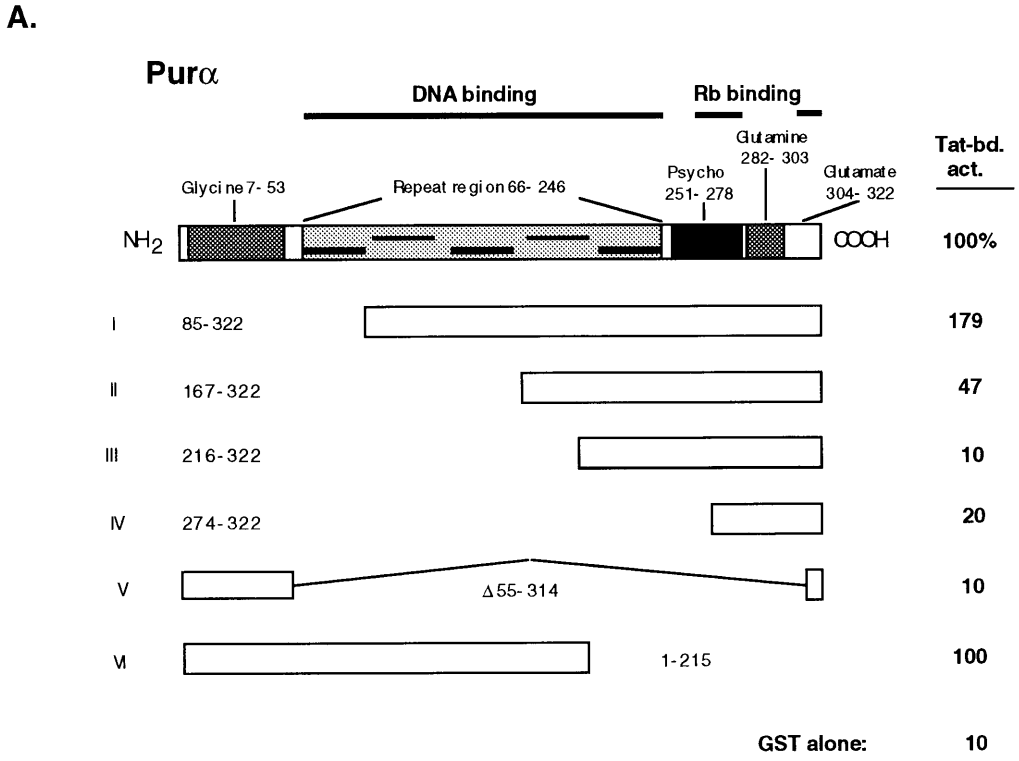
FIG. 2. (*A*) Binding of Purα to the JCV *upTAR PUR* element in the presence or absence of Tat. All gel shifts were performed as described (24, 25) using ^{32}P-end-labeled 24-mer oligonucleotide probe representing a dimer of the *upTAR* element. The purine-rich strand of this oligonucleotide is 5′-GGAGGCGGAGGCGGAGGCGGAGGC-3′. For both *A* and *B*, all Tat, Purα, and Purα deletion mutant proteins were employed as GST fusion proteins prepared by purification on glutathione-agarose columns as described for Fig. 1 and eluted with glutathione (34). In lanes labeled +, 7.5 pmol of either protein indicated was used. (*A Left*) Specificity of binding of Purα to the JCV *upTAR* element. The different oligonucleotide probes used for the indicated reactions are JCV+, the purine-rich single strand of the *upTAR* element; JCV ds, the double-stranded *upTAR* element, only the pyrimidine-rich strand of which is labeled; JCV−, the pyrimidine-rich strand of the *upTAR* element. (*A Right*) Effect of Tat upon binding of a series of Purα deletion mutants to the JCV *upTAR* element. Tat refers to GST–Tat. GST–PurA refers to full-length GST–Purα, in which Purα is 322 aa long. Numbers given for deletion mutants refer to the Purα aa remaining in the construct, beginning with the N terminus of Purα, except for PurA del 55–314, in which the indicated internal aa have been deleted. (*B*) Association of Tat and Purα proteins in the presence or absence of the JCV *upTAR* element. Nondenaturing gel electrophoresis of complexes between GST–Purα and GST–Tat (Tat–Pur) or GST–Tat72 (Tat72–Pur), in the presence or absence of the single-stranded 24-mer oligonucleotide representing *upTAR* (*upTAR* ol) was performed, and the gel was blotted and probed as described in the text. As controls purified GST (GST) and GST–Tat (Tat) were loaded alone. The filter was cut in sections. The left section was probed with ^{32}P-labeled *upTAR* ol. Binding of labeled *upTAR* indicates the position of GST–Purα on the filter. The right section, containing lanes run in parallel to those on the left, was probed with rabbit anti-Tat antibody 705, as described for Fig. 1, to indicate the position of Tat.

presence of GST–Purα alone, with JCV+, in the presence of Purα and Tat. In most studies Purα has approximately 10-fold lower affinity for its double-stranded element (JCV ds) than for its single-stranded element. The *upTAR* element of JCV is adjacent to the viral origin of replication and exists in an unwound configuration at certain times. Purα has little affinity for the pyrimidine-rich strand of the element (JCV−). Fig. 2*A Right* shows effects of Tat on DNA binding by a series of Purα deletion mutants. The enhancing effect of Tat on Purα DNA binding is abolished when the amino-terminal 84 aa of Purα are removed. Purα binds DNA as a series of multimeric aggregates (Fig. 2*A Right*). The amino terminus of Purα affects the association of Purα molecules, allowing more of the protein to exist unaggregated. As more of the amino terminus is deleted, only higher-order multimers of Purα are seen. Tat enhances the DNA binding of all multimeric forms of Purα >10-fold but does not appreciably shift the position of any of these bands. Since Tat and Purα may form a transient complex under standard gel shift conditions, we used an alternative system to analyze the interaction (31). In this system Purα and another protein, in this case Tat, are subjected to electrophoresis together under nondenaturing conditions, and Purα is subsequently probed using its binding oligonucleotide. One advantage of this new system is that the DNA recognition element oligonucleotide may or may not be included in the electrophoresis. The experiment in Fig. 2*B* shows that Tat and Purα do form a complex and that presence of the single-stranded *PUR* recognition element dissociates the complex. Fig. 2*B Left* shows that Purα alone migrates as a series of multimers and that these are only slightly shifted by presence of the JCV *PUR* element. In the presence of Tat, Purα is shifted to near the top of the gel and, as in Fig. 2*A*, the overall level of DNA binding is enhanced several fold. Addition of the unlabeled *PUR* element during electrophoresis disrupts the Tat–Purα complex, as seen in the lane +Tat+Pur. DNA binding by Purα is enhanced by Tat even after Tat and Purα are no longer associated. One possible explanation for this result is that binding to Tat could have a persisting effect on

the conformation of Purα. This would explain the strong enhancement of Purα DNA binding by Tat in Fig. 2*A* even though the Purα–DNA complexes are not supershifted by the Tat protein. We tested the ability of Tat to interact with another single-stranded DNA-binding protein, YB-1. YB-1 binds a pyrimidine-rich sequence element that is similar to the complement of the *PUR* element, and Purα and YB-1 both interact with JCV tumor antigen (29). Tat had no effect on the binding of YB-1 to DNA. The enhancement of Purα DNA binding by Tat is not limited to the JCV *PUR* element, since Tat also enhanced binding to an oligonucleotide representing a *PUR* element present upstream of the human c-*myc* gene.

Purα Binding to Tat Requires Two Purα Leucine-Rich Repeats (LRRs). Purα has a complex structure with two series of interspersed repeats, a glutamine-rich potential transactivation domain, a region of amphipathic helix, and a glycine-rich domain (Fig. 3*A*). The domain(s) of the Purα protein required for binding to Tat were mapped. To do this, purified Tat was labeled with [^{32}P]phosphate and incubated with either Purα or each of six Purα deletion mutants coupled to GST–glutathione-agarose. Results are presented in the column to the right in Fig. 3*A*, where radioactivity bound by full-length Purα is normalized to 100%. Removal of the amino terminus of Purα in mutant I, including one of three basic aromatic repeats (25), does not hinder Tat binding, actually increasing the amount of Tat retained. In contrast removal of one of two acidic LRRs (25) in mutant II reduces Tat binding by about half. Removal of the other acidic LRR in mutant III reduces binding to control levels. Implication of the acidic LRR in Tat binding is best demonstrated by comparison of mutant VI, which possesses 100% of original binding activity, with mutant II, which possess 47% of original binding activity. The only overlap between these two mutants is the second acidic LRR, and when this is removed in mutant III, Tat binding is lost. We conclude that the two acidic LRRs are equally required for binding to Tat. The amino terminus of Purα is also required for enhancement of DNA binding by Tat (Fig. 1*A*) although the amino terminus itself does not bind Tat (Fig. 3*A*).

Tat and Purα Act Synergistically at a Purα DNA Recognition Element to Enhance Transcription. To assess the consequences of Tat–Purα interaction with the JCV *upTAR* element *in vivo,* we transfected U-87MG glioblastoma cells with plasmids for expression of both proteins, together with a plasmid bearing the JCV *upTAR* element placed upstream of a heterologous *tk* promoter and a CAT reporter gene. Over the course of many studies, it became clear that results were dependent upon the cell cycle phase in which cells were transfected. Fig. 4 shows results of transfection of U-87MG cells released from



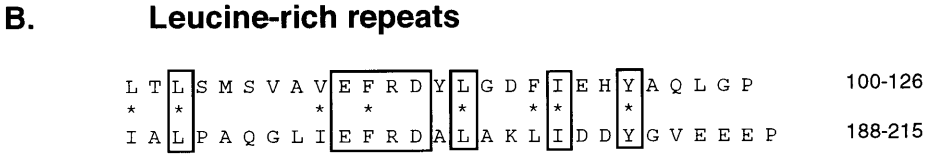


FIG. 3. Binding of purified Tat to Purα deletion mutants. (*A*) Depicted are GST–Purα deletion mutants with respect to structural features of the Purα protein, which is 322 aa in length. The amino-terminal GST portion of the molecule has been omitted from this diagram. DNA and Rb-binding regions are indicated above the diagram. Three basic aromatic repeats are indicated by heavy horizontal lower lines in the repeat region of the protein. Two acidic LRRs are indicated by light horizontal upper lines in the repeat region. Tat binding activity is presented in the column at right. After subtraction of background binding to glutathione-agarose beads alone, values were normalized to show binding to full-length Purα as 100%. Binding of Tat to control GST alone is shown at bottom. (*B*) Acidic LRRs implicated in Purα binding to Tat. Sequences are from human Purα (25, 26). Amino acid numbers are at right. Positions of conserved hydrophobic residues are starred. Homologies are boxed.

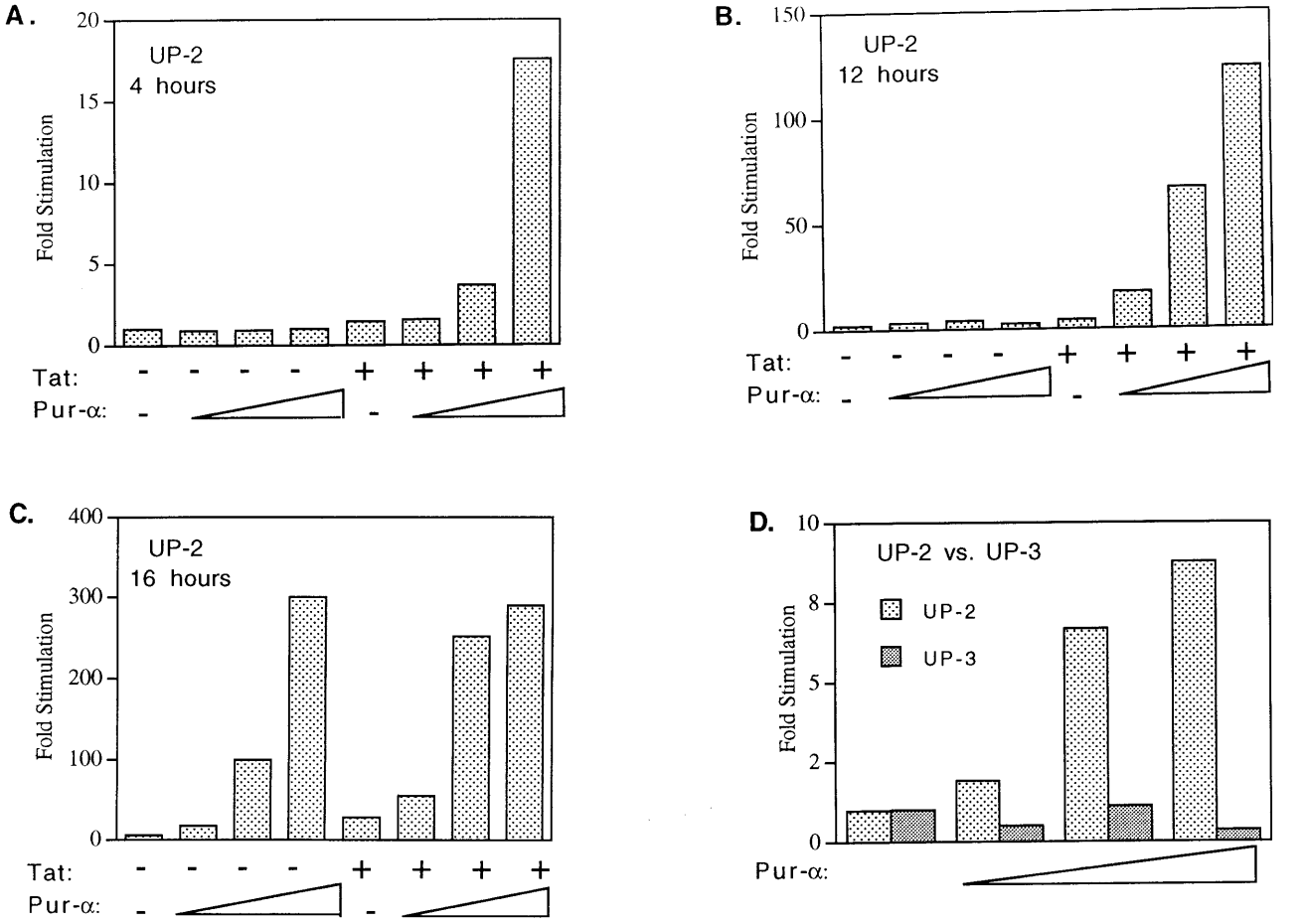


FIG. 4. Cooperative action of Tat and Purα at the JCV Tat-responsive transcriptional control element *upTAR*. U-87MG human glioblastoma cells were transfected. For *A–C*, cells were transfected, either singly or in combination as indicated, with CAT reporter plasmid UP-2, containing the *upTAR* element linked to a heterologous TK promoter and CAT gene, plasmid pActin-Tat, containing the full-length 86-codon Tat gene under control of a human β-actin promoter, and plasmid pEBV-Pur (0.01, 0.05, or 0.10 μg, as indicated by wedges), expressing the human Purα cDNA. For *D*, nonsynchronous cells were transfected with either plasmid UP-2 or UP-3, containing a mutated *upTAR* element, and plasmid pEBV-Pur as indicated. CAT assays were performed.

a G_0-phase serum-starvation block. At 4 hr (Fig. 4*A*) and 12 hr (Fig. 4*B*), corresponding to G_0/G_1 phase and G_1/S phase, respectively, Purα has no independent effect on transcription. However, Tat and Purα together display a striking synergism. At 12 hr, this synergism leads to stimulation of transcription >100-fold over basal levels (Fig. 4*B*). At 16 hr (Fig. 4*C*), corresponding to S phase, Purα alone has a stimulatory effect, and synergism with Tat is seen primarily at intermediate levels of Purα-expressing plasmid. The effects of Purα and Tat on transcription are dependent upon the *upTAR* element. In nonsynchronous cells, the effect of Purα upon the *upTAR* element is abolished by substituting a mutated version of the element (Fig. 4*D*). We have tested the effect of two Purα mutants lacking the LRRs on transactivation of the JCV late promoter *in vivo*. One mutant, Purα 216–322, is illustrated in Fig. 3. The other was PurαΔ71-216. U-87MG cells were transfected to express or not express Tat protein in the presence or absence of various amounts of Purα-mutant-expressing plasmid at 12 hr after release from serum starvation exactly as described for Fig. 4*B*. Neither of the Purα mutants displayed any significant stimulation of transcription. Note that both of these mutants lack both LRRs while retaining one version of the DNA-binding repeat. Mutant PurαΔ71-216 possesses all sequences both amino-terminal and carboxyl-terminal to the Purα repeat region. These results further implicate the LRR in Tat–Purα interaction.

DISCUSSION

While JCV, a DNA papovavirus, is normally latent in humans, it is activated in the central nervous systems of many individuals with AIDS and other immunocompromising conditions. There is a disproportionate incidence of progressive multifocal leukoencephalopathy in HIV-1-infected individuals, presently affecting 4–8% of AIDS patients. Evidence for a direct role for HIV in JCV activation is derived from studies showing transactivation of the JCV major late promoter by the HIV-1 Tat protein (22). Although both HIV-1 and JCV may infect various glial cell populations in the brain, it is not known whether the viruses simultaneously infect an individual cell. Mechanisms exist, however, aside from coinfection, for an action of the Tat protein in JCV-containing glial cells. Tat can be secreted from HIV-1-infected cells (3) and taken up by cells in proximity in a form capable of activating transcription (4, 5). While our results may implicate a Tat–Purα interaction in activation of JCV in the central nervous system, further work is necessary to detail the role of this interaction in HIV-1 pathogenesis.

We have not thus far been able to isolate a Tat–Purα–DNA ternary complex stably linked in any form. That does not imply that such a complex does not exist. The data of Fig. 2*B Right* suggest the presence of such a complex, but together with the data of Fig. 2*B Left*, further suggest that any such complex may be transient. Fig. 2*B Right* is an anti-Tat-probed blot of the nondenaturing gel. It can be seen that the position of Tat in the gel is

shifted by Purα. The position of this Tat–Purα complex is further shifted by coelectrophoresis with the 24-mer, upTAR, Purα-binding DNA element. It should be kept in mind that this native gel is not that of a standard gel-shift experiment, in which labeled oligonucleotide is used. It is important to note that the oligonucleotide-induced shift is seen with the anti-Tat antibody even though Tat itself does not bind the *PUR* oligonucleotide. If these data indicate presence of a ternary complex, such a complex is likely to be transient, as suggested by the data of Fig. 2*B Left*, showing that presence of the upTAR oligonucleotide actually dissociates a fraction of the Tat–Purα complex.

Tat displays a high affinity for a specific and intriguing sequence in Purα. The sequences of the two Purα LRRs, involved in Tat binding, are compared in Fig. 3*B*. These repeats are of the general structure of LRR found in a large number of functionally diverse proteins and have recently been implicated in protein–protein interactions (35). These repeats could provide a target for altering or blocking the interaction of Purα with Tat. Crystallographic studies of porcine RNase inhibitor, which contains many LRRs, show that each LRR consists of two segments, an amino-terminal β-sheet segment and a carboxyl-terminal α-helical segment, which form a loop with hydrophobic residues oriented inward. The charged residues that separate these segments are oriented outward and may form the basis for specific interactions. It is not known whether the LRRs in Purα form such structures, but the requisite aa configuration is present. We have now also examined the interaction of Purα with a series of Tat deletion mutants and point mutants. It is clear from these studies that Purα binding requires amino acids in the conserved core motif of the Tat protein and that Cys-22, while not essential, is important. These results, together with their functional significance, are to be reported in detail elsewhere.

The present data show that the Tat–Purα interaction has functional consequences for transcription *in vivo*. Any mechanism by which Tat and Purα influence transcription may not be limited to the JCV late promoter. The PUR element GGGAG is present in the loop structure of TAR RNA. Studies on the ability of Tat and Purα, in association with TAR, to modulate HIV-1 gene transcription are currently under way. Various cellular proteins have been reported to bind the HIV-1 Tat protein, including TBP-1 (14), SP1 (18), NF-κB (19), TAP (16), and the Tata-binding subunit of TFIID (20). Purα shares no extensive homology with any of these proteins. The Tat–Purα interaction links cellular activity of Tat to cell cycle timing. It has recently been reported that Tat-induced apoptosis of CD4 T lymphocytes is associated with enhanced activation of cyclin-dependent protein kinases (36). In one retroviral system, Rous sarcoma virus-infected avian cells, Purα reportedly mediates viral-activated clusterin gene expression accompanying apoptosis (28). Purα binds cyclin A/Cdk2 in S phase and stimulates associated kinase activity (H. Itoh, H. Liu, L. Y. Wang, S.B.-V., M. Kanovsky, R. Uson, R. E. Gordon, C. Chu, D. S. Kohtz & E.M.J., unpublished observations). Overexpression of Purα can delay progression to mitosis, as measured by flow cytometry analyses of transfected monkey CV-1 cells. Overexpression had no effect on progression through G_1. It is conceivable that certain transfected cells in the present study, expressing high levels of Purα, are altered in their cell cycle kinetics. This does not affect the interpretation that there is a cell cycle-dependent difference in the cooperative effects of Tat and Purα observed. The present data show highest levels of Purα activation, and Tat enhancement, at the JCV *upTAR* element in S phase (Fig. 4). However, at other times of the cell cycle, Tat and Purα act cooperatively on JCV transcription whereas Purα alone has no effect. The mechanistic basis for the cell cycle dependence of Tat and Purα cooperative effects is not known. The interaction of Tat and Purα transcends potential effects in glial cells. Purα is found in every human cell type tested, including lymphocytes. Since *PUR* elements are present near many genes and origins of DNA replication (25), the Tat–Purα interaction could be involved in a broad array of HIV-1 cellular effects.

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