We demonstrate that the interferon-induced, double-stranded (ds) RNA-activated kinase, PKR, is able to bind to and phosphorylate the human immunodeficiency virus type 1 (HIV-1) trans-activating protein, Tat. Furthermore, Tat can inhibit the activation and activity of the kinase. Phosphorylation of Tat by PKR is dependent on the prior activation of PKR by dsRNA and occurs on serine and threonine residues adjacent to the basic region important for TAR RNA binding and Tat function. Activated PKR efficiently phosphorylates both the two-exon form of Tat (Tat-86) and the single exon form (Tat-72). Mutagenesis indicates that the interaction between PKR and Tat requires the RNA-binding region of Tat. Tat competes with eukaryotic initiation factor 2, a well-characterized substrate of PKR, for phosphorylation by activated PKR. Tat also inhibits the autophosphorylation of PKR by dsRNA. This biochemical evidence of an intimate relationship between Tat, an important regulator of HIV transcription, and PKR, a pleiotropic cellular regulator, may provide insights into HIV-1 pathogenesis and, more generally, virus/host interactions.

The human immunodeficiency virus type 1 (HIV-1)\(^3\) tat gene product trans-activates viral gene expression and is essential for HIV-1 replication (1–3). Tat strongly activates transcription from the HIV-1 long terminal repeat by binding to the Tat-responsive region (TAR), an RNA stem-loop structure located at the 5’ end of HIV transcripts (4). Although the precise mechanism by which Tat exerts its effect is not yet known, it has been established that Tat regulates transcription at the level of initiation and elongation (5, 6). The Tat protein exists in two forms, which in the HXB2 viral isolate consists of 72 and 86 amino acids. The 86-amino acid protein (Tat-86) is encoded by two exons, whereas the 72-residue protein (Tat-72), which is identical except for lacking 14 residues from the C terminus, is the product of the first tat exon. The shorter form is sufficient for trans-activation (7). The second exon of Tat has been proposed to play a role in activation of integrated long terminal repeats, regulation of MHC class 1 gene promoter activity, and TAR-independent trans-activation (8–10). Mutational analysis of Tat has revealed three major regions that are important for function (Fig. 1); these include the N terminus, the cysteine-rich region which is important for metal binding, and a charged, arginine-rich region important for nuclear localization and for binding to the cis-acting TAR element (11–13).

One of the primary cellular responses to viral infection is the production of interferon (14). The RNA-dependent protein kinase PKR, also referred to as DAI, P1 kinase, and p68 kinase, is a serine/threonine kinase that is induced by interferon and activated in the presence of dsRNA. PKR exerts a well-established regulatory effect on initiation of protein synthesis. Activation of PKR by dsRNA, or polyribozymes such as heparin and some structured single-stranded RNAs (15), is accompanied by autophosphorylation (16). Following its activation, PKR in turn catalyzes the phosphorylation of the α subunit of eukaryotic initiation factor 2 (eIF2) on a serine residue at amino acid position 51 (17, 18). Phosphorylation of eIF2 results in the sequestration of a second initiation factor, the guanosine nucleotide exchange factor eIF2B, leading to the inhibition of protein synthesis (18). This mode of translational shut down provides a mechanism of host defense and, as such, is detrimental to the viral life cycle. Many viruses have developed strategies to circumvent the action of PKR activation. The mechanisms by which viruses prevent the action of PKR vary as follows: adenovirus (19), vaccinia virus (20), and influenza virus (21) directly reduce PKR activity via different means, whereas poliovirus infection leads to PKR degradation (22). PKR appears to be down-regulated by HIV-1 (23), but the mechanism remains to be elucidated. PKR has also been implicated in oncogenic transformation and tumorigenesis (24, 25) as well as differentiation (26) and apoptosis (27). The substrate specificity of PKR has been shown to extend beyond eIF2 to include IκB (28, 29) an inhibitor of the transcriptional activator NFκB, as well as histone H2A (30), and a 90-kDa protein found in rabbit reticulocytes (31) which can be phosphorylated by PKR in vitro.

Tat binds a variety of cellular factors including a putative ATPase and DNA helicase (32), and a 36-kDa nuclear factor (33), as well as the transcription factors TFIIID (34) and Sp1 (35). Several observations prompted us to evaluate the possibility of an interaction between Tat and PKR. First, both have been demonstrated to interact stably with TAR (14, 36). Moreover, the TAR RNA binding protein can interact with PKR, preventing the activation of PKR (37, 38). Second, recent reports indicate that Tat binds a novel cellular kinase (39, 40), and Tat-mediated transcription is sensitive to the kinase inhibitor 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole (41). Third, PKR has been shown to activate NFκB by phosphoryl-
**HIV-1 Tat Protein Is a Substrate and Inhibitor of PKR**

**EXPERIMENTAL PROCEDURES**

**Purification of HIV-1 Tat-72 Protein**—The region of Tat encoded by the first exon (amino acids 1–72) of the HIV-1 HXB2 isolate was overexpressed in *Escherichia coli* (43) and purified to greater than 90% homogeneity as assessed by gel electrophoresis on glass plates (30). HIV-1 Tat-72 (amino acids 1–130) and single exon forms of Tat-2 are illustrated, together with the first exon (amino acids 1–72) of the HIV-1 HXB2 isolate was overexpressed in *E. coli* (43) and purified to greater than 90% homogeneity as assessed by gel electrophoresis on glass plates (30).

**Expression of GST-Tat Fusion Proteins**—Plasmids expressing glutathione S-transferase (GST) fusions with wild-type Tat-1 86 or Tat-1 72 from the HIV-1 HXB2 isolate were generously donated by A. Rice. Wild-type Tat-2 130 (ROD isolate), mutant Tat-1, and Tat-2 fusion proteins (Tat-1, Tat-2, Tat-2/36, Tat-2/18, Tat-2/50, Tat-2/50, Tat-4A, Tat-4A/181S, Tat-4A/21C, Tat-4A/21C, Tat-4A, Tat-4A/181S, Tat-4A/21C, Tat-4A/21C, Tat-4A, Tat-4A/181S, Tat-4A/21C, Tat-4A/21C, Tat-4A, Tat-4A/181S, Tat-4A/21C, Tat-4A/21C) were obtained from the NIH AIDS Research Program, NIAID (Rockville, MD). GST-Tat constructs were expressed and purified as described previously (45). Competent *E. coli*, strain BL21 (Strategene, La Jolla, CA), was transformed with either pGEX2T or pGEX2TK vectors (Pharmacia Biotech Inc.) containing either wild-type or mutant forms of Tat (see Fig. 1). A GST construct lacking Tat protein was used as a control. Luria Bertani (LB) broth cultures (50 ml), supplemented with ampicillin (Sigma) 50 μg/ml, were incubated overnight with shaking at 37°C. Overnight cultures were diluted 1:10, and the incubation was allowed to continue for a further 3 h. Fusion protein expression was induced by the addition of isopropl-1-thio-β-D-galactoside (Sigma) (1 mM final concentration) and growth was continued for a further 1.5 h. Bacteria were pelleted by centrifugation at 5,000 rpm for 10 min at 4°C and resuspended in 4 ml of EBC buffer (50 mM Tris, pH 8.0, 120 mM NaCl, 0.5% Nonidet P-40) containing 5 mM DTT, 2 mM mg/l lysozyme, 1 μg/ml leupeptin, 2 μg/ml aprotinin, and 50 μg/ml phenylmethylsulfonyl fluoride. After incubation at 37°C on ice and three times for 30 s on ice, the lysed bacteria were centrifuged at 12,000 rpm for 15 min at 4°C. Supernatants were transferred to fresh tubes and stored at −70°C.

**Cleavage of GST-Tat Fusion Protein**—Bacterial extracts (50 μl) were mixed with 250 μl of EBC buffer and 25 μl of glutathione Sepharose beads (equilibrated in EBC buffer). Samples were incubated on a rocking platform for 30 min at 4°C, centrifuged for 10 s at 12,000 rpm in a microcentrifuge, and the supernatant discarded. Pelleted complexes were washed twice with 500 μl of EBC buffer containing DTT (5 mM) and SDS (0.075%). Precipitates were resuspended in 20 ml of thrombin cleavage buffer (50 mM Tris-HCl, pH 7.6, 20 mM KCl, 1 mM DTT). Suspensions were centrifuged at 3000 rpm for 3 min at room temperature. Supernatants were discarded, and pellets were resuspended in 100-μl volumes of cleavage buffer. Two units of human thrombin (Sigma) were added, and the reactions were allowed to proceed for 4 h at room temperature. Following the incubations, reactions were centrifuged in a microcentrifuge at 3000 rpm for 3 min and the supernatants collected. The concentration of Tat protein obtained following thrombin digestion was estimated by running samples of the supernatant on a 15% polyacrylamide gel containing SDS, staining the gel with Coomassie Blue, and comparing the band intensities with those of standard proteins of known concentrations.

**Kinase Assays**—Reactions (20 μl) containing 2.5 μCi of [γ-32P]ATP (ICN Biomedical Inc., Costa Mesa, CA) and 0.5 μl of PKR (approximately 5 ng) purified to the mono-S stage (46) were conducted as described previously (47) in the presence of dsRNA derived from reovirus (a gift from A. Shatkine). Kinase reactions (20 μl total volume) containing PKC (10 ng) were carried out as described by the manufacturer (Upstate Biotechnology Inc., Lake Placid, NY). Kinase reactions containing the β-insulin receptor kinase (a gift from A. Flint) were performed as described by Villalba et al. (48). Mammalian and yeast kinases involved in the Ras signal transduction pathway (STE 20, STE 11, MEK, ERK) were a gift from A. Polverino; activation assays were performed as described by Polverino et al. (49). In each case, the reaction was stopped last to the reaction components assembled on ice. Phosphorylation was visualized using SDS-polyacrylamide gel electrophoresis and autoradiography at −70°C with an intensifier screen.

**Phosphorylation of Tat and eIF2 by PKR**—Kinase assays in the presence of Tat were performed by the addition of 10 μl from a 20-μl PKR activation assay (described above) to 50 ng of purified Tat-72 or Tat proteins. Phosphorylation was carried out by the addition of 20 μl of PKR, 50 ng of recombinant GST-Tat protein, 30 μl of glutathione-Sepharose beads (equilibrated in EBC buffer) containing Tat protein, and 50 ng of ATP. Reactions were incubated for 90 min at 30°C and stopped by the addition of Laemmli sample buffer. Phosphorylated proteins were resolved in a 20% polyacrylamide gel containing SDS. Dried gels were exposed at −70°C to x-ray film (Eastman Kodak Co.) in the presence of an intensifier screen.

**Edman Degradation and Phosphoamino Acid Analysis**—Phosphate-labeled Tat-72 was excised from the gel and processed as described by Beemon and Hunter (50). Labeled protein was digested in-gel for 20 h at 30°C (51) with either trypsin or Achromobacter protease I (final concentration of 1 μg/reaction) and fractionated by HPLC. Fractions containing phosphate-labeled derivatives were subjected to Edman degradation using the modification of Russo et al. (52). Following acid hydrolysis, one-dimensional phosphoamino acid analysis was performed as described by Cooper et al. (53), using electrophoresis on glass thin layer chromatography plates (1. T. S. 2. Blue, 3. Buffer 3 5. 10:100:1890, pyridine:glacial acetic acid:H₂O), for 30 min at 1000 V.

**Binding of PKR to Tat**—Aliquots (100 μl) containing recombinant GST-Tat protein were mixed with 100-μl volumes of glutathione Sepharose beads (equilibrated in EBC buffer) and incubated for 30 min at 4°C. Complexes were sedimented by centrifugation in a microcentrifuge for 20 s at 12,000 rpm. The pellets were washed five times in...
PKR has been shown to phosphorylate histone 2A, and further studies indicated that PKR and PKC phosphorylated the HIV-1 trans-activating protein Tat, suggesting that PKR previously activated in the presence of dsRNA is subsequently able to phosphorylate highly purified Tat-72. Phosphorylation of Tat by PKR increased linearly between 0.25 and 25 μg/ml, but the intensity of phosphorylation was reduced at higher Tat concentrations (data not shown). No phosphorylation of either PKR or Tat occurred in the absence of either dsRNA (lanes 2 and 5) or PKR (lanes 3 and 6).

Additional tests were conducted to rule out the possibility that these observations resulted from contamination of either the Tat or PKR preparations. Initial attempts to confirm that the 32P-labeled protein is indeed Tat, by using antibodies directed against Tat in immunoprecipitation or immunoblotting experiments, were unsuccessful. Three different anti-Tat antibody preparations all failed to react with the phosphoprotein. However, Tat-72 purified by reverse phase chromatography using a C18 column (6; data not shown), and both Tat-72 and Tat-86 isolated from GST-Tat fusion proteins (see Fig. 4), also served as substrates for activated PKR. The latter differ from one another in electrophoretic mobility, thereby eliminating potential contaminants from consideration. Two other HIV-1 encoded regulatory proteins, Rev and Nef, were not phosphorylated detectably by PKR (data not shown). A variety of mono-S fractions containing PKR (46), as well as PKR purified by an immunoaffinity column containing monoclonal antibody against PKR (54), all phosphorylated Tat-72 in a dsRNA-dependent manner (data not shown). Therefore, Tat-72 is a substrate for activated PKR.

**Specificity of Tat Phosphorylation**—To further assess the specificity of the interaction between Tat and PKR, several kinases were tested for their ability to phosphorylate Tat. Activated kinases were incubated in the presence of 50 ng of purified Tat-72. The results shown in Fig. 3 (lanes 8, 10, 12, 14, 16, and 18) suggest that neither the β-insulin receptor kinase nor a series of kinases (ST20, ST11, MEK, Byr, and Erk) required for Ras-regulated signal transduction was able to phosphorylate the purified form of Tat (lanes 7–18). Confirmation that these kinases were activated prior to incubation with Tat was achieved by observing the autophosphorylation of PKR, PKC, BIRK, ST20, and Erk (not shown). Of the kinases tested, only PKR and PKC phosphorylated Tat (lanes 1 and 5). As expected, activated PKR and PKC also phosphorylated histones, histone 2A only in the case of PKR (lanes 3 and 6).
HIV-1 Tat Protein Is a Substrate and Inhibitor of PKR 8391

Several radiolabeled nucleotides were tested to further address the specificity of phosphorylation of Tat by activated PKR. Both \( \gamma^{32}\text{P}\)ATP and \( \gamma^{32}\text{P}\)GTP were utilized in Tat phosphorylation, but \( \alpha^{32}\text{P}\)ATP, \( \alpha^{32}\text{P}\)UTP, and \( \alpha^{32}\text{P}\)CTP failed to phosphorylate Tat in a PKR-dependent manner. However, when Tat was present at high concentrations (greater than approximately 10 \( \mu\)g/ml), it was nonspecifically labeled by all of these nucleotides even in the absence of PKR or dsRNA. This nonspecifically labeled Tat comigrated with the unphosphorylated Tat-86, Tat-72, or Tat-48A. The same material was subjected to sequential Edman degradation, and the release of radioactive derivatives was monitored. Peaks of radioactivity were observed at cycles 6, 8, and 12, indicative of phosphorylation sites at these distances following an arginine or lysine residue. The only place that such a pattern occurs in the Tat-72 molecule is at serine 64, threonine 68, and serine 69 (Fig. 5C). These residues follow a run of basic residues at positions 49–58; it seems that digestion took place preferentially after arginine 56, in accordance with previous observations (55). Digestion with an alternative protease in place of trypsin corroborated these assignments. 

**Identification of Tat-72 Phosphorylation Sites**—There are 5 serine residues and 4 threonine residues in Tat-72. To determine which amino acids on Tat-72 are phosphorylated in vitro by PKR, labeled Tat was digested with trypsin, and the products were resolved by HPLC. A single predominant peak resulted (Fig. 5A). One-dimensional phosphoamino acid analysis on material from the HPLC peak showed that the label is associated with both serine and threonine residues (Fig. 5B). The same material was subjected to sequential Edman degradation, and the release of radioactive derivatives was monitored. Peaks of radioactivity were observed at cycles 6, 8, and 12, indicative of phosphorylation sites at these distances following an arginine or lysine residue. The only place that such a pattern occurs in the Tat-72 molecule is at serine 64, threonine 68, and serine 69 (Fig. 5C). These residues follow a run of basic residues at positions 49–58; it seems that digestion took place preferentially after arginine 56, in accordance with previous observations (55). Digestion with an alternative protease in place of trypsin corroborated these assignments. 

**Correlation Between the Binding and Phosphorylation of Tat by Activated PKR**—To investigate the sequence requirements for the binding of Tat to PKR, similar amounts of wild-type and mutant GST-Tat proteins were bound to glutathione S-Sepharose beads and then incubated with \( ^{32}\text{P}\)-labeled PKR. Fig. 6 shows that GST alone is unable to bind PKR. GST-Tat 86, GST-Tat 72, and their mutant variants, p18IS and C22G, all bound PKR, whereas the 48A Tat truncation and its associated mutants failed to bind PKR. In addition the Tat construct Tat 86, successfully bound activated PKR. These data suggest that the Tat sequence contained between amino acids 49 and 72 is important for binding PKR, but the N-terminal and C-terminal residues 2–36 and 73–86 are dispensable. In similar experiments with HIV-2 Tat (see Fig. 1), the intact molecule bound labeled PKR with an efficiency comparable with that of HIV-1 Tat (GST-Tat 130; Fig. 6). Removal of residues from the N terminus of HIV-2 Tat had little effect on PKR binding (GST-
Tat 99, whereas a truncation in the basic region (GST 84–D) or the mutation of four consecutive arginine residues in this region to alanines (GST-Tat 99 8184A) abolished binding. These data emphasize the importance of the Tat basic region for PKR binding and raised the possibility that the interaction of the two proteins might be mediated by an RNA bridge. No support for this idea was obtained from experiments with RNases, however. RNase treatment of activated PKR, or of the bacterial extract containing GST-Tat, had no discernible effect on the interaction. Both single-stranded RNA (RNase A) and double-stranded RNA (RNase III)–specific RNases were tested, singly and in combination (data not shown).

Substrate Competition Between Tat and eIF-2—PKR regulates protein synthesis by phosphorylating eIF2 on serine 51 of its α subunit (17, 18). The ability of Tat to serve as a substrate for PKR raised the possibility that Tat might compete with eIF2 for phosphorylation by activated PKR. To address this hypothesis, activated PKR was incubated with Tat and increasing concentrations of purified eIF2 (Fig. 7, lanes 1–4). The results suggest that at high concentrations, eIF2 reduces Tat phosphorylation (lane 4). Conversely, increasing the concentration of Tat protein, while maintaining a fixed concentration of eIF2, resulted in a marked reduction in eIF2 phosphorylation (lanes 5–8). These data suggest that Tat and eIF2 can compete as substrates for phosphorylation by autophosphorylated PKR. Considering the relative molecular masses of Tat and eIF2 (about 10 and 125 kDa, respectively), they appear to serve as substrates for PKR and as competitors on a comparable molar basis.

Autophosphorylation of PKR Is Inhibited by Purified Tat 72—Experiments to this juncture have shown that Tat is not only a substrate for PKR but that it is also able to inhibit eIF2 phosphorylation by this kinase. The activation of PKR is closely associated with its autophosphorylation (14). To determine whether Tat can inhibit this reaction as well, Tat was added to kinase reactions either before or after the introduction of dsRNA (Fig. 8). In the absence of Tat, PKR was autophospho-
HIV-1 Tat Protein Is a Substrate and Inhibitor of PKR

DISCUSSION

The molecular mechanisms governing the control of HIV-1 gene expression are complex and not fully understood. In the present study our aim has been to further characterize the properties of the HIV-1 regulatory protein, Tat, specifically with regard to its relationship with the interferon-induced dsRNA-activated protein kinase PKR. Several reports link these two regulatory proteins. First, both of them can bind to TAR RNA, the structured RNA segment that is the target for Tat trans-activation (56, 57). Second, TAR RNA modulates the activity of PKR, although there is disagreement as to whether this RNA activates the kinase (57–59) or blocks its activation (36, 60). Third, Tat stimulates the translation of TAR-containing RNAs in vitro (61). Fourth, a synthetic Tat peptide that binds TAR RNA was reported to inhibit PKR activation (62). Finally, Tat appears to down-regulate PKR in cells infected with HIV-1 or stably expressing Tat (23). We therefore set out to test the hypothesis that there is a direct interaction between Tat and PKR. Three lines of evidence support this hypothesis.

The results of in vitro kinase assays demonstrated that dsRNA-activated PKR phosphorylates Tat purified according to a number of different protocols. Both the one-exon and two-exon forms of Tat (Tat-72 and Tat-86, respectively) are substrates for the kinase. Mutations that prevent Tat from trans-activating HIV-1 transcription (P18IS and C22G) do not affect Tat phosphorylation, but a mutation removing residues 49–72 (D48) eliminates phosphate labeling by activated PKR. Without exception, the phosphorylation of Tat proteins by PKR was shown to be dependent on the prior activation of PKR by dsRNA. Tat-72 could also serve as an inhibitor of PKR, both by blocking its ability to autophosphorylate in response to dsRNA (and hence to become activated for phosphorylation of substrates) and by competing with its natural substrate eIF2. In the competition assay, the affinity for Tat and eIF2 seemed to be comparable in molar terms. Binding studies showed that PKR can form a complex with either Tat-72 or Tat-86 and that the Tat sequences required for this interaction include residues 49–72. Similarly, the binding of PKR to HIV-2 Tat was dependent on the integrity of the basic region. Deletions in the N
terminus of both Tat proteins led to increased PKR binding, suggesting that a conformational change may render the interaction site more available to PKR. While this report was in preparation, McMillan and co-workers (63) reported the interaction of Tat with PKR. In their study, Tat-86 was phosphorylated but labeling of Tat-72 was not, although its ability to inhibit PKR was observed. Although we cannot provide an explanation for this discrepancy, we speculate that it might be attributable to the different sources of Tat employed (McMillan et al. (63) used synthetic Tat-72).

PKR phosphorylates two serine and one threonine residues immediately adjacent to the basic region of Tat, consistent with the known preference of PKR for serines in the context of a basic amino acid environment (17, 18, 64). This suggested that Tat phosphorylation might influence its ability to trans-activate via its interaction with TAR, or via effects on its cellular localization, or both. When tested in a Tat-dependent transcription assay (65), phosphorylation by PKR elicited no discernible effect on trans-activation; however, this negative result is difficult to interpret as only a fraction of the Tat was modified. On the other hand, in this study we demonstrate the phosphorylation of Tat by the mitogen-activated kinase PKC. PKC also phosphorylates Nef (66), although PKR did not. Several researchers have proposed that PKC plays a role in Tat-mediated trans-activation (67–69). Depletion of PKC in Jurkat and 293 cells resulted in a reduction in Tat trans-activation, and a PKC mutant lacking a functional ATP-binding site failed to support trans-activation (69). It is also possible that PKC influences HIV-1 transcription via the phosphorylation of IxB (70) or another unidentified PKC substrate (71). Since both PKR and PKC phosphorylate Tat and IxB in vitro, it will be important to learn whether they modify the same sites on these proteins and whether they mediate similar responses in vivo. In this connection, we note that a number of laboratories have reported their inability to detect Tat phosphorylation in vivo (67, 68). Our failure to observe reaction of antibodies with PKR-phosphorylated Tat suggests that these negative results may be due to the inability of anti-Tat antibodies to recognize the modified form of Tat. Indeed, phosphorylation of the Tat protein of HIV-2 has been observed both in vivo and in vitro (39).

The ability of Tat to inhibit PKR also has potential biological implications. Dominant negative forms of PKR can cause malignant transformation of 3T3 cells (24, 25). Therefore it is conceivable that down-regulation of the kinase by Tat could contribute to the deregulation of growth control seen in Tat-treated cells derived from Kaposis sarcoma lesions of HIV-1-infected individuals (72, 73). Furthermore, PKR plays a recognized role in the interferon-induced antiviral response (14). It has been reported that HIV-1 replication is sensitive to interferon and that Tat confers partial resistance to the effect of interferon (3). Our data suggest that Tat may exert its effect via its ability to inhibit the activation of the interferon-induced protein kinase PKR, thereby reducing the PKR-mediated phosphorylation of eIF2, or of IxB, or other substrates of this kinase. Moreover, the ability of Tat to form a complex with PKR provides a possible mechanism for the repression of PKR levels that have been observed in HeLa cells stably expressing Tat or in T-cells infected with HIV-1 (23).

Although both PKR and Tat are both RNA-binding proteins and can interact with TAR RNA, the PKR/Tat interaction documented here is evidently independent of TAR RNA and of RNA in general. In that both Tat and eIF2 are substrates for PKR and interact with similar affinities, it would appear that Tat resembles the vaccinia virus protein kinase K3L (74). This early protein displays sequence homology to the α subunit of eIF2 in the region of its phosphorylation site; lacking an appropriately placed serine residue, it acts as a pseudo-substrate for PKR. Like Tat, K3L down-regulates PKR by directly binding to the kinase and preventing its activation (i.e., its auto-phosphorylation) and inhibiting its activity (74). This mechanism is distinct from the competition that can occur between Tat and PKR for binding to TAR RNA but not dsRNA (59, 61, 62).

In conclusion, we have demonstrated that both the virally activated protein kinase PKR and the mitogen-activated kinase, PKC, are able to phosphorylate the HIV-1 trans-activation protein Tat. Protein phosphorylation constitutes an important mechanism for the regulation of intracellular events (75). Although the consequences of Tat phosphorylation with respect to the regulation of HIV-1 gene expression remain to be established, the ability of Tat to control the activity of PKR may have far-reaching implications. These potentially include effects on both transcription and translation, mediated by the phosphorylation of IxB and eIF2, respectively, and suggest a mechanism whereby Tat may contribute to the ability of HIV to evade the action of PKR. The regulation of Tat by a kinase may provide the trigger whereby latent integrated virus is activated and elicits exciting possibilities for targeted intervention.

Acknowledgment—We thank Nora Poppito for technical help.

REFERENCES

1. Arya, S. K., Guo, C., Josephs, S. J., and Wong-Staal, F. (1985) Science 229, 69–73
2. Dayton, A. I., Sodroski, J. G., Rosen, C. A., Goh, W. C., and Haseltine, W. A. (1986) Cell 44, 941–947
3. Shirazi, Y., Popik, W., and Fitha, P. M. (1994) J. Interferon Res. 14, 259–263
4. Rosen, C. A., Sodroski, J. G., and Haseltine, W. H. (1985) Cell 41, 813–823
5. Feinberg, M. B., Baltimore, D., and Frankei, A. D. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4045–4040
6. Lapins, M. F., Rice, A. P., and Mathews, M. B. (1989) Cell 59, 283–292
7. Sodroski, J. G., Patarca, R., Rosen, C. A., Wong-Staal, F., and Haseltine, W. A. (1985) Science 229, 74–77
8. Howcroft, K. T., Strebel, K., Martin, M. A., and Singer, D. S. (1993) Science 260, 1320–1322
9. Jeang, K.-T., Berkhourd, B., and Dropulic, B. (1993) J. Biol. Chem. 268, 24949–24949
10. Kim, Y.-S., and Panganiban, A. T. (1993) J. Virol. 67, 3739–3747
11. Hauber, J., Perkins, A., Heimer, E. P., and Cullen, B. R. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 6365–6368
12. Roy, S., Delling, U., Chen, C. H., Rosen, C. A., and Sonenberg, N. (1990) Genes Dev. 4, 1365–1373
13. Rubin, M., Perkins, A., Purcell, R., Young, K., Sia, R., Burghoff, R., Haseltine, W. B., and Rosen, C. A. (1989) J. Virol. 63, 1–8
14. Samuel, C. E. (1991) Virology 183, 1–11
15. Hovanessian, A. G., and Galaburda, J. (1987) Eur. J. Biochem. 167, 467–473
16. Farrell, P. J., Balkow, K., Hunt, T., Jackson, R. K., and Trachsel, H. (1977) Cell 11, 187–200
17. Proud, C. G. (1986) Trends Biochem. Sci. 11, 73–77
18. Hershey, J. W. B. (1991) Annu. Rev. Biochem. 60, 717–755
19. Mathews, M. B., and Shenk, T. (1991) J. Virol. 65, 5657–5662
20. Akkaraju, G. R., Whitaker-Douling, P., Younger, J., and Jugus, R. (1989) J. Biol. Chem. 264, 10321–10325
21. Lee, H. T., Tomita, J., Hovanessian, A. G., and Katze, M. G. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 6208–6212
22. Black, L. T., Safer, B., Hovanessian, A., and Katze, M. G. (1991) J. Virol. 65, 2244–2251
23. Roy, S., Katze, M. G., Parkin, N. T., Edery, I., Hovanessian, A. G., and Sonenberg, N. (1990) Science 247, 1216–1219
24. Barber, G. N., Wambach, M., Thompson, S., Jugus, R., and Katze, M. G. (1995) Mol. Cell. Biol. 15, 3138–3146
25. Koromilas, A. E., Roy, S., Barber, G. N., Katze, M. G., and Sonenberg, N. (1992) Science 257, 1685–1689
26. Judware, R., and Petryshyn, R. (1991) Mol. Cell. Biol. 11, 3259–3267
27. Lee, S. B., and Esteban, M. (1994) Virology 199, 491–496
28. Kumar, A., Haque, J., Learste, J., Hiscott, J., and Williams, B. R. G. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 6288–6292
29. Offermann, M. K., Zimring, J., Mellits, K. H., Hagan, M. K., Shaw, R., Judware, R., and Petryshyn, R. (1991) Mol. Cell. Biol. 11, 11–20
HIV-1 Tat Protein Is a Substrate and Inhibitor of PKR

33. Desai, K., Lowenstein, P. M., and Green, M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 8875–8879
34. Kashanchi, F., Pira, G., Radонович, M. F., Duval, J. F., Fattaey, A., Chain, C. M., Roeder, R. G., and Brady, J. N. (1994) Nature 367, 295–299
35. Jeang, K.-T., Chun, R., Lin, N. H., Gatignol, A., Glabe, C. G., and Fan, H. (1983) J. Virol. 67, 6224–6233
36. Gunnery, S., Rice, A. P., Robertson, H. D., and Mathews, M. B. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 8867–8861
37. Cosentino, G. P., Venkatesan, S., Serluca, F. C., Green, S. R., Mathews, M. B., and Sonenberg, N. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9445–9449
38. Park, H., Davies, M. V., Langland, J. O., Chang, H., Nam, Y. S., Tartaglia, J., Paoletti, E., Jacobs, B. L., Kaufman, R. J., and Venkatesan, S. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4713–4717
39. Herrmann, C. H., and Rice, A. P. (1993) J. Virol. 68, 1152–1156
40. Herrmann, C. H., and Rice, A. P. (1995) J. Virol. 69, 1612–1620
41. Marciniak, R. A., and Sharp, P. A. (1991) Cell 63, 781–802
42. Kawakami, K., Scheidereit, C., and Roeder, R. G. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4700–4704
43. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) Methods Enzymol. 185, 60–89
44. Frankel, A. D., and Pabo, C. O. (1988) Cell 55, 1189–1193
45. Rhim, H., Echeteu, C. O., Herrmann, C. H., and Rice, A. P. (1994) J. Acquired Immune. Defic. Syndr. 7, 1116–1121
46. Kostruba, M., and Mathews, M. B. (1989) Mol. Cell. Biol. 9, 1576–1586
47. Meltz, K. H., Pevé, T., Manche, L., Robertson, H. D., and Mathews, M. B. (1990) Nucleic Acids Res. 18, 5401–5406
48. Villalba, M., Wente, S. R., Russel, D. S., Atn, J., Reichelderfer, C. F., and Rosen, O. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 7848–7852
49. Polverino, A., Foote, J., Yang, P., Hutchinson, M., Neiman, A. M., Cobb, M. H., and Marcus, S. (1995) J. Biol. Chem. 270, 26067–26070
50. Beemon, K., and Hunter, T. (1978) J. Virol. 28, 551–566
51. Rosenfeld, J., Capdevielle, J., Guillemot, J. C., and Ferrara, P. (1992) Anal. Biochem. 203, 173–179
52. Russo, G. L., Vandenberg, M. T., Yu, I. J., Bae, Y.-S., Franza, B. R., Jr., and Marshak, D. R. (1992) J. Biol. Chem. 267, 20317–20325
53. Cooper, J. A., Selton, B. M., and Hunter, T. (1983) Methods Enzymol. 99, 368–402
54. Galabru, J., and Hovanessian, A. (1987) J. Biol. Chem. 262, 15538–15544
55. Cramer, R. E. (1965) J. Biol. Chem. 238, 269–274
56. McCormack, S. J., and Samuel, C. E. (1995) Virology 200, 511–519
57. SenGupta, D. N., and Silverman, R. H. (1989) Nucleic Acids Res. 17, 969–978
58. Edery, I., Petryshyn, R., and Sonenberg, N. (1989) Cell 66, 303–312
59. Maitra, R. K., McMillan, N. A., Jr., Desai, S., McSwiggen, J., Hovanessian, A. G., Sen, G. Williams, B. R. G., and Silverman, R. H. (1994) Virology 204, 825–827
60. Gunnery, S., Rice, A. P., and Mathews, M. B. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 11557–11561
61. SenGupta, D. N., Berkhour, B., Zhou, A., and Silverman, R. H. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7492–7496
62. Judware, R., Li, J., and Petryshyn, R. (1993) J. Interferon Res. 13, 153–160
63. McMillan, N. A., Chun, R. F., Siderovski, D. P., Galabru, J., Toone, W. M., Samuel, C. S., Mak, T. W., Hovanessian, A. G., Jeang, K. T., and Williams, B. R. G. (1995) Virology 213, 431–442
64. Taylor, D. B., Lee, S. B., Romano, P. R., Marshak, D. R., Hinnebusch, A. G., Esteban, M., and Mathews, M. B. (1996) Mol. Cell. Biol. 16, 6295–6302
65. Laspa, M. F., Wendel, P., and Mathews, M. B. (1993) J. Mol. Biol. 232, 732–746
66. Guy, B., Kienny, M. P., Riviere, Y., LePeuch, C., Dutt, K., Girard, M., Montangnier, L., and Leeoq, J. P. (1987) Nature 330, 266–269
67. Han, W.-M., Loras, A., Rousseville, M. P., Kumar, A., and Shank, P. R. (1992) J. Virol. 66, 4065–4072
68. Hauber, J., Bouvier, M., Malim, M. H., and Cullen, B. R. (1988) J. Virol. 62, 4901–4904
69. Jakobovits, A., Rosenthal, A., and Capon, D. (1990) EMBO J. 9, 1165–1170
70. Ghosh, S., and Baltimore, D. (1990) Nature 344, 678–682
71. Doppler, U., Schalasta, G., Atnmann, E., and Sauer, G. (1992) AIDS Res. Hum. Retroviruses 8, 245–252
72. Ensoli, B., Buonauro, L., Barillari, G., Fiorelli, V., Gentelman, R., Morgan, R. A., Wingfield, P., and Gallo, R. C. (1993) J. Virol. 67, 277–287
73. Ensoli, B., Barillari, G., Salahuddin, S. Z., Gallo, R. C., and Wong-Staal, F. (1990) Nature 345, 84–86
74. Beattie, E., Tartaglia, J., and Paolletti, E. (1991) Virology 183, 419–422
75. Krebs, E. G. (1985) Biochem. Soc. Trans. 13, 813–820