Biochemical and Biophysical Research Communications **286,** 587–594 (2001)
doi:10.1006/bbrc.2001.5442, available online at http://www.idealibrary.com on IDEAL®

HIV-1 Tat Can Substantially Enhance the Capacity of NIK to Induce IκB Degradation

Xuguang Li,* Juliana Josef,* and Wayne A. Marasco*,†,[1]

**Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, Massachusetts 02115; and †Department of Medicine, Harvard Medical School, 44 Binney Street, Boston, Massachusetts 02115*

Received July 12, 2001

The human immunodeficiency virus type 1 (HIV-1) Tat is a virally encoded protein that dramatically upregulates viral replication through interactions with the HIV-1 5′ long terminal repeat (LTR) and cellular transcription factors. The HIV-1 LTR is divided into three major regions: modulatory, core and TAR. The modulatory region contains numerous *cis*-acting sequences for the binding of transcription factors including NF-κB, NF-AT, and AP-1. In several reports, Tat has been found to induce NF-κB activation of the HIV-1 LTR, while in other studies Tat has been reported to have no effect on activation of NF-κB. These discrepancies may arise from differences in experimental conditions such as the source of Tat (exogenous versus endogenous), the detection methods for NF-κB activation (DNA binding capability versus IκB degradation), and the types of reporters used (HIV-1 versus non-HIV-1 derived). To reconcile these differences we examined the effect of endogenous Tat on NF-κB activation, on IκB degradation and its interaction with upstream MAP3Ks. We demonstrate that although an 80% reduction in Tat-induced HIV-1 LTR activity can be detected if the κB binding sites are mutated, surprisingly endogenous Tat (expressed intracellularly by transfection) lacks direct effect on IκB degradation. Further analysis demonstrates that although Tat alone lacks direct effect on IκBα degradation or dissociation from NF-κB, Tat can substantially enhance the capacity of NF-κB-inducing kinase (NIK), but not MEKK1, to accelerate degradation of IκB. We propose a model to explain these collective experimental findings.

Key Words: **HIV-1; Tat; NF-κB; IκB; NIK; HIV-1 LTR.**

The HIV-1 Tat protein is generated as one of the earliest viral proteins following infection of the $CD4^+$, co-receptor-positive cells. Tat drastically upregulates viral replication by promoting transcription initiation and elongation of viral mRNAs through interaction with TAR, an RNA stem-loop structure present at the 5′-end of all transcripts and cellular transcription factors (1–9). Tat also contributes to viral replication by *tar*-independent mechanisms such as potentiation of reverse transcription (10), and activation of binding elements in the viral promoter for transcription factors such as NF-κB, NFAT, and Sp1 (4, 5, 8, 9, 11–13). These DNA binding elements also constitute various promoters for a wide range of cytokine genes such as those for IL-2 and TNF-α (14–18). Tat is believed to greatly contribute to the pathogenesis of HIV-1-induced diseases by disturbing a delicate balance between stimulatory and inhibitory host factors (particularly endogenous cytokines) (18, 19).

NF-κB is a transcription factor that regulates a variety of cellular genes (20). NF-κB is normally sequestered in the cytoplasmic compartment by the inhibitory molecule IκB (20–22). Through physical interaction, IκB masks the nuclear localization signal and the DNA binding domain of NF-κB, which is, as a result of such interactions, rendered in an inactive state (20–22). Degradation and/or dissociation of IκB from the NF-κB complex is the prerequisite for NF-κB activation in response to various agents such as T-cell mitogens and proinflammatory cytokines (20–22). In the case of IκB degradation, IκB kinase (IKK) phosphorylates IκB on two serine residues (position 32/36), thereby triggering IκB degradation through the ubiquitin-proteasome pathway (20–22). Upstream of IKK, at least two MAP3Ks participate in the process of NF-κB activation, i.e., NF-κB-inducing kinase (NIK) and mitogen-activated protein kinase kinase kinase 1 (MEKK1) (23–32), both of which have been shown to activate IKKs, which in turn phosphorylate and trigger degradation of IκB (23–32). However, under certain circumstances dissociation instead of degradation of IκB is also sufficient to activate NF-κB when the ty-

[1] To whom correspondence should be addressed at Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, JFB 824, 44 Binney Street, Boston, MA 02115. Fax: (617) 632-3889. E-mail: wayne_marasco@dfci.harvard.edu.

0006-291X/01 $35.00

rosine residue (at 42 position in IκBα) is phosphorylated (33, 34).

Although the interaction between Tat and NF-κB has been intensely studied over the past several years, experimental results have been conflicting and the molecular mechanisms involved in Tat-induced NF-κB activation are not well understood. Indeed, different experimental approaches such as the use of different sources of Tat (exogenous versus endogenous), different assessment methods for NF-κB activation, i.e., DNA binding capability, IκB degradation, and the use of HIV-1 verses non-HIV-1 derived-reporter systems may have contributed to these discrepancies. For example, while increased NF-κB activity as assessed by DNA binding was detected in cells expressing Tat, no studies were performed to analyze IκB levels (36). In contrast, lack of responsiveness of some NF-κB containing promoters to transactivation by Tat has been recently reported (53). Furthermore, while degradation of IκBα was detected in HeLa cells treated with exogenous Tat protein (35), it is unclear whether the same effects occur in cells expressing intracellular Tat (endogenous Tat). To address these issues, we investigated the effect of endogenous Tat on NF-κB activation, on IκBα degradation and its interaction with upstream MAP3Ks. We report herein that endogenous Tat protein alone causes little effect on either degradation or dissociation of IκBα in HeLa cells, although mutations at the κB binding sites in HIV-1 LTR result in a dramatic reduction in Tat-induced HIV-1 LTR activation. In addition, although Tat alone lacks direct effect on IκBα degradation, it markedly enhances the capacity of NIK to accelerate IκBα degradation. Such synergistic effects between Tat and NIK were determined to occur through a ubiquitin-proteasome pathway since the mutant IκBα (S32A/S36A) could not be degraded under the same condition. Interestingly, although constitutively activated MEKK1 was found to degrade IκBα under similar conditions, its ability of inducing IκBα degradation was not influenced by Tat.

MATERIALS AND METHODS

Plasmids, chemicals, and other reagents. pSVTat was obtained from Dr. J. Sodroski (Dana-Farber Cancer Institute). pcDNA3-NIK-WT (wild-type) and the pcDNA-NIK-MUT (mutant KK429-430AA) were described (23). pFL-NIK-WT is the pcDNAs-NIK-WT with a FLAG tag; pFL-NIK-MUT is a pcDNA-NIK-MUT with a FLAG tag were provided by Dr. D. Wallach (The Weizmann Institute of Science, Rehovot, Israel) (23). HIV-LTR-CAT (wild-type) and HIV-LTR-mut- NF-κB-CAT are gifts from Dr. G. Nabel (12). Constitutively active MEKK1 (the C-terminal catalytic domain of $MEKK1_{(301-672)}$) was kindly provided by Dr. Jacques Pousségur (UMR 6543, CNRS, Université de Nice, Nice, France), and is denoted as MEKK1* in this report. The kinase defective MEKK1 mutant, MEKK1K432M was kindly provided by Christophe Marcireau (Aventis Pharma, Paris, France). It was cloned into pcDNA3 eucaryotic vector (37). IκBα with HA tag expression vectors, i.e., HA-IκBα and HA-IκBα-mut (S32A/S36A) were kindly provided by Dr. W. Greene, Gladstone Institute of Virology and Immunology, University of California, San Francisco, and were described (38). TNF-α was obtained from Genzyme (Cambridge, MA). Anti-HA monoclonal antibody was purchased from Boehringer-Mannheim (Indianapolis, IN). Other chemicals were also purchased from Sigma (St. Louis, MO).

Cell transfection, CAT assay, and Western blot. HeLa cells were routinely maintained in DMEM plus 10% FCS. Transfection of HeLa cells was performed using the calcium phosphate method as described (39). Jurkat cells were cultured in RPMI-1640 in the presence of 10% FCS. Transfection of Jurkat cells were performed as described by using LipofectAMINE (Life Technologies, Inc.) as transfection mediator (40).

CAT assay was performed as described briefly (41). Typically, each transfection 2 μg of CAT reporter, 2 μg of DNA constructs expressing exogenous proteins (see Results section) were used. In all transfection experiments, 1 μg of β-gal expression vector, pcDNA-LacZ (Invitrogen) was cotransfected and triplicate transfections were used to standardize transfection efficiency. Empty vectors were used to bring the total amount of DNA to μg. Data were presented as fold increase in CAT activity compared with the empty vector control (40, 41).

Western blot assays were performed as described (40). Briefly, equal amounts of proteins extracted from the transfected cells were fractionated on SDS–12% polyacrylamide gel, followed by transferring the samples to a nitrocellulose membrane. For detection of IκBα, MAb against HA (Boehringer-Mannheim) were used as primary antibody to detect HA-tagged IκBα expressed by eucaryotic vectors, while MAb against the IκBα (Santa Cruz Biotechnology, Santa Cruz, CA) was used for detection of endogenous IκBα. In some cases, cells were treated with TNF-α at a final concentration of 20 ng/ml (Genzyme, Cambridge, MA) for 20 min before the cells were harvested for protein extraction. To induce tyrosine phosphorylation of IκBα, cells were treated with 500 μM pervanadate (pV) as described (33, 34) for 20 min before cells were harvested for analysis of IκBα level in the aforementioned Western blot assays. In most cases, the blots were stripped and reprobed with anti-β-actin as controls for cellular proteins.

RESULTS

To verify the activity of Tat on HIV-1 LTR promoter activation under defined experimental conditions, 2 μg of pSV-Tat was co-transfected with 2 μg of either pHIV-LTR-CAT, or pHIV-LTR-mut-NK-κB-CAT and analyzed CAT activity after 48 h. Consistent with previous findings (12), Tat caused approximately 200-fold increase in CAT activity as compared with the vector control and mutation of the NF-κB binding sites caused about 85% reduction in CAT activity (Fig. 1). Transfection of either NIK or MEKK-1 resulted in 40- and 55-fold increase in CAT activity respectively, which is completely dependent on NF-κB activation since mutation of the NF-κB binding site results in a loss of CAT activity (Fig. 1). Clearly, mutation of the κB binding site did not completely abolish Tat-induced LTR activation as previously reported (4, 5, 12) and demonstrated here (Fig. 1) since Tat also stimulates the HIV-1 promoter by other mechanisms (9).

We next investigated the mechanism(s) involved in this Tat-mediated activation pathway. The ability of Tat to induce IκBα degradation was first analyzed. HeLa cells were co-transfected with pSV-Tat and HA-IκBα. As a positive control some of the cells were treated with TNFα (20 ng/ml), a potent NF-κB activa-

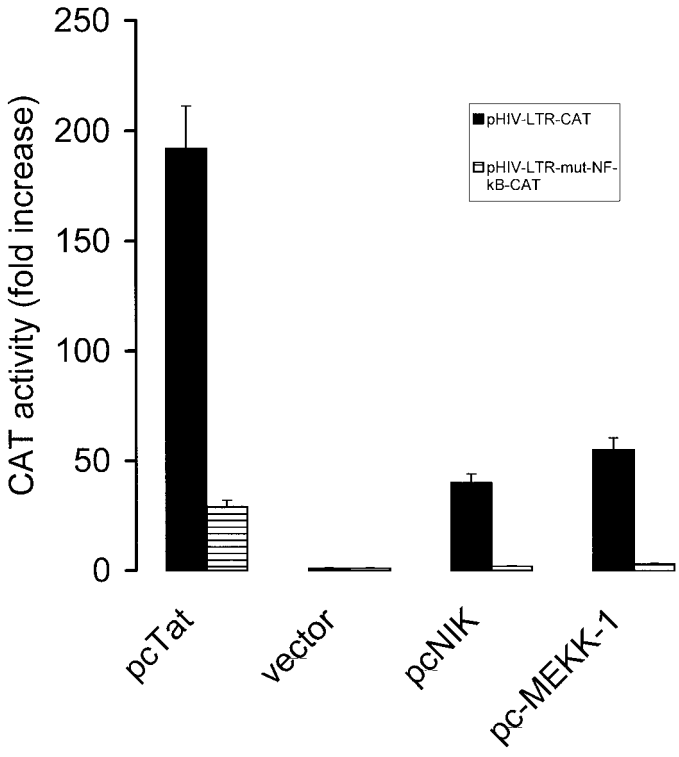

FIG. 1. Transactivation of HIV-1 CAT activity by Tat, NIK, or MEKK1. HeLa cells were transfected with 2 μg of pHIV-1-CAT plus 2 μg of eukaryotic expression plasmids for Tat, NIK, or MEKK1. In some of these transfections, HIV-LTR-mut-NFκB-CAT was used in place of HIV-LTR-CAT (12). Transfection efficiency is routinely monitored by cotransfection of pcDNA-LacZ in addition to triplicate transfections (40). Data were presented as fold increase in CAT activity compared with the empty vector control (for details, see Materials and Methods).

tor (20–22). The protein extracts from transfected cells were analyzed for IκBα level in Western Blot assays using anti-HA antibody to detect IκBα. As is shown in Fig. 2, Tat itself causes little effects on IκBα degradation (lanes 1–5), except in lane 1, where the highest amount of pSV-Tat was used (6 μg). In contrast, a dramatic degradation of IκBα was observed with the addition of TNFα (lanes 6–10). These results demonstrate that endogenous Tat displays negligible effects on IκBα degradation.

Because only a minor degradation of IκBα by Tat was detected, we next investigated whether interaction of Tat with NIK would accelerate IκBα degradation. As is shown in Fig. 3A, Tat (4 μg) alone did not cause detectable IκBα degradation (lane 5) as compared with the vector (lane 11), consistent with Fig. 2, lane 2. However, NIK itself was able to induce IκBα degradation as is shown in lanes 7 (pcDNA-NIK-WT) and 9 (WT NIK with FLAG tag) of Fig. 3A. As expected, mutant NIKs were unable to degrade IκBα (lanes 8 and 10) as compared with the vector control (lane 12). Importantly, the greatest level of IκBα degradation was observed in cells transfected with both Tat and NIK (lanes 1 and 3). No synergistic effects on IκBα degradation was observed between Tat and mutant NIKs (lanes 2 and 4). Figure 3B shows densitometry analysis of the IκBα levels (for clarity, the NIKs with FLAG tags were omitted since they have similar effects as the NIKs without any tags). As is shown in this figure, treatment with wt NIK caused approximately 50% reduction in the levels of IκBα. Despite its incapability of inducing IκBα degradation, Tat augments NIK-induced IκBα degradation, resulting in nearly 80% degradation of IκBα.

MEKK1 is another MAP3K implicated to act upstream of IKKs in inducing IκBα degradation. We next investigated whether interaction of Tat with MEKK1 could affect IκBα degradation. As is shown in Fig. 4, transfection of constitutively activated MEKK1 (MEKK1*) resulted in a decrease in the level of IκBα (lane 4) as compared to the vector control (lane 1). However, again little change in IκBα level was detected when pSV-Tat alone was transfected into the cells (lane 5). In contrast to observations from cotransfection of Tat with NIK (Fig. 3), we failed to detect an accelerated degradation of IκBα following cotransfection of Tat with MEKK1* (lane 2). As additional controls, no effect on IκBα degradation was detected with transfection of MEKK1-mut (lane 6) or cotransfection of Tat with MEKK1-mut (lane 3). From these results we conclude that there is no significant enhancement by Tat of MEKK1-induced IκBα degradation.

Because Tat was able to accelerate IκBα degradation in the presence of NIK (Fig. 3), we wished to further verify that IκBα degradation induced by Tat and NIK

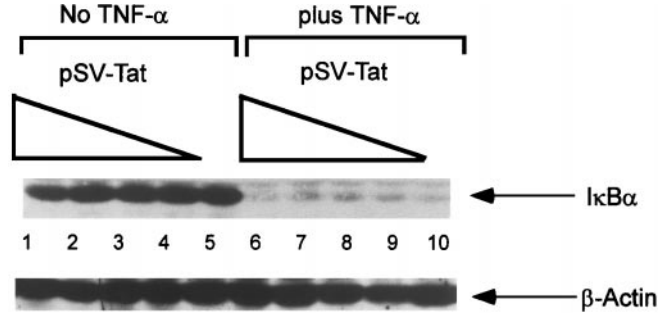

FIG. 2. Failure of endogenous Tat (transfected Tat) to stimulate degradation of IκBα. 1.5 μg of HA-tagged IκBα expression vector (38) was cotransfected with various amounts of pSV-Tat (6, 4, 2, 1, 0 μg) as shown in lanes 1 through 5. Forty-eight h later, the cells were lyzed in lysis buffer consisting of 50 mM Tris–Cl, pH 7.8; 50 mM KCl, 5 mM DTT, 1 mM EDTA, 0.1% SDS, 0.5% Triton X-100, 10 mM, NaF, 0.5 mM $NaVO_3$, and protease inhibitor tablets (Boehringer-Mannheim). The extracted proteins were then used in Western blot for detection of IκBα level using anti-HA antibody at 2 μg/ml (Boehringer-Mannheim). The secondary antibody (HRP-goat anti-mouse IgG, Sigma) was used at 1:2000 dilution. In parallel, the transfected cells were treated with TNF-α for 20 min prior to cell lysis (20 ng/ml, Genzyme, Cambridge, MA) in the presence of various amounts of Tat. Also shown in the figure is the control in which the same blot was probed with anti-β-actin monoclonal antibody (Sigma).

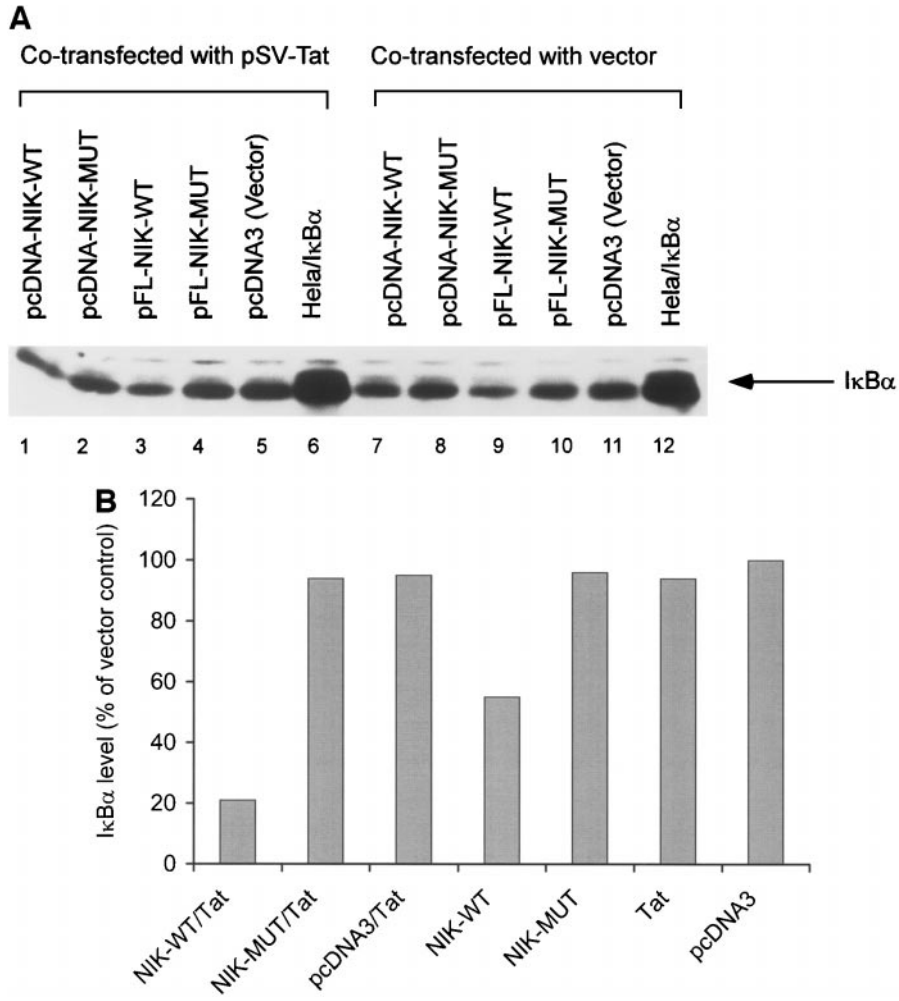

FIG. 3. Enhancement of NIK-induced IκBα degradation by transfected Tat. (A) IκBα level analyzed in Western blot. 1.5 μg of HA-tagged IκBα expression vector was cotransfected with 2 μg of various expression constructs (indicated in the figure), followed by analysis of IκBα as described in the legend to Fig. 2. Note that in lanes 6 and 12, excess amount of HA-tagged IκBα expression vector (6 μg) was transfected in order to serve as a positive control for IκBα itself. Note that pFL-NIK-WT is pcDNA-NIK-WT with a FLAG tag, while pFL-NIK-MUT is pcDNA-NIK-MUT with a flag. These expression plasmids have been described previously (23). (B) Densitometry analysis of the IκBα level. As is shown in this figure, approximately 50–60% of IκBα was detected by the treatment of wt NIK. Tat at this concentration (4 μg) lacks significant effect on IκBα degradation, but it can accelerate NIK-induced IκBα degradation, resulting in nearly 80% degradation of IκBα.

is indeed via the pathway in which phosphorylation of IκBα at S32/S36 takes place prior to its degradation. To this end, HA-IκBα-mut (S32A/S36A) was cotransfected with Tat/NIK, followed by analysis of IκBα level in a Western Blot assay. Consistent with results from Fig. 3, cotransfection of Tat and wt NIK resulted in a significantly lower level of IκBα (lane 1, Fig. 5A) as compared to Tat alone (lane 3, Fig. 5A). However, cotransfection of NIK and Tat failed to promote IκBα degradation if the two critical serine residues (S32/36) were mutated (lane 2, Fig. 5A), again Tat was unable to affect IκBα degradation (lane 3, Fig. 5A). Also in Fig. 5A, a control shows that the wild type IκBα was efficiently degraded by TNFα (lane 4), while another control shows that TNFα, as expected, does not cause IκBα degradation if the two serine residues were mutated (lane 5). These results confirm that Tat accelerates NIK-induced IκBα degradation through the classical ubiquitin-proteasome pathway. Figure 5B shows densitometry analysis of the data presented in Fig. 5A.

Another pathway leading to activation of NF-κB is through dissociation of IκBα after the tyrosine residue (position 42) is phosphorylated (33–34). We next investigated whether Tat could influence tyrosine phosphorylation of IκBα. Jurkat cells were transfected with increasing amounts of pSV-Tat. Forty-eight hours after transfection, cellular proteins were extracted for analysis of IκBα mobility in a Western blot assay, in which a positive control, i.e., treatment of Jurkat cells with pervanadate (pV), was included for comparison. As is shown in Fig. 5C, treatment of Jurkat cells with pV (lane 6) significantly slowed the migration of IκBα as a

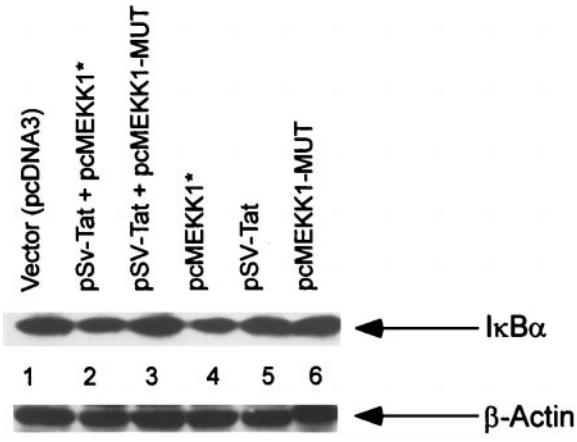

FIG. 4. Tat fails to enhance MEKK1-mediated IκBα degradation. 1.5 μg of HA-tagged IκBα expression vector was cotransfected with 4 μg of MEKK1* or MEKK1-mut in the presence or absence of Tat as indicated in the figure. The level of IκBα was determined using Western blot as described in the legend to Fig. 2.

typical result of tyrosine phosphorylation (position 42) (33, 34). However, Tat, tested here at even higher concentrations than that used in Figs. 1, 2, and 3, failed to affect IκBα mobility (lane 1 through 5). Although Tat, at highest concentration tested (10 μg), accelerated IκBα degradation in the presence of NIK, it still failed to slow the mobility of IκBα, thereby excluding a possibility that Tat might promote tyrosine phosphorylation of IκBα.

DISCUSSION

NF-κB is a transcription factor that has been intensively studied in terms of its interaction with HIV-1 Tat (35, 47–49). Several reports have demonstrated an increased NF-κB activity in cells treated by HIV-1 Tat while other studies have shown a lack of responsiveness of NF-κB-regulated promoters to Tat (35, 36, 41, 47–49, 53). Discrepancies in the interpretation of the results, which likely arise from the different experimental approaches used in these previous reports, have prevented a more complete understanding of the molecular mechanism(s) interposed between Tat and NF-κB.

The present study was undertaken to better understand the effects of intracellular Tat on NF-κB activation at the molecular level, especially with regard to IκB degradation versus dissociation. One of the critical steps in the activation of the transcription factor NF-κB involves serine phosphorylation of IκBα that consequently leads to its degradation (20–22, 33, 34). Two MAP3Ks, NIK and MEKK1, that have been found to function upstream of IKKs and to be responsible for serine phosphorylation of IκBα (23–32) were also examined. Consistent with previous observations (12, 13, 41, 47), we observed that mutations of the κB binding site in the HIV-1 LTR resulted in a dramatic reduction in Tat-induced CAT activities, reinforcing the notion that a NF-κB-associated pathway is involved in this process. However, our further investigations demonstrate that Tat alone causes little effect on IκBα degradation as compared with NIK (Figs. 3 and 4). The inability of endogenous Tat to accelerate IκBα degradation presented herein is partially in agreement with observations from a recent study in which lack of responsiveness of a NF-κB-regulated promoter to transactivation by endogenous Tat is reported (53).

The inability of Tat itself to significantly induce IκBα degradation may first be perceived as puzzling, giving that mutation of the κB binding sites in HIV-1 promoter results in a dramatic loss in Tat-induced promoter activity (Fig. 1), and exogenous Tat has been reported to cause IκBα degradation (35, 49). However, our observations provide new information regarding Tat interaction with the HIV-1 LTR that we believe are not fundamentally in conflict with these previous findings (35, 36, 47–49, 53). Based on data presented here together with previous reports, we propose several explanations toward these seemingly conflicting findings. First, the κB binding sites substantially overlap NF-AT binding sites in HIV-1 LTR (12, 13) and Tat is known to induce NF-AT activity as well (13, 40, 50, 51). Therefore mutations in this region are detrimental for both NF-κB and NF-AT factor binding and as a result synergistic interaction amongst Tat, NF-AT and NF-κB in transcriptional activation of HIV-1 can be largely compromised (13). Second, exogenous and endogenous Tat-induced NF-κB activation may have distinct pathways in the activation of NF-κB. For instance, exogenous Tat may interact with cell membrane proteins to exert its effects without a prerequisite need of being internalized (48, 52), while endogenous Tat, as reported here, may interact with different signal transduction factors inside the cell. Inefficient transfection and detection of IκB degradation by endogenous Tat (expressed intracellularly by transfection) as an explanation of our results seems to be very unlikely as the eukaryotic expression constructs for IκBα, NIK, MEKK-1, and Tat have been confirmed to produce adequate amounts of proteins in the transfected cells by Western blot (data not shown) and have been similarly described in previous publications (3, 38, 40). In addition, NIK and MEKKI* although much weaker activators for HIV-1 LTR compared to Tat, clearly demonstrate their capability to induce IκBα degradation. Based on the above discussions, the dramatic reduction in Tat-induced HIV-1 LTR activation (Fig. 1) following mutation in the κB regulatory element can be more adequately explained by a disruption of synergistic interaction amongst Tat, NF-AT, and NF-κB rather than a single block of NF-κB binding.

The mechanism involving in enhancement of NIK-, but not MEKK1-induced IκBα degradation by Tat is

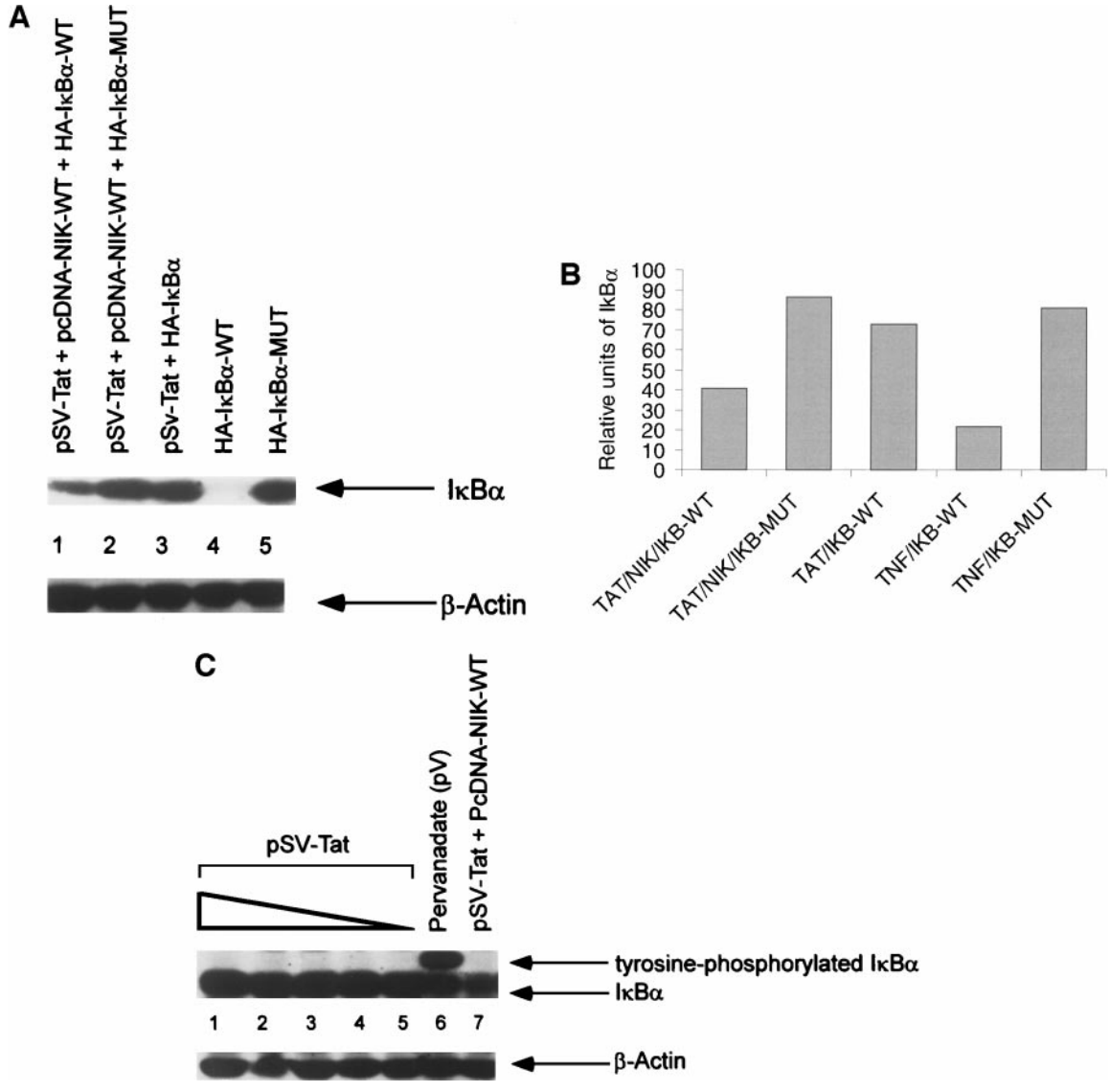

FIG. 5. Pathway involving enhancement of NIK-induced NF-κB activation by Tat. (A) Tat accelerates NIK-induced IκBα degradation through serine phosphorylation/ubiquitin-proteasome pathway. HA-tagged IκBα mutant (S32A/S36A) expression vector (32) was used in place of the wild type HA-tagged IκBα expression constructs. Transfection of the HeLa cells and detection of HA-tagged IκBα level is the same as described in the legend to Fig. 2. (B) Densitometry analysis of the IκBα level. The data in A were subjected to analysis and the values presented in relative units of IκBα were normalized to β-actin. (C) Tat shows no effect on tyrosine phosphorylation of IκBα. Jurkat cells were transfected with various amounts of pSV-Tat (10, 8, 5, 2, 0 μg) as shown in lanes 1 through 5. Lane 6 represents the positive control in which Jurkat cells were treated with 500 mM pervanadate (pV) for 20 min (33). Note the shift in mobility indicates tyrosine phosphorylation in IκBα has taken place (33, 34).

currently unclear. We did not observe physical association between Tat and NIK, suggesting Tat might act through other intermediate factors (data not shown). Interestingly, Tax, the transactivator of human T cell leukemia virus type 1 (HTLV-1) binds to MEKK1, but not NIK, to stimulate IκB kinase activity and NF-κB activation (32). Therefore, it seems that these two retroviral transactivators may use distinct pathways to effect NF-κB. However, our results are in partial agreement with observations from a recent study showing transfected Tat, albeit unable to directly activate some non-HIV-1-derived NF-κB responsive promoters, can enhance the responsiveness of these promoters to a transfected p65 (Rel A) expression vector (53). Obviously, further study aimed at revealing the identities of addition cellular factors will be needed to elucidate the complicated relationship between Tat and NF-κB. Lastly, we also excluded a possibility that Tat may promote tyrosine phosphorylation of IκBα in Jurkat cells, although we were able to detect NIK/Tat-induced degradation of IκBα in these cells. Tyrosine phosphorylation of IκBα may only take place under quite specific

circumstances such as in reoxygenated hypoxic cells or in cells treated with pervanadate (33, 34).

ACKNOWLEDGMENTS

We thank Drs. R. Geleziunas, W. Greene, C. Marcireau, and G. Nabel, J. Pousségur, S. C. Sun, and D. Wallach for providing valuable reagents used in this study. We also express our gratitude to Drs. J. Sodroski, A. Mhashilkar, and D. Biswas for provision of research materials and helpful discussion. We also thank Drs. J. Bai and R. He for their help in graphic preparations. We thank Ms. Y. McLaughlin for preparing the manuscript.

REFERENCES

1. Dayton, A. I., Sodroski, J. G., Rosen, C. A., Goh, W. C., and Haseltine, W. A. (1986) The trans-activator gene of the human T cell lymphotropic virus type III is required for replication. *Cell* **44,** 941–947.
2. Fisher, A. G., Feinberg, M. B., Josephs, S. F., Harper, M. E., Marselle, L. M., Reyes, G., Gonda, M. A., Aldovini, A., Debouk, C., Gallo, R. C., *et al.* (1986) The trans-activator gene of HTLV-III is essential for virus replication. *Nature* **320,** 367–371.
3. Sodroski, J., Patarca, R., Rosen, C., Wong-Staal, F., and Haseltine, W. (1985) Location of the trans-activating region on the genome of human T-cell lymphotropic virus type III. *Science* **229,** 74–77.
4. Berkhout, B., Silverman, R. H., and Jeang, K. T. (1989) Tat trans-activates the human immunodeficiency virus through a nascent RNA target. *Cell* **59,** 273–282.
5. Berkhout, B., Gatignol, A., Rabson, A. B., and Jeang, K. T. (1990) TAR-independent activation of the HIV-1 LTR: Evidence that tat requires specific regions of the promoter. *Cell* **62,** 757–767.
6. Zhou, M., Halanski, M. A., Radonovich, M. F., Kashanchi, F., Peng, J., Price, D. H., and Brady, J. N. (2000) Tat modifies the activity of CDK9 to phosphorylate serine 5 of the RNA polymerase II carboxyl-terminal domain during human immunodeficiency virus type 1 transcription. *Mol. Cell. Biol.* **20,** 5077–5086.
7. Kashanchi, F., Piras, G., Radonovich, M. F., Duvall, J. F., Fattaey, A., Chiang, C. M., Roeder, R. G., and Brady, J. N. (1994) Direct interaction of human TFIID with the HIV-1 transactivator tat. *Nature* **367,** 295–299.
8. Yu, L., Loewenstein, P. M., Zhang, Z., and Green, M. (1995) In vitro interaction of the human immunodeficiency virus type 1 Tat transactivator and the general transcription factor TFIIB with the cellular protein TAP. *J. Virol.* **69,** 3017–3023.
9. Cullen, B. R. (1995) Regulation of HIV gene expression. *AIDS* **9,** S19–S32.
10. Harrich, D., Ulich, C., Garcia-Martinez, L. F., and Gaynor, R. B. (1997) Tat is required for efficient HIV-1 reverse transcription. *EMBO J.* **16,** 1224–1235.
11. Jeang, K. T., Chun, R., Lin, N. H., Gatignol, A., Glabe, C. G., and Fan, H. (1993) In vitro and in vivo binding of human immunodeficiency virus type 1 Tat protein and Sp1 transcription factor. *J. Virol.* **67,** 6224–6233.
12. Nabel, G., and Baltimore, D. (1987) An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature* **326,** 711–713.
13. Kinoshita, S., Su, L., Amano, M., Timmerman, L. A., Kaneshima, H., and Nolan, G. P. (1997) The T cell activation factor NF-ATc positively regulates HIV-1 replication and gene expression in T cells. *Immunity* **6,** 235–244.
14. Fraser, J. D., Straus, D., and Weiss, A. (1993) Signal transduction events leading to T-cell lymphokine gene expression. *Immunol. Today* **14,** 357–362.
15. Rao, A. (1991) Signaling mechanisms in T cells. *Crit. Rev. Immunol.* **10,** 495–519.
16. Ullman, K. S., Northrop, J. P., Verweij, C. L., and Crabtree, G. R. (1990) Transmission of signals from the T lymphocyte antigen receptor to the genes responsible for cell proliferation and immune function: The missing link. *Annu. Rev. Immunol.* **8,** 421–452.
17. Schreiber, S. L., and Crabtree, G. R. (1992) The mechanism of action of cyclosporin A and FK506. *Immunol. Today* **13,** 136–142.
18. Ott, M., Emiliani, S., Van Lint, C., Herbein, G., Lovett, J., Chimule, N., McCloskey, T., Pahwa, S., and Verdin, E. (1997) Immune hyperactivation of HIV-1-infected T cells mediated by Tat and the CD28 pathway. *Science* **275,** 1481–1485.
19. Fauci, A. S. (1996) Host factors and the pathogenesis of HIV-induced disease. *Nature* **384,** 529–534.
20. Baldwin, A. S., Jr. (1996) The NF-kappa B and I kappa B proteins: New discoveries and insights. *Annu. Rev. Immunol.* **12,** 649–683.
21. May, M. J., and Ghosh, S. (1998) Signal transduction through NF-kappa B. *Immunol. Today* **19,** 80–88.
22. Karin, M. (1999) How NF-kappaB is activated: The role of the IkappaB kinase (*IKK*) complex. *Oncogene* **18,** 6867–6874.
23. Malinin, N. L., Boldin, M. P., Kovalenko, A. V., and Wallach, D. (1997) MAP3K-related kinase involved in NF-kappaB induction by TNF, CD95 and IL-1. *Nature* **385,** 540–544.
24. Shinkura, R., Kitada, K., Matsuda, F., Tashiro, K., Ikuta, K., Suzuki, M., Kogishi, K., Serikawa, T., and Honjo, T. (1999) Alymphoplasia is caused by a point mutation in the mouse gene encoding NF-kappa B-inducing kinase. *Nat. Genet.* **22,** 74–77.
25. Woronicz, J. D., Gao, X., Cao, Z., Rothe, M., and Goeddel, D. V. (1997) IkappaB kinase-beta: NF-kappaB activation and complex formation with IkappaB kinase-alpha and NIK. *Science* **278,** 866–869.
26. Hirano, M., Osada, S., Aoki, T., Hirai, S., Hosaka, M., Inoue, J., and Ohno, S. (1996) MEK kinase is involved in tumor necrosis factor alpha-induced NF-kappaB activation and degradation of IkappaB-alpha. *J. Biol. Chem.* **271,** 13234–13238.
27. Lee, F. S., Hagler, J., Chen, Z. J., and Maniatis, T. (1997) Activation of the IkappaB alpha kinase complex by MEKK1, a kinase of the JNK pathway. *Cell* **88,** 213–222.
28. Lee, F. S., Peters, R. T., Dang, L. C., and Maniatis, T. (1998) MEKK1 activates both IkappaB kinase alpha and IkappaB kinase beta. *Proc. Natl. Acad. Sci. USA* **95,** 9319–9324.
29. Meyer, C. F., Wang, X., Chang, C., Templeton, D., and Tan, T. H. (1996) Interaction between c-Rel and the mitogen-activated protein kinase kinase kinase 1 signaling cascade in mediating kappaB enhancer activation. *J. Biol. Chem.* **271,** 8971–8976.
30. Nakano, H., Shindo, M., Sakon, S., Nishinaka, S., Mihara, M., Yagita, H., and Okumura, K. (1998) Differential regulation of IkappaB kinase alpha and beta by two upstream kinases, NF-kappaB-inducing kinase and mitogen-activated protein kinase/ERK kinase kinase-1. *Proc. Natl. Acad. Sci. USA* **95,** 3537–3542.
31. Nemoto, S., DiDonato, J. A., and Lin, A. (1998) Coordinate regulation of IkappaB kinases by mitogen-activated protein kinase kinase kinase 1 and NF-kappaB-inducing kinase. *Mol. Cell. Biol.* **18,** 7336–7343.
32. Yin, M. J., Christerson, L. B., Yamamoto, Y., Kwak, Y. T., Xu, S., Mercurio, F., Barbosa, M., Cobb, M. H., and Gaynor, R. B. (1998) HTLV-I Tax protein binds to MEKK1 to stimulate IkappaB kinase activity and NF-kappaB activation. *Cell* **93,** 875–884.
33. Imbert, V., Rupec, R. A., Livolsi, A., Pahl, H. L., Traenckner, E. B., Mueller-Dieckmann, C., Farahifar, D., Rossi, B., Auberger, P., Baeuerle, P. A., and Peyron, J. F. (1996) Tyrosine phosphor-

ylation of I kappa B-alpha activates NF-kappa B without proteolytic degradation of I kappa B-alpha. *Cell* **86,** 787–798.

34. Beraud, C., Henzel, W. J., and Baeuerle, P. A. (1999) Involvement of regulatory and catalytic subunits of phosphoinositide 3-kinase in NF-kappaB activation. *Proc. Natl. Acad. Sci. USA* **96,** 429–434.
35. Demarchi, F., d'Adda di Fagagna, F., Falaschi, A., and Giacca, M. (1996) Activation of transcription factor NF-kappaB by the Tat protein of human immunodeficiency virus type 1. *J. Virol.* **70,** 4427–4437.
36. Westendorp, M. O., Shatrov, V. A., Schulze-Osthoff, K., Frank, R., Kraft, M., Los, M., Krammer, P. H., Droge, W., and Lehmann, V. (1995) HIV-1 Tat potentiates TNF-induced NF-kappa B activation and cytotoxicity by altering the cellular redox state. *EMBO J.* **14,** 546–554.
37. Pomerance, M., Multon, M.-C., Parker, F., Venot, C., Blondeau, J.-P., Tocque, B., and Schweighoffer, F. (1998) Grb2 interaction with MEK-kinase 1 is involved in regulation of Jun-kinase activities in response to epidermal growth factor. *J. Biol. Chem.* **273,** 24301–24304.
38. Sun, S. C., Elwood, J., Beraud, C., and Greene, W. C. (1994) Human T-cell leukemia virus type 1 Tax activation of NF-kappa B/Rel involves phosphorylation and degradation of I kappa B alpha and RelA (p65)-mediated induction of the c-rel gene. *Mol. Cell. Biol.* **14,** 7377–7384.
39. Li, X., Liang, C., Quan, Y., Chandok, R., Laughrea, M., Parniak, M. A., Kleiman, L., and Wainberg, M. A. (1997) Identification of sequences downstream of the primer binding site that are important for efficient replication of human immunodeficiency virus type 1. *J. Virol.* **71,** 6003–6010.
40. Li, X., Multon, M. C., Henin, Y., Schweighoffer, F., Venot, C., Josef, J., Zhou, C., LaVecchio, J., Stuckert, P., Raab, M., Mhashilkar, A., Tocque, B., and Marasco, W. A. (2000) Grb3-3 is upregulated in HIV-1-infected T-cells and can potentiate cell activation through NFATc. *J. Biol. Chem.* **275,** 30925–30933.
41. Mhashilkar, A. M., Biswas, D. K., LaVecchio, J., Pardee, A. B., and Marasco, W. A. (1997) Inhibition of human immunodeficiency virus type 1 replication in vitro by a novel combination of anti-Tat single-chain intrabodies and NF-kappa B antagonists. *J. Virol.* **71,** 6486–6494.
42. Garber, M. E., and Jones, K. A. (1999) HIV-1 Tat: Coping with negative elongation factors. *Curr. Opin. Immunol.* **11,** 460–465.
43. Taube, R., Fujinaga, K., Wimmer, J., Barboric, M., and Peterlin, B. M. (1999) Tat transactivation: A model for the regulation of eukaryotic transcriptional elongation. *Virology* **264,** 245–253.
44. Jeang, K. T., Xiao, H., and Rich, E. A. (1999) Multifaceted activities of the HIV-1 transactivator of transcription. *J. Biol. Chem.* **274,** 28837–28840.
45. Albini, A., Ferrini, S., Benelli, R., Sforzini, S., Giunciuglio, D., Aluigi, M. G., Proudfoot, A. E., Alouani, S., Wells, T. N., Mariani, G., Rabin, R. L., Farber, J. M., and Noonan, D. M. (1998) HIV-1 Tat protein mimicry of chemokines. *Proc. Natl. Acad. Sci. USA* **95,** 13153–13158.
46. Li, C. J., Friedman, D. J., Wang, C., Metelev, V., and Pardee, A. B. (1995) Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein. *Science* **268,** 429–431.
47. Biswas, D. K., Ahlers, C. M., Dezube, B. J., and Pardee, A. B. (1993) Cooperative inhibition of NF-kappa B and Tat-induced superactivation of human immunodeficiency virus type 1 long terminal repeat. *Proc. Natl. Acad. Sci. USA* **90,** 11044–11048.
48. Badou, A., Bennasser, Y., Moreau, M., Leclerc, C., Benkirane, M., and Bahraoui, E. (2000) Tat protein of human immunodeficiency virus type 1 induces interleukin-10 in human peripheral blood monocytes: Implication of protein kinase C-dependent pathway. *J. Virol.* **74,** 10551–10562.
49. Manna, S. K., and Aggarwal, B. B. (2000) Differential requirement for p56lck in HIV-tat versus TNF-induced cellular responses: Effects on NF-kappa B, activator protein-1, c-Jun N-terminal kinase, and apoptosis. *J. Immunol.* **164,** 5156–5166.
50. Vacca, A., Farina, M., Maroder, M., Alesse, E., Screpanti, I., Frati, L., and Gulino, A. (1994) Human immunodeficiency virus type-1 tat enhances interleukin-2 promoter activity through synergism with phorbol ester and calcium-mediated activation of the NF-AT cis-regulatory motif. *Biochem. Biophys. Res. Commun.* **205,** 467–474.
51. Macian, F., and Rao, A. (1999) Reciprocal modulatory interaction between human immunodeficiency virus type 1 Tat and transcription factor NFAT1. *Mol. Cell. Biol.* **19,** 3645–3653.
52. Gutheil, W. G., Subramanyam, M., Flentke, G. R., Sanford, D. G., Munoz, E., Huber, B. T., and Bachovchin, W. W. (1994) Human immunodeficiency virus 1 Tat binds to dipeptidyl aminopeptidase IV (CD26): A possible mechanism for Tat's immunosuppressive activity. *Proc. Natl. Acad. Sci. USA* **91,** 6594–6598.
53. Kelly, G. D., Morris, C. B., and Offermann, M. K. (1999) Lack of responsiveness of nuclear factor-kappaB-regulated promoter to transactivation by human immunodeficiency virus 1 Tat in HeLa cells. *Virology* **263,** 128–138.