The Association of HIV-1 Tat with Nuclei Is Regulated by Ca²⁺ Ions and Cytosolic Factors*

(Received for publication, January 31, 1997)

Paola Morgavi†, Neris Bonifaci‡,§, Massimiliano Pagani¶, Sara Costigliolo‡, Roberto Sitiat©, and Anna Rubartelli‡**

From the †National Institute of Cancer Research, 16132 Genova and the §National Institute of Cancer Research, 20132 Milano, Italy

Human immunodeficiency virus-1 (HIV-1) Tat, a nuclear transcription factor, has been shown to function extracellularly, implying that some Tat molecules escape nuclear import and are secreted. This raises the question of what regulates, in HIV-1-infected cells, the nuclear targeting of the polypeptide. Here we show that cytosolic components activated by Ca²⁺ ions are required to reveal the karyophilic properties of Tat: in vitro translated Tat molecules do not associate with isolated nuclei unless preincubated with Ca²⁺. Moreover, Ca²⁺ ions induce karyophilicity of chemically synthesized Tat molecules only upon addition of cytosolic extracts. The Ca²⁺-induced karyophilicity is prevented by inhibitors of either tyrosine kinases (herbimycin A and genistein) or tyrosine phosphatases (vanadate), suggesting the involvement of Ca²⁺-dependent phosphorylation/dephosphorylation events. In line with these observations, the transcriptional activity of Tat is inhibited by treatment with either vanadate or genistein. The same occurs with Tat mutants lacking either one or both the two tyrosine residues (positions 26 and 47). Hence, Ca²⁺-dependent tyrosine kinase(s) and phosphatase(s) act on accessory cellular protein(s), which in turn are responsible of Tat karyophilicity.

In eukaryotic cells, gene expression is often regulated by post-translational modifications such as phosphorylation (1) of the relevant transcription factors or associated proteins. These modifications may modulate the cellular localization of the transcription factors, their DNA binding capacity, or their transactivating activity. Examples of transcription factors able to undergo inducible nuclear import include steroid receptors (2), the v-jun oncogenic counterpart of the AP-1 complex member c-jun (3), the yeast SW15 (4), and NF-xB, which is imported to the nucleus following dissociation from the cytosolic anchoring protein IxB-a (5, 6).

In addition to this group of transcription factors, which have a different intracellular localization depending on their functional state, many examples of proteins with dual intracellular targeting have been reported (7). Among HIV¹ products, the matrix protein MA contains two subcellular localization signals with competing effects: a myristoylated N terminus that targets the protein to the plasma membrane and a nuclear localization sequence. Myristoylation is the dominant signal. However, a small subset of MA molecules undergo tyrosine phosphorylation, and this modification is sufficient to reveal their karyophilic properties (8). The case of human immunodeficiency virus-1 (HIV-1) Tat is still more complex, as in addition to its transactivating function, necessary for viral replication (9, 10), it can also be secreted by transfected or virus-infected cells (11) and exert several extracellular activities that interfere with growth regulation of different cells (12, 13). While much effort has been spent on elucidating the molecular details of transactivation of HIV-1, the mechanisms regulating nuclear import versus secretion of Tat protein are largely unknown.

In a previous study, we have shown that Tat molecules, synthesized in vitro in wheat germ extracts, do not associate with nuclei when added to lysed cells (14); however, we noted that after a period of incubation in culture medium, some Tat molecules become capable of associating posttranslationally with the nuclear fraction, suggesting that some components of the culture medium might induce modification(s) on Tat molecules that reveal its karyophilicity.

Here we exploit in vitro and in vivo assays to investigate the effects of Ca²⁺ and of cytosolic factors on the nuclear targeting of Tat.

MATERIALS AND METHODS

**Drugs—**PMA, A23187, ionomycin, sodium orthovanadate, and cyclosporin A (CsA) were obtained from Sigma (Milano, Italy); herbimycin A, bisindolylmaleimide I, okadaic acid, genistein, KN-62, staurosporine, calbiochem (Milano, Italy); and alkaline phosphatase from Boehringer (Mannheim, Germany).

**Plasmids—**Plasmids used in this study are: pcDNA1-Tat (in which the Tat coding region from pS2V2Tat (14) was subcloned in pcDNA1-neo, Invitrogen, San Diego, CA), pJK2 (Ref. 15, coding for β-galactosidase; and the complementary for Tat 47F and TatDF); 5′-CCAATTG and 5′-TGGCAGGAAG-3′ (Ref. 17, obtained from V. Zappavigna, Milano, Italy). The three Tat mutants, Tat26F, Tat47F, and TatDF, were generated by in vitro site-directed mutagenesis (QuickChange site-directed mutagenesis kit, Stratagene), using oligonucleotide primers that replace the two tyrosines into phenylalanines (5′-CCAAATTGCTTTTGGAAAG-3′ and the complementary for Tat26F and TatDF; 5′-CCACCAATGCTTTGCCAGGAAG-3′ and the complementary for Tat 47F and Tat DF). The mutations were

* This work was supported in part by grants from CNR (PF ACRO), Associazione Italiana Ricerca sul Cancro (AIRC), and Ministero Sanita®, Rome. This paper is available online at http://www-jbc.stanford.edu/jbc/

† Present address: Laboratory of Cell Biology, Howard Hughes Medical Inst., The Rockefeller University, New York, NY 10021.
‡ To whom correspondence should be addressed. Laboratory of Clinical Pathology, National Institute of Cancer Research, Largo Rosanna Benzi, 10, 16132 Genova, Italy. Tel.: 39-10-5600204; Fax: 39-10-5600210; E-mail: annarub@hp380.ist.unige.it.

¹ The abbreviations used are: HIV, human immunodeficiency virus; HIV-1, human immunodeficiency virus-1; CsA, cyclosporin A; LTR, long terminal repeat; CMV, cytomegalovirus; β-γ, β-γ-galactosidase; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; ONPG, o-nitrophenyl-β-D-galactopyranoside; TK, tyrosine kinase; PTPase, protein tyrosine phosphatase; PMA, phorbol 12-myristate 13-acetate.
different concentrations of CaCl₂ and nuclear association was determined. Analysis with Centricon-10 microconcentrators (Amicon, Beverly, MA).

HeLa cells were cultured in RPMI 1640 medium and Dulbecco’s modified Eagle's medium, respectively, supplemented with 10% fetal calf serum and 1 mM l-glutamine (all from Life Technologies, Inc., Milano, Italy) and cultured in complete medium at 10⁴/well in 96-well plates for the last 4 h, orthovanadate (100 \( \mu \)M) or CsA (12 \( \mu \)M) was added for the last 18 h. Six \( \times \) 10⁶ Jurkat cells were transfected by 30 \( \mu \)l of Lipofectin (Life Technologies, Inc.) with 5 \( \mu \)g of CMV-\( \beta \)-gal or 10 \( \mu \)g of pJRK2 alone or together with 10 \( \mu \)g of pcDNA1-Tat, pcDNA1-Tat26F, pcDNA1-Tat47F, or pcDNA1-TatDF by electroporation (250 mV, 960 microfarads) in a Bio-Rad gene pulser apparatus (Bio-Rad, Milano, Italy) and cultured in complete medium at 10⁴/well in 96-well plates for 48 h. When indicated, herbimycin A (2 \( \mu \)M) or genistein (180 \( \mu \)M) was added for the last 4 h of incubation, while orthovanadate (100 \( \mu \)M) was added for the last 18 h.

In Vitro Transcription and Translation—The cDNAs were transcribed in vitro with either SP6 or T7 polymerase (Promega, Firenze, Italy) and the transcripts translated in a wheat germ extract (Promega) or in a rabbit reticulocyte lysate (Amersham, Milano, Italy) as specified by the supplier. \[^{35}S\]Methionine or \[^{35}S\]cysteine (both by the supplier. [35S]Methionine or [35S]cysteine (both \( 0.7 \) M sucrose and spun 15 min at 800 \( g \)).

Nuclear Association Assay—Aliquots of radiolabeled Tat, Tat mutants, or HoxD9 proteins synthesized Tat (in which a biotin was added at the N terminus) (both provided by G. Fassina, Tecnogen, Piana di Monteverna (CE), Italy) were employed for labeling of HoxD9 and Tat or Tat mutants, respectively. Free radioactive amino acids were removed by dialysis with Centricon-10 microconcentrators (Amicon, Beverly, MA).

Preparation of Cytosol—10⁶ HeLa cells were washed three times in PBS, once in 25 mM Hepes, 125 mM KC₂H₃O₂, resuspended in 2.5 volumes of the same buffer containing a mixture of protease inhibitors, and kept on ice for 10 min, and the supernatant was collected as the cytoplasmic fraction. Nuclei were isolated and resolved by SDS-PAGE. The percentage of nuclear Tat was calculated from densitometric analyses of autoradiograms. Means of three different experiments and standard deviations are shown. The inset shows a representative fluorogram. N, nuclear fraction; C, cytosolic fraction. Panel B, 5 \( \times \) 10⁴ cpm of in vitro translated Tat was incubated 60 min at 37 °C without (–) or with 1 mM CaCl₂ or 1 mM CaCl₂ plus 10 mM EGTA (EGTA + Ca²⁺) or 1 mM CaCl₂ for 60 min followed by 10 mM EGTA for other 60 min (Ca²⁺-Ih + EGTA) or 1 mM CaCl₂ followed by 60 min at 4 °C. The rate of Tat nuclear association was determined as above. Panels C and D, 5 \( \times \) 10⁴ cpm of in vitro translated Tat were incubated with 1 mM CaCl₂ or for 1 h at different temperatures (panel C) or for different periods of time at 37 °C (panel D) and the percentage of Tat nuclear association was determined.

Cell Cultures and Transient Transfection Assays—Jurkat and COS7 cells were cultured in RPMI 1640 medium and Dulbecco’s modified Eagle’s medium, respectively, supplemented with 10% fetal calf serum and 1 mM l-glutamine (all from Life Technologies, Inc., Milano, Italy) in 5% CO₂ in humidified atmosphere. 2 \( \times \) 10⁶ COS7 cells in PBS/glucose (1 mg/ml) were transfected with 10 \( \mu \)g of CMV-\( \beta \)-gal or 10 \( \mu \)g of pJRK2 alone or together with 10 \( \mu \)g of pcDNA1-Tat, pcDNA1-Tat26F, pcDNA1-Tat47F, or pcDNA1-TatDF by electroporation (250 mV, 960 microfarads) in a Bio-Rad gene pulser apparatus (Bio-Rad, Milano, Italy) and cultured in complete medium at 10⁴/well in 96-well plates for 48 h. When indicated, herbimycin A (2 \( \mu \)M) or genistein (180 \( \mu \)M) was added for the last 4 h of incubation, while orthovanadate (100 \( \mu \)M) was added for the last 18 h.

At the end of the incubation, the proteins were added to 1.5 \( \times \) 10⁶ HeLa cells lysed in 50 \( \mu \)l of PBS, 1% Triton X-100 containing 10 mM EGTA, 10 mM NaF, 1 mM N-ethylmaleimide and protease inhibitors phenylmethylsulfonyl fluoride aprotinin, leupeptin) and kept on ice for 20 min as described (14). Samples were spun at 800 \( g \) for 10 min, and the supernatant was collected as the cytoplasmic fraction. Nuclei were prepared as described in Ref. 14. Briefly, the nuclear pellet was washed twice in the same buffer containing sucrose 0.4 M, loaded onto a cushion of PBS, 0.7 M sucrose and spun 15 min at 800 \( g \). Nuclei were treated with 50 

\( \mu \)g/ml of DNase for 10 min at 37 °C and boiled in Laemmli sample buffer (18).

Autoradiography and Western Blotting—Cytoplasmic and nuclear fractions were then resolved on 12% SDS-PAGE and processed for fluorography, when \[^{35}S\]labeled proteins were used, or transferred to nitrocellulose filters (Hybond C extra, Amersham) when synthetic or biotinylated Tat was used (14). Blots were probed with anti-Tat antibodies (Ref. 19, kind gift of B. Ensoli, Roma, Italy) followed by peroxidase-conjugated secondary antiserum (Dako, Milano, Italy) or with peroxidase-conjugated streptavidin (Amersham) and developed by chemiluminescence (Amersham).

Different exposures of the same film were scanned at a Utrasonic densitometer (Molecular Dynamics, Milano, Italy). The purity of the nuclear and cytoplasmic fractions was determined by probing the blots with anti-\( \beta \)-Gal and anti-calnexin antibodies or by localizing histones (14).

Fig. 1. Ca²⁺ specifically induces association of in vitro translated Tat to isolated nuclei. Panel A, 5 \( \times \) 10⁴ cpm of in vitro translated Tat (tat, first to sixth columns) or of in vitro translated HoxD9 (hoxD9, seventh and eighth columns) were treated 1 h at 37 °C without (–) or with 1 mM CaCl₂ (Ca²⁺), MgCl₂ (Mg²⁺), CaCl₂ + MgCl₂ (Ca²⁺ + Mg²⁺), ZnCl₂ (Zn²⁺), or SrCl₂ (Sr²⁺) and then incubated 20 min on ice with 10⁶ HeLa cells lysed in 1% Triton X-100. Nuclear and cytosolic fractions were isolated and resolved by SDS-PAGE. The percentage of nuclear Tat was calculated from densitometric analyses of autoradiograms. Means of three different experiments and standard deviations are shown. The inset shows a representative fluorogram. N, nuclear fraction; C, cytosolic fraction. Panel B, 5 \( \times \) 10⁴ cpm of in vitro translated Tat was incubated 60 min at 37 °C without (–) or with 1 mM CaCl₂ or 1 mM CaCl₂ plus 10 mM EGTA (EGTA + Ca²⁺) or 1 mM CaCl₂ for 60 min followed by 10 mM EGTA for other 60 min (Ca²⁺-Ih + EGTA) or 1 mM CaCl₂ followed by 60 min at 4 °C. The rate of Tat nuclear association was determined as above. Panels C and D, 5 \( \times \) 10⁴ cpm of in vitro translated Tat were incubated with 1 mM CaCl₂ or for 1 h at different temperatures (panel C) or for different periods of time at 37 °C (panel D) and the percentage of Tat nuclear association was determined.
In Vitro Translated Tat Molecules Become Karyophilic upon Treatment with Ca2+—As an in vitro assay to follow karyophility, in vitro translated Tat is added to detergent-lysed cells on ice, as such or after incubation at 37 °C with Ca2+ or other divalent ions, and the presence of radioactive Tat is evaluated in the cytoplasmic or in the nuclear fraction. As shown in Fig. 1 (panel A and inset), 35S-labeled Tat molecules, translated in wheat germ lysates, accumulate in the cytoplasm but are absent from the nuclear fraction. Upon treatment with CaCl2, Tat becomes able to associate with nuclei. Nuclear association is not observed following incubation with other divalent ions, such as Zn2+, Mg2+, or Sr2+ (panel A). Similar results are obtained with Tat translated in rabbit reticulocyte lysates and using nuclei isolated after mechanic disruption rather than detergent lysis of the cell (not shown). As expected, the effects of Ca2+ are prevented by the simultaneous addition of EGTA (Fig. 1, panel B). However, once Tat molecules have been rendered karyophilic by incubation with Ca2+ ions at 37 °C, they remain so even if rewarmed at 4 °C or treated with EGTA. The Ca2+-induced Tat karyophilicity is dependent on time, temperature, and concentration (Fig. 1, panels C–E); it is not observed at temperatures lower than 18 °C (panel C), and it requires at least 15 min at 37 °C (panel D). Karyophilicity is still induced with concentrations of Ca2+ as low as 30 μM (panel E).

The effects of Ca2+ ions are specific for Tat. In the same assay, the amount of the transcription factor HoxD9 that accumulates within the nuclear fraction is not modified by Ca2+ treatment (Fig. 1, panel A).

Synthetic Tat Does Not Become Karyophilic in the Presence of Ca2+—Unless Cytosolic Extracts Are Added—To investigate whether Ca2+ ions act directly on Tat molecules or rather through modifications of accessory cytosolic molecules, a full-length chemically synthesized Tat, modified by the addition of a biotin to the N-terminal (biotinyl-Tat), was incubated in the presence or absence of Ca2+ ions as above and added to Triton X-100-lysed cells. Its association with nuclear or cytoplasmic fractions was investigated by Western blotting with streptavidin-conjugated peroxidase. As shown in Fig. 2, panels A and B, there is only a small increase in the amount of biotinyl-Tat that associates with nuclei following Ca2+ treatment. In the absence of Ca2+ the addition of wheat germ extracts or HeLa cells cytosol does not promote significantly nuclear association; however, when CaCl2 is added to the mix of biotinyl-Tat and cell extracts, the karyophilicity of biotinyl-Tat is unveiled. EGTA completely prevents Tat nuclear association. Similar results were obtained when unmodified synthetic Tat was revealed by anti-Tat antibodies (data not show).

Taken together, these results suggest that cytosolic components play a role in modifying the fate of Tat.

Tyr Kinases (TK) and Phosphatases (PTPase) Are Involved in the Increase of Tat Karyophilicity—The possible involvement of Ca2+-dependent phosphatases or kinases on Ca2+-induced Tat karyophilicity was investigated by studying the effects of drugs affecting either phosphorylation or dephosphorylation. When added to in vitro translated Tat together with CaCl2, staurosporin, KN-62 (Fig. 3, panel A) and bisindolylmaleimide (not shown), which inhibit, with different specificity, serine/threonine kinases such as calmodulin, protein kinase A, and protein kinase C, do not affect significantly the nuclear association of Tat. Only a slight decrease is observed with the Ser-Thr phosphatase inhibitor okadaic acid. However, no effect is detected by treating with alkaline phosphatase either in the presence or absence of Ca2+. In contrast, an almost complete abrogation of Tat nuclear targeting is obtained with herbinycin A and genistein, two TK inhibitors, and with sodium orthovanadate (Na3VO4), that inhibits PTPases.

The nuclear association of Hox D9 is not influenced by Na3VO4 or herbinycin A (Fig. 3, panel B).

Tat-mediated HIV-1-LTR Transactivation Is Inhibited by TK and PTPase Inhibitors—To investigate whether Ca2+-mediated phosphorylation/dephosphorylation events play a role also...
on the transcriptional activity of Tat, COS7 cells were co-transfected with cDNAs encoding Tat (pcDNA-Tat) and its reporter gene HIV-1-LTR β-galactosidase (pJK2), and the levels of transactivation were evaluated after culture in the absence or presence of Na3VO4 or genistein. Both drugs inhibit the transactivation of the reporter gene (Fig. 4, panel A). As a control, neither drug affects the expression of β-galactosidase under the control of a CMV promoter in the cells (Fig. 4, panel A). Fig. 4, panel B, shows that in a T cell line (Jurkat) co-transfected with Tat and pJK2, activation by PMA/ionomycin results in an increase in LTR transactivation. Na3VO4 and genistein inhibit transactivation both in resting and in activated cells, while the immunosuppressant CsA has an inhibitory effect on activated cells only (panel B). As a control, panel C shows that Na3VO4, genistein, or CsA does not alter the expression of CMV-β-gal in resting or PMA/ionomycin-activated Jurkat cells. No detectable β-galactosidase activity was observed in resting or activated Jurkat cells transfected with the reporter gene pJK2 alone (not shown).

Tat Tyrosine Residues Are Not Essential for Acquisition of Karyophilicity—The two tyrosine residues present in Tat (positions 26 and 47) were replaced so as to determine whether they are the targets of the Ca2+-dependent phosphorylation/dephosphorylation events leading to karyophilicity. The three mutants obtained (Tat26F, Tat47F, or TatDF, in which both tyrosine have been replaced) were analyzed for their acquisition of karyophilicity in response to Ca2+-treatment as well as for their transactivation activity.

Fig. 5, panel A, shows that the karyophilicity of the three in vitro translated 35S-labeled Tat mutants is unveiled by Ca2+ and inhibited by vanadate as in the case of wild type Tat. Moreover, when co-transfected with the reporter gene pJK2, Tat mutants are able to induce β-galactosidase expression at approximately the same extent as wild type Tat (Fig. 5, panel B) and are similarly inhibited by Na3VO4 and genistein.

**DISCUSSION**

In this paper we describe a novel mechanism of control of HIV-1 Tat activity, involving tyrosine phosphorylation/dephosphorylation of cytosolic component(s), whose activation, dependent on Ca2+, unveils the karyophilicity of Tat.

The kinetics and the irreversibility of the Ca2+-induced karyophilicity (Fig. 1) suggest that Ca2+ ions do not act directly on Tat itself; this notion is further supported by the observation that chemically synthesized Tat does not associate to nuclei after treatment with Ca2+ unless cytosolic factors are added. Ca2+ may thus activate cellular protein(s) which in turn confer karyophilicity to Tat. As drugs blocking either TK or PTPase inhibit karyophilicity, Ca2+-dependent tyrosine phosphorylation/dephosphorylation events seem to be involved in controlling the nuclear association of Tat. The similar inhibitory effects of drugs acting on targets with opposite functions (TK and PTPase) is not surprising as kinases may be activated by dephosphorylation and vice versa (1, 20). The nature of the TK(s) and PTPase(s) involved, as well as the order of their activation, remains to be investigated. Whatever their nature, the factors are highly conserved through evolution, as demonstrated by the similar activity of wheat germ and HeLa cell cytosolic extracts. Several examples of Ca2+-induced serine/threonine protein kinases or phosphatases involved in activation of transcription factors have been provided (1, 5, 21); in contrast, while the importance of TKs and PTPases in the early steps of signal transduction is well established, their direct involvement in regulating the activity of transcription factors is less

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**FIG. 4.** The transactivating activity of Tat is increased in stimulated Jurkat cells and is inhibited by Na3VO4 and herbimycin A. Panel A, COS cells transfected with CMV-β-gal (open bars) or co-transfected with pJK2 and pcDNA Tat (closed bars) were cultured for 48 h in the absence (−) or presence of Na3VO4 for the last 18 h or genistein (gen) for the last 4 h prior to lysis in the presence of ONPG. β-Galactosidase activity was determined by colorimetric assays (16). Panel B, Jurkat cells co-transfected with pJK2 and pcDNA Tat, resting (closed bars) or activated with PMA/ionomycin (open bars), were cultured for 48 h in the absence (−) or presence of Na3VO4 for the last 18 h or genistein (gen) or CsA for the last 4 h and β-gal activity was determined as above. Panel C, Jurkat cells transfected with CMV-β-gal, resting (closed bars) or activated with PMA/ionomycin (open bars) were cultured for 48 h with or without Na3VO4 for the last 18 h or genistein (gen) or CsA for the last 4 h. The β-galactosidase activity was determined as above.

**FIG. 5.** Tyr residues are dispensable for Tat nuclear association and transcriptional activity. Panel A, in vitro translated Tat mutants in which Tyr-26, Tyr-47, or both were substituted with phenylalanine residues (26F, 47F, and DF, respectively) were treated with Ca2+ in the absence (lane 2) or presence (lane 3) of Na3VO4 as in Fig. 3, and their nuclear association was evaluated. Panel B, COS cells co-transfected with pJK2 and the three Tat mutants were cultured in the absence (closed bars) or presence of genistein (GEN, open bars) or Na3VO4 (gray bars) as in Fig. 4, and β-galactosidase activity was determined.
defined. Tyrosine phosphorylation of the three subunits of the interferon-stimulated gene factor 3 appears to be responsible for their translocation into the nucleus and hence their activation (22); the regulatory enzyme involved is TYK-2, a cytoplasmic, Ca\(^{2+}\)-independent TK induced by interferon-\(\alpha\) (23). Another example of differential targeting modulated by tyrosine phosphorylation is HIV-1 MA protein (8).

In the case of Tat, substitution of either one or both Tyr-26 or Tyr-47 results in mutant Tat molecules that undergo the same control of nuclear association as wild type Tat. These observations confirm that the Ca\(^{2+}\)-activated TK/PTPases involved do not act directly on Tat molecules. Rather, these data suggest that the target of the phosphorylation is accessory protein(s), perhaps involved in chaperoning Tat molecules to the nucleus. In agreement with this, the functional activity is only slightly inhibited when Tyr-26 and/or Tyr-47 are replaced: these mutants are able to transactivate a reporter gene at approximately the same extent as wild type Tat. Three Tat mutants bearing single substitution of Tyr-26 with Ala or Tyr-47 with Ala or histidine have been reported previously (24, 25); in these mutants substitution of either tyrosine did not abolish Tat transcriotional activity, although Ala-26 had a weaker activity in HeLa cells. The maintainance of the full activity by the Tat mutants described here may be due to the more conservative substitution of Tyr with Phe.

The TK/PTPases-mediated induction of Tat karyophilicity has a functional correspondence in the finding that genstein and vanadate inhibit HIV-1 LTR transactivation in transfected cells, of either lymphoid or non-lymphoid origin. Genistein and vanadate ihibit HIV-1 LTR transactivation in transfected mutants, confirming that the TK/PTPases involved in Tat nuclear targeting and transcriptional activity act on accessory protein(s).

Our observations of a more effective transactivation of LTR-\(\beta\) galactosidase in stimulated rather than in resting Jurkat T cells are in line with previous reports (26, 27) and support the hypothesis of a role for intracellular [Ca\(^{2+}\)]\(^{-}\) in regulating Tat activity. The immunosuppressive drug CsA was shown to block the Tat-mediated increase of IL-2 promoter activity in activated T cells (28). Similarly, we show that CsA inhibits LTR transactivation in stimulated, but not resting, T cells, suggesting that, in activated cells, additional, CsA-sensitive, cellular factors act in concert with Tat with resulting activation of HIV-1 gene expression.

Altogether, these findings suggest that, depending on their activation state, HIV-1-infected cells have the possibility of modifying the intracellular fate and the transcriptional activity of Tat by modulating its karyophilicity through activation of TK/PTPase, with obvious influence on viral replication and on the development of HIV-associated syndromes. Compounds that block the activity of these enzymes may lead to novel strategies to slow the spread of the virus in HIV-infected individuals.

Acknowledgments—We thank Drs. F. Blasi, B. Ensoli, M. Emerman, and V. Zappavigna for reagents and advice and Dr. G. Fassina for generously supplying chemically synthesized Tat and biotinylated Tat. We also thank Dr. C. E. Grossi for support and suggestions.

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