Evidence for Cell-Surface Association Between Fusin and the CD4-gp120 Complex in Human Cell Lines

Cheryl K. Lapham,* Jun Ouyang, Bhaskar Chandrasekhar, Nga Y. Nguyen, Dimiter S. Dimitrov, Hana Golding

Accessory cell-surface molecules involved in the entry of human immunodeficiency virus–type 1 into cells have recently been identified and shown to belong to the family of chemokine receptors. Treatment of human cell lines with soluble monomeric gp120 at 37°C induced an association between the surface CD4-gp120 complex and a 45-kilodalton protein, which can be down-modulated by the phorbol ester phorbol 12-myristate 13-acetate. The three proteins were coprecipitated from the cell membranes with antibodies to CD4 or to gp120. The 45-kilodalton protein comigrated with fusin on sodium dodecyl sulfate gels and reacted with rabbit antisera to fusin in protein immunoblots. No 45-kilodalton protein could be coprecipitated from similarly treated non-human cells. However, infection of 3T3.CD4.401 cells with vaccinia-fusin recombinant virus (vCBYF1), followed by gp120 treatment, resulted in coprecipitation of fusin and CD4.401 molecules from their membranes. Together these data provide evidence for physical association between fusin and the CD4-gp120 complex on cell membranes.

It was recently found that seven-transmembrane heterotrimeric GTP-binding protein (G protein)–coupled receptors (specifically, members of the chemokine receptor family) can function as coreceptors with CD4 for human immunodeficiency virus–type 1 (HIV-1) entry. Different members of this family are involved in cell fusion with T cell line–tropic and macrophage-tropic strains (*1–3*). The exact mechanism of action of these coreceptors during the post-binding stages of HIV-1 membrane fusion has not yet been elucidated, although it has been demonstrated that the V3 loop of gp120 is involved in the cell tropism and the selection of coreceptor (*1–3*).

We have shown that an accessory membrane component required for HIV-1 envelope–mediated fusion is down-modulated by phorbol myristate acetate (PMA) (*4*). We also showed that truncated CD4 molecules (CD4.401) are resistant to surface down-modulation by PMA or by cross-linking with antibody to CD4. However, preincubation of these cells with soluble monomeric gp120 at 37°C, followed by PMA treatment, resulted in significant (50 to 70%) down-modulation of their surface CD4 molecules (*5*) (Tables 1 and 2). This phenomenon was strictly temperature-dependent and was restricted to human cells. We postulated that the interaction between gp120 and CD4 induces conformational changes that promote the association of the complex with another membrane component that is susceptible to PMA-induced down-modulation (*5*).

To test this model, human T cell lines expressing CD4 were surface-biotinylated, incubated with soluble monomeric gp120 at 37°C, and lysed. The cell lysates were immunoprecipitated with either OKT4 or rabbit antiserum to gp120 (Fig. 1). Immunoprecipitation with either antibody resulted in coprecipitation of surface CD4 with a protein that was initially estimated to be 40 kD in size (Fig. 1). However, in subsequent experiments using human leukocyte antigen (HLA) class I (45 kD) as an internal control, it was determined that this protein component is 45 kD in size (Fig. 2). The 45-kD protein was unlikely to be a breakdown product of CD4, because it did not react with polyclonal antibodies to CD4 in protein immunoblots. Addition of gp120 to the cell lysates did not induce this association and coprecipitation (Fig. 1A), which suggests that the association can occur only in the membranes of intact cells. In some experiments, this component was minimally coprecipitated with surface CD4 molecules from cells not treated with gp120 (Fig. 2, U937). However, gp120 treatment always increased the amount of the coprecipitated 45-kD protein relative to the amount of CD4 as confirmed by densitometry of an exposure where CD4 was in the linear range. Commonly, additional background bands were seen after immunoprecipitation with OKT4 (Fig. 1C). However, only the 45-kD band showed consistently enhanced coprecipitation with CD4 after treatment of cells with soluble monomeric gp120. In all experiments, the CD4 band was much more intense than the 45-kD band. This may reflect different efficiencies of biotinylation of the two surface molecules, difference in their surface densities, or disruption of the

Table 1. Soluble gp120 can prime 3T3.CD4.401 cells infected with vCBYF1 for PMA-induced down-modulation of CD4. 3T3.CD4.401 cells were infected overnight with control vaccinia virus vSC8 or with vCBYF1 (*3*) at 10 PFU per cell. Cells (1×10^6 per group) were treated with soluble gp120 (Intracel, MA) (10 μg/ml) or with medium for 45 min and then treated with PMA (100 ng/ml) for three hours at 37°C. Alternatively, cells were pretreated with PMA for 3 hours, washed extensively, and treated with gp120 for 1 hour. All groups were stained with fluorescein isothiocyanate (FITC)–OKT4. Mean fluorescent units (MFU) were determined with fluorescinated beads as described to generate standard curves (*5*). The percentage of down-modulation of surface CD4 expression was calculated after subtraction of background fluorescence. Dashes indicate no treatment.

Cell	Treatment	Staining with FITC-OKT4	
		MFU (*n*)	Down-modulation (%)
A2.01.CD4.401	– –	2700	
	– PMA	2700	0
	gp120 –	1900	30
	gp120 → PMA	1000	63
	PMA → gp120	2350	13
3T3.CD4.401 (infected with vSC8)	– –	3800	
	– PMA	3700	3
	gp120 –	3900	0
	gp120 → PMA	3700	3
3T3.CD4.401 (infected with vCBYF1)	– –	3300	
	– PMA	3300	0
	gp120 –	2700	18
	gp120 → PMA	1300	41
	PMA → gp120	3300	0

C. K. Lapham and H. Golding, Division of Viral Products, Center for Biologics Evaluation and Research (CBER), Food and Drug Administration, Building 29B, Room 3G21, HFM 454, 8800 Rockville Pike, Bethesda, MD 20892, USA.
J. Ouyang and D. S. Dimitrov, Section on Membrane Structure and Function, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.
B. Chandrasekhar and N. Y. Nguyen, Facility for Biotechnology Resources, CBER, Food and Drug Administration, Bethesda, MD 20892, USA.
*To whom correspondence should be addressed.

multimolecular complexes during the extraction and precipitation procedures. It may also suggest that only a fraction of the surface CD4 molecules are associated with the 45-kD protein after gp120 treatment. When a panel of human and nonhuman cell lines was tested, it was found that the gp120-induced coprecipitation of CD4 and the 45-kD protein was restricted to human cell lines (Fig. 2). If A2.01.CD4.401 cells were treated with PMA before the addition of gp120, no coprecipitation of CD4 and the 45-kD protein was observed (Fig. 3). Without PMA treatment, coprecipitation of the 45-kD protein and the tailless CD4 molecules was seen after gp120 treatment. This suggests that PMA treatment depleted the 45-kD protein from the cell surface.

After the recent identification by Feng *et al.* (2) of LESTR/HUMSTR (also called fusin) as the coreceptor for T cell line–tropic HIV-1 viruses, we attempted to determine if the 45-kD protein identified in our studies is fusin. The 45-kD protein that coprecipitated with CD4 after gp120 treatment (Fig. 4A) comigrated with the fusin protein expressed in CEM cells infected with the fusin-vaccinia recombinant virus (vCBFY1) (Fig. 4A). In CEM and U937 cells, gp120 treatment and OKT4 precipitation resulted in coprecipitation of gp120, CD4, and a 45- to 50-kD band reactive with rabbit antibodies to fusin (but not with normal rabbit serum) (Fig. 4B) (6). This reactivity could be specifically blocked by preincubation of the anti-fusin rabbit immunoglobulin G (IgG) with a peptide derived from the NH_2-terminus of fusin, but not with a control peptide derived from the NH_2-terminus of CCR-5 (7). The slightly slower migration of fusin detected by antibodies to fusin, as compared with that of the 45-kD biotinylated band observed in Figs. 1 through 3, may be explained by the 5- to 10-fold higher protein concentrations used in the anti-fusin protein immunoblots (Fig. 4B).

Table 2. Rabbit anti-fusin IgG partially blocks syncytium formation and CD4.401 down-modulation. A2.01.CD4.401 cells were treated with gp120 (10 μg/ml), followed by PMA treatment (100 ng/ml) as described in Table 1, in the presence (at 10 μg/ml each) of no serum, of rabbit preimmune IgG, or of IgG derived from a rabbit immunized with a branched peptide containing the NH_2-terminal 15 amino acids of fusin (6). Syncytium formation was determined after 6 hours in co-cultures of A2.01.CD4.401 cells and 12E1 cells infected overnight with vPE16 recombinant vaccinia expressing gp120-41 (IIIB) in triplicates as described (4).

Rabbit IgG	CD4 down-modulation after gp120 + PMA treatment (%)	Syncytia (*n*)
None	53	157 ± 22
Preimmune	52	155 ± 13
Anti-fusin	28	68 ± 9

After infection of the nonhuman cell line 3T3.CD4.401 with fusin vaccinia (vCBYF1), but not with a control vaccinia (vSC8), gp120 could induce the association and coprecipitation of CD4.401 molecules with fusin (Fig. 4C). We also tested the ability of fusin-reconstituted 3T3.CD4.401 cells to down-modulate their surface tailless CD4. After infection of the murine fibroblast line with the fusin-expressing vaccinia recombinant vCBYF1 but not with the control virus vSC8, it was possible to induce down-modulation of the tailless CD4 (Ta-

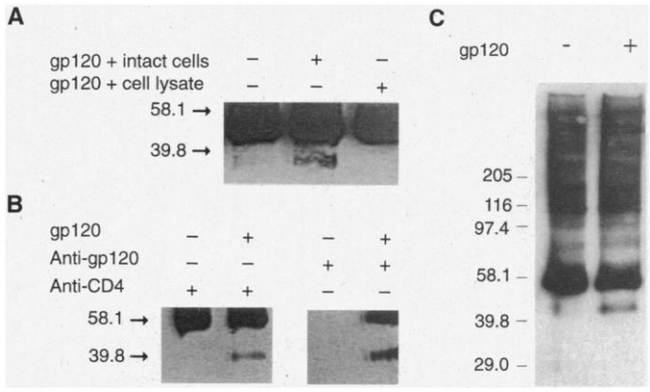

Fig. 1. Coprecipitation of CD4 or gp120 and a 45-kD protein from the surface of CEM cells pretreated with soluble gp120 at 37°C. Cem surface proteins were biotinylated by addition of 2 mM sulfo-NHS-LC-Biotin (Pierce) to 5×10^7 cells per milliliter in phosphate-buffered saline (PBS) for 1 hour on ice. The reaction was quenched with 20 mM glycine for 15 min. Washed cells were suspended in RPMI and 10% fetal bovine serum (5×10^7 cells per milliliter) and incubated in the presence or absence of gp120 (10 μg/ml) (LAI, Intracel, MA) for 2 hours at 37°C. Cells were washed with PBS and lysed in a buffer containing 1% Brij 97, 150 mM NaCl, 20 mM tris (pH 8.2), 5 mM iodoacetamide, and protease inhibitors. After 20 min on ice, nuclei were pelleted by centrifugation at 13,000*g* for 5 min. (**A**) CD4 was immunoprecipitated with OKT4-containing ascites and 50 μl of protein G–Sepharose beads (diluted 1:2 in PBS) that were added to lysates and mixed overnight at 4°C. (**B**) Lysates were immunoprecipitated with either OKT4 ascites (left two lanes) or with anti-gp120 polyclonal rabbit serum (40 μl; Intracel) (right two lanes). (**C**) As in (A), but a full-sized gel is shown. The beads were washed five times with lysis buffer and boiled for 5 min with 30 μl of 2× Laemmli sample buffer. Samples (from 1 to 2×10^7 cells per lane) were run on a 10% gel with SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and were electrophoretically transferred to nitrocellulose membranes. Membranes were blocked with 20 mM tris-HCl (pH 7.6) containing 140 mM NaCl, 0.1% Tween-20, and 5% nonfat powdered milk and were incubated with streptavidin-conjugated horseradish peroxidase (HRP) in blocking buffer for 30 min. After washing, the blots were incubated with supersignal chemiluminescent substrate (Pierce) for 1 min and exposed to film. In Figs. 1 through 4, numbers at left or right of panels indicate positions of biotinylated molecular weight markers.

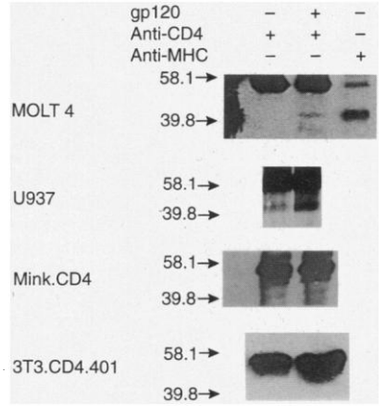

Fig. 2. After pretreatment with gp120, antibody to CD4 coprecipitates a 45-kD protein from human cell lines but not from nonhuman cells expressing human CD4. Human cell lines (U937 and MOLT 4) and nonhuman cells (Mink.CD4 and 3T3.CD4.401) were pretreated with or without gp120 (10 μg/ml for 2 hours). After cell lysis, precipitation of CD4 was carried out as described in Fig. 1. Major histocompatibility complex (MHC) class I molecules were precipitated from biotinylated MOLT 4 cells with the W6/32 monoclonal antibody (mAb). Samples were subjected to SDS-PAGE as described in Fig. 1.

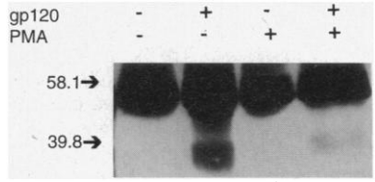

Fig. 3. The 45-kD protein is down-modulated by PMA. A2.01.CD4.401 cells were treated with PMA (100 ng/ml, Sigma) or with control medium for 3 hours at 37°C. The cells were washed extensively, biotinylated, and incubated with gp120 for 2 hours. Cell lysis and immunoprecipitation with OKT4 ascites were conducted as described in Fig. 1, except that the eluates were run on long gels for a longer time to allow good resolution between the 45-kD band and the truncated CD4 molecules.

ble 1) by incubation of the cells with gp120 and then with PMA. In contrast, if the cells were first treated with PMA, no down-modulation was observed. Similar data were obtained with the human cell line A2.01.CD4.401 (Table 1) (*5*). The effect of PMA pretreatment can be explained by depletion of fusin from the cell surface before gp120 addition, as demonstrated in Fig. 3. The direct role played by fusin in the down-modulation of CD4.401 molecules was further examined with anti-fusin rabbit IgG (*6*). Anti-fusin IgG at 10 μg/ml partially blocked the down-modulation of CD4.401 molecules from cells treated with gp120 and PMA (Table 2). In parallel experiments, this anti-fusin IgG (but not preimmune IgG) blocked syncytium formation between A2.01.CD4.401 cells and 12E1-vPE16 (expressing the IIIB envelope) cells by 67% (Table 2). These findings suggest that the NH_2-terminus of fusin is directly involved in the intermolecular interactions required for the co–down-modulation of CD4 and for the HIV-1 envelope fusion process. The incomplete blocking may be explained by the specificity of the serum used. Other extracellular domains of fusin may contribute to its HIV-1 coreceptor function, as was shown for binding of interleukin-8 to its receptor (*8*).

These data suggest that after binding of monomeric gp120, CD4.401 molecules associate (either directly or indirectly) with fusin. This association may occur in membrane compartments (microdomains), rendering them susceptible to PMA-induced down-modulation, probably via clathrin-coated pits as previously described (*5*). This study provides evidence that fusin is recruited into a molecular complex with CD4-gp120 at the cell surface, as we previously postulated (*5*, *9*). This association may stabilize the post-binding conformational state of the gp120-41 envelope required for exposure of the gp41 hydrophobic NH_2-terminus and its insertion into the target membrane.

The biological and biochemical approaches described here can be used to determine if similar interactions occur with the coreceptors for macrophage-tropic virus strains (such as CCR-3 and CCR-5). They can also be applied for further elucidation of the regions in gp120, CD4, and fusin that are directly involved in these interactions. It has been predicted that the V3 loop in gp120 is involved in these interactions (*1*–*3*). However, a direct contact between surface CD4 and fusin most likely occurs at low frequency in the absence of gp120 and is significantly increased after gp120 binding, as suggested by our findings. Such studies will assist in the rational design of agents capable of blocking the association between fusin (or other coreceptors) and the CD4-gp120 complex without affecting the normal function of these molecules.

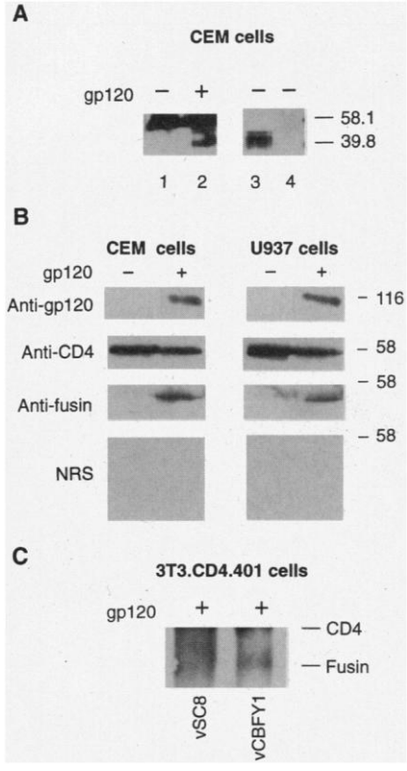

Fig. 4. Antibody to fusin recognizes a 45-kD protein that coprecipitates with CD4 after gp120 pretreatment of cells. (**A**) Surface-biotinylated CEM cells were treated without (lane 1) or with (lane 2) gp120 and lysed in lysis buffer containing 1% Brij 97. Samples were precipitated with OKT4 linked to protein G–Sepharose beads and processed as in Fig. 1. On the same gel, nonbiotinylated whole-cell extracts from cells infected overnight with vCBYF1 (lane 3) or control vSC8 (lane 4) were run in parallel. Vaccinia-infected cells were lysed at a concentration of 5×10^6 cells per milliliter with buffer containing 1% NP-40, 150 mM NaCl, 10 mM tris-HCl (pH 7.4), and protease inhibitors. After 20 min on ice, nuclei were pelleted by centrifugation at 13,000*g* for 5 min. Cell extracts (10 μl) were mixed with 30 μl of Laemmli sample buffer supplemented with 8 M urea and incubated at 37°C overnight, then boiled for 3 min. Blots were reacted with rabbit polyclonal antiserum to fusin (*6*) at a 1:500 dilution (lanes 3 and 4) or with normal rabbit serum (no reactivity) (*11*), followed by HRP-conjugated goat anti-rabbit IgG. The blots were incubated with ultra supersignal chemiluminescent substrate (Pierce) for 5 min and exposed to film. (**B**) Unlabeled CEM and U937 cells were incubated with gp120 (10 μg/ml for 2 hours at 37°C) or in control medium. Cell lysates were immunoprecipitated with OKT4 antibodies covalently linked to protein G–Sepharose beads. Eluted samples were concentrated, electrophoresed (1×10^8 cell equivalent per lane), and blotted onto nitrocellulose membranes. Blots were reacted with either rabbit polyclonal antiserum to gp120 (Intracel, MA), polyclonal antiserum to CD4, rabbit anti-fusin IgG (*6*), or normal rabbit IgG, followed by horse HRP-conjugated goat anti-rabbit or anti-mouse IgG (Amersham). The blots were incubated with ultra supersignal substrate for 5 min and exposed to film. All the protein immunoblots shown are from the same immunoprecipitates that were run in adjacent wells on the same gel. The experiment was repeated four times. (**C**) Fusin can be coprecipitated with CD4.401 molecules from the surface of 3T3.CD4.401 cells infected with vCBYF1. 3T3.CD4.401 cells were infected overnight with vCBYF1 or control vaccinia vSC8 at 10 plaque-forming units (PFU) per cell. Infected cells were biotinylated (1.2×10^7 per milliliter), treated with gp120 (10 μg/ml for 2 hours at 37°C), and lysed in buffer containing 1% Brij 97 (5×10^6 cells per milliliter). Immunoprecipitation with OKT4 mAb and processing of the eluted samples were done as in Fig. 3, except that 5×10^6 cell equivalents were loaded per lane on long 10% SDS gels. The experiment was repeated three times.

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6. Two synthetic peptides corresponding to the predicted extracellular NH_2-terminus of fusin were synthesized by standard fluorenyl methoxycarbonyl chemistry: a 15–amino acid (MEGISIYTSDNYTEE) (*10*) branched peptide [MAP-8, according to J. P. Tam, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5409 (1988)] and a 38–amino acid linear peptide (MEGISIYTSDNYTEEMGSGDYDSMKEPCFREENANFNK) (*10*). The linear peptide contained an additional cysteine at the NH_2-terminus and was purified by reversed-phase high-performance liquid chromatography (HPLC). The purified peptides were characterized by analytical HPLC, capillary electrophoresis, mass spectrometry analysis, and NH_2-terminal amino acid sequence analysis. The purified peptide was conjugated to keyhole limpet hemocyanin (KLH) through the NH_2-terminal cysteine residue, using *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester as the cross-linking agent. New Zealand White rabbits were immunized with the branched peptide or with KLH linear peptide at 1 mg per dose, emulsified in complete Freund's adjuvant followed by two to three boosts (3 weeks apart) in incomplete Freund's adjuvant. Immune and preimmune IgG fractions were prepared by two rounds of saturated ammonium sulfate precipitations.
7. Anti-fusin polyclonal IgG (*6*) was preincubated for 2 hours with a 50-fold molar excess of peptide derived from the NH_2-terminus of fusin or with a control peptide derived from the NH_2-terminus of CCR-5. These peptide-treated immune IgGs were used in protein immunoblots. Complete blocking of binding to the fusin band was obtained with the fusin-derived peptide. The CCR-5–derived peptide had no blocking

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10. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
11. C. K. Lapham *et al.*, data not shown.
12. Human A2.01.CD4.401 and mouse 3T3.CD4.401 cell lines expressing tailless CD4 molecules were produced in the laboratory of D. Littman. We thank C. C. Broder for helpful discussions, C. C. Broder and E. A. Berger for providing us with vCBFY1, R. Blackburn for the generation of rabbit immune antisera to fusin and purified IgG, J. Manischewitz for cell line propagation and vaccinia virus infections, and B. Golding and K. Peden for critical review of the manuscript.

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Tat-SF1: Cofactor for Stimulation of Transcriptional Elongation by HIV-1 Tat

Qiang Zhou and Phillip A. Sharp*

Tat may stimulate transcriptional elongation by recruitment of a complex containing Tat-SF1 and a kinase to the human immunodeficiency virus–type 1 (HIV-1) promoter through a Tat-TAR interaction. A complementary DNA for the cellular activity, Tat-SF1, has been isolated. This factor is required for Tat trans-activation and is a substrate of an associated cellular kinase. Cotransfection with the complementary DNA for Tat-SF1 specifically modulates Tat activation. Tat-SF1 contains two RNA recognition motifs and a highly acidic carboxyl-terminal half. It is distantly related to EWS and FUS/TLS, members of a family of putative transcription factors with RNA recognition motifs that are associated with sarcomas.

Tat activation of HIV-1 transcription is mechanistically different from conventional DNA sequence-specific transcription factors. Most activators affect transcription by increasing the rate of initiation, although some DNA sequence-specific transcription factors such as GAL4-VP16 stimulate both initiation and elongation (*1*). In contrast, Tat predominantly stimulates elongation (*2*). Whereas most activators interact with promoter or enhancer DNA, Tat interacts with the trans-acting responsive (TAR) RNA element (*2*). Located at the 5′ end of the nascent viral transcript, TAR forms a stem-loop structure. The specific binding of Tat to TAR is dependent on the bulge loop and immediately flanking sequences in the double-stranded RNA. Sequences in the apical loop of TAR are also important for Tat activation of transcription in vivo (*3*).

Mechanisms regulating the efficiency of elongation by RNA polymerase II have not been extensively studied. The necessity for control of elongation is highlighted by the finding that an elongation factor, Elongin, is probably the functional target of the von Hippel–Lindau tumor suppressor protein (*4*–*6*). Furthermore, regulation of elongation by Tat is essential for HIV replication. We have used the Tat trans-activation system to characterize cellular cofactors critical for Tat activation of elongation.

Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

*To whom correspondence should be addressed.

We have developed a partially reconstituted transcription reaction that supports a Tat-specific and TAR-dependent activation of HIV transcription (*7*) (Fig. 1A). This reaction requires a Tat-SF (Tat stimulatory factor) activity that is specific for Tat stimulation of elongation, a phosphocellulose 0.5 to 1.0 M KOAc fraction of HeLa nuclear extract (the pc-D fraction), and the purified basal factors TFIID, TFIIA, and transcription factor Sp1. Reactions with these components, but without Tat-SF activity, support activation by Sp1 and GAL4-VP16 (*7*) but not by Tat (Fig. 1A). With the inclusion of a partially purified Tat-SF fraction, Tat increased the number of transcripts elongating beyond 1000 nucleotides from an HIV-1 promoter containing the wild-type TAR element (pHIV+TAR-G400) (*7*), but not from an internal control promoter with a mutant TAR (pHIVΔTAR-G100) (Fig. 1A). The pc-D fraction contains the basal transcription factors TFIIB, TFIIE, TFIIF, TFIIH, and RNA polymerase II (*7*). Because pc-D cannot be substituted for by highly purified basal transcription factors (*8*), it probably contains other activities necessary for Tat function. With the use of this reconstituted reaction, Tat-SF was further purified (*9*).

Phosphorylation of RNA polymerase II has been implicated in regulation of the processivity of elongation (*10*). To investigate whether protein phosphorylation might be associated with Tat-SF, we examined proteins absorbed on immobilized HIV TAR RNA from a reconstituted transcription reaction in the presence of [γ-^{32}P]ATP (adenosine triphosphate) (Fig. 1B). In reactions with either the pc-D fraction or the Tat-SF fraction alone, addition of Tat did not consistently affect the phosphorylation of proteins on immobilized TAR. When both fractions were incubated together in the presence of Tat, phosphorylation of a protein of ~140 kD, termed pp140, was observed (Fig. 1B). In the absence of Tat,

Table 1. The effect of Tat-SF1 overexpression on Tat and VP16 trans-activation. Tat-SF1 gene was cloned into the mammalian expression vector pSV7d (*28*) to create pSV-Tat-SF1. pSV-Tat-SF1 or pSV7d and a reporter construct pBennCAT (*29*) containing HIV-1 LTR linked to the bacterial CAT gene (1 μg each) and an internal control plasmid pCMVβ-Gal were cotransfected into HeLa cells, either in the presence or absence of a Tat-expressing plasmid pcTat (0.3 μg) (*30*). CAT activity was measured 48 hours later as described (*31*). In control experiments, pSV-Tat-SF1 or pSV7d and the reporter construct pMyc3E1BLuc (*18*) were introduced into HeLa cells together with the plasmids pRCCMV-TFEB-VP16 (0.3 μg) expressing the TFEB-VP16 fusion protein (*18*). pMyc3E1BLuc contained the luciferase gene downstream of the adenovirus E1B promoter with three binding sites for TFEB. Reporter construct pG5E1BCAT (*19*) containing five GAL4-binding sites inserted upstream of the E1B promoter and the CAT gene was used to assay GAL4-VP16 trans-activation.

Exp.	Vector			Tat-SF1			Fold enhancement†
	−*	+	Fold act.	−	+	Fold act.	
Tat							
1	100	7,228	72.3	31.7	7,699	242.9	3.36
2	100	15,779	157.8	17.0	16,548	973.4	6.17
3	100	4,899	49.0	35.3	10,353	293.3	5.99
TFEB-VP16							
1	100	30,229	302.3	118	20,782	176.1	0.58
2	100	18,241	182.4	179	15,080	84.2	0.46
GAL4-VP16							
1	100	132,208	1,322	95.0	129,960	1,368	1.03

*CAT or luciferase activity measured in cells transfected with the empty vector plus the reporter plasmid only were normalized to a value of 100. Values shown in the second, fourth, and fifth columns were adjusted accordingly. †The fold enhancement represents the fold activation by Tat or VP16 observed in cells expressing Tat-SF1 divided by the fold activation in cells containing the empty vector.