Nuclear Receptor Coactivator p160 Proteins Enhance the HIV-1 Long Terminal Repeat Promoter by Bridging Promoter-bound Factors and the Tat-P-TEFb Complex*

Received for publication, July 6, 2001, and in revised form, September 27, 2001
Published, JBC Papers in Press, November 9, 2001, DOI 10.1074/jbc.M106312200

Tomoshige Kino‡§, Olga Slobodskaya¶, George N. Pavlakis¶, and George P. Chrousos‡

From the ¶Pediatric and Reproductive Endocrinology Branch, NICHD, National Institutes of Health, Bethesda, Maryland 20892 and the §§Human Retrovirus Section, Center for Cancer Research, National Cancer Institute-Frederick, Frederick, Maryland 21702

We report that p160 nuclear receptor coactivators potentiate the transactivating activity of Tat, the most potent virally encoded transactivator of HIV-1. One of the p160 proteins (GRIP1) is tethered to the HIV-1 long terminal repeat (LTR) through κB-responsive elements, most likely via NF-κB, with which it also associates through its coactivator motifs (LXXLL motifs, “NR boxes”). Indeed, the Tat-stimulated κB-defective HIV-1 LTR had a markedly impaired response to GRIP1, whereas NR box-defective GRIP1 proteins lost part of their Tat coactivator effect on the HIV-1 LTR. Through its N-terminal basic helix-loop-helix and C-terminal domains, GRIP1 binds to the N-terminal region of Tat and to the host cell protein cyclin T1, respectively, which is normally complexed with CDK9 as P-TEFb. Thus, NF-κB is crucial for tethering p160 coactivator molecules to the HIV-1 LTR, allowing full activation of this promoter by Tat. Interestingly, cotransfection of Tat, GRIP1, and cyclin T1 enhanced not only the activity of the HIV-1 LTR, but also the glucocorticoid receptor-mediated stimulation of the mouse mammary tumor virus (MMTV) promoter, suggesting that Tat can also attract the P-TEFb complex to the MMTV LTR through GRIP1. Thus, it appears that the coactivator complexes of the HIV-1 and MMTV LTRs both include p160 coactivators and use similar coactivator and elongation complexes for their transcription. Tat may function as an adaptor molecule, efficiently stimulating the processes of transcription initiation and elongation through potentiation of the coupling of p160 coactivators and the P-TEFb complex.

Human immunodeficiency virus type 1 (HIV-1) transcription and replication are strongly stimulated by the unique transcriptional activator of this virus, Tat (1, 2). Tat, an 86-amino acid HIV-1-encoded protein, is tethered to the HIV-1 long terminal repeat (LTR) by binding to a small transcribed viral RNA stem-loop structure called TAR (transactivation response region) (2, 3). The transactivation domain of Tat, localized in the N-terminal region of the molecule, strongly interacts with cyclin T1, a component of P-TEFb (positive-acting transcription elongation factor-b), which also contains CDK9 (cyclin-dependent kinase-9) or PIALRE (4, 5). This complex phosphorylates the C-terminal domain of the large subunit of RNA polymerase II and is required for the elongation of transcription of many genes (6). Thus, Tat enhances transcription by tethering P-TEFb to the HIV-1 LTR and by facilitating the transcription elongation step.

Coactivators play an essential role in the transactivating activity of many transcription factors (7). They accomplish this by transducing transcription signals to the transcription initiation complex and to downstream transcription machinery. Coactivators also possess histone acetyltransferase activity, by which they loosen DNA revolutions around nucleosomes (7, 8) and enhance transcription by increasing accessibility of other transcription factors and machinery to the promoter region. Among them, one family of coactivator molecules consisting of the homologous p300 and CBP (cAMP-responsive element-binding protein-binding protein) may serve as macromolecular docking “platforms” for transcription factors from several signal transduction cascades, including nuclear receptors, cAMP-responsive element-binding protein, AP-1, NF-κB, p53, Ras-dependent growth factor, and the STAT (signal transducer and activator of transcription) proteins (8–17). Because of their central position in many signal transduction cascades, the p300/CBP coactivators have also been called co-integrators. pCAF, originally reported as a human homolog of yeast Gcn5 that interacts with p300/CBP, is also a broad coactivator with histone acetyltransferase activity (8, 18, 19). Recently, Tat was shown to interact with p300/CBP and pCAF, which play an important role in the transactivation of the HIV-1 LTR. p300 enhances the transcription of the HIV-1 LTR incorporated in the host genome (20–22). They also modulate the binding affinity of Tat for TAR and cyclin T1 by acetylating Tat at specific lysine residues.

Coactivator molecules interacting preferentially with nuclear receptors have been described and include members of the p160 family of proteins: SRC1 (steroid receptor coactivator-1); TIF-II (transcription intermediary factor) or GRIP1 (glucocorticoid receptor-interacting polypeptide-1), also called SRC2; and p/CIP (p300/CBP/co-integrator-associated protein), activator of thyroid receptor, or RAC3, also called SRC3 (7, 8, 23). They contain one or more copies of the coactivator signature motif sequence LXXLL, which is necessary for interaction with the nuclear hormone receptors (8, 16, 23, 24). On the nuclear receptor-responsive promoters, p300/CBP, p/CIP, and p160 coactivators form complexes via their mutual interaction domains and activate the downstream transcription cascade. Among them, p160 coactiva-
tors are first tethered to the promoter region and play a central role in efficiently attracting p300/CBP and possibly p/CAF to the transcriptosome (25). Vpr potentiates Tat-enhanced HIV-1 LTR activity (26–29). Also, we have shown that Vpr markedly potentiates glucocorticoid receptor action on glucocorticoid-responsive element-containing promoters such as the mouse mammary tumor virus (MMTV) LTR, acting as a nuclear receptor coactivator (30). Like p300/CBP and the p160 coactivators, Vpr contains the coactivator signature motif LXXLL at amino acids 64–68. Vpr binds to p300 at the region between amino acids 2045 and 2191, which also interacts with p160 coactivators, and efficiently

**A: Jurkat Cells**

**HIV-LTR**

|        | Luciferase Activity (RLU) |
|--------|---------------------------|
| (-)    |                           |
| Tat (-) |                           |
| Tat (+) |                           |
| SRC1a  | *                         |
| GRIP1  | *                         |
| p/CIP  | *                         |

**MMTV-LTR**

|        | Luciferase Activity (RLU) |
|--------|---------------------------|
| (-)    |                           |
| Dexamethasone (-) |                     |
| Dexamethasone (+) |                     |
| SRC1a  | *                         |
| GRIP1  | *                         |
| p/CIP  | *                         |

**B: HeLa Cells**

**HIV-LTR**

|        | Luciferase Activity (RLU) |
|--------|---------------------------|
| (-)    |                           |
| Tat (-) |                           |
| Tat (+) |                           |
| SRC1a  | *                         |
| GRIP1  | *                         |
| p/CIP  | *                         |

**MMTV-LTR**

|        | Luciferase Activity (RLU) |
|--------|---------------------------|
| (-)    |                           |
| Dexamethasone (-) |                     |
| Dexamethasone (+) |                     |
| SRC1a  | *                         |
| GRIP1  | *                         |
| p/CIP  | *                         |

**C: A204 Cells**

**HIV-LTR**

|        | Luciferase Activity (RLU) |
|--------|---------------------------|
| (-)    |                           |
| Tat (-) |                           |
| Tat (+) |                           |
| SRC1a  | *                         |
| GRIP1  | *                         |
| p/CIP  | *                         |

**MMTV-LTR**

|        | Luciferase Activity (RLU) |
|--------|---------------------------|
| (-)    |                           |
| Dexamethasone (-) |                     |
| Dexamethasone (+) |                     |
| SRC1a  | *                         |
| GRIP1  | *                         |
| p/CIP  | *                         |

Fig. 1. p160 coactivators SRC-1a, GRIP1, and p/CIP differently increase the Tat-stimulated HIV-1 LTR and the dexamethasone-stimulated MMTV LTR in Jurkat (A), HeLa (B), and A204 (C) cells. Jurkat, HeLa, and A204 cells were transfected with the indicated coactivator-expressing plasmids: pSG5-SRC1a, pSG5-GRIP1-fl or pSG5-p/CIP, or pSG5 with L3-Luc or MMTV-LTR-Luc, and pSV40-β-gal. Bars show the means ± S.E. of the luciferase activity normalized for β-galactosidase activity in the absence or presence of Tat or dexamethasone. *, p < 0.01, in comparison with the base line; RLU, relative luciferase units.
G. L. Hager (National Institutes of Health, Bethesda, MD). (the full-length MMTV LTR promoter, which contains four glucocorticoid-responsive element (GRE)-containing promoter, and βB-responsive promoter in HeLa cells. HeLa cells were transfected with increasing amounts of pSG5-GRIP1-Ⅲ, HIV-LTR-Luc or mB-HIV-LTR-Luc, and pSV40-β-gal. Bars show the means ± S.E. of the chloramphenicol acetyltransferase activity normalized to β-galactosidase activity in the absence or presence of Tat or dexamethasone. *, p < 0.01, in comparison with the base line. B, NR box-mutated GRIP1 molecules differentially modulate the HIV-1 LTR, glucocorticoid-responsive element (GRE)-containing promoter, and βB-responsive promoter in HeLa cells. HeLa cells were transfected with pSG5-GRIP1-WT or NR box mutant GRIP1 expression vectors; L3-Luc (HIV-1-LTR), MMTV-LTR-Luc (GRE-Promoter), or (βB)3-Luc (βB-Promoter); and pSV40-β-gal. Bars show the means ± S.E. of the luciferase activity normalized to β-galactosidase activity in the absence or presence of Tat, NF-κB, or dexamethasone. *, p < 0.01, in comparison with the base line; WT, wild-type; RLU, relative luciferase units.

FIG. 2. A, GRIP1 partially loses its enhancing effect on the βB-defective HIV-1 LTR in HeLa cells. HeLa cells were transfected with increasing amounts of pSG5-GRIP1-Ⅲ, HIV-LTR-Luc or mB-HIV-LTR-Luc, and pSV40-β-gal. Bars show the means ± S.E. of the chloramphenicol acetyltransferase activity normalized to β-galactosidase activity in the absence or presence of Tat or dexamethasone. *, p < 0.01, in comparison with the base line. B, NR box-mutated GRIP1 molecules differentially modulate the HIV-1 LTR, glucocorticoid-responsive element (GRE)-containing promoter, and βB-responsive promoter in HeLa cells. HeLa cells were transfected with pSG5-GRIP1-WT or NR box mutant GRIP1 expression vectors; L3-Luc (HIV-1-LTR), MMTV-LTR-Luc (GRE-Promoter), or (βB)3-Luc (βB-Promoter); and pSV40-β-gal. Bars show the means ± S.E. of the luciferase activity normalized to β-galactosidase activity in the absence or presence of Tat, NF-κB, or dexamethasone. *, p < 0.01, in comparison with the base line. B, wild-type; RLU, relative luciferase units.

Attracts p300 to the promoter, similar to p160 coactivators (30).2 Interestingly, Vpr also enhances the HIV-1 LTR activity using its LXXLL motif and p300-binding site, suggesting that Vpr enhances both the glucocorticoid-responsive promoter and the HIV-1 LTR through a similar mechanism.2 Therefore, we examined the possibility that the HIV-1 LTR employs a similar coactivator complex for its transactivation as a glucocorticoid-responsive promoter. Here, we show that a host nuclear receptor coactivator p160 protein functions as a coactivator of Tat on the HIV-1 LTR, bridging promoter-bound proteins and the Tat-P-TEFb complex.

MATERIALS AND METHODS

Plasmids—BS-Tat contains Tat cDNA, which encodes amino acids 1–86 (two exons) of Tat. L3-Luc possesses the full-length HIV-1 LTR promoter, which drives the luciferase reporter gene. HIV-LTR-Luc and mB-HIV-LTR-Luc, which possess the luciferase gene under the control of the wild-type HIV-1 LTR or an HIV-1 LTR mutant with its two βB sites deleted, respectively, were kind gifts from Dr. M. K. Calame (Columbia University, New York). MMTV-LTR-Luc contains the full-length MMTV LTR promoter, which contains four glucocorticoid-responsive element sites, and was a generous gift from Dr. G. L. Hager (National Institutes of Health, Bethesda, MD). (βB)3-Luc, which contains three synthetic βB-responsive elements upstream of the luciferase gene, was a kind gift from Dr. S. Miyamoto (University of Wisconsin, Madison, WI). pGEX-2T-Tat-(1–86), pGEX-2T-Tat-(22–86), and pGEM2-Tat were donated by the National Institutes of Health AIDS Research and Reference Reagent Program (Rockville, MD). pm-Tat-(1–72), pGEX-4T3-Tat-(1–72), pGEX-4T3-Tat-(1–40), pGEX-4T3-Tat-(22–61), and pGEX-4T3-Tat-(41–72) were constructed by subcloning PCR-amplified cDNA fragments of the corresponding polypeptides into pM (CLONTECH, Palo Alto, CA) or pGEX-4T3 (Amersham Biosciences, Inc.) using L3-Tat (which encodes amino acids 641–645 with alanines using the PCR-assisted mutagenesis reaction with pGS5-GRIP1-WT and pGS5-GRIP1-NRB-IIm, which express wild-type GRIP1 and the corresponding NR (nuclear receptor-binding) box-defective GRIP1 proteins, were generous gifts from Dr. M. R. Stallcup (University of Southern California, Los Angeles). pSG5-GRIP1-Ⅲ, which express wild-type GRIP1 and the corresponding NR (nuclear receptor-binding) box-defective GRIP1 proteins, were generous gifts from Dr. D. H. Price (University of Iowa, Iowa City, IA). pGEX-4T3-cyclin T1-(1–255), pcDNA3-HA-cyclin T2a, and pcDNA3-HA-cyclin T2b were kind gifts from Dr. D. H. Price (University of Iowa, Iowa City, IA). pGEX-4T3-cyclin T1-(1–255),

2 T. Kino, A. Gragerov, G. P. Chrousos, and G. N. Pavlakis, submitted for publication.
p160 is a Coactivator of the HIV-1 LTR

p160 Coactivators Potentiate the Tat-stimulated HIV-1 LTR in Three Cell Lines—Vpr potentiates both the Tat-stimulated HIV-1 LTR and the dexamethasone-stimulated MMTV LTR by efficiently attracting p300 through two distinct domains, an LXXLL motif and a p300-binding site. This prompted us to examine the contribution of p160 coactivators, which also contain LXXLL motifs and a p300-binding site, to the Tat-stimulated HIV-1 LTR activity by 3–5-fold depending on the cell lines and coactivators employed. Of the three coactivators, GRIP1 showed the most potent effect on the HIV-1 LTR. As expected, these coactivators similarly increased dexamethasone-stimulated MMTV LTR activity in the same cell lines (Fig. 1, A–C, right panels), suggesting that the HIV-1 LTR requires coactivators for its transcriptional activation, similar to the glucocorticoid-responsive MMTV LTR.

Contribution of NF-κB-responsive Elements of the HIV-1 LTR and Nuclear Receptor-binding Domains of GRIP1—p160 coactivators interact with the p50 subunit of NF-κB and enhance its responsive promoters (33, 34). Because the HIV-1 LTR contains two NF-κB-responsive elements, we examined the possibility that p160 coactivators are attracted to the HIV-1 LTR through NF-κB. In HeLa cells, GRIP1 partially lost its enhancing effect on a κB-mutated HIV-1 LTR, suggesting that κB sites are in part necessary for the attraction of GRIP1 (Fig. 2A). We tested the contribution of nuclear receptor-binding sites of p160 coactivators to the HIV-1 LTR by employing four GRIP1 mutants defective in one or all of the LXXLL motifs. GRIP1 proteins defective in NR boxes II and III partially lost their enhancing effect on the Tat-stimulated wild-type HIV-1 LTR in HeLa cells, whereas the NR box I mutant showed derepression. The GRIP1 mutant defective in all three NR boxes showed a partial effect (Fig. 2B). We compared the activities of these GRIP1 NR box mutants on the HIV-1 LTR with those on the MMTV LTR and a κB-responsive promoter to further investigate the mechanism of contribution. All NR box-mutated GRIP1 mutants dramatically lost their enhancing effect on the MMTV LTR, whereas they similarly lost some of their effect on the HIV-1 LTR, suggesting that NR boxes of GRIP1 are functional regarding its effect on the HIV-1 LTR, possibly through the transcriptional activity of NF-κB (Fig. 2B).

Tat Binds to p160 Coactivators in Vivo—Because p160 coactivators strongly enhanced the Tat-stimulated HIV-1 LTR, we next examined the possibility that Tat and p160 coactivators form complexes in human cells. Because Tat is known to associate with cyclin T1, we also examined the involvement of this protein in the complex. We transfected cells with vectors expressing Gal4 DNA-binding domain-fused Tat and hemagglutinin-tagged cyclin T1 and precipitated a potential complex using anti-Gal4 or anti-hemagglutinin antibody (Santa Cruz Biotechnology, Santa Cruz, CA) bound to protein A/G-agarose (Santa Cruz Biotechnology). After blotting on nitrocellulose membrane, Gal4-fused Tat or hemagglutinin-tagged cyclin T1 was visualized using anti-Gal4 or anti-hemagglutinin antibody (Santa Cruz Biotechnology), respectively.

In Vitro Binding Assay—S-Labeled SRC1a, pCIP, wild-type and mutant GRIP1 proteins, Tat, cyclin T1, and glucocorticoid receptor-α were generated in vitro translation using pSG5-SRC1a, pCMX/pCIP, pSG5-GRIP1, pGEM2-Tat, pDNA3-HA-cyclin T1, and pGR107 as templates, respectively. Interaction was tested with GST-fused Tat, GRIP, and cyclin T1 fragments or GST protein immobilized on glutathione-Sepharose beads in buffer containing 50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 1 mM EDTA, 0.1% Nonidet P-40, 10% glycerol, and 0.1 mg/ml bovine serum albumin at 4 °C for 1.5 h. For Tat or GST-Tat, interactions were tested in buffer containing 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA, 0.1% Nonidet P-40, 10% glycerol, 0.1 mg/ml bovine serum albumin, and 1 mM dithiothreitol. After vigorous washing with the buffer, proteins were eluted and separated on an SDS-polyacrylamide gel. Gels were fixed, exposed to Enlightening (PerkinElmer Life Sciences) for 30 min, dried up, and exposed on films.

RESULTS

p160 Coactivators Potentiate the Tat-stimulated HIV-1 LTR in Three Cell Lines—Vpr potentiates both the Tat-stimulated HIV-1 LTR and the dexamethasone-stimulated MMTV LTR by efficiently attracting p300 through two distinct domains, an LXXLL motif and a p300-binding site. This prompted us to examine the contribution of p160 coactivators, which also contain LXXLL motifs and a p300-binding site, to the Tat-stimulated HIV-1 LTR activity by 3–5-fold depending on the cell lines and coactivators employed. Of the three coactivators, GRIP1 showed the most potent effect on the HIV-1 LTR. As expected, these coactivators similarly increased dexamethasone-stimulated MMTV LTR activity in the same cell lines (Fig. 1, A–C, left panels), suggesting that the HIV-1 LTR requires coactivators for its transcriptional activation, similar to the glucocorticoid-responsive MMTV LTR.

Contribution of NF-κB-responsive Elements of the HIV-1 LTR and Nuclear Receptor-binding Domains of GRIP1—p160 coactivators interact with the p50 subunit of NF-κB and enhance its responsive promoters (33, 34). Because the HIV-1 LTR contains two NF-κB-responsive elements, we examined the possibility that p160 coactivators are attracted to the HIV-1 LTR through NF-κB. In HeLa cells, GRIP1 partially lost its enhancing effect on a κB-mutated HIV-1 LTR, suggesting that κB sites are in part necessary for the attraction of GRIP1 (Fig. 2A). We tested the contribution of nuclear receptor-binding sites of p160 coactivators to the HIV-1 LTR by employing four GRIP1 mutants defective in one or all of the LXXLL motifs. GRIP1 proteins defective in NR boxes II and III partially lost their enhancing effect on the Tat-stimulated wild-type HIV-1 LTR in HeLa cells, whereas the NR box I mutant showed derepression. The GRIP1 mutant defective in all three NR boxes showed a partial effect (Fig. 2B). We compared the activities of these GRIP1 NR box mutants on the HIV-1 LTR with those on the MMTV LTR and a κB-responsive promoter to further investigate the mechanism of contribution. All NR box-mutated GRIP1 mutants dramatically lost their enhancing effect on the MMTV LTR, whereas they similarly lost some of their effect on the HIV-1 LTR, suggesting that NR boxes of GRIP1 are functional regarding its effect on the HIV-1 LTR, possibly through the transcriptional activity of NF-κB (Fig. 2B).

Tat Binds to p160 Coactivators in Vivo—Because p160 coactivators strongly enhanced the Tat-stimulated HIV-1 LTR, we next examined the possibility that Tat and p160 coactivators form complexes in human cells. Because Tat is known to associate with cyclin T1, we also examined the involvement of this protein in the complex. We transfected cells with vectors expressing Gal4 DNA-binding domain-fused Tat and hemagglutinin-tagged cyclin T1 and precipitated a potential complex using specific antibodies against GRIP1 or Gal4. The precipitates were electrophoresed on SDS-polyacrylamide gels, and proteins were visualized by probing with the indicated antibodies. Tat and cyclin T1 were co-immunoprecipitated by the anti-GRIP1 antibody (Fig. 3), indicating that these proteins form a complex with GRIP1 in HeLa cells.

To further examine Tat/p160 coactivator binding, we next used a GST pull-down assay. In vitro translated and labeled p160 coactivators SRC1a, GRIP1, and pCIP all bound to bacterially produced GST-fused Tat protein (Fig. 4A). We constructed GST-fused GRIP1 fragment-expressing plasmids to localize the binding site of Tat on this molecule (Fig. 4B). We also examined the binding of cyclin T1 to GRIP1 fragments. Tat interacted strongly with GRIP1(1–301) and weakly with GRIP1(740–1217). Cyclin T1 also interacted with GRIP1(1–301) and GRIP1(1101–1462), but not with GRIP1(740–1217). Neither Tat nor cyclin T1 bound to the domains of GRIP1 that interact with nuclear receptors (Fig. 4C) (2, 4). As positive controls, we examined the interactions of dexamethasone-bound glucocorticoid receptor-α and GRIP1. The former bound
FIG. 4. *In vitro* binding of p160 coactivators and Tat or cyclin T1. A, SRC1a, GRIP1, and p/CIP bind to Tat *in vitro*. *In vitro* translated and labeled SRC1a, GRIP1, and p/CIP were incubated with bacterially produced GST-Tat(1–86) or GST to test their interaction with this protein. Samples were run on a 6% SDS-polyacrylamide gel and detected by autoradiography. B, localization of functional domains in a linearized GRIP1 molecule and the fragments of GRIP1 fused with GST employed to localize the domain of GRIP1 that interacts with Tat. bHLH, basic helix-loop-helix; PAS, Period/aryl hydrogen receptor/single-minded; NRB, nuclear receptor-binding box. C, Tat and cyclin T1 bind to specific GRIP1 fragments *in vitro*, suggesting interactions with both the N- and C-terminal regions of the molecule. *In vitro* translated and labeled Tat, cyclin T1, and glucocorticoid receptor-H9251 (GR-H9251) were incubated with bacterially produced GST-fused GRIP1 fragments or GST. The samples were run on 16, 10, and 8% SDS-polyacrylamide gels, respectively, and detected by autoradiography. Dexamethasone (10^{-5} M) was added to the reaction for glucocorticoid receptor-a. D, Tat and cyclin T1 bind to GRIP1(1–97) *in vitro*, suggesting that the coactivator's first 100 amino acids are necessary for these interactions. *In vitro* translated and labeled Tat and cyclin T1 were incubated with bacterially produced GST-fused GRIP1 fragments or GST. The samples were run on 16 and 10% SDS-polyacrylamide gels, respectively, followed by autoradiography.
to GRIP1-(596–774) strongly and to GRIP1-(740–1217) weakly, as previously reported (35–38). Because GRIP-(1–301) interacts strongly with both Tat and cyclin T1, we determined a narrower region containing the specific binding site(s). Interestingly, both Tat and cyclin T1 efficiently bound to the GRIP1-(1–97) sequence, which has a basic helix-loop-helix polypeptide conformation (Fig. 4D) (7, 23). We also examined their in vitro binding in a mammalian two-hybrid assay and obtained similar results (data not shown).

To determine the Tat- and cyclin T1-specific binding sites for GRIP1, we made a series of GST-Tat and GST-cyclin T1 fragment-expressing plasmids. Fig. 5 (A and B) shows the functional domains of Tat and cyclin T1 and the GST-Tat and GST-cyclin T1 fragment fusion proteins employed, respectively. GRIP1 interacted with Tat-(1–86), Tat-(1–72), and Tat-(1–40) (Fig. 5C), i.e., the N terminus, which also contains its transactivation domain and the binding site for cyclin T1 (Fig. 5A) (2, 4). GRIP1 interacted with cyclin T1-(1–308) and cyclin T1-(1–255), which also contain the domain that binds to the Tat molecule (Fig. 5D) (4).

To examine the in vivo relevance of the in vitro Tat/GRIP1 binding, we examined the effect of GRIP1 fragments defective in binding to Tat and/or cyclin T1 on the Tat-stimulated HIV-1 LTR in HeLa cells (Fig. 6A). GRIP1-(300–1462) defective in its N-terminal portion dramatically lost its enhancing effect on the Tat-stimulated HIV-1 LTR. GRIP1-(1–1217), which did not have its C-terminal portion, however, also partially lost its stimulatory effect. GRIP1-(300–1217), defective in both the N and C termini, became completely inactive for the Tat-stimulated HIV-1 LTR. We next examined the effect of shorter GRIP1 fragments on the GRIP1 enhancement of the Tat-stimulated HIV-1 LTR. GRIP1-(300–640), which did not interact with Tat and cyclin T1, did not show such an effect.

**Involvement of P-TEFb in the Glucocorticoid Transactivation System**—Because GRIP1 interacted with both Tat and cyclin T1 and functioned as a coactivator of Tat on the HIV-1 LTR, we examined the possibility that P-TEFb may be involved in transactivation of the glucocorticoid-responsive promoter. Three different P-TEFb proteins containing three different isoforms of cyclin T (T1, T2a, and T2b) are known, formed by each of these cyclin T proteins and CDK9. All three P-TEFb proteins significantly increased the dexamethasone-stimulated MMTV LTR in HeLa cells (Fig. 7A). Cyclin T2b was the most potent of the cyclin T isoforms on the MMTV LTR. Interestingly, Tat transfection moderately increased the luciferase activity of the dexamethasone-stimulated MMTV LTR, whereas P-TEFb alone did not.

When we cotransfected Tat, P-TEFb components cyclin T1 and CDK9, and GRIP1 together, we observed strong synergy in increasing dexamethasone-stimulated MMTV LTR activity (Fig. 7C). These results indicate that Tat and P-TEFb function as enhancer molecules in the glucocorticoid receptor transactivation system, cooperating with p160 coactivators. This also supports the hypothesis...
that Tat modulates the association of p160 coactivators and the P-TEFb complex.

**DISCUSSION**

**Role of p160-type Coactivators in the HIV-1 LTR**—All three subtypes of p160 coactivators, including SRC1a, GRIP1, and p/CIP, enhanced the Tat-stimulated HIV-1 LTR in three different cell lines, functioning as coactivators of the HIV-1 LTR (Fig. 1, A–C). Tat bound to all three p160 coactivators and interacted strongly with the amino-terminal region of GRIP1, which contains a basic helix-loop-helix domain, and to a lesser degree with the GRIP1-(740–1217) region, which contains one of the transactivation domains of GRIP1 (Fig. 4, A, C, and D) (7, 23). Cyclin T1 also bound GRIP1 at the same basic helix-loop-helix domain and moderately at GRIP1-(740–1217) region, which contains one of the transactivation domains of GRIP1 (Fig. 4, A, C, and D) (7, 23). All these GRIP1 fragments were necessary for the Tat enhancement of the HIV-1 LTR (Fig. 6). Because p300 and p/CAF are known to be present in the HIV-1 LTR (20–22, 40), the HIV-1 promoter appears to develop a coactivator complex, which is reminiscent of the classic nuclear receptor coactivator complex formed on the MMTV LTR. Tat/HIV-1 LTR and nuclear receptor-driven promoters also share Tip60 (Tat-interacting protein of 60 kDa), which possesses histone acetyltransferase activity and functions as a coactivator in both promoters (41, 42). Therefore, the extended coactivator complex formed on the HIV-1 or MMTV LTR appears to use similar components and configurations. In the nuclear receptor-responsive promoters, p160 coactivators are first tethered to the promoter via nuclear hormone receptors and play an essential role in attracting other coactivators such as p300/CBP and p/CAF (25). Because p160 coactivators are accumulated on the HIV-1 LTR, they might play a similar role in this promoter. p300 and p/CAF acetylate the Tat molecule, modulating the binding affinity of Tat for TAR and cyclin T1 (40). Because p160 coactivators contain histone acetyltransferase activity, they might also acetylate the Tat molecule and hence regulate its transactivating activity as well.

The *Hin-2* gene (GenBank™/EBI Data Bank accession number U19179), which is the marker of an insertion point of the HIV-1 LTR into the human genomic DNA, encodes a protein almost identical in sequence to amino acids 1102–1462 of the human SRC1a and GRIP1 coactivators. This region of GRIP1 contains the binding site(s) for cyclin T1, necessary for the full activation of the HIV-1 LTR. Therefore, it is possible that HIV-1 might increase the expression of this protein by inserting its LTR in front of its coding sequence. In this situation, the expressed protein could enhance this promoter’s activity, which may be beneficial for viral replication and proliferation.

---

**Fig. 6.** A, GRIP1 fragments defective in the N and/or C terminus lose the enhancing activity of the wild-type protein on the Tat-stimulated HIV-1 LTR in HeLa cells. HeLa cells were transfected with the indicated GRIP fragment-expressing vectors, L3-Luc, and pSV40-β-gal. Bars show the means ± S.E. of the luciferase activity normalized to β-galactosidase activity in the absence or presence of Tat. B, specific GRIP1 fragments compete with wild-type GRIP1 for the enhancement of the Tat-stimulated HIV-1 LTR in HeLa cells. HeLa cells were transfected with the indicated GRIP fragment-expressing vectors (2.0 μg/well) in the presence of 1.0 μg/well pSG5-GRIP1-fl, L3-Luc, and pSV40-β-gal. Bars show the means ± S.E. of the luciferase activity normalized to β-galactosidase activity in the absence or presence of Tat. RLU, relative luciferase units.
GRIP1 is tethered to the HIV-1 LTR partially through NF-kB—

GRIP1 is indirectly tethered to the promoter partially through NF-kB-responsive elements (Fig. 2A). p60, a component of NF-κB, binds p160 coactivators and attracts them to an NF-kB-responsive promoter (33, 34). Thus, it is quite likely that one of the major mechanisms of tethering p160 proteins to the HIV-1 LTR is via NF-kB. However, because the impact of destruction of NF-kB-responsive elements was partial, other as yet unknown factor(s) may also contribute to the attraction of p160 proteins. Because the HIV-1 LTR contains binding sites for several transcription factors in addition to those for NF-kB, one or more of such transcription factors might also bind to and attract p160 proteins.

Involvement of the LXXLL Motif in the Transcriptional Activity of the HIV-1 LTR—The HIV-1 LTR appears to employ the LXXLL motifs of p160 coactivators for its transcriptional activation (Fig. 2B). In our hands, the effect of NR box-defective p160 coactivators on the activity of the HIV-1 LTR was similar to that observed on the κB-defective promoter. We demonstrated that NR boxes II and III positively contributed to transcription, whereas NR box I showed the opposite effect. A previous report suggested the positive involvement of the NR box II LXXLL motif of SRC1 in NF-κB-stimulated transcription (34). The reason for the discrepancy between our results and those of the previous report is not known; however, it may be explained by the difference in the p160 coactivators employed: we used GRIP1, whereas the previous study employed SRC1. Alternatively, it may be because we used a different experimental system. We employed transient expression of GRIP1 mutant molecules, whereas the other study employed microinjection of neutralizing antibody for NR box domains. Although the mechanistic implication of LXXLL in the HIV-1 LTR remains unclear, it is likely that the HIV-1 LTR accumulates as yet unknown protein(s), which may interact with the LXXLL motifs of p160 coactivators. One such candidate protein may be a nuclear receptor. Indeed, several nuclear receptors are known to be associated with the HIV-1 LTR (43–46). Also recently, the glucocorticoid receptor was found in the transcription complex formed on an NF-κB-responsive promoter by a chromatin immunoprecipitation method (47). Because only the heterodimer form of NF-κB, composed of the p65 and p50 subunits, associates with p160 proteins in vivo (34), it is likely that interaction of NF-κB with p160 coactivators at the promoter region is necessary for Tat and/or cyclin T1 to properly regulate the HIV-1 LTR.

GRIP1 Is Important for HIV-1 LTR Transactivation by Tat—p160 coactivators contain two transactivation domains, activa-
p160 is a Coactivator of the HIV-1 LTR

Fig. 8. Schematic models of HIV-1 (A) and MMTV (B) LTR transcriptional activation by p160 coactivators. These models do not show the dynamic sequential interactions between the reactants, which would be essential to explain the presence of the multiple overlapping binding sites that these proteins have for each other. RNPH, RNA polymerase II; CTD, C-terminal domain.

Tat enhances transcription from both the HIV-1 LTR and a glucocorticoid-responsive promoter (Fig. 8). p160 coactivators may provide a binding domain for Tat, functioning as a platform for the regulation of the HIV-1 LTR activity by Tat, TAR, and P-TEFb, and of the MMTV LTR activity by Tat and P-TEFb.

Acknowledgments—We thank Drs. M. R. Stallcup, D. H. Price, K. L. Calame, D. M. Livingston, M. G. Parker, M. G. Rosenfeld, R. M. Evans, and G. L. Hager for kindly providing plasmids; Dr. A. Gragerov for helpful discussion; and K. Zachman and J. Wong for superb technical assistance.

REFERENCES

1. Pavlakis, G. N. (1996) in AIDS: Diagnosis, Treatment and Prevention (DeVita, V. T., Hellman, S., and Rosenberg, S. A., eds) 4th Ed., pp. 45–74, Lippincott Raven, Philadelphia

2. Tang, H., Ruben, K. L., and Wong-Staal, F. (1999) Annu. Rev. Genet. 33, 133–170

3. Feng, S., and Holland, E. C. (1988) Nature 334, 165–167

4. W. P. Garber, M. E., Pang, S. M., Fischer, W. H., and Jones, K. A. (1998) Cell 92, 451–462

5. Fujinaga, K., Cuje, T. P., Peng, J., Garriga, J., Price, D. H., Grana, X., and Peterlin, B. M. (1998) J. Virol. 72, 7154–7159

6. Price, D. H. (2000) Mol. Cell. Biol. 20, 2629–2634

7. McKenna, N. J., Lanz, R. B., and O’Malley, B. W. (1999) Endocr. Rev. 20, 321–344

8. Glass, C. K., and Rosenfeld, M. G. (2000) Genes Dev. 14, 121–141

9. Kwok, R. P., Lundblad, J. R., Chiriva, J. C., Richards, J. P., Bachinger, H. P., Brennan, R. G., Roberts, S. G., Green, M. R., and Goodman, R. H. (1994) Nature 370, 223–226

10. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S. C., Heyman, R. A., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1996) Cell 85, 403–414

11. Gerretnsen, M. E., Williams, J. M., Neish, A. S., Moore, S., Shi, Y., and Collins, T. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2927–2932

12. Avantaggiati, M. L., Ogryzko, V., Gardner, K., Giordano, A., Levine, A. S., and Kelly, K. (1997) Cell 89, 1175–1184

13. Horvai, A. E., Xu, L., Korzus, E., Brard, G., Kalafus, D., Mullen, T. M., Rose, D. W., Rosenfeld, M. G., and Glass, C. K. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1074–1079

14. Zhang, J. J., Vinkemeier, U., Gu, W., Chakravarti, D., Horvath, C. M., and Darnell, J. E., Jr. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 15902–15906

15. Bhattacharya, S., Eckner, R., Grossman, S., Oldread, E., Arany, Z., D’Andrea, A., and Livingston, D. M. (1996) Nature 383, 344–347

16. McKenna, N. J., Xu, J., Nawaz, Z., Tsai, S. Y., Tsai, M. J., and O’Malley, B. W. (1999) J. Steroid Biochem. Mol. Biol. 69, 3–12

17. Goodman, R. H., and Smolik, S. (2000) Genes Dev. 14, 1553–1577

18. Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. W., and Nakatani, Y. (1998) Nature 382, 319–324

19. Blanco, J. C., Minucci, S., Lu, J., Yang, J. X., Walker, K. K., Chen, H., Evans, R. M., Nakatani, Y., and Ogryzko, V. V. (1998) Genes Dev. 12, 1638–1651

20. Marzio, G., Tyagi, M., Gutierrez, M. I., and Giacca, M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 13519–13524

21. Hettiger, M. O., and Nabel, G. J. (1998) J. Virol. 72, 8252–8256

22. Benkirane, M., U. F., Xiao, H., Ogryzko, V. V., Howard, B. W., Nakatani, Y., and Jeang, K. T. (1998) J. Biol. Chem. 273, 24988–24995

23. Lee, C., and Chen, J. D. (2000) Genes Immun. 1, 1–11

24. Heery, D. M., Kalkhoven, E., Hoare, S., and Parker, M. G. (1997) Nature 387, 733–736

25. Shang, Y., Hu, X., Difrenzo, J., Lazar, M. A., and Brown, M. (2000) Cell 103, 843–852

26. Sawaya, B. E., Khaliili, K., Gordon, J., Taube, R., and Amini, S. (2000) J. Biol. Chem. 275, 35209–35214

27. Felzen, I. K., Wolfenden, C., Hettiger, M. O., Subramanian, R. A., Cohen, E. A., and Nabel, G. J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5281–5286

28. Agostini, I., Navarro, J. M., Rey, F., Bouhmand, M., Spire, B., Vigne, R., and Sire, J. (1996) J. Mol. Biol. 261, 599–606

29. Sherman, M. P., de Noronha, C. M., Pearse, D., and Greene, W. C. (2000) J. Virol. 74, 8159–8165

30. Kino, T., Gragerov, A., Kopp, J. B., Stauber, R. H., Pavlakis, G. N., and Chrousos, G. P. (1999) J. Exp. Med. 189, 51–62

31. Chen, S. L., Dohman, D. H., Hosking, B. M., and Buscat, G. E. (2000) Genes Dev. 14, 1209–1228

32. Kino, T., Norde, S., Chrousos, G. P. (1999) J. Steroid Biochem. Mol. Biol. 70, 15–25

33. Na, S. Y., Lee, S. K., Han, S. J., Choi, H. S., Im, S. Y., and Lee, J. W. (1998) J. Biol. Chem. 273, 10831–10834

34. Shugay, A., R. A., Rose, D. W., Haque, Z. K., Kurokawa, R., Mincer, E., Westin, S., Thoson, D., Rosenfeld, M. G., Glass, C. K., and Collins, T. (1999) Mol. Cell. Biol. 19, 6367–6378

35. Ding, X. F., Anderson, C. M., Ma, H., Hong, H., Ubi, R. M., Kusner, P. J., and Stallcup, M. R. (1998) Mol. Endocrinol. 12, 302–313

36. Hong, H., Darmont, B. D., Ma, H., Yang, L., Yamamoto, K. R., and Stallcup, M. R. (1999) J. Biol. Chem. 274, 8496–8502

37. Wible, P., Nguyen, P., Shinsako, J., Anderson, C., Feng, W., Nguyen, M. P., Chen, D., Huang, M. S., Subramanian, S., McInerney, E., Katsenellenbo- gen, B. S., Stallcup, M. R., and Kushner, P. J. (1998) Mol. Endocrinol. 12, 1605–1618
38. Torchia, J., Rose, D. W., Inostroza, J., Kamei, Y., Westin, S., Glass, C. K., and Rosenfeld, M. G. (1997) *Nature* **387**, 677–684
39. Deleted in proof
40. Kiernan, R. E., Vanhulle, C., Schiltz, L., Adam, E., Xiao, H., Maudoux, F., Calomme, C., Burny, A., Nakatani, Y., Jeang, K. T., Benkirane, M., and Van Lint, C. (1999) *EMBO J.* **18**, 6106–6118
41. Brady, M. E., Ozanne, D. M., Gaughan, L., Waite, I., Cook, S., Neal, D. E., and Robson, C. N. (1999) *J. Biol. Chem.* **274**, 17599–17604
42. Kamine, J., Elangovan, B., Subramanian, T., Coleman, D., and Chinnadurai, G. (1996) *Virology* **216**, 357–366
43. Sawaya, B. E., Rohr, O., Aunis, D., and Schaeffer, E. (1996) *J. Biol. Chem.* **271**, 23572–23576
44. Desai-Yajnik, V., Hadzie, E., Medinger, P., Malhotra, S., Gecchik, G., and Samuels, H. H. (1995) *J. Virol.* **69**, 5103–5112
45. Desai-Yajnik, V., and Samuels, H. H. (1993) *Trans. Assoc. Am. Physicians* **106**, 15–32
46. Recio, J. A., Martinez de la Mata, J., Martin-Nieto, J., and Aranda, A. (2000) *FEBS Lett.* **469**, 118–122
47. Nissen, R. M., and Yamamoto, K. R. (2000) *Genes Dev.* **14**, 2314–2329
48. Chen, D., Ma, H., Hong, H., Koh, S. S., Huang, S. M., Schurter, B. T., Aswad, D. W., and Stallcup, M. R. (1999) *Science* **284**, 2174–2177
Nuclear Receptor Coactivator p160 Proteins Enhance the HIV-1 Long Terminal Repeat Promoter by Bridging Promoter-bound Factors and the Tat-P-TEFb Complex

Tomoshige Kino, Olga Slobodskaya, George N. Pavlakis and George P. Chrousos

J. Biol. Chem. 2002, 277:2396-2405.
doi: 10.1074/jbc.M106312200 originally published online November 9, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M106312200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 46 references, 25 of which can be accessed free at http://www.jbc.org/content/277/4/2396.full.html#ref-list-1