Poly(ADP-ribose) Polymerase-1 Is a Negative Regulator of HIV-1 Transcription through Competitive Binding to TAR RNA with Tat-Positive Transcription Elongation Factor b (p-TEFb) Complex*

Received for publication, July 26, 2004, and in revised form, October 13, 2004 Published, JBC Papers in Press, October 21, 2004, DOI 10.1074/jbc.M408435200

Marianne Parent‡§, Tetsu M. C. Yung‡§, Ann Rancourt‡, Erick L. Y. Ho‡, Stéphane Vispé‡, Fumihiko Suzuki-Matsuda¶, Aki Uehara¶, Tadashi Wada¶, Hiroshi Handa¶, and Masahiko S. Satoh‡‡

From the ‡Division of Health and Environmental Research, Laval University Medical Center (CHUL), and Department of Anatomy and Physiology, Faculty of Medicine, Laval University, 2705 Blvd Laurier, Ste-Pey, Quebec G1V 4G2, Canada and the ¶Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8503, Japan

Human immunodeficiency virus, type 1 (HIV-1) transcription is regulated by a virus-encoded protein, Tat, which forms a complex with a host cellular factor, positive transcription elongation factor b (P-TEFb). When this complex binds to TAR RNA synthesized from the HIV-1 long terminal repeat promoter element, transcription is trans-activated. In this study we showed that, in host cells, HIV-1 transcription is negatively regulated by competition of poly(ADP-ribose) polymerase-1 (PARP-1) with Tat-P-TEFb for binding to TAR RNA. PARP-1, which has a high affinity for TAR RNA (Kd = 1.35 × 10^{-10} M), binds to the loop region of TAR RNA and displaces Tat or Tat-P-TEFb from the RNA. In vitro transcription assays showed that this displacement leads to suppression of Tat-mediated trans-activation of transcription. Furthermore in vivo expression of luciferase or destabilized enhanced green fluorescent protein genes under the control of the HIV-1 long terminal repeat promoter was suppressed by PARP-1. Thus, these results suggest that PARP-1 acts as a negative regulator of HIV-1 transcription through competitive binding with Tat or the Tat-P-TEFb complex to TAR RNA.

Human immunodeficiency virus, type 1 (HIV-1)1 transcription is regulated by a virus-encoded protein, Tat, through its binding to an RNA stem-loop, TAR RNA (for reviews, see Refs. 1 and 2). This 59-bp RNA stem-loop is transcribed from a promoter located within the long terminal repeat (LTR) element by RNA polymerase II (Pol II). The synthesis of TAR RNA, however, stalls Pol II, resulting in the production of non-processive RNA transcripts (1, 2). To rescue the stalled Pol II, Tat binds to a uracil-rich bulge in TAR RNA (+22 to +24) (3–6). This binding is stabilized by the interaction of Tat with a host cellular factor, positive transcription elongation factor (P-TEFb) (3, 4). The cyclin T1 subunit of P-TEFb is involved in the interaction (7–10), and the other P-TEFb subunit, CDK9, hyperphosphorylates the C-terminal domain of the stalled Pol II to trans-activate transcription (11, 12).

As HIV-1 can establish a latent infection, HIV-1 transcription is also thought to be negatively regulated in host cells, although the molecular mechanisms of the negative regulation remain to be elucidated. However, several factors that might be involved in this negative regulation have been identified, one being 7SK RNA, an abundant small nuclear RNA (13, 14), which, in association with P-TEFb (15, 16), inhibits CDK9 activity (15, 16). Thus, the 7SK RNA is likely to have a negative regulatory role in HIV-1 gene expression (15, 16). Another identified factor is 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole sensitivity-inducing factor (DSIF), comprising the two subunits hSpt5 and hSpt4 (17). 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole sensitivity-inducing factor acts in concert with negative elongation factor (NELF) to suppress transcription at the level of elongation (18) and is suggested to be involved in suppression of HIV-1 transcription (19).

In addition to these factors, we have previously found evidence (20, 21) suggesting that the abundant nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1) might act as a negative regulator of HIV-1 transcription. PARP-1 is an enzyme known to bind to DNA breaks and to subsequently catalyze a post-translational modification of various proteins with ADP-ribose polymers (22–24). PARP-1 molecules have three major domains: DNA binding, automodification, and catalytic domains (22, 25). The DNA binding domain, located at the N-terminal end of PARP-1, contains two homologous zinc finger motifs (25), which possess a high binding affinity for DNA breaks (26). We have previously reported that the zinc finger motifs also bind to various RNA stem-loops, including those in TAR RNA (21). Although the specific TAR RNA substructure recognized by PARP-1 is not yet known, it is plausible that, if the binding site of PARP-1 on TAR RNA is in close proximity to that of Tat-P-TEFb, PARP-1 may compete with Tat-P-TEFb in binding to TAR RNA, leading to HIV-1 transcription suppression.

To test this hypothesis, we studied the effects of the binding of PARP-1 to TAR RNA on Tat-mediated trans-activation of...
transcription. We showed that PARP-1 binds to the loop region of TAR RNA with high affinity ($K_D$ of 1.35 $\times 10^{-10}$ m), even higher than the binding affinity of Tat-P-TEFb for TAR RNA (10). Thus, PARP-1 is able to displace Tat-P-TEFb from TAR RNA. In addition, PARP-1 suppressed Tat-mediated trans-activation of transcription in cell-free transcription assays. Furthermore, using in vivo luciferase (Luc) reporter assays, we observed a similar suppression in Tat-mediated trans-activation of transcription by PARP-1. Using a stable cell line expressing Tat and destabilized enhanced green fluorescent protein (d1EGFP) under the control of HIV-1 LTR, we found that PARP-1 was able to suppress the expression of d1EGFP in vivo. Thus, our results suggest that PARP-1 is, to the best of our knowledge, the first identified host cellular factor that negatively regulates HIV-1 transcription by directly competing with Tat-P-TEFb for binding to TAR RNA.

**Materials and Methods**

**Preparation of PARP-1 and Tat**—The Excherichia coli expression construct for PARP-1, pET32a-PARP-1-His, was provided by Dr. Y. Matsumoto. pET32a-PARP-1-His. PARP-1 was expressed and purified as described previously (20, 21, 27). Tat was purified from E. coli BL21 (DE3) transformed with pGEX2T (11, 28) following the method of Herrmann and Rice (11, 28).

**Gel Retardation Assay**—The sequences corresponding to TAR RNA, 5'-GGGGGGCTTC TCTGCTGATA CGACTGCTG CAAGGGAAC CGAGCCGTTACC A3'-; a mutant of TAR RNA (M-TAR RNA) (29), 5'-GGGGGGCTTC TCTGCTGATA CCA GTCCTG CTTCTGGGCTA ACTAGGAAAC CGACCGTACC A3'-; and Stem-loop (Fig. 1, A and B), 5'-GGGGGGCTTC CTCTTGGGATA CGACTGCTG CAAGGGAAC CGAGCCGTTACC A3'-, were cloned downstream to a T3 RNA polymerase promoter at the HindIII site of pBluescript K/S (pBS/TAR, pBS/M-TAR, respectively). These plasmids with HindIII, uniformly 32P-labeled TAR RNA, M-TAR RNA, and stem-loop RNA were prepared as described previously (20). Alternatively pBS/M-TAR was digested with NarI to produce an RNA 139 bases in length, 5'-GGGGGGCTTC TCTGCTGATA CCA GTCCTG CTTCTGGGCTA ACTAGGAAAC CGAGCCGTTACC AAGCTT GGCACCGTTACC A-3', which was cloned downstream to a T7 RNA polymerase promoter (TAR-Luc) constructed previously with an expectation that PARP-1 would interact with d1EGFP expression construct for PARP-1 tagged with red fluorescent protein (DsRed), provided as pPARP-1-DsRed (35), was transfected into cells with high glucose containing 10% fetal bovine serum, antibiotics, and 1 mg/ml G418; cells were cloned from the resulting colonies. Expression of d1EGFP was confirmed by ImageJ software.

**Surface Plasmon Resonance Biosensor**—The binding affinities of Tat and PARP-1 to TAR RNA were determined using BIACORE 3000. A biotinylated single-stranded oligodeoxynucleotide (ssBI; 100 ng/ml, 20 bases in length), dissolved in 25 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM MgCl2, 0.1% Nonidet P-40 (Bi buffer), was immobilized onto streptavidin-coated biosensor tips (SA5) at a flow rate of 30 nl/min. Then a 53-base-long single-stranded RNA (ssRNA) or TAR RNA was synthesized with a complementary sequence to the ssBI by T7 RNA polymerase (21), dissolved in Bi buffer, and immobilized onto the sensor tips at a flow rate of 1 ml/min. Tat or PARP-1 was dissolved in Bi buffer, and the interaction of Tat or PARP-1 with TAR RNA was measured by applying the protein or enzyme to sensor tips at a flow rate of 1 ml/min. Association and dissociation constants were determined with BIA Evaluation Version 3.1 (BIACORE).

**Competition of PARP-1 with Tat or Tat-P-TEFb in TAR RNA Binding**—TAR RNA/Tat complexes were preformed by incubation of 300 pmol of Tat, 50 fmoI of [32P]TAR RNA, and 0.5 µg of anti-Tat antibody (NT3 2D11 (29)) at 30 °C for 20 min in a 15-µl reaction containing 25 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM MgCl2, and 0.5 mM dithiothreitol (DTT) buffer and immobilized according to the method of Dingwall et al. (29) with minor modifications. Briefly 5 µl of protein G-Sepharose was added, and the mixture was incubated at 4 °C for 1 h. The protein G-Sepharose was then washed twice with 10 µl of Bi buffer. Then 14 µl of C buffer containing 0.2 mM ZnCl2 was incubated with the protein G-Sepharose (the presence or absence of P-TEFb), which was washed as described previously (30), for 10 min at 30 °C. Next PARP-1 was added and incubated at 30 °C for 20 min. The protein G-Sepharose was centrifuged, and the radiolabeled activity remaining on and released from the protein G-Sepharose was measured by scintillation counting.

**Cell-free Transcription Assay**—pHIV-1LTR/Luc (31, 32) and pCMV/Luc, containing the Luc gene under the control of HIV-1 LTR or CMV promoters, respectively, were linearized by EcoRI. These linearized templates were used in a run-off transcription assay with HeLa nuclear extracts (Promega). Reactions were carried out for 1 h at 30 °C using 4 µg/ml linearized pHIV-1LTR/Luc or pCMV/Luc and 1000 transcription units of HeLa nuclear extract as described previously (21). The expected sizes of the run-off products from linearized pHIV-1LTR/Luc and pCMV/Luc were 674 and 680 basepairs, respectively. After termination of the reaction and purification of transcription, RNA was fractionated on a 5 or 10% polyacrylamide, 8 m urea gel. After gel drying, 32P activity was visualized by autoradiography.

**Western Blotting**—Western blots were done using anti-hs57 (17) or anti-PARP-1 antibodies (C-II-10, provided by Dr. Guy Poirier).

**Luc Assays**—Either pHIV-1LTR/Luc (0.5 µg/ml) or pCMV/Luc (0.5 µg/ml) and p31 (1 µg/ml), a mammalian expression construct of PARP-1 (with a CMV promoter, provided by Dr. J.-H. Küpper), were co-transfected into HeLa S3 cells or HeLa-tat-III cells (33, 34) as described previously (21). Instead of p31, pcDNA 3.1/24 kDa (a mammalian expression construct of the N-terminal 24-kDa fragment of PARP-1) or pcDNA 3.1/89 kDa (a mammalian expression construct of the C-terminal 89-kDa fragment of PARP-1) was also used. Cells were cultured for 24 h and were then lysed. Luc activity was measured using a Luc assay kit (Promega). pHIV-1LTR Tat/Luc, in which the sequence corresponding to Tat was removed by Sacl-HindIII digestion of pHIV-1LTR/Luc, and Northern blot analysis was carried out using a standard method.

**RNA Protection Assay**—pHIV-1LTR/Luc was digested with XbaI, and the fragment containing the sequence for TAR RNA and the following 5' portion of the Luc gene were cloned into pBluescript KS. A 32P-labeled probe, complementary to the sequence from +1 to +156 was synthesized by T3 RNA polymerase. An RNA protection assay was carried out using the RPA III kit (Ambion). Protected fragments were fractionated on a 10% polyacrylamide, 8 m urea gel and visualized by autoradiography.

**Establishment of Cells Constitutively Expressing d1EGFP**—pID1EGFP-N1, containing d1EGFP under the control of a CMV promoter, was constructed from pEGFP-N1. The CMV promoter was substituted into the HIV-1 LTR promoter of pCMV/d1EGFP using site-directed mutagenesis. The resulting construct was digested with Sacl and HincII and cloned into the HIV-1 LTR promoter of pCMV/d1EGFP using T4 DNA ligase.

**Transfection of pPARP-1-DsRed and Confocal Microscopy**—HeLa-tat-III (CMV/d1EGFP or HeLa-tat-III-LTR/d1EGFP cells, respectively) were transfected with pID1EGFP-N1 or pID1EGFP-LTR to create HeLa-tat-III-CMV/d1EGFP and HeLa-tat-III-LTR/d1EGFP cells, respectively. The cells were grown in Dulbecco's modified Eagle's medium with high glucose containing 10% fetal bovine serum, antibiotics, and 1 mg/ml G418; cells were cloned from the resulting colonies. Expression of d1EGFP was confirmed by fluorescence microscopy.

**Transfection of pPARP-1-DsRed and Confocal Microscopy**—HeLa-tat-III (CMV/d1EGFP or HeLa-tat-III-LTR/d1EGFP cells, respectively) were seeded in Delta T4 culture dishes (Biotechn) in Dulbecco's modified Eagle's medium with high glucose containing 10% fetal bovine serum and antibiotics. Then each pPARP-1-DsRed (1.5 µg/ml), a mammalian expression construct for P-TEFb, or p24kDa-DsRed, a mammalian expression construct for P-TEFb, was transfected into HeLa-tat-III-LTR/d1EGFP using Fugene 6. The expected sizes of the run-off products from linearized pHIV-1LTR/Luc or pCMV/Luc were 674 and 680 basepairs, respectively. After termination of the reaction and purification of transcription, RNA was fractionated on a 5 or 10% polyacrylamide, 8 m urea gel. After gel drying, 32P activity was visualized by autoradiography.

**RESULTS**

PARP-1 and RNA—PARP-1 has the ability to bind various types of RNA stem-loops, including TAR RNA (20, 21), although the recognized site of this RNA stem-loop is not yet known. Thus, in this study, we first determined the binding site of PARP-1 on TAR RNA using gel retardation assays. Consistent with our previous observations (20, 21), PARP-1 reduced the mobility of TAR RNA on a native polyacrylamide
PARP-1. After the binding reaction, samples were fractionated on a native 6% polyacrylamide gel, and the 32P activity was visualized by autoradiography. Within this 68-base RNA stretch, 10 putative ssRNA regions were found. RNA), a single retarding band was still found (Fig. 1A). Even when the loop sequence of TAR RNA was altered (M-TAR), while the mobility of M-TAR RNA +68 fur-
terted with RNase T1, which digests ssRNA (Fig. 1B), stem-loop. As shown in Fig. 1B (stem-loop), PARP-1 formed a single retarding band with the stem-loop RNA, while no obvious retardation was observed with the digested stem-loop (Fig. 1B, stem). These results suggest that PARP-1 binds to the loop region of RNA stem-loops. Previously we demonstrated that PARP-1 forms two discrete retarding bands with RNA synthesized from the cystic fibrosis transmembrane conductance regulator gene (20). This RNA contains two stem-loops, and thus the formation of two retarding bands is likely related to the number of stem-loops presented in the RNA. Thus, we then tested longer M-TAR RNA with an extra RNA stretch of 68 bases (M-TAR RNA +68). Based on the structure prediction using RNA Structure Version 3.1 (Scripps Institute), we found 10 putative ssRNA regions in M-TAR RNA +68. As described, only one retarding band was formed by incubating M-TAR RNA with PARP-1 (Fig. 1A), while the mobility of M-TAR RNA +68 further decreased with increasing amounts of PARP-1 (Fig. 1C). This retardation can be explained by the presence of multiple PARP-1 binding sites in M-TAR RNA +68. As PARP-1 also reduced the mobility of ssRNAs, including poly(A), poly(C), poly(U), and poly(G) (data not shown), these results suggest that PARP-1 has an affinity for ssRNA and thus recognizes loop regions of RNA stem-loops.

**Binding Affinity of PARP-1 for TAR RNA**—Next we determined the equilibrium dissociation constant, $K_D$, between PARP-1 and TAR RNA using a surface plasmon resonance biosensor. As a control, the $K_D$ between Tat and TAR RNA was also determined. Previously, using fluorescence resonance energy transfer, Zhang et al. (10) showed that the $K_D$ between Tat and TAR RNA is $8.2 \times 10^{-9}$ M. The $K_D$ that we obtained using a surface plasmon resonance biosensor between Tat and TAR RNA was $3.01 \times 10^{-9}$ M (Fig. 2A). Thus, although our method showed a 2.7-fold lower $K_D$ than that obtained by fluorescence resonance energy transfer, our results are, overall, in agreement with the results obtained by Zhang et al. (10). We then determined the $K_D$ between PARP-1 and TAR RNA, which was $1.35 \times 10^{-10}$ M (Fig. 2B), showing that PARP-1 has an affinity for TAR RNA 22 times stronger than Tat. Zhang et al. (10) reported that the affinity of Tat for TAR RNA increases about 10-fold after forming the Tat-P-TEFb complex. Even when taking this increase into account, PARP-1 still has a 2.2 times higher affinity for TAR RNA than does Tat-P-TEFb.

**Competition between PARP-1 and Tat or Tat-P-TEFb in Binding to TAR RNA**—Binding of Tat-P-TEFb to TAR RNA primarily requires Tat recognition of a TAR RNA bulge region. Given that the bulge is located near the loop region (Fig. 1A), we next examined whether PARP-1 binding to TAR RNA inhibits Tat or Tat-P-TEFb binding to TAR RNA. As illustrated in Fig. 3A, [32P]TAR RNA was incubated with Tat in the presence of anti-Tat antibody (29). Then the anti-Tat antibody-Tat-[32P]TAR RNA complexes were precipitated with protein G-Sepharose. PARP-1 was added to the complexes under the presumption that if PARP-1 displaces Tat from the complexes, [32P]TAR RNA might be released. This predicted release of [32P]TAR RNA by PARP-1 (Fig. 3A) did in fact occur, suggesting that PARP-1 was able to displace Tat from TAR RNA. We then added P-TEFb to anti-Tat antibody-Tat-[32P]TAR RNA complexes to allow the formation of Tat-P-TEFb complexes and
studied the effect of PARP-1 on the release of [32P]TAR RNA from these complexes. P-TEFb was previously reported to stabilize Tat-TAR RNA complexes by increasing the binding affinity of Tat by about 10-fold (10). Accordingly the addition of 0.8 pmol of PARP-1, which released more than 60% of [32P]TAR RNA from the anti-Tat antibody-Tat-[32P]TAR RNA complexes (Fig. 3B, Tat, 0 versus 0.8 pmol of PARP-1) only released 10% of the [32P]TAR RNA when the complexes were incubated with P-TEFb (Fig. 3B, Tat + P-TEFb, 0 versus 0.8 pmol of PARP-1).

However, an increased amount of PARP-1 (2.0 pmol) resulted in a 75% release of [32P]TAR RNA (Fig. 3B, Tat + P-TEFb, 2.0 pmol of PARP-1). These results thus indicate that PARP-1 also has the ability to displace Tat-P-TEFb from TAR RNA.

**Suppression of Tat-promoted trans-Activation of Transcription by PARP-1 in Vitro**—In cell-free transcription assays with HeLa nuclear extracts, the addition of Tat has been shown to have a promoting effect on transcription initiated from the HIV-1 LTR because of the formation of Tat-P-TEFb and trans-
activation of transcription through its binding to TAR RNA (36). In fact, in a run-off transcription assay with a template DNA containing the HIV-1 LTR together with HeLa nuclear extracts (Promega), 32P-Labeled transcripts were analyzed on a 5% polyacrylamide, 8 M urea gel and visualized by autoradiography. Tat and/or PARP-1 was also added to the reactions. Quantified data are shown. B, in vitro transcripts were fractionated on a 10% polyacrylamide, 8 M urea gel to analyze RNA of 50–80 bases in length, which would encompass TAR RNA. [32P]TAR RNA was loaded as a marker. C, after a run-off transcription reaction, the mixture was fractionated on a 7.5% SDS-polyacrylamide gel to separate the phosphorylated and non-phosphorylated forms of hSpt5, which were detected by Western blotting with anti-hSpt5 antibody. A HeLa S3 extract prepared by the method of Lavoie et al. (49) was used as marker of non-phosphorylated and phosphorylated hSpt5.

FIG. 4. Suppression of Tat-mediated trans-activation of transcription by PARP-1 in vitro. A, a run-off transcription assay was carried out with a template DNA containing the HIV-1 LTR together with HeLa nuclear extracts (Promega). 32P-Labeled transcripts were analyzed on a 5% polyacrylamide, 8 M urea gel and visualized by autoradiography. Tat and/or PARP-1 was also added to the reactions. Quantified data are shown. B, in vitro transcripts were fractionated on a 10% polyacrylamide, 8 M urea gel to analyze RNA of 50–80 bases in length, which would encompass TAR RNA. [32P]TAR RNA was loaded as a marker. C, after a run-off transcription reaction, the mixture was fractionated on a 7.5% SDS-polyacrylamide gel to separate the phosphorylated and non-phosphorylated forms of hSpt5, which were detected by Western blotting with anti-hSpt5 antibody. A HeLa S3 extract prepared by the method of Lavoie et al. (49) was used as marker of non-phosphorylated and phosphorylated hSpt5.

If this suppression is in some way caused by inhibition of Tat-P-TEFb binding to TAR RNA by PARP-1, accumulation of TAR RNA would be expected. Thus, we analyzed RNA transcripts similar in length to TAR RNA (50–80 bases in length). As shown in Fig. 4B, RNA showing a similar mobility to 32P-labeled transcripts containing TAR RNA (76 bases in length, Fig. 4B, lane 1) were found after transcription reactions with a template DNA containing HIV-1 LTR but in the absence of Tat (Fig. 4B, lane 2). Thus, these results suggest that TAR RNA accumulates during the reaction. Adding Tat to the cell-free transcription assays reduced the amount of TAR RNA found in the reactions because of the trans-activation of transcription (Fig. 4B, lane 3). When PARP-1 was added to the reaction containing Tat, TAR RNA again accumulated (Fig. 4B, lanes 4–6). These results indicate that trans-activation of transcription is suppressed by PARP-1 and that this suppression results from inhibition of the binding of Tat-P-TEFb to TAR RNA.

It has been proposed that phosphorylation of the hSpt5 subunit of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole sensitivity-inducing factor by CDK9 occurs during Tat-mediated trans-activation of transcription (19). If PARP-1 competes with Tat-P-TEFb in binding to TAR RNA, suppression of hSpt5 phosphorylation would also be expected. To further confirm whether PARP-1 suppresses Tat-mediated trans-activation of transcription, we decided to study the effect of PARP-1 on hSpt5 phosphorylation using Western blot. As a marker of both non-phosphorylated and phosphorylated hSpt5, we prepared whole cell extracts from HeLa S3 cells according to published methods (17), and two bands were visualized (Fig. 4C, lane 1). Nuclear extracts (Promega) used for the transcription assay contained only non-
suggest that binding of Tat-P-TEFb to TAR RNA is inhibited by
phosphorylated hSpt5 (Fig. 4C, lane 2). Adding Tat to the
transcription assay resulted in phosphorylated hSpt5 (Fig. 4C,
lane 3). Then we added both PARP-1 and Tat in the transcrip-
tion reactions and found that PARP-1 reduced the formation of
phosphorylated hSpt5 (Fig. 4C, lanes 5–7). Thus, these results
suggest that binding of Tat-P-TEFb to TAR RNA is inhibited by
PARP-1.

Luc Reporter Assay—We then studied whether PARP-1 sup-
presses Tat-mediated trans-activation of transcription in vivo
using a Luc reporter assay. pHIV-1/LTR/Luc, containing a Luc
gene downstream of the HIV-1 LTR, was transfected into HeLa-tat-III cells, which constitutively express Tat (33, 34), or
HeLa S3 cells, and Luc activity was measured. In these cells,
Luc activity was over 150-fold higher in the HeLa-tat-III cells
compared with the HeLa S3 cells (Fig. 5A, HeLa S3, pHIV-1/
LTR/Luc versus HeLa-tat-III, pHIV-1/LTR/Luc). As transfec-
tion of pHIV-1/LTR ΔTAR/Luc, which lacks the sequence cor-
responding to TAR RNA, into HeLa-tat-III cells did not result in
Luc activity increase (Fig. 5A, HeLa-tat-III, pHIV-1/LTR
ΔTAR/Luc), Tat-mediated trans-activation of transcription ap-
pears to be occurring from transfection of pHIV-1/LTR/Luc into
HeLa-tat-III cells (Fig. 5A, HeLa-tat-III, pHIV-1/LTR/Luc). By
co-transfecting pHIV-1/LTR/Luc with p31, a PARP-1 expres-
sion construct (resulting in a 2-fold increase in cellular content
of PARP-1), Luc activity was reduced by 85% (Fig. 5A, HeLa-
tat-III, pHIV-1/LTR/Luc + p31). Expression of the N-terminal
24-kDa PARP-1 fragment, which contains two zinc finger mo-
tifs, also resulted in reduced Luc activity (Fig. 5A, HeLa-tat-III,
pHIV-1/LTR/Luc + p24). Although the reduction was less
significant than that resulting from PARP-1 overexpression.
This could reflect a reduced RNA binding affinity of the 24-kDa
PARP-1 fragment (21). On the other hand, expression of the
C-terminal 89-kDa PARP-1 fragment, containing the auto-
maintenance and the catalytic domains of PARP-1, showed only
negligible reduction of Luc activity (Fig. 5A, HeLa-tat-III,
pHIV-1/LTR/Luc + p89). These results suggest that
PARP-1 overexpression leads to the reduction of Luc activity
and that the DNA binding domain of PARP-1, containing two
zinc finger motifs, is involved in the reduction.

We then measured the amount of Luc mRNA in HeLa-tat-III
cells. As shown in Fig. 5B, p31 transfection reduced the amount of Luc mRNA in HeLa-tat-III cells, indicating that reduced Luc
activity by overexpression of PARP-1 resulted from suppres-
sion of Luc mRNA transcription initiated from HIV-1 LTR.

Effect of PARP-1 Overexpression on Transcription from
HIV-1 LTR and CMV Promoters—We have previously reported
that PARP-1 reduces the rate of RNA synthesis by Pol II at the
level of elongation (20, 21). Thus, PARP-1 might suppress tran-
scription of Luc gene under the control of HIV-1 LTR (Fig. 5B)
by inhibiting Tat-P-TEFb binding to TAR RNA and/or by re-
ducing the overall mRNA synthesis rate. To further elucidate
these mechanisms, we utilized a CMV promoter in addition to
the HIV-1 LTR. As shown in Fig. 6A, the Luc activity found in
cells transfected with pCMV/Luc was reduced by PARP-1 over-
expression. Thus, it appears that this reduction could be caused
by a decreased rate of mRNA synthesis. However, a more
prominent effect in reducing Luc activity was observed when
pHIV-1/LTR/Luc was used. Furthermore similar results were
obtained by expression of the 24-kDa PARP-1 fragment instead
of PARP-1 (Fig. 6B). Thus, while PARP-1 can suppress HIV-1
gene expression by reducing the overall rate of mRNA synthe-
sis, these results suggest that formation of TAR RNA leads to
prominent suppression of transcription initiated from HIV-1 LTR.

Accumulation of TAR RNA in Vivo—In vitro, we found that
addition of PARP-1 to the cell-free transcription reaction
caused TAR RNA to accumulate by inhibiting Tat-mediated
trans-activation of transcription (Fig. 4B). If, in vivo, PARP-1

FIG. 5. Suppression of transcription initiated from HIV-1 LTR by PARP-1 in vivo. A, PARP-1 expression construct (p31) and/or
pHIV-1/LTR/Luc containing the Luc gene under the control of HIV-1 LTR were transfected into HeLa S3 or HeLa-tat-III cells, and Luc activity was
measured. As a control, pcDNA3.1/GFP (containing a non-related cDNA under the control of a CMV promoter), pcDNA3.1/89 kDa (coding for the
89-kDa PARP-1 fragment, p89 kDa), or pcDNA3.1/24 kDa (coding for the 24-kDa PARP-1 fragment, p24 kDa) was used instead of p31. pHIV-1/LTR
ΔTAR/Luc was also used instead of pHIV-1/LTR/Luc. Standard deviations from three independent experiments are shown. B, total RNA was
extracted after the transfection, and a Northern blot was carried out with 32P-labeled probes for Luc mRNA. Recovery of rRNA is also shown.
indeed suppresses mRNA synthesis initiated from the HIV-1 LTR by inhibiting trans-activation, TAR RNA would be expected to accumulate in cells that overexpress PARP-1. We thus used a RNA protection assay to test this in vivo. We designed a probe that has a sequence complementary to the LTR-Luc mRNA from +1 to +156 that covers the R region coding for TAR RNA and part of a Luc coding sequence as illustrated in Fig. 7. Thus, mRNA transcribed through the R region can be detected as a 156-base-long protected fragment. In fact, the protected fragments were found in RNA extracted from HeLa-tat-III cells transfected with the pHIV-LTR/Luc (Fig. 7, 156 bases, lane 3), while these fragments were not found in HeLa S3 cells (Fig. 7, 156 bases, lane 2) because of transcription terminating the R region prematurely, as reported previously (37). The amount of protected 156-base-long fragments was also reduced by overexpression of PARP-1 in HeLa-tat-III cells that were transfected with pHIV-LTR/Luc (Fig. 7, 156 bases, lane 3 versus lane 4), suggesting that RNA synthesis is terminated by PARP-1 at an upstream location of +156. We then analyzed RNA fragments that have a length corresponding to TAR RNA. In HeLa S3 cells transfected with pHIV-LTR/Luc, an accumulation of TAR RNA (or its digested products (37)) was observed (Fig. 7, TAR RNA, lane 2), while in HeLa-tat-III cells, the amount of fragments was reduced due to Tat-mediated trans-activation of transcription (Fig. 7, TAR RNA, lane 3) as reported by Laspia et al. (37). Interestingly overexpression of PARP-1 in HeLa-tat-III cells transfected with pHIV-LTR/Luc led to the accumulation of these fragments (Fig. 7, TAR RNA, lane 4), suggesting that Tat-mediated trans-activation of transcription is inhibited by PARP-1.

Inhibition of Tat-mediated trans-activation of transcription by PARP-1 in live cells—We then investigated the effect of PARP-1 expression on Tat-mediated trans-activation of transcription in live cells. For this purpose, we established HeLa-tat-III cell lines stably expressing d1EGFP under the control of either the CMV promoter (HeLa-tat-III/CMV/d1EGFP cells) or HIV-1 LTR (HeLa-tat-III/LTR/d1EGFP cells). Then an expression construct for PARP-1 tagged with DsRed was created (pPARP-1-DsRed), and both d1EGFP and DsRed emissions from these cells were captured after sequential excitations of these fluorescent proteins by laser as described previously (35). As shown in Fig. 8A (HeLa-tat-III/CMV/d1EGFP), d1EGFP was expressed in HeLa-tat-III/CMV/d1EGFP cells, and PARP-1-DsRed-expressing cells could be identified by monitoring fluorescence emission from DsRed. The level of d1EGFP in over 100 cells was then quantified to determine the effect of PARP-1-DsRed expression on d1EGFP transcription. On the basis of this quantitation, we found that the average intensity of d1EGFP in non-PARP-1-DsRed-expressing cells (Fig. 8B, Control) was 61 arbitrary units, while the intensity was reduced to 39 arbitrary units by PARP-1-DsRed expression (Fig. 8B, PARP-1-DsRed expressed). Thus, consistent with the results obtained from the Luc reporter assay (Fig. 6A), PARP-1 expression leads to an inhibition of transcription initiated by the CMV promoter. Then when pPARP-1-DsRed was transfected into HeLa-tat-III/LTR/d1EGFP cells, we found a prominent suppression of d1EGFP expression by PARP-1-DsRed (Fig. 8A, HeLa-tat-III/LTR/d1EGFP). As shown in Fig. 8C, the average intensity of d1EGFP was in fact reduced from 75 arbitrary units (Control) to 9 arbitrary units (PARP-1-DsRed expressed) by PARP-1-DsRed expression. Furthermore similar results were obtained by using an expression construct of the 24-kDa PARP-1 fragment tagged with DsRed (p24 kDa-DsRed) instead of pPARP-1-DsRed (Fig. 8, D, E, and F). Thus, taken together, these results consistently suggest that PARP-1 suppresses Tat-mediated trans-activation of transcription and that its zinc finger motifs located within the 24-kDa fragment play a role in the suppression.

**DISCUSSION**

Possible links between PARP-1 and HIV-1 have been reported previously. In 1991, for example, Yamagoe et al. (38) observed a promotion of UV irradiation-induced HIV-1 gene expression by PARP-1 inhibitors, although it is not yet known whether PARP-1 per se is indeed involved in the promotion. More recently, Ha et al. (39) reported a role for PARP-1 in HIV-1 integration, although Siva and Bushman (40) later showed that PARP-1 is not strictly required for integration as they observed efficient HIV-1 integration in cells lacking PARP-1. Thus, the role of PARP-1 in HIV-1 infection has remained elusive. In this report, we show that PARP-1 is a host cellular factor that negatively regulates HIV-1 transcription by directly competing with Tat-P-TEFb for binding to TAR RNA. Tat-P-TEFb-mediated transcription regulation and PARP-1—In infected cells, while HIV-1 actively transcribes its genes
during the lytic phase, this transcription is suppressed in the latent phase. Thus, HIV-1 transcription seems to be controlled by opposing positive and negative regulators that control the existence of the virus in host cells. During the lytic phase, the effect of a negative regulator, PARP-1, on HIV-1 transcription can therefore be overcome by a positive regulator, Tat/P-TEFb. How HIV-1 overcomes PARP-1-mediated suppression of transcription can be explained by comparing the binding affinities of PARP-1 and Tat-P-TEFb for TAR RNA. As shown in Fig. 2, PARP-1 has a $K_d$ for TAR RNA in the order of $10^{-10}$ M, which enables the formation of a highly stable complex with TAR RNA. Thus, factors that have significantly less affinity for TAR RNA may not be able to compete with PARP-1 for binding to TAR RNA. In fact, the viral protein Tat, having a 22-fold lower $K_d$ for TAR RNA, is able to overcome PARP-1-mediated suppression of transcription.

**Fig. 8. Suppression of Tat-mediated trans-activation of transcription by PARP-1 in living cells.**

A, pPARP-1-DsRed was transfected into either HeLa-tat-III/CMV/d1EGFP or HeLa-tat-III/LTR/d1EGFP cells stably expressing d1EGFP under the control of the CMV promoter or HIV-1 LTR, respectively. Cells were mounted onto a microscope stage 24 h after transfection. d1EGFP and DsRed were sequentially excited by helium-neon and argon lasers, respectively, using a confocal laser scanning unit. Fluorescence emissions from d1EGFP and DsRed were captured sequentially to eliminate cross-talk. Images were prepared from one optical section. B and C, the level of d1EGFP fluorescence in PARP-1-DsRed-non-expressing and PARP-1-DsRed-expressing HeLa-tat-III/CMV/d1EGFP (B) or HeLa-tat-III/LTR/d1EGFP (C) cells was determined. Results obtained from over 100 cells are shown. D, p24 kDa-DsRed was used instead of pPARP-1-DsRed. E and F, quantified results obtained from HeLa-tat-III/CMV/d1EGFP (E) or HeLa-tat-III/LTR/d1EGFP (F) cells are shown.
affinity for TAR RNA than PARP-1 does, was displaced from TAR RNA by PARP-1 (Fig. 3A). Therefore, without mechanisms to promote the binding affinity of Tat for TAR RNA, HIV-1 may not be able to compete with PARP-1 in binding to TAR RNA. Interestingly, by forming a complex with P-TEFb, the affinity of Tat for TAR RNA increases 10-fold (10) and becomes only a fewfold less than that of PARP-1. In fact, Tat-P-TEFb was more resistant to displacement from TAR RNA by PARP-1 than Tat alone due to the stabilization of Tat-TAR RNA complexes (Fig. 3B). Because of this stabilization by P-TEFb, Tat possibly becomes capable of competing with PARP-1 in binding to TAR RNA. Although one of the major functions of P-TEFb on HIV-1 gene expression is to transactivate HIV-1 transcription by phosphorylating hSpt5 and the C-terminal domain of Pol II (11, 12, 19), the stabilization of Tat-TAR RNA complexes per se, through promotion of Tat binding affinity for TAR RNA, must therefore be another critical role of P-TEFb in overcoming the negative regulatory effect of PARP-1 on HIV-1 transcription.

If HIV-1 transcription is regulated by the relative levels of negative and positive regulators, any factor capable of reversing favorable situations for HIV-1 transcription could lead to the suppression of HIV-1 transcription. For example, P-TEFb sequestration by 7SK RNA (15, 16) may increase the probability of PARP-1 binding to TAR RNA, leading to the suppression of HIV-1 transcription. Alternatively if greater amounts of PARP-1 become available to bind TAR RNA, HIV-1 transcription could also be suppressed. In fact, as shown in Fig. 3B, Tat-P-TEFb complexes, even if resistant to displacement from TAR RNA by 0.8 pmol of PARP-1, were eventually removed from the stem-loop by adding greater amounts of PARP-1 (Fig. 3B). Furthermore we observed reduced expression of Luc and d1EGFP genes under HIV-1 LTR control when PARP-1 was expressed (Figs. 5, 6, and 8). HIV-1 establishes latent infections in CD4+ memory T cells capable of survival for a significantly long period of time, and latent HIV-1 infections are often found in cell populations that survived antiviral therapy (41). It is possible that favorable conditions for the suppression of HIV-1 transcription are established in these cells, and PARP-1 may only be one of many factors involved in the establishment of these conditions.

**PARP-1 and HIV-1 Transcription**—The function of PARP-1 to suppress HIV-1 transcription through TAR RNA binding is apparently related to its role in the regulation of host cellular genes. We have previously shown that PARP-1 has the ability to regulate RNA synthesis by interacting with topoisomerase I (35), an enzyme required for the progression of Pol II during transcription (42, 43), by regulating DNA superhelical tension with its topoisomerase I-like activity (27) and by binding to nascent RNA (20, 21). In particular, binding of PARP-1 to nascent RNA reduced the rate of RNA synthesis, and therefore it was proposed that PARP-1 could be a novel type of negative elongation regulator (20, 21). Suppression of HIV-1 transcription through binding of PARP-1 to TAR RNA is thus likely to be related to the function of PARP-1 as a negative elongation regulator.

In addition to the function of PARP-1 as a negative transcription elongation regulator, PARP-1 may also be involved in HIV-1 transcription at the level of initiation as it interacts with NF-κB (44, 45), a factor regulating the initiation of HIV-1 transcription (46). In fact, Hassa et al. (44) suggested that NF-κB-promoted transcription by interacting with PARP-1. Furthermore Kameoka et al. (47) recently reported an inactivation of HIV-1 LTR in cells treated with small interfering RNA directed against PARP-1, suggesting that PARP-1 is required for HIV-1 transcription initiation. However, contrary to the report from Hassa et al. (44), Chang et al. (45) demonstrated that NF-κB is unable to bind to its target sequence when NF-κB is interacting with PARP-1, which suggests that PARP-1 negatively regulates NF-κB-dependent transcription initiation. Of note, although the reason for this discrepancy is not clear, Gwack et al. (48) reported a similar negative regulation of transcription where PARP-1 forms a complex involving a transcription activator of γ-2 herpesvirus. Although the role of PARP-1 in HIV-1 transcription initiation through its interaction with NF-κB may need clarification, PARP-1 appears to be involved in regulation of HIV-1 transcription at both the levels of initiation and elongation.

**Conclusion**—HIV-1 transcription is regulated at the level of elongation via Tat and TAR RNA (1, 2). As reported here, Tat-P-TEFb plays a critical role as a positive regulator, and PARP-1 acts as a negative regulator of HIV-1 gene expression. Because these regulations occur through binding of PARP-1 or Tat-P-TEFb to TAR RNA, this unique stem-loop RNA plays a central role in both positive and negative regulation of HIV-1 transcription.

In infected cells, positive regulation of HIV-1 transcription leads to the lytic phase, while HIV-1 can enter the latent phase by suppressing its transcription. On the other hand, HIV-1 in latently infected cells can be reactivated by various viral stimuli. Interestingly P-TEFb and PARP-1 activities are controlled in cells by exposure to viral stimuli, including UV and reactive oxygen species. For example, P-TEFb, which is sequestered by 7SK RNA, is released upon cell exposure to UV (15, 16), and PARP-1 is converted to its inactive form (automodified PARP-1) by cell exposure to reactive oxygen species (22, 24). The P-TEFb release from 7SK RNA and PARP-1 inactivation primarily occur in host cells to protect cells from stresses induced by reactive oxygen species or UV. HIV-1 might perhaps monitor the host cellular response to these stresses as well as the relative activities of P-TEFb and PARP-1 as a measure to control its own life cycle and may use TAR RNA as a sensor to detect these activities by creating a binding site for both positive and negative regulators.

**Acknowledgments**—We thank T. Lindahl and S. Sato for comments. We are also grateful to the AIDS Research and Reference Reagent Program, Division of AIDS, NIADDK, National Institutes of Health for providing Tat antibody (NT3 2D1.1, J. Karn), Tat expression construct (pGEX2T, A. Rice), HIV-LTR/Luc (also known as pLTRWT-lite, S. Zeichner), and HeLa-tat-III (W. Haseltine and E. Tewari).
Negative Regulation of HIV-1 Transcription by PARP-1

19. Ping, Y. H., and Rana, T. M. (2001) *J. Biol. Chem.* 276, 12951–12958

20. Vispe, S., Yung, T. M. C., and Satoh, M. S. (2000) *Proc. Natl. Acad. Sci. U. S. A.* 97, 9886–9891

21. Yung, T. M. C., and Satoh, M. S. (2000) *J. Biol. Chem.* 276, 11279–11286

22. Cleaver, J. E., and Morgan, W. F. (1991) *Mutat. Res.* 257, 1–18

23. D'Amours, D., Desnoyers, S., D'Silva, I., and Poirier, G. G. (1999) *Biochem. J.* 342, 249–268

24. Althaus, F. R., and Richter, C. (1987) *ADP-ribosylation of Proteins: Enzymology and Biological Significance*, Springer-Verlag, Berlin

25. de Murcia, G., Menissier-de Murcia, J., and Schreiber, V. (1991) *Bioessays* 13, 455–462

26. D'Silva, I., Pelletier, J. D., Lagueux, J., D'Amours, D., Chaudhry, M. A., Weinfield, M., Lees-Miller, S. P., and Poirier, G. G. (1999) *Biochim. Biophys. Acta* 1430, 119–126

27. Yung, T. M., Parent, M., Ho, E. L., and Satoh, M. S. (2004) *J. Biol. Chem.* 279, 11992–11999

28. Rhim, H., Echeteau, C. O., Herrmann, C. H., and Rice, A. P. (1994) *J. Acquir. Immune Defic. Syndr.* 7, 1116–1121

29. Dingwall, C., Ernberg, I., Gait, M. J., Green, S. M., Heaphy, S., Karn, J., Lowe, A. D., Singh, M., Skinner, M. A., and Valerio, R. (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 6925–6929

30. Wada, T., Takagi, T., Yamaguchi, Y., Watanabe, D., and Handa, H. (1998) *EMBO J.* 17, 7395–7403

31. Zeichner, S. L., Kim, J. Y., and Alwine, J. C. (1991) *J. Virol.* 65, 2436–2444

32. Zeichner, S. L., Hirka, G., Andrews, P. W., and Alwine, J. C. (1992) *J. Virol.* 66, 2268–2273

33. Rosen, C. A., Sodroski, J. G., Campbell, K., and Haseltine, W. A. (1986) *J. Virol.* 57, 379–384

34. Terwilliger, E., Proulx, J., Sodroski, J., and Haseltine, W. A. (1988) *J. Acquir. Immune Defic. Syndr.* 1, 317–323

35. Yung, T. M. C., Sato, S., and Satoh, M. S. (2004) *J. Biol. Chem.* 279, 39686–39696

36. Marciniak, R. A., Calnan, B. J., Frankel, A. D., and Sharp, P. A. (1990) *Cell* 63, 791–802

37. Laspia, M. F., Rice, A. P., and Mathews, M. B. (1989) *Cell* 59, 283–292

38. Yamagoe, S., Kohda, T., and Oishi, M. (1991) *Mol. Cell. Biol.* 11, 3522–3527

39. Hass, C., Juluri, K., Zhou, Y., Leung, S., Herrmannova, M., and Snyder, S. H. (2001) *Proc. Natl. Acad. Sci. U. S. A.* 98, 3364–3368

40. Siva, A., and Bushman, F. (2002) *Mol. Cell. Biol.* 76, 11904–11910

41. Piersig, T., McArthur, J., and Siliciano, R. (2000) *Annu. Rev. Immunol.* 18, 665–708

42. Champoux, J. J. (2001) *Annu. Rev. Biochem.* 70, 369–413

43. Wang, J. C. (1996) *Annu. Rev. Biochem.* 65, 635–692

44. Hass, P. O., Covic, M., Hasan, S., Imhof, R., and Hottinger, M. O. (2001) *J. Biol. Chem.* 276, 45588–45597

45. Chang, W. J., and Alvarez-Gonzalez, R. (2001) *J. Biol. Chem.* 276, 45664–45670

46. Cullen, B. R. (1991) *FASEB J.* 5, 2361–2368

47. Kameoka, M., Nukuzuma, S., Itoya, A., Tanaka, Y., Ota, K., Ikuta, K., and Yoshimura, K. (2004) *J. Virol.* 78, 8931–8934

48. Gwack, Y., Nakanura, H., Lee, S. H., Souvias, J., Yustein, J. T., Gygi, S., Kung, H.-J., and Jung, J. U. (2003) *Mol. Cell. Biol.* 23, 8282–8294

49. Lavoie, S. B., Albert, A. L., Handa, H., Vincent, M., and Bensaude, O. (2001) *J. Mol. Biol.* 312, 675–685
Poly(ADP-ribose) Polymerase-1 Is a Negative Regulator of HIV-1 Transcription through Competitive Binding to TAR RNA with Tat-Positive Transcription Elongation Factor b (p-TEFb) Complex

Marianne Parent, Tetsu M. C. Yung, Ann Rancourt, Erick L. Y. Ho, Stéphane Vispé, Fumihiko Suzuki-Matsuda, Aki Uehara, Tadashi Wada, Hiroshi Handa and Masahiko S. Satoh

J. Biol. Chem. 2005, 280:448-457.
doi: 10.1074/jbc.M408435200 originally published online October 21, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M408435200

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 48 references, 27 of which can be accessed free at http://www.jbc.org/content/280/1/448.full.html#ref-list-1