FACT Proteins, SUPT16H and SSRP1, Are Transcriptional Suppressors of HIV-1 and HTLV-1 That Facilitate Viral Latency*

Received for publication, March 18, 2015, and in revised form, September 14, 2015. Published, JBC Papers in Press, September 16, 2015, DOI 10.1074/jbc.M115.652339

Huachao Huang†, Netty Santos‡, Derek Power†, Sydney Simpson†, Michael Dieringer†, Hongyu Miao†, Katerina Gurova†, Chou-Zen Giam**, Stephen J. Elledge†‡§§, and Jian Zhu**†

From the Departments of† Microbiology and Immunology, ‡ Biochemistry and Biophysics, and §§ Department of Biostatistics and Computational Biology, University of Rochester Medical Center, Rochester, New York 14624, the §§ Department of Arts and Sciences, University of Rochester, Rochester, New York 14627, the † Department of Cell Stress Biology, Roswell Park Cancer Institute, Buffalo, New York 14263, the ** Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814, the §§ Division of Genetics, Brigham and Women’s Hospital, Howard Hughes Medical Institute, Boston, Massachusetts 02115, and the §§ Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115

Background: FACT proteins SUPT16H and SSRP1 are identified as host factors that restrict HIV-1 replication. FACT interacts specifically with histones H2A/H2B to affect assembly and disassembly of nucleosomes, as well as transcription elongation. We further investigated the suppressive role of FACT proteins in HIV-1 transcription. First, depletion of SUPT16H or SSRP1 protein enhances Tat-mediated HIV-1 LTR (long terminal repeat) promoter activity. Second, HIV-1 Tat interacts with SUPT16H but not SSRP1 protein. However, both SUPT16H and SSRP1 are recruited to LTR promoter. Third, the presence of SUPT16H interferes with the association of Cyclin T1 (CCNT1), a subunit of P-TEFb, with the Tat-LTR axis. Removing inhibitory mechanisms to permit HIV-1 transcription is an initial and key regulatory step to reverse post-integrated latent HIV-1 proviruses for purging of reservoir cells. We therefore evaluated the role of FACT proteins in HIV-1 latency and reactivation. Depletion of SUPT16H or SSRP1 protein affects both HIV-1 transcriptional initiation and elongation and spontaneously reverses latent HIV-1 in U1/HIV and J-LAT cells. Similar effects were observed with a primary CD4+ T cell model of HIV-1 latency. FACT proteins also interfere with HTLV-1 Tax-LTR-mediated transcription and viral latency, indicating that they may act as general transcriptional suppressors for retroviruses.

Results: Biochemical and genetic evidences that SUPT16H and SSRP1 affect HIV-1/HTLV-1 transcription and latency are provided.

Conclusion: SUPT16H and SSRP1 suppress transcription of HIV-1/HTLV-1, and their presence may promote HIV-1 latency.

Significance: Identification of host factors necessary for HIV-1 latency is critical, which may benefit the development of novel HIV-1 latency-reversing agents.

We conclude that FACT proteins SUPT16H and SSRP1 play a key role in suppressing HIV-1 transcription and promoting viral latency, which may serve as promising gene targets for developing novel HIV-1 latency-reversing agents.

The global rate of HIV-1 infection and the number of AIDS-related deaths have dramatically declined because of expanding access to highly active antiretroviral therapy (HAART). However, HIV-1 epidemic remains unsolved, and there is still no cure for HIV-1 infection, as well as a lack of valid HIV-1 vaccines (1). The HAART regimen remains the most effective treatment, but this only blocks active HIV-1 replication, leaving residual viremia in most AIDS patients (2, 3). Viral loads readily rebound once the antiretroviral regimen is interrupted. Also, lifelong HAART is associated with significant adverse effects and increases the risk of multiple end organ diseases.

Ultrasonic measurements confirm that residual viremia is present with <50 copies/ml in the plasma of HAART-treated patients, initiating from a small set of cells harboring latent HIV-1 (4). Resting memory CD4+ T cells are the major, well defined latent reservoir, although other cells, including macrophages and hematopoietic stem cells, are permissible for low HIV-1 replication. HIV-1 infects activated CD4+ T cells and leads to their rapid death through cytopathic effects. Rarely (1/106), HIV-infected CD4+ T cells revert to a resting memory state, leading to silence of HIV-1 gene expression (5). In these resting cells, a comprehensive set of changes in the cellular environment prevents viral gene expression, and multiple cel...

* This work is supported by National Institutes of Health Grants R21AI116180 and R01GM117838 (to J. Z.) and in part by the University of Rochester Centers for AIDS Research Grant P30AI078498 from National Institutes of Health and the University of Rochester Center for Integrative Bioinformatics and Experimental Mathematics pilot grant. The authors declare that they have no conflicts of interest with the contents of this article.

† To whom correspondence should be addressed: Dept. of Microbiology and Immunology, Dept. of Biochemistry and Biophysics, University of Rochester Medical Center, 601 Elmwood Ave., Box 672, Rochester, NY 14642. Tel.: 585-275-6916; E-mail: Jian_Zhu@urmc.rochester.edu.

‡ The abbreviations used are: HAART, highly active antiretroviral therapy; HDAC, histone deacetylase; RIGER, RNAi gene enrichment ranking; LTR, long terminal repeat; SAHA, suberoylanilide hydroxamic acid; NT, nontargeting; qPCR, quantitative PCR; IP, immunoprecipitation; mIgG, mouse IgG; BRD4, bromodomain-containing protein 4.
lular mechanisms facilitate HIV-1 latency, mostly through suppression of transcription. Cell surface receptors that inhibit T cell proliferation and differentiation, including PD-1, CTLA-4, and TIM-3, are turned on as negative feedback during T cell activation (6, 7). Key transcriptional initiating factors (NF-κB, NFAT) for HIV-1 gene expression are excluded from the nucleus. The P-TEFb (CDK9/Cyclin T) protein complex required for HIV-1 Tat-mediated RNA polymerase II activation is sequestered in the 7SK small nuclear ribonucleoprotein complex as an inactive form by HEXIM1 (8). The HIV-1 cDNAs tend to integrate within actively transcribed host genes in resting CD4+ T cells from HIV-1-infected individuals (9). In this scenario, transcriptional interference may occur that promotes HIV-1 latency (10, 11). Epigenetic regulation of the proviral 5’ long terminal repeat (LTR) region places another layer to control HIV-1 latency, which includes the events of chromatin modification and nucleosome reorganization by recruiting histone deacetylases (HDACs), histone methyltransferases, and DNA methyltransferases coordinately to silence the viral promoter (12, 13).

Thus, persistent HIV-1 latency is the major obstacle for HIV-1 elimination. Reversing HIV-1 latency for eradication requires the reactivation of integrated proviruses to induce a cytopathic effect to the reservoir cells, followed by reinforced HIV-specific immune responses to purge these cells. To reactivate latent HIV-1 proviruses, the cellular restrictive mechanisms suppressing HIV-1 transcription should be removed. A pharmacological approach seems effective for this purpose. Epigenetic suppression is reversed by inhibiting chromatin remodeling enzymes, such as HDACs, histone methyltransferases, and DNA methyltransferases. The HDAC inhibitors are currently one of the most promising HIV-1 latency-reversing agents, such as vorinostat (suberoylanilide hydroxamic acid (SAHA)) (14, 15). The NF-κB pathway is turned on by activating protein kinase C. Several drugs, such as TNF-α (16), prostratin (17), and bryostatin (18), are currently under investigation for treating HIV-1 latency through this mechanism. P-TEFb is activated by some small compounds to trigger its transcriptional interference which may occur that promotes HIV-1 latency (10, 11). Epigenetic regulation of the proviral 5’ long terminal repeat (LTR) region places another layer to control HIV-1 latency, which includes the events of chromatin modification and nucleosome reorganization by recruiting histone deacetylases (HDACs), histone methyltransferases, and DNA methyltransferases coordinately to silence the viral promoter (12, 13).

Experimental Procedures

**Cells and Plasmids**—HEK293 and HEK293T cells were maintained in DMEM supplemented with 10% FBS. The monocytic THP89GFP cells were kindly provided by David Levy (New York University) (27), which were cultured in complete medium (RPMI 1640, 10% FBS, 1× glutamine, 1× MEM non-essential amino acid solution, 20 mmol/liter HEPES). U1/HIV, J-LAT A2, and MT-2 cells were obtained from the National Institutes of Health AIDS reagent repository and cultured in RPMI 1640 medium with 10% FBS. Primary CD4+ T cells were purchased (Sanguine Biosciences, Lonza). pCDNA-Tat, pQCXIP-FLAG-Tat, HIV-LTR-luciferase, and pRL-TK-Renilla vectors were described previously (22). HTLV-1 LTR-luciferase, BC12-Tax, and pB-His6-Tax, were kindly provided by Chou-Zen Giam (Uniformed Services University of the Health Sciences) (28). pCDNA-V5-SSRP1 was constructed through Gateway® LR cloning technology using pDONR223-SSRP1 entry vector and pCDNA-DEST40 destination vector (Life Technologies). SUP16H, SSRP1, or nontargeting (NT) shRNAs were cloned into pAPM lentiviral vector (29) or pInducE10 vector (30), using Xhol and EcoRl sites.

**Small Molecules**—Prostratin (SC-203422) and SAHA (sc-220139) were purchased (Santa Cruz Biotechnology). Compounds were used at the following concentrations: Prostratin (1 μM) and SAHA (0.5 μM). Drug-treated cells were cultured in the presence of compounds for 24 h and subjected to flow cytometry assays on a FACSCalibur flow cytometer (Becton Dickinson), and results were acquired using BD CellQuest software and analyzed using FlowJo vX.0.7 program. Puromycin and doxycycline for cell treatment were purchased from Fisher Scientific. Propidium iodide for cell cycle assay was purchased from MP Biomedicals and used according to the manufacturer’s manual.

**Viruses**—Lentiviruses were produced by transfecting plasmids in HEK293T cells using TransIT®-293 transfection reagent (Mirus). VSV-G pseudotyped HIV-1 NL4–3 viruses were produced by co-transfecting pCG-VSV-G vector (22) with HIV-1 NL4–3-Luc (dEnv) plasmid (pNL4–3.Luc.R.E–), National Institutes of Health AIDS reagent repository, no. 3418). pAPM or pInducE10 shRNA expression vectors were transfected in HEK293T cells with packaging vectors pSAX2

**SUPT16H and SSRP1 Regulate Latency of HIV-1 and HTLV-1**

**SUPT16H and SSRP1 Regulate Latency of HIV-1 and HTLV-1**

**SUPT16H and SSRP1 Regulate Latency of HIV-1 and HTLV-1**
SUPT16H and SSRP1 Regulate Latency of HIV-1 and HTLV-1

and pMD2.G (Addgene) (22). Cell supernatants containing lentiviruses were harvested and filtered through 0.45-μm filters (Millipore). Viruses were stored in aliquots at −80 °C for later use. To generate cell lines for stable expression of shRNAs, lentiviruses were transduced into cells (HEK293, J-LAT A2, U1/HIV, THP89GFP, or MT-2 cells). At 72 h post-transduction, puromycin (1 μg/ml) was added to the medium for stable selection.

Antibodies—The following antibodies were used in this study: mouse anti-SUPT16H (A-1), mouse anti-SSRP1 (D-7), rabbit anti-Cyclin T1 (CCNT1, H-245), rabbit anti-GAPDH (FL-335), goat anti-mouse IgG-HRP, and goat anti-rabbit IgG-HRP (Santa Cruz Biotechnology); mouse anti-V5 (Invitrogen); rabbit anti-FLAG (RoCKland); mouse anti-FLAG (Sigma); and unlabeled rabbit and mouse IgGs (Southern Biotech). Monoclonal mouse anti-Tax antibody (4C5) was kindly provided by Chou-Zen Giam (Uniformed Services University of the Health Sciences).

Luciferase Reporter Assays—HEK293 cells that stably express SUPT16H or SSRP1 or NT shRNA were infected with VSV-G pseudo-typed HIV-1 NL4–3-Luc (dEnv) viruses. At 24 h postinfection, the luciferase unit was measured using the One-Glo® luciferase assay system (Promega) and normalized to total cell numbers. To measure the HIV-1 LTR activity, HEK293 cells that stably express SUPT16H or SSRP1 or NT shRNA were transfected with HIV-1 LTR-luciferase, pRL-TK-Renilla, and pCDNA-Tat vectors. At 48 h post-transfection, luciferase and Renilla units were measured using the Dual-Glo® luciferase assay system (Promega), and the relative luciferase unit was calculated. For HTLV-1 study, these cells were transfected with HTLV-1 LTR-luciferase, pRL–TK Renilla, and BC12-Tax vectors (28). At 48 h post-transfection, the relative luciferase unit was measured. All results were collected on a Luminoskan Ascent Microplate Luminometer (Thermo).

Chromatin Immunoprecipitation—ChIP assays followed the previously described protocol (32) with minor changes. Briefly, HEK293 cells in 10-cm tissue culture dishes were infected with VSV-G pseudo-typed HIV-1 NL4–3-Luc (dEnv) viruses. At 48 h postinfection, the cells were cross-linked using 0.5% formaldehyde. After quenching the cross-linking reaction with 125 mM glycine, the cells were washed with cold 1 × PBS and lysed in 1 × CE buffer (10 mM HEPES-KOH, pH 7.9, 60 mM KCl, 1 mM EDTA, 0.5% Nonidet P-40, 1 mM DTT, and protease inhibitor mixture). Cell lysate was centrifuged at 700 × g for 10 min at 4 °C to pellet the nuclei. Nuclei pellet was resuspended in 1 × SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.1, and protease inhibitor mixture) to a final concentration of 3 × 107 cells/ml, and nuclear lysate was sonicated for 2 min using Fisher Scientific® model 505 sonic dismembrator (1-s on and 1-s off cycles at 50% amplitude) to fragment DNA to an average size of ~500 bp. Cellular debris was spun down, and the supernatant was diluted 10-fold with 1 × ChIP dilution buffer (0.01% SDS, 1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, 1.50 mM NaCl, and protease inhibitor mixture) and incubated with 5 μg of antibodies against SUPT16H, SSRP1, CCNT1, or control mIgG or rabbit IgG antibody, with rotation overnight at 4 °C. 50 μl of protein A/G beads were pre-equilibrated with 0.5 mg/ml BSA (Fisher Scientific) and 0.125 mg/ml calf thymus DNA ( Trevigen) for 1 h at 4 °C and then added to each sample for incubation for another 2 h at 4 °C. Beads were collected, washed once each with low salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl), high salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 500 mM NaCl), and LiCl buffer (0.25 M LiCl, 1% Nonidet P-40, 1% sodium deoxycholate, 1 Mm EDTA, 10 mM Tris-HCl, pH 8.1) and twice with 1 × TE buffer (10 mM Tris-HCl pH 8.1, 0.1 mM EDTA). Immunoprecipitated protein-DNA complexes were eluted twice with fresh elution buffer (1% SDS, 0.1 M NaHCO3) for 1 h and 15 min, respectively, at room temperature. Eluates and nuclear lysates (“input”) were heated at 65 °C for overnight to reverse cross-links in the presence of 0.2 M NaCl. Samples were then treated with 1 μl of 20 mg/ml proteinase K (Life Technologies), 10 μl of 2′ Tris-HCl (pH 6.5), and 10 μl of 0.5 mM EDTA for 2 h at 50 °C. Released DNA was extracted by phenol/chloroform, precipitated by ethanol, and resuspended in 100 μl of water. 5 μl of each sample was used for semiquantitative PCR using primer sets for amplifying theLTR or nef region of HIV-1 genome. To investigate the effect of SUPT16H on P-TEFb LTR association, HEK293 cells stably expressing SUPT16H or NT shRNA were infected with VSV-G pseudo-typed HIV-1 NL4–3-Luc (dEnv) viruses and subjected to ChIP assays using rabbit CCNT1 or rabbit IgG antibody.
Real Time Quantitative PCR—To measure shRNA-mediated gene depletion or level of viral transcripts, total RNA was extracted from cells that stably express shRNAs using the RNeasy mini kit (Qiagen) and subsequently reverse transcribed using random hexamers (0.1 μM) and iScript™ cDNA synthesis kit (Bio-Rad). The gene-specific primers in Table 1 (0.1 μM each) were mixed with reverse transcribed cDNA templates and iTaq™ Universal SYBR® Green supermix (Bio-Rad). The qPCR was performed on the CFX Connect™ real time PCR detection system (Bio-Rad), in a 20-μl volume using the following program: 95 °C for 1 min and 40 cycles of 95 °C for 15 s and 60 °C for 30 s. GAPDH was used as an internal control.

Generation of Primary CD4+ T Cell Model of HIV-1 Latency—To investigate the role of FACT protein in HIV-1 latency in primary cells, we used the primary cell model established by Dr. Vicente Planelles’s group (33, 34) with slight modifications. Briefly, naïve CD4+ T cells (Sanguine Biosciences, Lonza) were stimulated with anti-human CD3 and anti-human CD28 antibodies (eBioscience) precoated on a Nunc-Immuno MaxiSorp plate (Thermo Scientific). Cells were incubated with complete medium supplemented with 10 ng/ml of TGF-β, 2 μg/ml of anti-human IL-12, and 1 μg/ml of anti-human IL-4 (R & D Systems) for 3 days. After activation, shRNA transduction was performed by spinoculation of cells with pINDUCER10-shNT/shSUPT16H/shSSRP1 lentiviruses at 1,741 g for 2 h at 37 °C. The cells were then resuspended in complete medium supplemented with 100 IU/ml of rIL-2 and kept in culture for 4 days. The medium was changed every 2 days. On day 7, cells were infected with VSV-G pseudo-typed HIV-1 NL4–3-Luc (dEnv) viruses and selected with puromycin (1 μg/ml) for stable transduction of pINDUCER10 shRNAs. The cells were kept in culture for another 10 days and then treated with doxycycline (0.1 μg/ml) for 4 days to induce shRNA expression. On day 21, cells were

FIGURE 1. Depletion of FACT proteins increases HIV-1 transcription. A, RIGER method was applied to analyze screens performed using multiple orthologous RNAi reagents. Genes were ranked in order of their RIGER scores, from lowest to highest. RIGER analysis of these screens recognized several known host restriction factors (CCNK, BRD4, and NELFCD), as well as new ones, such as SUPT16H and SSRP1 FACT proteins. B and C, shRNAs (sh1, sh2) targeting SUPT16H or SSRP1 in lentiviral pAPM vector were transduced in HEK293 cells. HEK293 cells stably expressing shRNAs were lysed, separated by SDS-PAGE, and analyzed by Western blots using anti-SUPT16H (B) or anti-SSRP1 (C) antibody. GAPDH protein level was determined using an anti-GAPDH antibody to indicate equal loading of protein samples. The results were one representative from three independent experiments. D and E, HEK293 cells depleted of FACT proteins were infected with VSV-G pseudo-typed HIV-1 NL4–3-Luc (dEnv) viruses. At 24 h postinfection, the cells were subjected to measurement of luciferase activity that was normalized by cell numbers. F and G, vectors of HIV-1 LTR-luciferase, pTK-Renilla, and pCDNA-Tat were co-transfected in HEK293 cells stably expressing shSUPT16H (F) or shSSRP1 (G). At 48 h post-transfection, luciferase activity was measured and normalized to the Renilla signal. The relative light unit (RLU) of shSUPT16H (F) or shSSRP1 (G) expressing cells was normalized to shNT cells. The results throughout are the means of three independent experiments ± S.D. *, p < 0.05 using Student’s t test.
analyzed by the luciferase reporter assays. An aliquot of activated CD4+/H11001 T cells were subjected to transduction, selection, and induction of pINDUCER10 shRNAs, and these cells were further used for RNA extraction to measure SUPT16H or SSRP1 depletion by reverse transcription and real-time qPCR. This study was approved by University of Rochester Medical Center Institutional Review Board for Protection of Human Subjects (no. RSRB00053667).

Results

RNAi Screens Identify FACT Proteins as Top HIV-1 Restriction Factors—We identified FACT proteins, SUPT16H and SSRP1, as top host restrictive modulators of HIV-1 replication, using multiple orthologous RNAi reagents coupled with integrated analytical tools (23). To calculate a gene-specific enrichment score based on the rank distribution of each individual RNAi reagent among all three screens performed by our group (Silencer Select, esiRNA, and SMARTpool), we used the RNAi gene enrichment ranking (RIGER) method that denotes the likelihood that the selected gene plays a role in the phenotype of interest (35, 36). We generated the average RIGER score (RIGER3) from three RIGER integrative approaches (the second best, weighted sum, and Kolmogorov-Smirnov) and ranked genes from the most likely host dependence factors to host restriction factors.

FIGURE 2. FACT proteins associate with Tat-LTR. A, HEK293 cells were transiently transfected with a pQCXIP-FLAG-Tat vector. At 48 h post-transfection, cells were lysed for IP assays using an anti-FLAG or a mlgG antibody. Cell lysate and precipitated protein samples were separated by SDS-PAGE. Protein levels of SUPT16H, SSRP1, or FLAG-Tat were determined by Western blots using their respective antibody. B, IP assays were performed for HEK293 cells that were co-transfected with pcDNA-V5-SSRP1 and pQCXIP-FLAG-Tat vectors. At 48 h post-transfection, cells were lysed and incubated with an anti-V5 or mlgG antibody. Protein level of V5-SSRP1 or FLAG-Tat in cell lysate and precipitated samples was determined using their respective antibody. C and D, HEK293 cells were infected with VSV-G pseudo-typed HIV-1 NL4–3-Luc (dEnv) virus. At 48 h postinfection, cells were cross-linked using formaldehyde, and nuclei were isolated and lysed. Nuclear lysate was sonicated, precleared, and subjected to ChIP assays using anti-SUPT16H (C), anti-SSRP1 (D), or mlgG antibody. Precipitated DNA samples were released, extracted, and analyzed by semiquantitative PCR using primer sets amplifying the HIV-1 LTR promoter or nef region.

FIGURE 3. SUPT16H interferes with association of P-TEFb with Tat-LTR. A, HEK293 cells stably expressing shSUPT16H (sh2) or shNT were transiently transfected with a pQCXIP-FLAG-Tat vector and subjected to IP assays using an anti-FLAG antibody. Endogenous CCNT1 protein level in cell lysate and precipitated samples was analyzed by Western blots using an anti-CCNT1 antibody. B, ChIP assays were performed against CCNT1 in shSUPT16H (sh2) or shNT expressing HEK293 cells that were infected with VSV-G-pseudo-typed HIV-1 NL4–3-Luc (dEnv) viruses. The results throughout were from one representative from three independent experiments.

SUPT16H and SSRP1 Regulate Latency of HIV-1 and HTLV-1
according to the RIGER3 score (Fig. 1A). Both FACT components, SUPT16H and SSRP1, were ranked as top HIV-1 restriction factors, among which were several known HIV-1 restriction factors including CCNK (37), BRD4 (22), and NELFCD (38).

Depletion of FACT Proteins Enhances HIV-1 LTR Promoter Activity—To confirm the effect of FACT proteins on HIV-1 replication, two sequence-unique shRNAs targeting SUPT16H or SSRP1 were synthesized and cloned in the pAPM lentiviral
expression vector (29). SUPT16H or SSRP1 shRNAs were transduced in HEK293 cells, which were subjected to selection of shRNA stable expression. HEK293 cells expressing NT shRNA were created as a negative control. The NT shRNA expression in these cells did not affect the luciferase activity from VSV-G pseudo-typed HIV-1 NL4–3-Luc (dEnv) viruses as well as the HIV-1/HTLV-1 LTR-luciferase constructs (data not shown). The endogenous SUPT16H or SSRP1 protein expression was mostly silenced by their respective shRNA (Fig. 1, B and C). These cells were infected with VSV-G pseudo-typed HIV-1 NL4–3-Luc (dEnv) viruses and then subjected to luciferase assay at 24 h postinfection. Depletion of SUPT16H or SSRP1 clearly permitted better HIV-1 replication in HEK293 cells. The results throughout are the means of three independent experiments. GFP-expressing cells were sorted using a defined gate, and a percentage of GFP-positive cells was measured and normalized to shNT-expressing cells. The protein level was determined using an anti-GAPDH antibody to indicate equal loading of protein samples. The results were from one representative from three independent experiments.

**Figure 1.** Depletion of FACT proteins enhances reversal of HIV-1 latency in monocytes. A. U1/HIV cells were transduced with pAPM-shSUPT16H (sh2), shSSRP1 (sh1), or shNT. Cell lysate was prepared, separated by SDS-PAGE, and analyzed by Western blotting using anti-SUPT16H or anti-SSRP1 antibody. GAPDH protein level was determined using an anti-GAPDH antibody to indicate equal loading of protein samples. The results were from one representative from three independent experiments. B and D. cDNA samples from the aforementioned cells were subjected to qPCR assays to measure HIV-1 gag mRNA. The level of gag transcripts was normalized to shNT-expressing cells. C and D. cDNA samples from the aforementioned cells were subjected to qPCR assays to measure the HIV-1 initiation and elongation transcripts. The level of viral transcripts was normalized to shNT-expressing cells. E. THP899GP cells were transduced with pAPM-shSUPT16H (sh2), shSSRP1 (sh1), or shNT. Cells were lysed, separated by SDS-PAGE, and analyzed by Western blot using anti-SUPT16H or anti-SSRP1 antibody. GAPDH protein level was determined using an anti-GAPDH antibody to indicate equal loading of protein samples. The results were from one representative from three independent experiments. F and G, the aforementioned cells were treated with DMSO or SAHA (0.5 μM) for 24 h. Cells were analyzed by flow cytometry. GFP-expressing cells were sorted using a defined gate, and a percentage of GFP-positive cells was measured and normalized to shNT-expressing cells. The results throughout are the means of three independent experiments ± S.D. *p < 0.05 using Student’s t test.
shSUPT16H or shSSRP1 in these cells efficiently depleted FACT proteins (Fig. 4A). Depletion of SUPT16H or SSRP1 increased the level of HIV-1 gag viral transcript (Fig. 4B). To further discriminate whether FACT proteins affect HIV-1 transcriptional initiation or elongation, primer sets recognizing distinguished viral transcripts were used for SYBR® Green-based RT-qPCR (Table 1), following a previously reported protocol (22). Interestingly, both transcriptional initiation and elongation of HIV-1 were enhanced in U1/HIV cells depleted of FACT proteins (Fig. 4, C and D), indicating that FACT proteins interfere with HIV-1 transcription through a P-TEFb independent route, considering that P-TEFb mainly affects elongation (42).

We also evaluated the effects of FACT proteins on HIV-1 latency in another monocytic cell line of HIV-1 latency, THP89GFP (27). Depletion of FACT proteins mildly increased HIV-1 reactivation (2–3-fold) with or without the presence of SAHA (Fig. 4, E and F).

**Depletion of FACT Proteins Reverses Latent HIV-1 in CD4+ T Cells**—Although monocytes are one source of residual HIV-1, resting CD4+ T cells count for the majority of HIV-1 latent reservoirs (43, 44). Thus, we then determined the role of FACT proteins in HIV-1 latency in CD4+ T cells. Using a J-LAT cell line (Jurkat cells latently infected with a HIV-1 virus encoding GFP), J-LAT A2, we confirmed that SUPT16H and SSRP1 were
SUPT16H and SSRP1 Regulate Latency of HIV-1 and HTLV-1

**FIGURE 6. Depletion of FACT proteins enhances reversal of HIV-1 latency in primary T cells.** A, procedures are illustrated for generation of primary CD4+ T cell model of HIV-1 latency to study the latency-reversing effect of FACT proteins. It was adapted from Refs. 33 and 34. Colored bars indicate T cell differentiation and activation states. B, primary CD4+ T cells isolated from three donors were cultured ex vivo. Activated CD4+ T cells were transduced with pINDUCER10-shSUPT16H (sh2), shSSRP1 (sh1), or shNT. Cells stably expressing shRNAs were selected by treating cells with puromycin. shRNA expression was induced with doxycycline. Total RNAs were extracted from these cells and analyzed by reverse transcription and qPCR for measuring the transcripts of FACT proteins. Level of SUPT16H or SSRP1 transcript was normalized to shNT-expressing cells for individual donor. C, pINDUCER10-shSUPT16H or shSSRP1 stably transduced primary memory CD4+ T cells were infected with VSV-G pseudo-typed HIV-1 NL4–3-Luc (dEnv) viruses. Cells were kept in long term culture to permit HIV-1 latency. HIV-1 latently infected cells were treated with doxycycline to induce shRNA expression. Luciferase activity was measured for cells depleted of SUPT16H or SSRP1 and normalized to shNT-expressing cells. The results throughout are the means of three independent experiments ± S.D. * indicates p < 0.05 using Student’s t test.

Depletion of FACT proteins enhances reversal of HIV-1 latency in primary T cells.

**FIGURE 7. FACT Proteins Elicit Similar Effects on HTLV-1 Transcription.** FACT Proteins Elicit Similar Effects on HTLV-1 Transcription—HTLV-1 is a human retrovirus that shares common features of transcriptional regulation with HIV-1, including the use of P-TEFBs (46, 47). We expect that FACT proteins elicit similar effects on HTLV-1 transcription. Depletion of FACT proteins increased the luciferase expression driven by the HTLV-1 LTR promoter in HEK293 cells that were transfected with HTLV-1 LTR-Luciferase, pTK-Renilla, and BC12-Tax vectors (Fig. 7, A and B). The co-immunoprecipitation experiments in HEK293 cells transfected with pB-His6-Tax also con-

Depletion of FACT proteins as well as TGF-β and anti-IL-4 antibodies. To allow the timing of the FACT shRNA expression after establishment of HIV-1 latency, we cloned shSUPT16H (sh2) and shSSRP1 (sh1) into a tet-inducible shRNA lentiviral expression vector, pINDUCER10 (30). These shRNAs were stably transduced in activated CD4+ T cells. These cells were then spinoculated with VSV-G pseudo-typed HIV-1 NL4–3-Luc (dEnv). Latent infection was established through a long term culture of cells with recombinant rIL-2. SUPT16H or SSRP1 shRNA expression was induced by treating cells with doxycycline. Latency-reversing effects were determined by measuring luciferase activity (Fig. 6A). Our results using CD4+ T cells isolated from three donors showed that the mRNA levels of SUPT16H and SSRP1 were efficiently depleted by induced shRNA expression (Fig. 6B). Furthermore, depletion of FACT proteins indeed spontaneously facilitated reversing of latent HIV-1 in nonpolarized, memory CD4+ T cells (Fig. 6C).
HIV-1 only encodes 12 genes and undertakes comprehensive interplay with host cellular machineries to fulfill its life cycle, especially silencing the gene expression of integrated proviral HIV-1 resulting in latency. Consequentially, the fate of HIV-1 proviruses is mostly dependent upon the cellular environment and host genetic function. Host factors regulating HIV-1 transcription are the key to control the switch between latency and reactivation of HIV-1 proviruses. Earlier studies focusing on host genes that are required for HIV-1 transcription have lead to the identification of major regulators that promote either initiation or elongation of LTR transcription. The LTR promoter alone is able to initiate transcription very efficiently. Host transcriptional activators, such as NF-κB, NFAT, and C/EBPβ, recognize binding sites at the LTR promoter and further recruit chromatin-remodeling proteins (HATs, SWI-SNF complexes, and demethylases). These proteins modify nucleosomes at the LTR and lead to induction of transcription (50–52). The LTR promoter establishes only nonprocessive (basal) transcription in the absence of Tat and exclusively depends on Tat for transcriptional elongation. Tat recruits P-TEFb, a critical host protein complex required for HIV-1 transcription, to phosphorylate the paused RNA polymerase II at the LTR and activates it for efficient elongation. However, host cells also develop the mechanisms to silence HIV-1 transcription, which is necessary for establishing and maintaining latency. There are a few factors reported previously, such as CBF1 (53), NELFCD (38), and HDACs (54), but they play a relatively general role in transcriptional suppression with a lack of specificity to HIV-1.

From our unbiased RNAi screens and further characterization of prioritized genes, we were able to identify a set of host factors that restrict HIV-1 replication through transcriptional suppression, including BRD4 and FACT proteins (SUPT16H and SSRP1) (Fig. 1A). These endeavors have allowed us to identify novel host regulators for HIV-1 transcription and further study their functions in HIV-1 latency and reactivation. Our goal is to improve the understanding of host–HIV interactions at latent phase, so that we can manage or manipulate them for elimination of residual HIV-1 in reservoir cells.

Discussion

HIV-1 and HTLV-1

**FIGURE 7. FACT proteins demonstrate similar effect on HTLV-1 transcription.** A and B, the vectors HTLV-1 LTR-luciferase, pTK-Renilla, and BC12-Tax, were co-transfected in HEK293 cells stably expressing shRNAs of SUPT16H (A) or SSRP1 (B). At 48 h post-transfection, luciferase activity was measured and normalized to the Renilla signal. The relative light unit (RLU) of shSUPT16H or shSSRP1 expressing cells was normalized to shNT cells. C, HEK293 cells were transfected with p8-His5-Tax vector. At 48 h post-transfection, cells were lysed and subjected to IP assays using an anti-SUPT16H or mlgG antibody. Cell lysate and precipitated protein samples were separated by SDS-PAGE. Protein level of His5-Tax was determined by Western blots using a mouse anti-Tax antibody (4C5). The results were one representative from three independent experiments. The results were one representative from three independent experiments. D, HTLV-1 transformed MT-2 cells were stably transduced with pAPM-shSUPT16H (sh2), shSSRP1 (sh1), or shNT. Cells were lysed, separated by SDS-PAGE, and analyzed by Western blots using anti-SUPT16H or anti-SSRP1 antibody. The GAPDH protein level was determined using an anti-GAPDH antibody to indicate equal loading of protein samples. The results were one representative from three independent experiments. E, cDNA samples from the aforementioned cells treated with SAHA (0.5 μM) were subjected to qPCR assays to measure the HTLV-1 gag/pol mRNA. Level of viral transcripts was normalized to shNT cells. The results throughout are the means of three independent experiments ± S.D., *p < 0.05 using Student’s t test.

firmed that exogenously expressed Tax protein associated with endogenous SUPT16H (Fig. 7C). We further investigated the activity of FACT proteins on HTLV-1 transcription and latency in a human T cell leukemia cell line, MT-2, which is transformed with HTLV-1 (48). Stable expression of shSUPT16H or shSSRP1 in these cells efficiently depleted FACT proteins (Fig. 7D). Depletion of SUPT16H or SSRP1 increased the expression level of the HTLV-1 gag/pol gene assayed by qPCR (49), when the cells were treated with SAHA (Fig. 7E).
believed that the FACT complex facilitates transcriptional elongation based upon these studies. However, it is not completely understood how FACT can coordinate its assembly and disassembly activity to facilitate the sliding of RNA polymerase. Two major models describe the mechanism of FACT-mediated nucleosome reorganization: dimer eviction model and global accessibility/noneviction model (59). More intriguingly, studies in yeast have indicated that SPT16/CDC68 is important for the maintenance of chromatin-mediated repression in the absence of transactivation (58), but there is no such evidence in mammalian cells. Because of a lack of current studies focusing on this complex, we cannot rule out that FACT proteins may exert both promptive and suppressive effects on gene expression in human cells. Nevertheless, it is still surprising that the FACT complex, a general transcriptional facilitator, imposes an inhibitory effect on HIV-1 transcription, opposing earlier speculation (60). Our results have indicated that the presence of SUPT16H may interfere with interactions of P-TEFb with Tat-LTR (Fig. 3, A and B), which provides a novel and plausible explanation for FACT-mediated inhibition of HIV-1 transcription. Our tentative model indicates that LTR recruitment of FACT by Tat may generate a steric hindrance for P-TEFb association, resulting in reduced efficiency of RNA polymerase II (Fig. 8). Tax plays a very similar role as Tat, recruiting P-TEFb for stimulation of HTLV-1 transcription (46, 47, 61), which leads us to believe that FACT proteins may also interfere with P-TEFb required for HTLV-1 transcription through association with Tax (Fig. 7C). Further study of FACT proteins in human retroviral transcription, as well as more profound analysis of FACT-mediated transcriptional regulation at the genome-wide scale in retrovirus-infected cells, may help to unravel the complicated functions of FACT that could be modulated by viral components. In addition, FACT is also a multifunctional protein complex that regulates other cellular processes, such as DNA replication (62–64), DNA damage response (65, 66), and cell cycle progression (67, 68). Whether FACT-mediated nontranscriptional functions may affect HIV-1/HTLV-1 replication is unclear and requires further investigation.

The direct evidence that the FACT proteins affect HIV-1 transcription is their association with Tat and the LTR promoter. Our protein interaction studies have indicated that Tat associates with SUPT16H but not SSRP1, either endogenous or transiently expressed (Fig. 2, A and B). Interestingly, we found that the presence of Tat did not disturb the association between SUPT16H and SSRP1 (Fig. 2F). These studies suggest that SUPT16H directly binds to Tat. This binding may explain how FACT suppresses both HIV-1 transcriptional initiation and elongation (Fig. 4, C and D), because Tat increases initiation complex formation on LTR and stabilizes the complex during elongation (69, 70). Furthermore, our chromatin association studies have confirmed that both SUPT16H and SSRP1 are recruited to the LTR (Fig. 2, C and D). An intriguing explanation for SSRP1 association with LTR but not Tat could be that either SSRP1 may indirectly associate with Tat through SUPT16H but to a much lesser degree or that SSRP1 contains a high mobility group box so that its recruitment to LTR may be Tat-independent.

Because FACT proteins suppress HIV-1 transcription, we speculate that their presence may silence viral gene expression and promote HIV-1 latency. Earlier studies demonstrate that nucleosomes are precisely positioned at the HIV-1 LTR, and their organization is conserved (71–73). We expect that FACT proteins may impose a similar effect on the HIV-1 LTR chromatin across various cell models of HIV-1 latency. We used multiple cell models of HIV-1 latency to confirm that depletion of FACT proteins alleviates transcriptional suppression and reverses latent HIV-1 (Figs. 4–6). Most compellingly, we were able to validate FACT functions in HIV-1 latently infected primary CD4+ T cells (Fig. 6). In this model, activated CD4+ T cells were nonpolarized to mimic central memory CD4+ T cells to establish HIV-1 latency (33, 34). Depletion of FACT proteins in these cells spontaneously reversed HIV-1 latency, indicating that FACT proteins are main host factors for maintaining HIV-1 latency, and their removal may directly activate some of latent HIV-1 without need of other cell signaling activation. Another significant discovery is that depletion of FACT proteins synergized with the known latency-reversing agent, SAHA (Fig. 5C). Because SAHA blocks local histone deacetylation and allows loading of the transcriptional initiation complex onto the viral LTR to induce transcription, whereas depletion of FACT proteins enhances HIV-1 transcriptional elongation by increasing binding of P-TEFb to Tat-LTR, this may explain the enhanced reversing of latent HIV-1. Failure to produce any synergistic effects between FACT depletion and prostratin treatment may be due to prostratin quenching HIV-1 reversal, masking the inhibitory effect of FACT proteins. Future studies should also investigate the role of FACT proteins in establishment of HIV-1 latency in activated primary CD4+ T cells once they return to the resting stage. Although these
results from primary cells are convincing, the more physiologically relevant HIV-1 latent reservoir cells to test ex vivo are CD8-depleted peripheral blood mononuclear cells isolated from HAART-treated AIDS patients with undetectable HIV-1 viral loads (22). However, it is difficult to perform genetic manipulation (shRNA-mediated knockdown or CRISPR/Cas9-mediated knockout) in these cells because the frequency of HIV-1 latently infected cells is extremely low (<1 of 1–5 million). The limited transduction efficiency of lentiviruses is unable to guarantee the successful delivery of shRNAs or sgRNAs (CRISPR/Cas9) into cells that actually harbor latent HIV-1 proviruses. Hence, we shall initiate the development and/or screening of small molecule compounds that are able to specifically disrupt the interaction of FACT proteins with Tat-LTR so that their suppressive effect on viral transcription is removed. These FACT inhibitors can be further tested in HIV-1 latent reservoirs of AIDS patients.

Author Contributions—J. Z. and S. E. conceived the project, J. Z. and H. H. designed the experiment and wrote the paper. H. H., N. S., D. P., S. S., and M. D. conducted the experiments. J. Z., H. H., N. S., and H. M. analyzed the results. K. G. and C.-Z. G. provided the reagents and advised the study. All authors reviewed the results and approved the final version of the manuscript.

Acknowledgments—We thank Dr. David Levy at New York University for the gift of THP89GFP cells. We also acknowledge Drs. Stephen Dewhurst and Sanjay Maggirwar (University of Rochester Medical Center) for helpful discussions and preparation of the manuscript.

References
1. Maartens, G., Celum, C., and Lewin, S. R. (2014) HIV infection: epidemiology, pathogenesis, treatment, and prevention. Lancet 384, 258–271
2. Finzi, D., Hermannkova, M., Pierson, T., Carruth, I. M., Buck, C., Chaisson, R. E., Quinn, T. C., Chadwick, K., Margolick, J., Brookmeyer, R., Gallant, J., Markowitz, M., Ho, D. D., Richman, D. D., and Siliciano, R. F. (1997) Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 278, 1295–1300
3. Wong, J. K., Hezareh, M., Günthard, H. F., Havlir, D. V., Ignacio, C. C., Markowitz, M., Ho, D. D., Richman, D. D., and Siliciano, J. D. (1997) Comparative analysis of measures of viral reservoirs in HIV-1 eradication studies. J. Virol. 71, 6060–6064
4. Shan, L., and Siliciano, R. F. (2013) From reactivation of latent HIV-1 to elimination of the latent reservoir: the presence of multiple barriers to viral eradication. BioEssays 35, 544–552
5. Eriksson, S., Grad, E. H., Dahl, V., Strain, M. C., Yukl, S. A., Lyensen, E. K., Bosch, R. J., Lai, J., Chioma, S., Emad, F., Abdel-Mohsen, M., Hoh, R., Hecht, F., Hunt, P., Somsouk, M., Wong, J., Johnston, R., Siliciano, R. F., Richman, D. D., O’Doherty, U., Palmer, S., Deeks, S. G., and Siliciano, J. D. (2013) Comprehensive analysis of measures of viral reservoirs in HIV-1 eradication studies. PLoS Pathog. 9, e1003174
6. Kutsch, O., Benveniste, E. N., Shaw, G. M., and Levy, D. N. (2002) Direct inhibition in HIV-1 infection. Cell Host Microbe 2, 807–816
7. Ott, M., Geyer, M., and Zhou, Q. (2011) The control of HIV transcription: keeping RNA polymerase II on track. Cell Host Microbe 10, 426–435
8. Han, Y., Lassen, K., Monie, D., Sedaghat, A. R., Shimoji, S., Lue, T., Pierson, T. C., Margolick, J. B., Siliciano, R. F., and Siliciano, J. D. (2004) Resting CD4+ T cells from human immunodeficiency virus type 1 (HIV-1)-infected individuals carry integrated HIV-1 genomes within actively transcribed host genes. J. Virol. 78, 6122–6133
9. Koizumi, K., Lin, Y. B., An, W., Xu, J., Yang, H. C., O’Connell, K., Dordai, D., Boeke, J. D., Siliciano, J. D., and Siliciano, R. F. (2008) Orientation-dependent regulation of integrated HIV-1 expression by host gene transcriptional readthrough. Cell Host Microbe 4, 134–146
10. Siliciano, R. F., and Greene, W. C. (2011) HIV latency. Cold Spring Harb. Perspect. Med. 1, a007096
11. Coiras, M., López-Huertas, M. R., Pérez-Olmeda, M., and Alcamí, J. (2009) Understanding HIV-1 latency provides clues for the eradication of long-term reservoirs. Nat. Rev. Microbiol. 7, 798–812
12. Williams, S. A., Chen, L. F., Kwon, H., Fenard, D., Biggrove, D., Verdin, E., and Greene, W. C. (2004) Prostratin antagonizes HIV latency by activating NF-κB. J. Biol. Chem. 279, 42008–42017
13. Sánchez-Duффuhrs, G., Vo, M. Q., Pérez, M., Calzado, M. A., Moreno, S., Appendino, G., and Muñoz, E. (2011) Activation of latent HIV-1 expression by protein kinase C agonists: a novel therapeutic approach to eradicate HIV-1 reservoirs. Curr. Drug Targets 12, 348–356
14. Klichko, V., Archin, N., Kaur, R., Lehrman, G., and Margolis, D. (2006) Hexamethylenebisacetamide remodels the human immunodeficiency virus type 1 (HIV-1) promoter and induces Tat-independent HIV-1 expression but blunts cell activation. J. Virol. 80, 4570–4579
15. Duh, E. J., Maury, W. J., Folks, T. M., Fauci, A. S., and Rabson, A. B. (1989) Cytomegalovirus infection in humans: a target of aggressive cancers. Nature 342, 375–378
16. Xing, S., Bullen, C. K., Shroff, N. S., Shan, L., Yang, H. C., Manucci, J. L., Bhat, S., Zhang, H., Margolick, J. B., Quinn, T. C., Margolis, D. M., Siliciano, J. D., and Siliciano, R. F. (2011) Disulfiram reactivates latent HIV-1 in a Bel-2-transduced primary CD4+ T cell model without inducing global cell activation. J. Virol. 85, 6060–6064
17. Ho, Y. C., Shan, L., Hosmane, N. N., Wang, J., Laskey, S. B., Rosenbloom, D. I., Lai, J., Blankson, J. N., Siliciano, J. D., and Siliciano, R. F. (2013) Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. Cell 155, 540–551
18. Zhu, J., Gaiha, G. D., John, S. P., Pertel, T., Chin, C. R., Gao, G., Qu, H., Walker, B. D., Elledge, S. J., and Brass, A. L. (2012) Reactivation of latent HIV-1 by inhibition of BRD4. Cell Reports 2, 807–816
19. Zhu, J., Davoli, T., Perriera, J. M., Chin, C. R., Gaiha, G. D., John, S. P., Sigliottot, D. F., Gao, G., Xu, Q., Qu, H., Pertel, T., Sims, J. S., Smith, J. A., Baker, R. E., Maranda, L., Ng, A., Elledge, S. J., and Brass, A. L. (2014) Comprehensive identification of host modulators of HIV-1 replication using multiple orthogonal RNAi reagents. Cell Reports 7, 752–766
20. Orphanides, G., Wu, W. H., Lane, W. S., Hampsey, M., and Reinberg, D. (1999) The chromatin-specific transcription elongation factor. FACT comprises human SPT16 and SSRP1 proteins. Nature 400, 284–288
21. Belotserkovskaya, R., Oh, S., Bondarenko, V. A., Orphanides, G., Studitsky, V. M., and Reinberg, D. (2003) FACT facilitates transcription-dependent nucleosome alteration. Science 301, 1090–1093
22. Garcia, H., Miezczkowski, J. C., Safina, A., Commene, M., Ruusulehto, A., Kilpinnen, S., Leach, R. W., Attwood, K., Li, Y., Degan, S., Omilian, A. R., Guryanova, O., Papantonopoulos, O., Wang, J., Buck, M., Liu, S., Morrisson, C., and Gurova, K. V. (2013) Facilitates chromatin transcription complex is an "accelerator" of tumor transformation and potential marker and target of aggressive cancers. Cell Rep. 4, 159–173
23. Kutsch, O., Benveniste, E. N., Shaw, G. M., and Levy, D. N. (2002) Direct
and quantitative single-cell analysis of human immunodeficiency virus type 1 reactivation from latency. J. Virol. 76, 8776–8786

38. Zhang, Z., Blattner, K., Dillingham, C. M., and Yuan, L. (2015) Mechanistic insights into the regulation of HIV-1 expression by the Tat-activated transcriptional activator. J. Mol. Biol. 443, 1115–1123

39. Kupfer, M. D., and Botchan, M. (2015) The chromatin remodeller ORC1 regulates HIV-1 latency. Nat. Struct. Mol. Biol. 22, 591–597

40. Nakamura, Y., and Kameyama, H. (2015) Histone deacetylase 6 regulates HIV-1 latency. J. Virol. 89, 4246–4257

41. Hsu, M., and Koromilas, A. (2015) The histone demethylase KDM5 regulates HIV-1 latency. J. Virol. 89, 8583–8592

42. Zhang, Z., Blattner, K., Dillingham, C. M., and Yuan, L. (2015) Mechanistic insights into the regulation of HIV-1 expression by the Tat-activated transcriptional activator. J. Mol. Biol. 443, 1115–1123

43. Kupfer, M. D., and Botchan, M. (2015) The chromatin remodeller ORC1 regulates HIV-1 latency. Nat. Struct. Mol. Biol. 22, 591–597

44. Nakamura, Y., and Kameyama, H. (2015) Histone deacetylase 6 regulates HIV-1 latency. J. Virol. 89, 4246–4257

45. Hsu, M., and Koromilas, A. (2015) The histone demethylase KDM5 regulates HIV-1 latency. J. Virol. 89, 8583–8592

46. Zhang, Z., Blattner, K., Dillingham, C. M., and Yuan, L. (2015) Mechanistic insights into the regulation of HIV-1 expression by the Tat-activated transcriptional activator. J. Mol. Biol. 443, 1115–1123

47. Kupfer, M. D., and Botchan, M. (2015) The chromatin remodeller ORC1 regulates HIV-1 latency. Nat. Struct. Mol. Biol. 22, 591–597

48. Nakamura, Y., and Kameyama, H. (2015) Histone deacetylase 6 regulates HIV-1 latency. J. Virol. 89, 4246–4257

49. Hsu, M., and Koromilas, A. (2015) The histone demethylase KDM5 regulates HIV-1 latency. J. Virol. 89, 8583–8592

50. Zhang, Z., Blattner, K., Dillingham, C. M., and Yuan, L. (2015) Mechanistic insights into the regulation of HIV-1 expression by the Tat-activated transcriptional activator. J. Mol. Biol. 443, 1115–1123

51. Kupfer, M. D., and Botchan, M. (2015) The chromatin remodeller ORC1 regulates HIV-1 latency. Nat. Struct. Mol. Biol. 22, 591–597

52. Nakamura, Y., and Kameyama, H. (2015) Histone deacetylase 6 regulates HIV-1 latency. J. Virol. 89, 4246–4257

53. Hsu, M., and Koromilas, A. (2015) The histone demethylase KDM5 regulates HIV-1 latency. J. Virol. 89, 8583–8592

54. Zhang, Z., Blattner, K., Dillingham, C. M., and Yuan, L. (2015) Mechanistic insights into the regulation of HIV-1 expression by the Tat-activated transcriptional activator. J. Mol. Biol. 443, 1115–1123

55. Kupfer, M. D., and Botchan, M. (2015) The chromatin remodeller ORC1 regulates HIV-1 latency. Nat. Struct. Mol. Biol. 22, 591–597

56. Nakamura, Y., and Kameyama, H. (2015) Histone deacetylase 6 regulates HIV-1 latency. J. Virol. 89, 4246–4257

57. Hsu, M., and Koromilas, A. (2015) The histone demethylase KDM5 regulates HIV-1 latency. J. Virol. 89, 8583–8592

58. Zhang, Z., Blattner, K., Dillingham, C. M., and Yuan, L. (2015) Mechanistic insights into the regulation of HIV-1 expression by the Tat-activated transcriptional activator. J. Mol. Biol. 443, 1115–1123

59. Kupfer, M. D., and Botchan, M. (2015) The chromatin remodeller ORC1 regulates HIV-1 latency. Nat. Struct. Mol. Biol. 22, 591–597

60. Nakamura, Y., and Kameyama, H. (2015) Histone deacetylase 6 regulates HIV-1 latency. J. Virol. 89, 4246–4257

61. Hsu, M., and Koromilas, A. (2015) The histone demethylase KDM5 regulates HIV-1 latency. J. Virol. 89, 8583–8592

62. Zhang, Z., Blattner, K., Dillingham, C. M., and Yuan, L. (2015) Mechanistic insights into the regulation of HIV-1 expression by the Tat-activated transcriptional activator. J. Mol. Biol. 443, 1115–1123

63. Kupfer, M. D., and Botchan, M. (2015) The chromatin remodeller ORC1 regulates HIV-1 latency. Nat. Struct. Mol. Biol. 22, 591–597

64. Nakamura, Y., and Kameyama, H. (2015) Histone deacetylase 6 regulates HIV-1 latency. J. Virol. 89, 4246–4257

65. Hsu, M., and Koromilas, A. (2015) The histone demethylase KDM5 regulates HIV-1 latency. J. Virol. 89, 8583–8592

66. Zhang, Z., Blattner, K., Dillingham, C. M., and Yuan, L. (2015) Mechanistic insights into the regulation of HIV-1 expression by the Tat-activated transcriptional activator. J. Mol. Biol. 443, 1115–1123

67. Kupfer, M. D., and Botchan, M. (2015) The chromatin remodeller ORC1 regulates HIV-1 latency. Nat. Struct. Mol. Biol. 22, 591–597

68. Nakamura, Y., and Kameyama, H. (2015) Histone deacetylase 6 regulates HIV-1 latency. J. Virol. 89, 4246–4257

69. Hsu, M., and Koromilas, A. (2015) The histone demethylase KDM5 regulates HIV-1 latency. J. Virol. 89, 8583–8592

70. Zhang, Z., Blattner, K., Dillingham, C. M., and Yuan, L. (2015) Mechanistic insights into the regulation of HIV-1 expression by the Tat-activated transcriptional activator. J. Mol. Biol. 443, 1115–1123

71. Kupfer, M. D., and Botchan, M. (2015) The chromatin remodeller ORC1 regulates HIV-1 latency. Nat. Struct. Mol. Biol. 22, 591–597

72. Nakamura, Y., and Kameyama, H. (2015) Histone deacetylase 6 regulates HIV-1 latency. J. Virol. 89, 4246–4257

73. Hsu, M., and Koromilas, A. (2015) The histone demethylase KDM5 regulates HIV-1 latency. J. Virol. 89, 8583–8592

74. Zhang, Z., Blattner, K., Dillingham, C. M., and Yuan, L. (2015) Mechanistic insights into the regulation of HIV-1 expression by the Tat-activated transcriptional activator. J. Mol. Biol. 443, 1115–1123

75. Kupfer, M. D., and Botchan, M. (2015) The chromatin remodeller ORC1 regulates HIV-1 latency. Nat. Struct. Mol. Biol. 22, 591–597

76. Nakamura, Y., and Kameyama, H. (2015) Histone deacetylase 6 regulates HIV-1 latency. J. Virol. 89, 4246–4257

77. Hsu, M., and Koromilas, A. (2015) The histone demethylase KDM5 regulates HIV-1 latency. J. Virol. 89, 8583–8592

78. Zhang, Z., Blattner, K., Dillingham, C. M., and Yuan, L. (2015) Mechanistic insights into the regulation of HIV-1 expression by the Tat-activated transcriptional activator. J. Mol. Biol. 443, 1115–1123

79. Kupfer, M. D., and Botchan, M. (2015) The chromatin remodeller ORC1 regulates HIV-1 latency. Nat. Struct. Mol. Biol. 22, 591–597

80. Nakamura, Y., and Kameyama, H. (2015) Histone deacetylase 6 regulates HIV-1 latency. J. Virol. 89, 4246–4257

81. Hsu, M., and Koromilas, A. (2015) The histone demethylase KDM5 regulates HIV-1 latency. J. Virol. 89, 8583–8592
chromatin transcription (FACT) protein maintains normal replication fork rates. J. Biol. Chem. 286, 30504–30512

63. Han, J., Li, Q., McCullough, L., Kettelkamp, C., Formosa, T., and Zhang, Z. (2010) Ubiquitylation of FACT by the cullin-E3 ligase Rtt101 connects FACT to DNA replication. Genes Dev. 24, 1485–1490

64. VanDemark, A. P., Blanksma, M., Ferris, E., Heroux, A., Hill, C. P., and Formosa, T. (2006) The structure of the yFACT Pob3-M domain, its interaction with the DNA replication factor RPA, and a potential role in nucleosome deposition. Mol. Cell 22, 363–374

65. Heo, K., Kim, H., Choi, S. H., Choi, J., Kim, K., Gu, J., Lieber, M. R., Yang, A. S., and An, W. (2008) FACT-mediated exchange of histone variant H2AX regulated by phosphorylation of H2AX and ADP-ribosylation of Spt16. Mol. Cell 30, 86–97

66. Dinant, C., Ampatziadis-Michailidis, G., Lans, H., Tresini, M., Lagarou, A., Grosbart, M., Theil, A. F., van Cappellen, W. A., Kimura, H., Bartek, J., Fousteri, M., Houtsmlner, A. B., Vermeulen, W., and Marteijn, J. A. (2013) Enhanced chromatin dynamics by FACT promotes transcriptional restart after UV-induced DNA damage. Mol. Cell 51, 469–479

67. Tan, B. C., and Lee, S. (2004) Nek9, a novel FACT-associated protein, modulates interphase progression. J. Biol. Chem. 279, 9321–9330

68. Morillo-Huesca, M., Maya, D., Muñoz-Centeno, M. C., Singh, R. K., Oreal, V., Reddy, G. U., Liang, D., Géli, V., Gunjan, A., and Chávez, S. (2010) FACT prevents the accumulation of free histones evicted from transcribed chromatin and a subsequent cell cycle delay in G1. PLoS Genet. 6, e1000964

69. Laspi, M. F., Rice, A. P., and Mathews, M. B. (1989) HIV-1 Tat protein increases transcriptional initiation and stabilizes elongation. Cell 59, 283–292

70. Brady, J., and Kashanchi, F. (2005) Tat gets the “green” light on transcription initiation. Retrovirology 2, 69

71. Verdin, E. (1991) DNase I-hypersensitive sites are associated with both long terminal repeats and with the intragenic enhancer of integrated human immunodeficiency virus type 1. J. Virol. 65, 6790–6799

72. Verdin, E., Paras, P., Jr., and Van Lint, C. (1993) Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. EMBO J. 12, 3249–3259

73. Colin, L., and Van Lint, C. (2009) Molecular control of HIV-1 postintegration latency: implications for the development of new therapeutic strategies. Retrovirology 6, 111
FACT Proteins, SUPT16H and SSRP1, Are Transcriptional Suppressors of HIV-1 and HTLV-1 That Facilitate Viral Latency
Huachao Huang, Netty Santoso, Derek Power, Sydney Simpson, Michael Dieringer, Hongyu Miao, Katerina Gurova, Chou-Zen Giam, Stephen J. Elledge and Jian Zhu

J. Biol. Chem. 2015, 290:27297-27310.
doi: 10.1074/jbc.M115.652339 originally published online September 16, 2015

Access the most updated version of this article at doi: 10.1074/jbc.M115.652339

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 73 references, 34 of which can be accessed free at http://www.jbc.org/content/290/45/27297.full.html#ref-list-1