FEBS Letters 588 (2014) 1630–1636

journal homepage: www.FEBSLetters.org

7SK small nuclear ribonucleoprotein complex is recruited to the HIV-1 promoter via short viral transcripts

Taketoshi Mizutani [a,b,c,*], Aya Ishizaka [a,b], Yutaka Suzuki [d], Hideo Iba [a]

[a] Division of Host-Parasite Interaction, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
[b] Laboratory of Basic Science, Institute of Microbial Chemistry;BIKAKEN, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141-0021, Japan
[c] RNA and Biofunctions, Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency (JST), 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan
[d] Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa-shi, Chiba 277-8568, Japan

ARTICLE INFO

Article history:
Received 9 December 2013
Revised 13 January 2014
Accepted 14 January 2014
Available online 6 March 2014

Edited by Ivan Sadowski

Keywords:
Virology
Viral transcription
RNA virus
Chromatin regulation
Molecular biology

ABSTRACT

In this study, we demonstrate that the 7SK small nuclear ribonucleoprotein (snRNP) complex is recruited to the HIV-1 promoter via newly-synthesized HIV-1 nascent transcripts (short transcripts) in an hnRNP A1-dependent manner and negatively regulates viral transcript elongation. Our deep-sequence analysis showed these short transcripts were mainly arrested at approximately +50 to +70 nucleotides from the transcriptional start site in the U1 cells, an HIV-1 latent model. TNF-α treatment promptly disrupted the 7SK snRNP complex on the nascent transcripts and viral elongated transcripts were increased. This report provides insight into how 7SK snRNP complex is recruited to HIV-1 promoter in the absence of Tat.

1. Introduction

Under antiretroviral therapy (ART), latent infections of HIV-1 are often caused by repression of transcriptional initiation of the viral genome and elongation of nascent transcripts. A useful clue in the study of latent infection is the presence of abortive transcripts, approximately 60 nucleotides long, in resting T cells of patients undergoing ART, even though latently infected T cells may not produce virions and are difficult to distinguish from uninfected cells [1,2]. Generally, these observations are not viral-specific, as genome-wide studies have suggested that cellular RNA polymerase II (RNAPII) generates many abortive transcripts [3,4].

Transcription of the HIV-1 provirus is characterized by early Tat-independent and late Tat-dependent phases. In the early Tat-independent phase, HIV-1 transcription depends upon the interaction of host transcription factors with cis-regulatory DNA elements within the viral 5′ long terminal repeat (LTR) as well as the assembly of the transcription apparatus, including RNAPII, on these sequences. In the steady state, Tat-independent HIV-1 basal promoter activity is weak, because some host factors, which are reported to function as negative regulators of viral LTRs, are known to restrict HIV-1 basal promoter activity [5]. One of these host factors, the 7SK small nuclear ribonucleoprotein complex (snRNP), functions as a negative regulator of HIV-1 transcription by interacting with the positive elongation factor b (p-TEFb), which is composed of cyclin dependent kinase 9 (CDK9) and Cyclin T1 or Cyclin T2, to mask RNAPII-directed kinase activity. Main components of the 7SK snRNP complex are 7SK snRNA, hexamethylene bis-acetamide inducible 1 (HEXIM1), La-related protein (LARP7) and methylphosphate capping enzyme (MePCE). LARP7 is a crucial component of the 7SK snRNP complex and acts as an integral molecule in 7SK-mediated negative regulation [6,7]. However the viral accessory protein, Tat interacts with viral promoter proximal transactivation responsive element (TAR) RNA element and

Abbreviations: ART, antiretroviral therapy; RNAPII, RNA polymerase II; LTR, long terminal repeat; TAR, transactivation responsive element; snRNP, small nuclear ribonucleoprotein complex; p-TEFb, positive elongation factor b; CDK9, cyclin dependent kinase 9; LARP7, La-related protein; HEXIM1, hexamethylene bis-acetamide inducible 1; MePCE, methylphosphate capping enzyme; hnRNP A1, heterogeneous nuclear ribonucleoprotein A1

* Corresponding author at: Laboratory of Basic Science, Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141-0021, Japan. Fax: +81 3 3441 7589.
E-mail address: mizutanit@bikaken.or.jp (T. Mizutani).

http://dx.doi.org/10.1016/j.febslet.2014.01.067
0014-5793/

recruits p-TEFb from the nucleoplasm or 7SK snRNP complex to promote elongation of RNAPII. After loss of p-TEFb, the 7SK snRNP complex is known to release HEXIM1 by changing its conformation [8]. A previous report showed that 7SK snRNP complex, with or without Tat, are recruited to the HIV-1 *cis* regulatory enhancer element (SP1) before transcription initiation, although it is unclear how HIV-1 transcription is selectively negative regulated by the 7SK snRNP complex [9].

Heterogeneous nuclear ribonucleoprotein A1 (HnRNP) A1 is a known as an RNA-binding protein that associates with pre-mRNA, and functions in viral and host mRNA splicing or metabolism, including the splicing machinery involved in Tat expression in HIV-1 [10,11]. HnRNP A1 shuttles from the nucleus to cytoplasm and is involved in HIV-1 mRNA transport [12]. HnRNP A1 also plays a role in stabilizing the 7SK snRNP complex through interacting with 7SK snRNA lacking p-TEFb and HEXIM1 [8].

In this study, we report that hnRNP A1 facilitates recruitment of the 7SK snRNP complex to the HIV-1 promoter region through short viral transcripts generated by promoter-paused RNAPII.

2. Materials and methods

2.1. Plasmid construction and cell culture

A 0.7-kb PCR fragment of the 5′ LTR region of the NL43 HIV-1 molecular clone was inserted into a pWLG plasmid [13] to generate pHIV-LTR-GFP. The region from the 5′ LTR to the splicing acceptor region (0.9 kb) of pHIV-LTR-GFP was inserted into the *Cla*I-*Bam*HI minimal promoter region of the pNF-κB-MinP-Luc plasmid to generate pLTR-Luc-pA (HLpA6), which has been previously described in detail [14]. The transiently transfected shRNA expressing plasmid (pmU6) produces shRNAs from the mouse U6 RNA polymerase III promoter. Control shGFP and sh-hnRNP A1 transduced U1 derived cells were generated by stably infecting with pSSSP (SIN type-shRNA expressing SV40-puro) retrovirus vectors and puromycin drug selection for 2 weeks. The shRNA sequence used in this study are listed in Supplemental Table S1.

2.2. Quantitation of HIV-1 viral production by TNF-α stimulation

U1-derived cells (1×10^6/24-well plate) were stimulated by TNF-α (10 ng/ml; R&D Systems), and culture supernatants were then collected by centrifugation at 0, 0.5, 1, 3, 6, 12, or 24 h after stimulation. Viral transcript and particle production were monitored by qRT-PCR and HIV-1 p24 antigen ELISA (ZeptoMetrix) according to the manufacturer's instructions.

2.3. Antibodies

Antibodies used in the experiments were as follows: HEXIM1 [ab25388] (Abcam); CDK9 [C12F7]; hnRNP A1 [R196] (Cell Signaling Technology); LARP7 [A303-723A] (Bethyl Laboratories); MePCE [14917-1-AP] (proteintech); CyclinT1 [SC10750]; GAPDH [SC25778] (Santa Cruz) (BD Transduction Laboratories); β-actin [013-24553] (Wako).

2.4. Chromatin immunoprecipitation (ChIP) assay

Cells were cross-linked with 1% formaldehyde. The lysates were then sonicated on ice to shear the DNA into fragments with an average length of less than 0.5 kb by using ELESTAIN035SD (ELECON Science. Corp). The lysates were incubated overnight on a rotating platform at 4 °C with the respective antibodies (5 mg each), which were previously bound to Dynabeads Protein G (Invitrogen). After washing, the DNA was purified and quantified according to the same protocol described in the RT-PCR section.

2.5. RNA preparation and quantitative RT-PCR

Total RNA was prepared from cells using the Isogen II isolation kit (Wako). For short transcripts, the small RNA fraction (<200 nt) was purified using the same kit. The isolated RNA was treated with Turbo DNase (Ambion) in accordance with the manufacturer's instructions. For long transcripts (>200 nt), cDNA was synthesized from total RNA by first-strand cDNA synthesis using the PrimeScript RT Master Mix (Takara Bio). Quantitative PCR was performed with a 7300 Real-Time PCR System (Applied Biosystems) using Premix Ex Taq (Probe qPCR) or SYBR Premix Ex Taq (Takara Bio). For short transcripts, cDNA was synthesized using the miScript Reverse Transcription Kit (Qiagen). Quantitative PCR was performed with a 7300 Real-Time PCR System (Applied Biosystems) using Premix Ex Taq (Probe qPCR; Takara Bio). The elongated transcript was amplified as the region between the 5′ LTR and splicing donor site. Threshold values (*Ct*) were calculated, and all reactions were run in triplicate. The specific primer pairs and probes used in this study are listed in Supplemental Table S1.

2.6. RNA immunoprecipitation (RIP) assay

RIP assays were performed and modified using the RiboCluster Profiler™/RIP-Assay Kit (MBL), according to the manufacturer's instructions. RNA was purified from the precipitates with Isogen II (Nippon Gene) reagent. These isolated RNAs were treated with Turbo DNase (Ambion) in accordance with the manufacturer's instructions. The cDNA was synthesized and quantified according to the same protocol described in the RT-PCR section.

2.7. Deep-sequence analysis of viral short transcripts

We prepared a small RNA fraction (<200 nt) from U1 cells and amplified viral short transcripts. Deep sequencing was performed using the Illumina GAIIx genome analyzer (Illumina) according to the manufacturer's instructions. Generated sequences were mapped to the reference HIV proviral genomic sequence (HIV-NL43; GenBank accession No. M19921-2). Sequence data that met the criteria of a quality value greater than 30 (100 000 sequences) were analyzed. Only sequences that perfectly matched the first 20 nt (GGU CUC UCU GGU CCA UAG GA) of the reference HIV genomic sequence were used, and we then analyzed the following sequence of the short transcripts (total 82 000 reads). All sequence data are deposited at the DNA Databank of Japan (DDBJ) (http://www.ddbj.nig.ac.jp) (accession number DRA000547).

2.8. Statistical analyses

We performed *t* test. All statistical tests were two-sided. We considered *P* values less than 0.05 to be statistically significant.

3. Results

3.1. HnRNP A1-knockdown releases suppression of HIV production in U1 cells

To elucidate the role of hnRNP A1 in the transcriptional regulation of HIV-1 proviral expression, retroviral vectors mediating either hnRNP A1 shRNA or control (GFP) shRNA expression vectors were stably transduced into U1 cells. This cell line harbors 2 copies of intact latent HIV-1 with defective Tat genes, and are often used as a cell model of latent HIV-1-infection [15]. The expression level of hnRNP A1 was reduced in U1 cells transduced with the hnRNP A1 shRNA vector (Fig. 1A). Interestingly, we found that, when hnRNP A1 was knocked down, albeit only partially, the basal viral