

COMMUNICATION

YB-1 stabilizes HIV-1 genomic RNA and enhances viral production

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ABSTRACT

HIV-1 utilizes cellular factors for efficient replication. The viral RNA is different from cellular mRNAs in many aspects, and is prone to attacks by cellular RNA quality control systems. To establish effective infection, the virus has evolved multiple mechanisms to protect its RNA. Here, we show that expression of the Y-box binding protein 1 (YB-1) enhanced the production of HIV-1. Downregulation of endogenous YB-1 in producer cells decreased viral production. YB-1 increased viral protein expression by stabilizing HIV-1 RNAs. The stem loop 2 in the HIV-1 RNA packaging signal was mapped to be the YB-1-responsive element. Taken together, these results indicate that YB-1 stabilizes HIV-1 genomic RNA and thereby enhances HIV-1 gene expression and viral production.

KEYWORDS Y-box binding protein 1, HIV-1, RNA stability

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) infection starts from binding to the cell surface receptors. By fusing with cellular membrane or by endocytosis (Miyachi et al., 2009), the viral particle is delivered into the recipient cells. The single-stranded viral genomic RNA is reverse-transcribed into double-stranded DNA by reverse transcriptase, followed by integration of the viral DNA into host chromosome. Viral accessory proteins Tat, Rev and Nef are the first produced viral proteins, whose nascent mRNAs in the nucleus are fully spliced by the cellular splicing machinery. Tat dramatically enhances the transcription, resulting in robust production of the viral RNAs. Rev modulates the splicing manner of viral transcripts and their exporting to the cytoplasm (Cochrane et al., 2006). Singly spliced RNAs encode HIV-1 Vif, Vpr, Vif and Envelope proteins. The

full-length RNA encodes Gag and Gag-Pol polyproteins, and also serves as the viral genome for progeny virus. Gag and Gag-Pol proteins assemble at the plasma membrane, packaging two copies of full-length viral RNA to produce immature viral particles. Cleavage of Gag proteins by the viral protease restructures the viral particles into mature virions (Ramezani and Hawley, 2002).

The HIV-1 genomic RNA is highly structured and very different from cellular mRNAs, rendering the viral RNA prone to attacks by the cellular RNA quality control system. For example, the 5'UTRs of multiply spliced viral mRNAs are targeted by zinc-finger antiviral protein (ZAP), which inhibits the translation and promotes the degradation of the viral mRNAs (Zhu et al., 2011). The 3'UTR of HIV-1 genomic RNA, which is much longer than that of most cellular mRNAs, is associated with Upf1 in a length-dependent manner. Upf1 binding of the viral RNA promotes mRNA decay (Hogg and Goff, 2010). How the virus protects its RNAs to establish effective replication is largely unknown.

Y-box-binding protein 1 (YB-1) is a DNA- and RNA-binding protein (Didier et al., 1988; Evdokimova et al., 1995), involved in a variety of cellular activities, including activating target gene transcription, engaging in pre-mRNA splicing, and stabilizing mRNAs (Eliseeva et al., 2011). YB-1 has been reported to interact with many viruses. It binds to the 3'UTR of Dengue Virus and inhibits the viral replication (Paranjape and Harris, 2007), interacts with the NS3/4A of hepatitis C virus to regulate viral RNA replication and particle production (Chatel-Chaix et al., 2011), and binds to the vRNP of influenza virus to increase progeny virus production (Kawaguchi et al., 2012). We recently reported that YB-1 increases the production of Murine leukemia virus- based vector by increasing the stability of the viral RNAs in producer cells, thus the available gRNA for packaging (Li et al., 2012).

In the present study, we show that YB-1 expression en-

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hances HIV-1 gene expression and viral production. Furthermore, we provide evidence indicating that YB-1 stabilizes the viral RNA in a sequence-specific manner.

RESULTS

Overexpression of YB-1 promotes HIV-1 production

To determine the effect of YB-1 expression on the production of HIV-1, a plasmid expressing YB-1 was co-transfected with the HIV-1 vector production constructs in HEK293T cells. Two HIV-1-based vectors were used, NL4-3luc and HR'luc (Zhu et al., 2011). Both vectors carry *firefly luciferase* coding sequences substituting the *nef* gene in the genome, whose transcription is driven by HIV-1 LTR (Fig. 1A). pNL4-3luc was derived from the wild-type HIV-1 isolate NL4-3, with a frame shift in the 5' end of the envelope coding sequence. Pseudotyped NL4-3luc can be produced by coexpression of pNL4-3luc and a plasmid expressing VSV-G, pVSV-G. To produce pseudotyped HR'luc, pHR'luc is co-expressed with pVSV-G and the plasmid pCMVΔR8.2, which expresses HIV-1 structural proteins (Gag), enzymes (Pol) and other accessory proteins (Fig. 1A). At two days posttransfection, the supernatants were collected to infect recipient cells. The luciferase activity was measured in the HEK293T viral producing cells and in the recipient cells. Overexpression of YB-1 increased the luciferase activity expressed from both pNL4-3luc and pHR'luc in the producer cells (Fig. 1B). In line with these results, the luciferase activity in the recipient cells infected with vectors produced in the presence of YB-1 was also increased (Fig. 1C). To test whether YB-1 promotes the production of replication-competent HIV-1, we used NLEnvLuc (Dang et al., 2008), an infectious HIV-1 clone derived from wild-type NL4-3 with the luciferase coding sequences inserted into *nef* to facilitate virions detection. As expected, the expression of YB-1 increased the luciferase activity encoded by NLEnvLuc in both producer cells and recipient cells (Fig. 1D). These results indicate that YB-1 expression enhances HIV-1 production.

Downregulation of YB-1 reduces HIV-1 production

To test whether endogenous YB-1 regulates the production of HIV-1, the expression of YB-1 was downregulated by RNA interference in the producer cells and the production of HIV-1 vectors was evaluated. Two shRNAs against YB-1 were constructed. The ability of the shRNAs to downregulate the expression of YB-1 was first confirmed by cotransfection of plasmids expressing the shRNA and Flag-tagged YB-1 in HEK293T cells (Fig. 2A). To analyze the effect of the downregulation of endogenous YB-1 on HIV-1 production, the shRNA against YB-1 was coexpressed with the NL4-3luc vector-producing constructs. The pseudoviruses were collected to infect recipient cells. Indeed, downregulation of endogenous YB-1 reduced the luciferase activity in both the producer cells (Fig. 2B) and recipient cells (Fig. 2C). The results indicate that endogenous YB1 facilitates HIV-1 production.

YB-1 stabilizes the viral genomic RNA

We next analyzed whether YB-1 enhances HIV-1 production through increasing the viral RNA levels. To analyze the viral genomic RNA levels in virion particles, the pseudovirus produced in the absence or presence of YB-1 were purified and the viral genomic RNA was extracted. The viral genomic RNA levels were analyzed by RT-qPCR. Indeed, the RNA level in the virions produced from YB-1-expressing cells is significantly higher than that from the control cells (Fig. 3A). Consistently, expression of YB-1 significantly increased the viral genomic RNA levels in the producer cells (Fig. 3B).

The increase in the viral genomic RNA levels could result from increased transcription or mRNA stabilization. To test whether YB-1 enhances HIV-1 transcription in HEK293T cells, we used a *firefly luciferase* reporter system, in which the *firefly luciferase* gene transcription is driven by HIV-1 LTR (U3, R and U5). Data show that YB-1 failed to increase the luciferase activity in HEK293T cells with or without the presence of the viral transcription activator Tat (Fig. 3C). These results suggest that in HEK293T cells, YB-1 does not increase the viral RNA level through enhancing viral transcription.

We next analyzed whether YB-1 stabilized the viral genomic RNA. A plasmid expressing YB-1 was cotransfected with pNL4-3luc. The global transcription was blocked by treatment of actinomycin D, and total RNA levels were measured by Northern blotting at different time points thereafter. Data show that the decay rate of the viral genomic RNA was significantly reduced in the presence of YB-1 (Fig. 3D), implicating that YB-1 expression stabilizes the viral genomic RNA.

Stem loop 2 of HIV-1 5'UTR is the target sequence of YB-1

YB-1 stabilized the viral genome RNA, which encodes Gag protein. Hence, YB-1 is expected to improve Gag protein levels in the producer cells. To test the idea, Gag expression was analyzed by Western blotting. In the NL4-3luc producer cells, Gag was translated from the viral genomic RNA while in the HR'luc producer cells Gag was encoded by pCMVΔR8.2. Data show that in the cells producing NL4-3luc, Gag expression was significantly enhanced by YB-1. In contrast, in the cells producing HR'luc, Gag expression was little affected (Fig. 4A). Comparison between the sequences of pNL4-3luc and pCMVΔR8.2 reveals that pNL4-3luc contains the viral 5' UTR upstream of the Gag coding sequence, which is absent in pCMVΔR8.2. To explore whether the 5'UTR is responsible for the difference between pNL4-3luc and pCMVΔR8.2 in the response to YB-1, it was cloned upstream of *firefly luciferase* coding sequence in the reporter, pGL3-linker (Guo et al., 2004). The transcription of this luciferase reporter is driven by an SV40 promoter. Luciferase expression from pGL3-linker was not affected by YB-1. In contrast, luciferase expression from the reporter containing the HIV-1 5'UTR was significantly increased (Fig. 4B). These results suggest that the 5'UTR of HIV-1 is sufficient to be targeted by YB-1. To further substantiate this notion, the Gag coding sequence with or without the

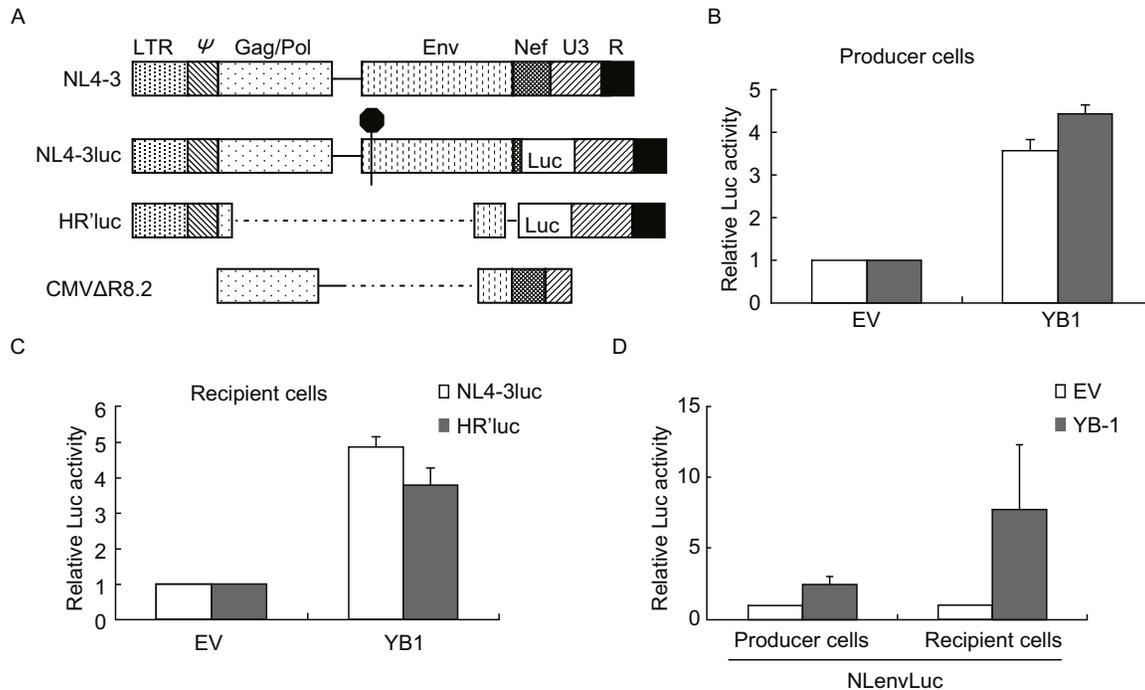


Figure 1. Overexpression of YB1 promotes HIV-1 production. (A) Schematic representation of HIV-1 vectors. A filled cycle in the coding sequence of Envelope in NL4-3luc represents a frame shift-derived stop codon. (B and C) HEK293T cells were transfected with plasmids indicated to produce HIV-1 vectors. A plasmid expressing renilla luciferase was included to serve as a control for transfection efficiency and sample handling. At 48 h post-transfection, supernatants were harvested to infect 293T (recipient) cells. (B) The luciferase activities in HEK293T (producer) cells were measured. (C) At 48 h post-infection, *firefly luciferase* activity was measured in the 293T (recipient) cells. *Firefly luciferase* activity expressed from the viral vectors was normalized by renilla luciferase activity. (D) HEK293T cells were transfected with pNLenvLuc to produce replication-competent HIV-1 in the presence or absence of pCMV-HF-YB-1. At 48 h post-transfection, supernatants were collected to infect Hela-CD4CCR5 cells. Luciferase activity in producer cells and recipient cells were measured. The data are means + SD of three independent measurements. The relative luciferase expression level in control cells was set 1. EV, empty vector.

5'UTR was cloned into pcDNA4/TO/myc-HisB vector (Fig. 4C), and their response to YB-1 was analyzed. Indeed, expression of Gag from the coding sequence alone was not affected by YB-1. However, the expression of 5'UTR-Gag was significantly enhanced by YB-1 (Fig. 4D). These results indicate that the 5'UTR of HIV-1 is the responsive sequences to YB-1.

The 5'UTR of HIV-1 includes R, U5, primer binding site (PBS), and the packaging signal which is composed of stem loop 1 (SL1), stem loop 2 (SL2) and stem loop 3 (SL3). To further map the region responsive to YB-1, a series of deletion mutants were constructed (Fig. 4C). Deletion from the 5'end up to SL1 (SL2-3-Gag) did not change the sensitivity to YB-1, while further deletion of SL2 (SL3-Gag) compromised the reporter's sensitivity to YB-1 (Fig. 4D). To determine whether SL2 is required for YB-1, SL2 was deleted in UTR-Gag [(-)SL2] (Fig. 4C). The resulting Gag level from (-)SL2 was not affected by YB-1 (Fig. 4E), indicating that SL2 is required for YB-1 to enhance Gag expression. To test whether SL2 is sufficient, SL2 was cloned upstream of the luciferase coding sequence. Data show that the luciferase activity was increased by SL2 in the response to YB-1 (Fig. 4F). Consistently, cloning

of both SL2 and SL3 (SL23) upstream of the luciferase coding sequence increased luciferase expression in the presence of YB-1 (Fig. 4F). These results indicate that SL2 is the target sequence for YB-1.

DISCUSSION

Here we report that the expression of YB-1 enhances HIV-1 production (Fig. 1) while downregulation of endogenous YB-1 decreases viral production (Fig. 2). Viral RNAs are stabilized by YB-1 (Fig. 3). Comparison of different Gag expression vectors revealed that the HIV-1 5'UTR is required for YB-1 to enhance Gag expression. The stem loop 2 region in the packaging signal was further mapped to be the YB-1-targeting sequences (Fig. 4).

It has been reported that in human astrocytic cell lines, YB-1 binds to both the TAR sequence and Tat protein to increase the activity of Tat, yet whether YB-1 increases HIV-1 production was not tested (Ansari et al., 1999). In our assays, however, such stimulation was not observed (Fig. 2). It is noted that here we used HEK293T cells, a cell line that is widely used to

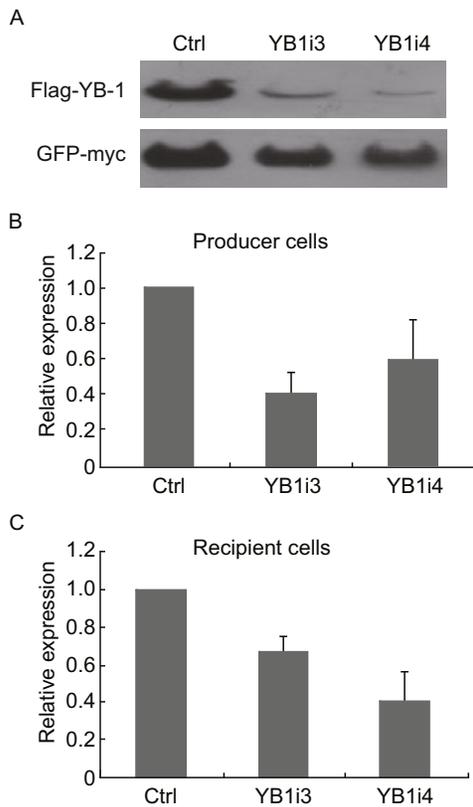


Figure 2. Downregulation of YB-1 reduces NL4-3luc production. (A) A scrambled control shRNA (Ctrl) or the shRNA directed against YB-1 (YB1i3 and YB1i4) was cotransfected into HEK293T cells with pCMV-HF-YB-1. A plasmid expressing myc-tagged GFP was included to serve as a control for transfection efficiency and sample handling. At 48 h post-transfection, cells were lysed and the expression levels of YB-1 were analyzed by Western blotting. (B and C) Plasmids pNL4-3luc and pVSV-G were cotransfected with the shRNA indicated into HEK293T cells. A plasmid expressing renilla luciferase was included to serve as a control for transfection efficiency and sample handling. The luciferase activities in producer cells (B) and in recipient cells (C) were measured as described in the legend to Fig. 1. *Firefly luciferase* activity was normalized by renilla luciferase activity. The data are means + SD of three independent measurements. The relative luciferase expression level in control cells was set 1.

produce HIV-1 (Ramezani and Hawley, 2002). The discrepancy between our results presented here and those previously reported by others suggest that YB-1 may behave differently in different cell lines.

YB-1 has been reported to be involved in pre-mRNA splicing (Hartmuth et al., 2002; Soop et al., 2003; Deckert et al., 2006), mRNA translation (Evdokimova et al., 1995; Davydova et al., 1997; Evdokimova et al., 1998) and mRNA stabilization (Evdokimova et al., 2001). These functions rely on the ability of YB-1 to bind target RNA. The binding specificity of YB-1 towards RNA is not strictly defined although it has been reported that YB-1 specifically stabilizes certain cellular mRNAs through

binding to the CU-rich elements, like *TGF β* mRNA (Jenkins et al., 2010). In the case of Dengue virus, YB-1 targets the RNA bearing the sequence 5'-UCCAGGCA-3'. The recently identified YB-1 targeting sequence in MLV is in the R region. The HIV-1 SL2 sequence (5'-GGCGACTGGTGAGTACGCC-3') identified here provides an additional target sequence of YB-1. However, alignment of these YB-1 targeting sequences displayed no significant sequence similarity. It is noted that for Dengue virus, YB-1 acts as an inhibitory factor repressing viral RNA translation. For MLV and HIV-1, YB-1 stabilizes viral mRNAs. What determines the outcome of YB-1 binding to its target RNA awaits further investigation.

HIV-1 based lentivectors are widely used for gene transfer. The most commonly encountered problem is the low titer of the vectors. Our results indicated that overexpression of YB-1 enhances the production of HIV-1-based vectors. Such a strategy may be used in the preparation of lentivectors.

MATERIALS AND METHODS

Plasmids

The plasmid expressing Flag-tagged YB-1, pCMV-HF-YB-1, has been described previously (Li et al., 2012). To generate the shRNAs directed against YB-1, oligonucleotides were designed and cloned into pSuper following the manufacturer's instruction (OligoEngine). The sequences of the shRNAs directed against YB-1 are listed below: YB-1-3i F, 5'-GATCCCCCAGTTCAAGGCAGTAAATATTTCAGAGAATATTTACTGCCTTGAAGTGGTTTTTA-3'; YB-1-3i R, 5'-AGCTTAAAAACAGTTCAA-GGCAGTAAATATTCTCTTGAATATTTACTGCCTTGAAGTGGGGG-3'; YB-1-4i F, 5'-GATCCCCGACGGCAATGAAGAAGATAAATTCAGAGATTTATCTTCTTCATTGCCGTCTTTTTTA-3'; YB-1-4i R, 5'-AGCTTAAAA-GACGGCAATGAAGAAGATAAATCTCTTGAATTTATCTTCTTCATTGCCGTCCGGG-3'.

HIV-1 producing plasmids pCMV-VSV-G, pHR'luc, pCMV Δ R8.2 and pNL4-3luc have been described previously (Zhu et al., 2011). pNLenvLuc was kindly provided by Dr. Yonghui Zheng of Michigan State University.

HIV-1 5'LTR, which is composed of U3, R and U5, was PCR-amplified from pNL4-3luc and cloned into pGL3-Basic (Promega). The U3 region serves as a promoter for transcription of *firefly luciferase* and the R-U5 region in the mRNA serves as part of the 5'UTR. Primer sequences are listed below: LTR-F, 5'-GGTACCCTGGAA-GGGCTAATTCACCTC-3'; LTR-R, 5'-CTCGAGCTGCTAGAGATTTTCACAC-3'.

HIV-1 5'UTR (R-U5-packaging signal) was PCR-amplified from pNL4-3luc and cloned into pGL3-linker (Guo et al., 2004) serving as the 5'UTR in the transcripts. Primer sequences are listed below: UTR-F, 5'-CCATGGGGTCTCTCTGGTTAGACCAG-3'; UTR-R, 5'-CCATGGCTCTCTCTCTAGCCTCCG-3'.

A *firefly luciferase* CDS was inserted into pcDNA4/TO/myc-HisB vector to generate pcDNA4-luc (luc), using XhoI and SacII sites. The fragments of HIV-1 SL2 and SL2-SL3 were annealed and inserted into pcDNA4-luc using BamHI and XhoI sites, to generate SL2-luc and SL2-SL3-luc (SL23-luc). The sequences are listed below: SL2, 5'-GGGCGGCGACTGGTGAGTACGCC-3'; SL23, 5'-GGGCGGCGACTGGTGAGT ACGCCAAAAATTTGACTAGCGGAGGCTAGAGAGAGAG-3'.

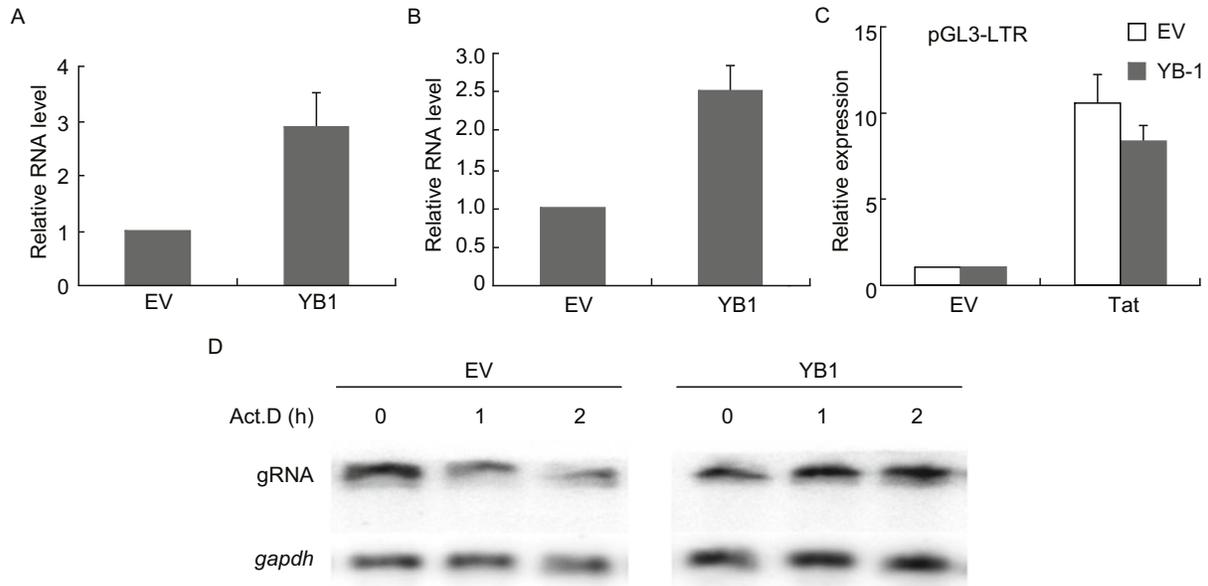


Figure 3. YB-1 stabilizes the viral genomic RNA. NL4-3luc-producing constructs were cotransfected into HEK293T cells with empty vector or pCMV-HF-YB-1. (A) At 48 h post-transfection, supernatants were collected and purified by sucrose ultracentrifugation. Viral genomic RNA was extracted and 10 Hg of the total RNA of naïve HEK293T cells was added to each sample before RNA-extraction to serve as a control for sample handling and detection. Viral genomic RNA levels were measured by quantitative RT-PCR with primers for *firefly luciferase* coding sequences. *GAPDH* mRNA from the added cellular RNA was used as an internal control. Data presented are means + SD from three independent measurements. (B) The viral RNA levels in the producer cells were measured by Northern blotting and Phosphoimager. The HIV-1 RNA level was normalized by that of *GAPDH*. Data presented are means + SD of three independent measurements. (C) pGL3-LTR was cotransfected into HEK293T cells with plasmids expressing YB-1 and HIV-1 Tat. A plasmid expressing renilla luciferase served as internal control. At 48 h post-transfection, cells were lysed for luciferase activity measurement. Data presented are means + SD of three independent measurements. All the relative expression of controls was set to 1. (D) pNL4-3luc was cotransfected with pCMV-HF-YB-1 into HEK293T cells. At 36 h post-transfection, cells were treated with actinomycin D (act. D) to stop global transcription. Cells were lysed with Trizol at the time points indicated. Total cellular RNA was extracted and the viral genomic RNA levels were detected by Northern blotting and Phosphoimager.

Five segments of HIV-1 were PCR-amplified from pNL4-3luc and cloned into pcDNA4/TO/myc-HisB vector (Invitrogen). The primers are listed below: UTRGag F, 5'-GCTCGGATCCGGTCTCTCTGGTTAGAC-CAG-3'; SL1-2-3 F, 5'-GCTCGGATCCGAGCTCTCTCGACGCAG-GAC-3'; SL2-3 F, 5'-GCTCGGATCCGGGCGGCGACTGGTGAG-TAC-3'; SL3 F, 5'-GCTCGGATCCAAAATTTTGACTAGCGGAG-3'; GAG F, 5'-CTCGGATCCGCCACCATGGGTGCGAGAGCGTCAG-3'. The reverse primer is Gag R, 5'-AGACTCGAGTTATTGTGAC-GAGGGTTCGCT-3'.

To generate (-)SL2, two overlapping PCR fragments were mixed to serve as templates and PCR-amplified using primers UTRGag F and Gag R. The 5' fragment was amplified from pUTRGag using primers UTRGag F and (-)SL2 R. The 3' fragment was amplified from pUTRGag using primers (-)SL2 F and Gag R. The sequences were cloned into pcDNA4/TO/myc-HisB (Invitrogen) for protein expression. The primers are listed below: (-)SL2 F, 5'-GCACGGCAAGAGC-GAGGGGCAAAAATTTTGACTAGCGGAG-3'; (-)SL2 R, 5'-CTC-CGCTAGTCAAAAATTTT GCCCTCGCCTCTTGCCGTGC-3'.

pCMV-HF-Tat (101 amino acids in length), which expresses HIV-1 Tat protein, was generated by cloning PCR-derived coding sequences of HIV-1 Tat into pCMV-HF vector (Guo et al., 2007) using BamHI and EcoRI sites.

Cell culture and viral infection

HEK 293T cells (ATCC CRL-11268) were maintained in DMEM supplemented with 1% antibiotics and 10% fetal bovine serum (Invitrogen). Hela-CD4CCR5 cells have been described previously (Deng et al., 1996). To produce pseudotyped HIV-1 vectors, the producing constructs were transfected into 293T cells. pRL-TK, a plasmid expressing renilla luciferase, was included to serve as a control for transfection efficiency and sample handling. At 48 h post-transfection, producer cells were lysed with Passive Lysis Buffer (Promega) for dual-luciferase activity assay. The supernatants were collected and filtered through 0.45 μ m filters (Millipore) to remove cell debris. The supernatants were then diluted three times with fresh medium for infection of 293T cells in the presence of polybrene at a final concentration of 8 μ g/mL. At 48 h post-infection, cells were lysed with Cell Culture Lysis Buffer (Promega) for luciferase activity measurement. For NLenvLuc vector production, pNLenvLuc was transfected into HEK293T cells. At 48h post-transfection, the supernatants were harvested to infect Hela-CD4CCR5 cells.

Measurement of viral RNA levels

The methods extracting virion RNAs and detecting RNA levels by real-time PCR has been described previously (Li et al., 2012). The RNA

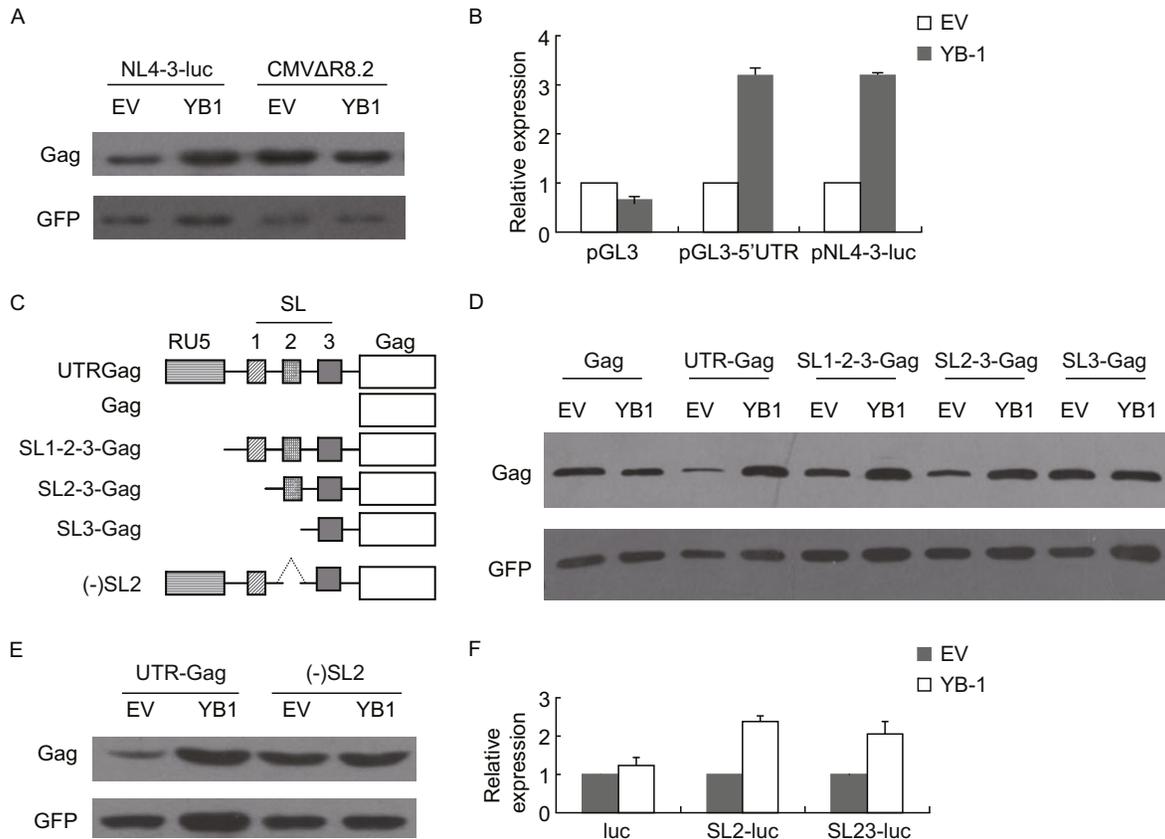


Figure 4. Mapping stem loop 2 of HIV-1 5'UTR as the YB-1-responsive element. (A) pNL4-3luc or pCMVDR8.2 was cotransfected with pCMV-HF-YB-1. A plasmid expressing myc-tagged GFP served as an internal control. At 48 h post-transfection, cells were lysed for Western blotting analysis of HIV-1 Gag expression. Anti-p24 antibody was used for the detection of Gag. Anti-myc antibody was used for the detection of GFP. (B) pGL3-linker (pGL3), pGL3-linker-5'UTR (pGL3-5'UTR) or pNL4-3luc was cotransfected with an empty vector or pCMV-HF-YB-1. A plasmid expressing renilla luciferase was included to serve as an internal control. At 48 h post-transfection, cells were lysed for luciferase activity measurement. Data presented are means + SD of three independent measurements. All the relative expression of controls was set to 1. (C) A schematic representation of HIV-1Gag expression constructs. SL, stem loop. (D) The Gag-expressing constructs were each cotransfected into HEK293T cells with an empty vector or pCMV-HF-YB-1. A plasmid expressing myc-tagged GFP served as an internal control. At 48 h post-transfection, cells were lysed for Western blotting analysis. (E) The plasmid indicated was cotransfected with pCMV-HF-YB-1. A plasmid expressing myc-tagged GFP served as an internal control. At 48 h post-transfection, cells were lysed for Western blotting analysis of Gag expression. (F) pcDNA4-luc (luc), pcDNA4-SL2-luc (SL2-luc) or pcDNA4-SL2SL3-luc (SL23-luc) was cotransfected with an empty vector or pCMV-HF-YB-1. A plasmid expressing renilla luciferase was included to serve as an internal control. At 48 h post-transfection, cells were lysed for luciferase activity measurement. Data presented are means + SD of three independent measurements. The relative luciferase expression level in control cells was set 1.

levels in producer cells were measured by Northern blotting following the procedure described previously (Li et al., 2012). A fragment of the HIV-1 Matrix gene that is PCR-amplified from pNL4-3luc was used as a template for the probe preparation.

Antibodies

Anti-myc monoclonal antibody 9E10 (Santa Cruz Biotechnology) and anti-Flag antibody M2 (Sigma) are commercially available. The anti-p24 mouse monoclonal antibody is a generous gift from Dr. Yong-Tang Zheng of Kunming Institute of Zoology, China.

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ABBREVIATIONS

HIV-1, human immunodeficiency virus type 1; LTR, long terminal repeat; SL2, stem loop 2; UTR, untranslated regions; YB-1, Y-box binding protein 1

COMPLIANCE WITH ETHICS GUIDELINES

Xin Mu, Wei Li, Xinlu Wang and Guangxia Gao declare that they have no conflict of interest.

The article does not contain any studies with human or animal sub-

jects performed by the any of the authors.

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