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Nuclear Matrix protein SMAR1 represses HIV-1 LTR mediated transcription through chromatin remodeling

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ABSTRACT

Nuclear Matrix and MARs have been implicated in the transcriptional regulation of host as well as viral genes but their precise role in HIV-1 transcription remains unclear. Here, we show that >98% of HIV sequences contain consensus MAR element in their promoter. We show that SMAR1 binds to the LTR MAR and reinforces transcriptional silencing by tethering the LTR MAR to nuclear matrix. SMAR1 associated HDAC1-mSin3 corepressor complex is dislodged from the LTR upon cellular activation by PMA/TNFα leading to an increase in the acetylation and a reduction in the trimethylation of histones, associated with the recruitment of RNA Polymerase II on the LTR. Overexpression of SMAR1 lead to reduction in LTR mediated transcription, both in a Tat dependent and independent manner, resulting in a decreased virion production. These results demonstrate the role of SMAR1 in regulating viral transcription by alternative compartmentalization of LTR between the nuclear matrix and chromatin.

Introduction

Human Immunodeficiency Virus Type 1 (HIV-1) is the etiological agent of AIDS and AIDS-related complex (ARC) (Barre-Sinoussi et al., 1983). Among the various steps of viral life cycle, the transcription from HIV-1 provirus is essential for amplification of the viral genes and considered crucial for viral replication. HIV-1 transcription is directed by the promoter located at 5′ long terminal repeat (LTR) of the integrated provirus and is basically segmented into three well defined regions; a negative regulatory element (NRE) region, an enhancer region, and a trans-acting region (Naghavi et al., 1999). The NRE contains a number of recognition sequences for cellular transcription factors, the deletion of which results in an increase of both reporter gene transcription and viral replication (Gaynor, 1992). However, information regarding the mechanism by which NRE exerts its negative effect on viral transcription is unknown because most of the transcription factors that bind to NRE are transcriptional activators. The DNA elements important for the LTR activity are located between −454 and +1884 nucleotides, where +1 is the transcription start site. This region contains the TATA box and binding sites for host transcription factors Sp1 and NF-κB, etc (Copeland, 2005; Korner et al., 1990). These binding sites and their relative orientations together mediate DNA–protein and protein–protein interactions that form a complex regulatory network. While activation of the LTR is associated with changes in chromatin structure, the mechanisms governing repression of LTR are not completely understood.

Multiple cellular factors like YY-1, c-Myc, p50, CTIP2, CBF-1 and AP-4 that recruit histone deacetylase (HDAC) to LTR are known to negatively regulate HIV transcription (Imai and Okamoto, 2006; Du et al., 2007; Jiang et al., 2007; Marban et al., 2007; Tyagi and Karn, 2007; Williams et al., 2007). Analysis of latently infected virus as compared to actively transcribing ones reveal epigenetic restrictions involving chromatin modifications responsible for subduing of transcription in the latent state. Pearson et al. (2008) show that upon activation of latent virus with TNF-α, there is significant reduction in the histone methylation with concomitant increase in acetylation and also reduced association of HP1-α. Thus, although the signal for activation or repression of transcription at LTR seems to be regulated to some extent by external stimuli, the actual ground rules are set by epigenetic modifications at the LTR.

In this context, the role of nuclear milieu and *cis* regulatory elements proximal to the sites of viral integration assumes importance (Copeland, 2005; Lassen et al., 2004). MARs are typically ~200 bp long AT-rich DNA sequences, characterized by duplex instability, duplex flexibility due to AT-richness, apart from DNaseI hypersensitivity, Topoisomerase II cleavage sites and a high affinity for binding to

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isolated nuclear matrix (NM) (Bode et al., 1992; Schubeler et al., 1996; Bisgrove et al., 2005; Dijkwel and Hamlin, 1988). NM is usually associated with inner nuclear membrane (INM) which is constituted by a distinct set of proteins such as LBR, LAP2 and Emerin as well as an underlying nuclear lamina, which have been proposed to interact with transcriptional repressors such as Sp1, SATB1 etc. (Guelen et al., 2008; Zhang et al., 1996). Another function of the NM is in concentrating and localizing various transcription factors which are present in limited amounts in the nucleus (Heessen and Fornerod, 2007). Moreover, recent studies have shown that transcriptional repression is mediated by repositioning of genes to the nuclear lamina and activation is mediated by repositioning to transcription factories (Reddy et al., 2008). There are several reports showing that a MAR from one species can bind to nuclear matrix preparations from another species (Breyne et al., 1992; Seo et al., 2005).

The expression of integrated HIV in infected latent cells is regulated at the level of transcription by cellular factors and the viral transactivator Tat. The HIV LTR contains *cis*-acting elements required for transcriptional initiation and binding sites for various transcription factors like Sp1, NF-κB, and NF-AT etc. These factors are known to regulate transcription based on the stimulus they get. Although signaling mechanisms that lead to viral activation have been worked out, the chromatin modifying factors and mechanisms involved therein are not clear.

In this context, the current study evaluates the role of MAR elements in LTR and a specific MAR binding protein SMAR1 in regulating viral transcription. This study stems from the fact that HIV-1 integration sites are flanked by potential MARs (Kulkarni et al., 2004). The transcriptional repressor function of a nuclear matrix attachment region binding protein SMAR1 has been well documented for genes like Cyclin D1 and CK8 through recruitment of Sin3/HDAC1 complex to the target sequence (Rampalli et al., 2005; Pavithra et al., 2009).

Our studies for the first time confirm the presence of conserved MARs in the 5′ LTRs of most of the HIV strains and indicate the binding of SMAR1 to this MAR in latent state. Interestingly, these MARs are capable of binding to nuclear matrix in unactivated but not in activated cells, suggesting a direct role of nuclear matrix in regulating viral latency. We demonstrate that SMAR1 acts as a repressor of HIV-1 LTR promoter both in presence and absence of transactivator Tat. Further, SMAR1 maintains the latent state of viral transcription by recruiting HDAC1-mSin3a corepressor complex, which is dislodged from the LTR MAR upon activation by PMA and TNFα.

Results

LTRs of all HIV isolates have conserved MAR in their 5′ LTR and SMAR1 anchors HIV- LTR to the Nuclear Matrix

Several reports highlight the importance of MARs in viral integration and their role in viral transcription (Kulkarni et al., 2004) but the mechanism by which they elicit these effects is not yet understood. Although NRE of LTR is known to bind to nuclear matrix, whether there are conserved matrix attachment regions in all the HIV strains is not known. To address this, we analyzed all the LTRs available in NCBI nucleotide database for the presence of MARs. Among the 3742 hits obtained for HIV LTR in GenBank, 223 represented distinct LTRs and were used for further analysis. The rest were multiple isolates of the same sequence. Analysis of these 223 LTR sequences for the presence of MARs using MARWIZ software showed the presence of one or more potential MARs ($\rho = 1$) in >98% of the LTRs analyzed (219 out of 223) (details of sequences are given in Supplementary information). We next checked for the possible consensus elements present in these LTR MARs. For this, MAR sequences from all the HIV LTRs were analyzed by MEME (Multiple EM for Motif Elicitation) software (Bailey et al., 2006). MEME analysis of these sequences revealed the presence of a conserved 50-mer motif spanning -258 to -208 region of HIV LTR with an error value of $3.3e^{-810}$ (Supplementary figure S1A). A further division of this 50-mer sequence into distal (WT-A), middle (WT-B) and proximal (WT-C) sequences revealed the presence of a conserved hexanucleotide (CAAAGA) in most of the 219 unique LTR- MARs that were analyzed.

Having verified the presence of conserved MAR elements in these LTRs, we next addressed if these LTR-MARs contribute to functional partitioning of the viral genome into chromatin and matrix fractions upon stimuli. For this, nuclear matrix and chromatin fractions were isolated from PMA activated or control CEM-GFP cells. Nuclear matrix-bound or unbound fractions (chromatin) were used as template in the PCR to determine the relative amounts of LTR bound to nuclear matrix under these conditions. The amplification of LTR in unbound chromatin fractions was 5-fold higher in activated as compared to unactivated cells (Fig. 1A, lanes 1 and 2). In contrast, LTR amplicon was ~3-fold less in nuclear matrix from activated cells compared to unactivated cells (Fig. 1A, upper panel, lanes 3 and 4, Fig. 1B). Similar experiment with p53-AIP promoter which does not have any MAR element revealed that the promoter mostly associated with chromatin fraction in control cells and there was no change in its association upon activation by PMA (Fig. 1A, lower panel, lanes 1 and 2). This shows that the LTR is sequestered in the nuclear matrix under latent conditions while upon cellular activation, it is displaced to the active chromatin.

Considering that matrix associated proteins play a major role as transcriptional repressors, we checked if SMAR1 which is known to be a MAR-binding protein is a component of nuclear matrix. Immunoblot analysis showed the presence of SMAR1 in nuclear matrix under endogenous conditions both in Jurkat and HeLa cells (Fig. 1C). Further, *in vitro* and *in vivo* matrix binding assays in SMAR1 overexpressed/knockdown/PMA activated cells were performed to check if SMAR1 is essential for anchoring LTR to the nuclear matrix. Overexpression of SMAR1 led to an increase in the amount of LTR-MAR bound to nuclear matrix (Fig. 1D, lane 3), whereas PMA-mediated activation and SMAR1-SiRNA led to a lower amount of LTR-MAR bound to nuclear matrix (Fig. 1D, lanes 4 and 5). Knock-down and overexpression of SMAR1 was confirmed by immunoblotting (Supplementary figure S1B). Upon immunoblot analysis of SMAR1 in TZM-bl cells, we observed a reduction of SMAR1 protein in the nuclear matrix fraction upon activation while there was no change in the chromatin fraction (Fig. 1E, lanes 2 and 4). This is consistent with our earlier observations. Purity of the nuclear matrix and chromatin fractions was confirmed by immunoblotting for Lamin B1 and Histone H1 respectively. Histone H1 levels in chromatin fraction are reduced upon activation, since it is evicted from the chromatin during transcriptional activation to maintain active chromatin state. To visualize the levels of SMAR1 in nuclear matrix in control and activated TZM-bl cells, *in-situ* nuclear matrix staining for SMAR1 was performed. There was a time dependent reduction of SMAR1 in nuclear matrix upon activation by PMA. Interestingly, we find that SMAR1 localizes to nuclear periphery upon activation by PMA (Supplementary figure S1D). Further, South-Western blotting in HeLa nuclear matrix with LTR-MAR as probe showed a band at 55 kDa that corresponds to SMAR1 band in immunoblot analysis (Supplementary figure S1C). Taken together, these results are suggestive of the binding of nuclear matrix component SMAR1 to the HIV LTR MAR.

SMAR1 binds to conserved MAR sequence within HIV-1 LTR

We next evaluated the direct binding of SMAR1 to the HIV LTR MAR. Gel shift assays using the recombinant protein revealed binding of SMAR1 to the 200 bp full length LTR-MAR (-350 to -150) *in vitro* (Fig. 2A, lanes 3, 4 and 5). One hundred-fold excess non-specific competitor (NS) could not reduce the complex formation (Fig. 2A, lane 6) while there was a significant reduction in the complex

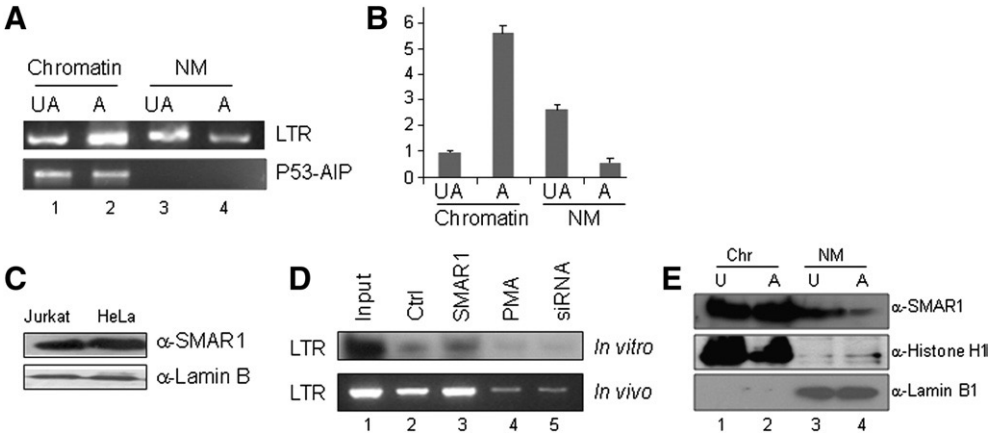

Fig. 1. SMAR1 anchors HIV-1 LTR to the nuclear matrix. (A) *In vivo* matrix binding assay showing displacement of LTR to Chromatin from nuclear matrix upon activation. Nuclear matrix and chromatin fractions were isolated from control CEM-GFP cells or cells activated with PMA and DNA associated with these fractions was isolated and LTR and p53-AIP promoter amplified using specific primers. (B) The densitomertric analysis of the same showing fold changes in LTR association in various fractions. (C) Immunoblot analysis of SMAR1 with Lamin B1 as control in nuclear matrix. Nuclear matrix isolated from Jurkat and HeLa cells was analyzed for presence of SMAR1 by Immunoblot analysis. Lamin B1 was used as control. (D) *In vitro* (top) and *in vivo* (bottom) matrix binding assays showing SMAR1 mediated anchoring of LTR to nuclear matrix. For *in vitro* matrix binding assay, radiolabeled LTR-MAR was incubated with nuclear matrix fraction isolated from control CEM-GFP cells (Ctrl), SMAR1 overexpressed (SM), SMAR1 knockdown (Si) or PMA activated cells for 1 h at 37 °C, washed three times with Tris-buffer containing 2 M NaCl, pH 7.8. The matrix bound DNA fragments were resolved on 10% native PAGE. In *in vivo* assay, LTR-MAR was PCR amplified from the same samples. (E) Immunoblot analysis of SMAR1 in different cellular fractions upon activation by 50 ng/ml of PMA. Forty micrograms each of chromatin and nuclear matrix fractions from control CEM-GFP cells or cells activated with PMA were resolved in 12% SDS-PAGE and analyzed for expression of SMAR1. Lamin B1 and Histone H1 were used as controls for nuclear matrix and chromatin fractions respectively.

formation upon 10 fold excess cold self-competitor (Fig. 2A, lane 7). The upstream non-MAR LTR (δ-MAR) region used as control did not show any complex formation with SMAR1 (Fig. 2B). In an attempt to map the exact SMAR1 binding sequence in the LTR-MAR, we synthesized oligos corresponding to WT-A (nucleotides towards 5′ end of LTR-MAR, WT-B (sequence at the middle of LTR-MAR), WT-C (nucleotides towards 3′ end of LTR-MAR) and WT-6 as described in Table 1. WT-A and WT-B failed to show any binding to GST-SMAR1 (Supplementary Fig. S2A, B), whereas the probe WT-C which spans the 3′ end of the consensus MAR showed a strong complex formation with SMAR1 (Fig. 2C, lanes 3 and 4). A DNA-binding mutant of SMAR1 protein (160–350 aa) did not show any complex formation with the probe (Fig. 2C, lane 5) showing the specificity of the binding. Further, to identify the exact bases which are necessary for binding of SMAR1 to LTR-MAR, we introduced more mutations in WT-C. Detailed description of these oligos and the mutants used are documented in Table 1. The mutation of all three 'A's to 'G' (Mut3xA), mutation of 'G' at position 7 to 'A' (MutG) (Fig. 2D and S1C, D) resulted in the loss of SMAR1 binding to the probe. Further, the mutation of 'T' to 'C' (MutT-C) and mutation of all 'A's to 'G' (MutA-G) also resulted in loss of SMAR1binding (Supplementary Fig. S2 D, E). Therefore, the bases 'G' at position 7, and three 'A's from position 4 seem to be very critical for binding of SMAR1 to LTR-MAR. To validate SMAR1 binding to this consensus element, gel shift assays were performed with tandem repeats of the conserved hexanucleotide sequence (WT-6) present in the proximal end of LTR-MAR described in the earlier section. We observed a strong nucleoprotein complex formation of SMAR1 and WT-6, confirming that this sequence is the actual SMAR1 binding sequence in LTR-MAR (Fig. 2D).

SMAR1 remodels chromatin structure at HIV LTR

Recent studies have shown that the presence of HDACs at the HIV LTR is strongly correlated with transcriptional repression. SMAR1 is known to recruit HDAC1/Sin3A corepressor complex to various promoters and repress gene expression (Rampalli et al., 2005;

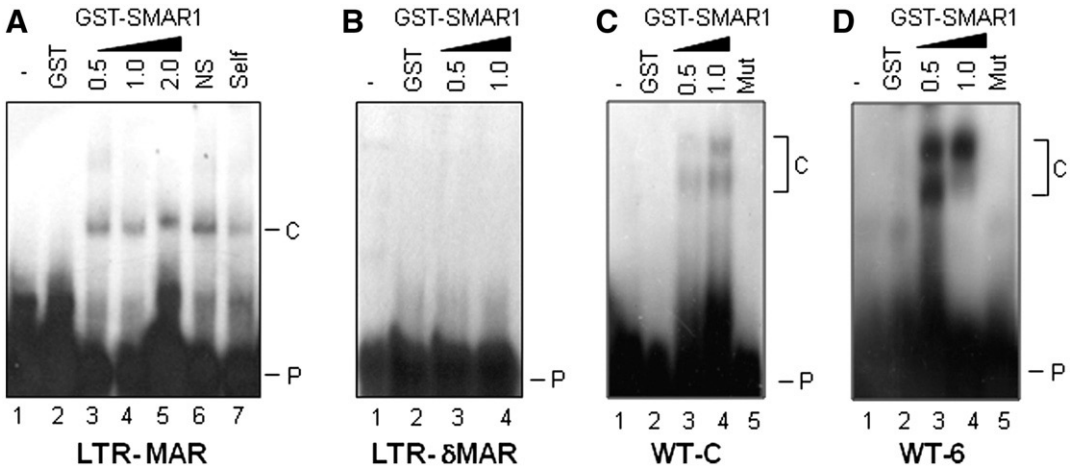

Fig. 2. SMAR1 binds to MAR in HIV-1 LTR *in vitro*. (A) Gel-shift assay showing binding of recombinant SMAR1 protein to HIV LTR. 200 bp full length radiolabeled MAR from HIV-1_{pNL4-3} was used as probe. The binding specificity was verified using 100-fold excess non-specific competitor DNA (Lane 6, NS). Ten fold excess unlabelled LTR-MAR led to reduction in complex formation (Lane 7, self). (B) Probe made from LTR DNA upstream of MAR did not show any complex formation (LTR δ-MAR). (C) 16-mer oligonucleotide from consensus MAR sequence from LTR shows complex formation with recombinant SMAR1. (D) A minimal hexanucleotide sequence CAAAGA shows strong complex formation with recombinant SMAR1.

Table 1
Sequences of probes used in Electrophoretic Mobility Shift Assays (EMSAs).

Name of probe	Sequence (5′ to 3′)
WT-A	CACATGAGCCCAAAGA (−240 to −224)
WT-B	GCACATCCGGAGTATC (−257 to −241)
WT-C	TACAAAGACTGCTGAC (−275 to −258)
WT-6	(CAAAGA)×4
Mut3xA	TACGGGGACTGCTGA
MutG	TACAAAAACTGCTGAC
Mut1C	TATAAAGACTGCTGAC
MutT-C	CACAAAGACCGCCGAC
MutA-G	TGCGGGGGCTGCTGGC

Pavithra et al., 2009). Since SMAR1 binds to HIV-1 LTR *in vitro*, we tested if both SMAR1 and HDAC1 are corecruited to the LTR-MAR by checking for the amplification of LTR-MAR in chromatin fraction pulled with SMAR1 and subsequently HDAC1. Sequential ChIP experiments performed in chromatin fractions from Jurkat cells infected with HIV-$1_{pNL4\text{-}3}$ confirmed the recruitment of SMAR1/HDAC1 complex to the LTR (Fig. 3B, upper panel). The sequence downstream of TATA box (LTR-DS shown in Fig. 3A) served as negative control (Fig. 3B, lower panel). Immunoprecipitation of chromatin extracts from HIV-$1_{pNL4\text{-}3}$ infected Jurkat cells transfected with SMAR1 siRNA showed a reduced association of HDAC1 with LTR (Fig. 3C, lane 3), revealing that SMAR1 is important for recruiting HDAC1 to LTR.

To study the role of SMAR1 in HIV-1 transcription, ACH-2 cells were used as an experimentally relevant model of transcriptional latency. To confirm the induction of virus production, supernatant from untreated or PMA/TNF-α treated ACH-2 cells were assayed for p24 antigen. There was approximately a 6 to 7-fold induction of p24 counts upon activation by PMA or TNF-α 24 h post-treatment (Fig. 3D). To map chromatin changes associated with the recruitment of SMAR1 and Sin3A-HDAC1 co-repressor complex on LTR, ACH-2 cells were activated with PMA for different time intervals. Amplification of LTR-MAR region (Fig. 3A) from PMA activated chromatin extracts showed a time-dependent dissociation of SMAR1 from LTR. The amount of SMAR1 associated with LTR in control cells was high. Upon activation by PMA, the association reduces rapidly in a time-dependent manner and after 90 min there was no SMAR1 associated with LTR (Fig. 4A). Similar pattern was observed in case of HDAC1 and Sin3A which associate with LTR in unactivated cells and dissociate at 90 min post activation showing that SMAR1-HDAC1-Sin3A exist as a complex at LTR and dissociate at the same time upon activation by PMA. This is correlated to an increased acetylation of histones at H3K9 and H4K16 and decreased histone tri-methylation at H4K20 (Fig. 4A, left panel), essential signatures of a transcriptionally active promoter (Sims and Reinberg, 2008). Further, there was increased recruitment of RNA Pol II with corresponding increase in phosphorylation at Serine 2 and Serine 5 indicating an active transcription. Amplification of the region downstream of the TATA box showed no SMAR1 binding to LTR both in activated and control cells, although a marked time-dependent increase in the acetylation of histones and RNA Pol II recruitment, indicative of a spreading effect of histone modifying enzymes was observed upon activation (Fig. 4A, right panel). To provide a control for the specificity of the ChIP assay, the same samples were analyzed for actin promoter. There was no difference in RNA Pol II and histones acetylated at H3K9 and H4K16 present at the actin promoter before and after activation by PMA. Also immunoprecipitates of SMAR1, HDAC1 and Sin3A did not yield any amplification (Supplementary Fig. S3, left panel).

PMA is known to activate many cellular pathways through regulation of diverse transcriptional activators like AP-1, Sp1, NF-κB etc. Therefore, to pinpoint the exact molecular mediators leading to LTR activation and SMAR1 dissociation, we performed similar ChIP experiment with 20 ng/ml of TNF-α which activates specifically NF-κB pathway. Similar to PMA, TNF-α treatment also lead to dissociation of SMAR1-HDAC1 complex from LTR MAR, albeit with a delay of ~15 min in the kinetics (Fig. 4B). Thus, SMAR1, HDAC1 and Sin3A bind to LTR causing deacetylation of histones which leads to repression of LTR-mediated transcription (Fig. 4B, left panel). Upon activation of the cells by PMA or TNF-α, SMAR1 and its associated repressor complex dissociates from LTR making way for NF-κB to recruit p300 on to LTR which in turn activates LTR-mediated transcription through acetylation of histones at LTR. It is known that histone modifications at promoters determine the nature of transcription factors that are recruited to the promoter. Since SMAR1 recruits HDAC1/Sin3A complex to LTR, it causes deacetylation of histones which leads to recruitment of other factors that enforce transcriptional repression. Therefore, SMAR1 plays a major role in creating a repressive chromatin structure at HIV LTR.

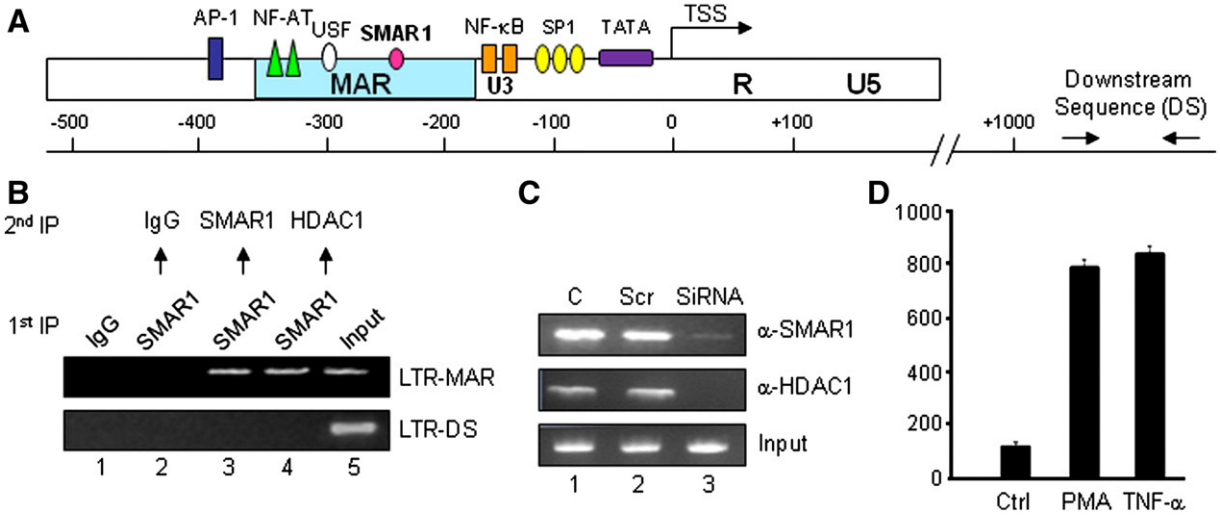

Fig. 3. SMAR1 binds to MAR in HIV-1 LTR *in vivo*. (A) Map of HIV LTR and location of primers used in the study along with major protein binding sites. (B) Sequential ChIP analysis of SMAR1 and HDAC1 on LTR. Cross-linked chromatin fragments from TZM-bl cells pulled with α- SMAR1 antibody were eluted and sequentially pulled with α-HDAC1 antibody from which LTR-MAR was amplified. (C) ChIP experiment showed knockdown of SMAR1 abrogates HDAC1 recruitment to LTR-MAR. (D) p24 ELISA in ACH-2 cells upon activation by PMA or TNF-α.

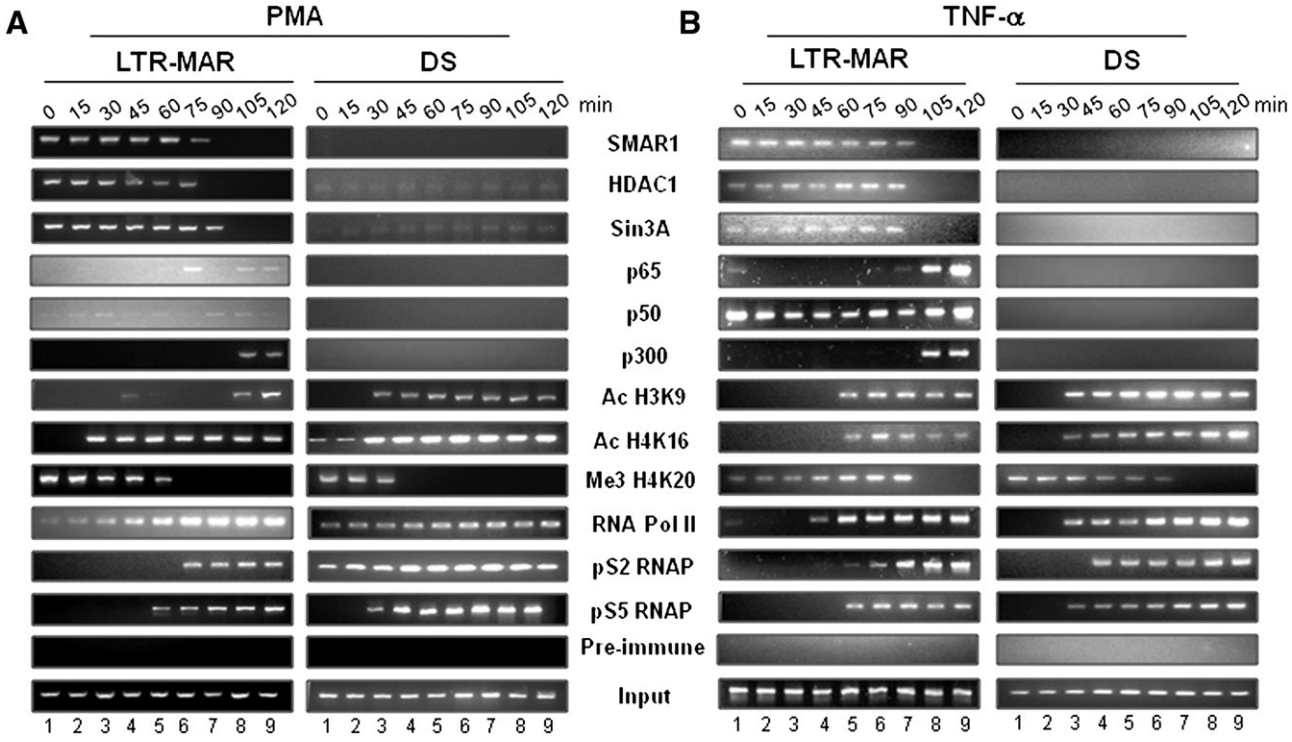

Fig. 4. SMAR1 binds and alters chromatin architecture at HIV LTR. (A) Time kinetics of dissociation of SMAR1/HDAC1/Sin3 complex from latent LTR upon activation by PMA or TNF-α, creating decondensed chromatin. ACH-2 cells were activated with 50 ng/ml of PMA and chromatin changes associated with transcriptional activation at LTR was followed at every 15 min interval using chromatin immunoprecipitation using antibodies specific for SMAR1, HDAC1, Sin3A, acetyl H3K9, acetyl H4K16, trimethyl H4K20 and RNA Pol II. The regions downstream of TATA box in HIV genome and Actin promoter were used as controls. (B) Chromatin immunoprecipitation assay as in (A) with activation by TNF.

SMAR1 inhibits HIV-1 transcription

Transcription factors which are part of chromatin remodeling complex can affect transcription in two ways; one by recruiting repressor complexes, other by modifying the chromatin structure through direct binding. Therefore we next assessed the role of SMAR1 in LTR-mediated transcription in TZM-bl cells. There was 2–3 fold reduction in basal transcription of HIV-1 LTR upon SMAR1 transfection (Fig. 5A). Moreover, overexpression of SMAR1 could over-ride Tat or PMA mediated transactivation of LTR, as shown by a 2-fold reduction in luciferase activity upon overexpression of SMAR1 in presence of Tat or PMA (Figs. 5B, C). Knockdown of SMAR1 using siRNA resulted in a significant increase in the basal as well as Tat-mediated transcription (Fig. 5D). There was a 2-fold increase in basal promoter activity and 1.5-fold higher Tat-mediated luciferase activity upon knockdown of SMAR1. Some chromatin remodeling proteins like SWI/SNF can affect expression of genes within chromatin and cannot affect transcription of genes expressed episomally. Therefore, we evaluated if SMAR1 affected LTR mediated transcription when the reporter was transfected exogenously. In transient transfection assays, LTR-Luc construct was designed to quantitate the LTR driven reporter activity upon SMAR1 expression in the presence or absence of either Tat or PMA. Our results showed that SMAR1 overexpression lead to 3-fold reduction in LTR-mediated transcription (Fig. 5E). We further observed that SMAR1 overexpression could over-ride Tat or PMA mediated transactivation (Figs. 5F, G) of transfected LTR-luciferase. Knockdown of SMAR1 lead to a 2-fold increase in both basal and Tat-mediated LTR-Luciferase activity (Fig. 5H). Taken together, our results suggest that SMAR1 represses viral transcription of both integrated and unintegrated LTRs.

SMAR1 regulates basal HIV transcription

In view of the constitutive binding of SMAR1 to the transcriptionally silent HIV LTR, we investigated if SMAR1 might be involved in regulating basal transcription from a transcriptionally silent LTR promoter. SMAR1 knock-down using specific siRNA or overexpression in latent ACH-2 cells was confirmed by Western blot analysis using SMAR1 specific antibody (Fig. 6A). SMAR1 knockdown resulted in ~2.5 fold induction in p24 counts, while its overexpression led to a 2-fold decrease in the p24 levels (Fig. 6B).

The role of SMAR1 in repressing HIV-1 virion production was further demonstrated by transfection of HeLa cells with infectious molecular clone pNL4-3 or pYU-2. The culture supernatant was then tested for the presence of viral protein $p24^{gag}$. Co-transfection with SMAR1 caused a dose-dependent inhibition of both CXCR4 (pNL4-3) and CCR5 (pYU-2)—tropic virus production, whereas cotransfection of vector control failed to inhibit HIV production. Transfection of siRNA specific for SMAR1 increased pYU2 virus production by 2-fold and pNL4-3 virus production by 2.5-fold compared to control cells. Co-transfection of pcTat resulted in an increased virion production and was used as positive control (Figs. 6C, D). These findings point out at the role of SMAR1 in the inhibition of HIV-1 replication and virion production.

Discussion

Transcription from the proviral form, which is a coordinated interplay of multiple DNA-bound activators, coactivators, inhibitors and basal transcription components, is one of the most important steps in the life cycle of HIV (Bisgrove et al., 2005). Although factors binding to HIV LTR have been well studied, little is known about factors binding to NRE and their effects thereof. In this report, we identify the presence of at least one MAR in the 5′ LTR of different strains of HIV and provide a consensus sequence present in their MARs. In this context, it would be interesting to look for similar sequences in 3′ LTR. Since 3′ LTR does not drive HIV gene expression, how existence of such MARs affects HIV transcription is debatable. It is apparent that the presence of *cis*

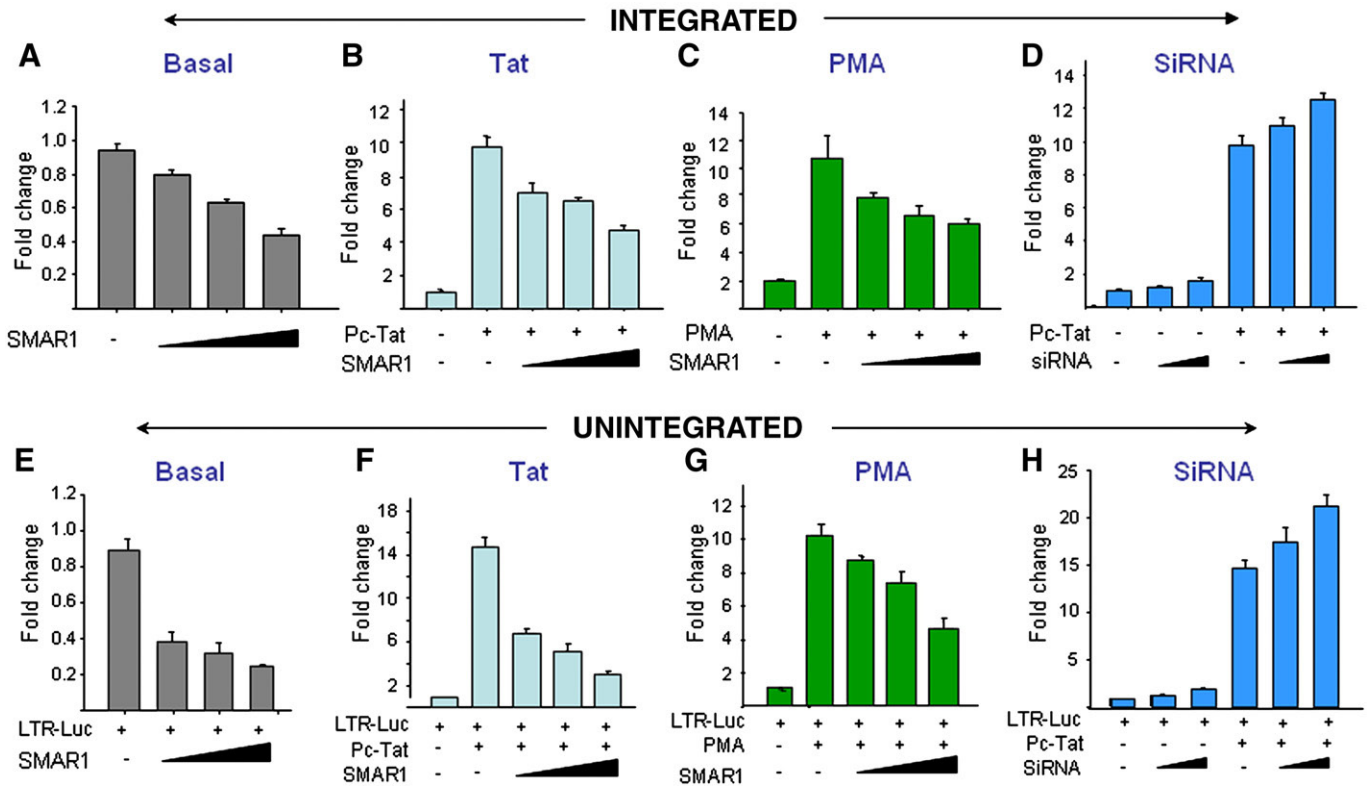

Fig. 5. SMAR1 downregulates HIV-1 transcription. Luciferase assays in SMAR1 overexpressed TZM-bl cells showing repression of (A) basal, (B) Tat mediated and (C) PMA activated LTR mediated transcription. (D) Luciferase assays upon SMAR1 knockdown in TZM-bl cells. SMAR1 downregulates basal (E), Tat-mediated (F) and PMA activated (G) transcription in 293T cells transiently transfected with LTR-Luc reporter. (H) Luciferase assays upon SMAR1 knockdown.

acting elements like MAR in 5′ LTR confers a regulated transcriptional control dependent on the nuclear milieu. This is similar to, the Human Papilloma Viruses (HPVs) that harbor MARs in both the 5′ LCR and the genome that serves to regulate the viral gene expression. Our observation suggests that binding to nuclear matrix is a general phenomenon of many viral promoters, a mechanism that may be adopted by viruses for conferring selective transcriptional activation.

We further hypothesize that the affinity of LTR-MAR to the nuclear matrix might determine the transcriptional activity of the LTRs of different strains of HIV. We show that SMAR1, a MARBP is responsible for tethering of LTR to nuclear matrix causing repression of LTR-

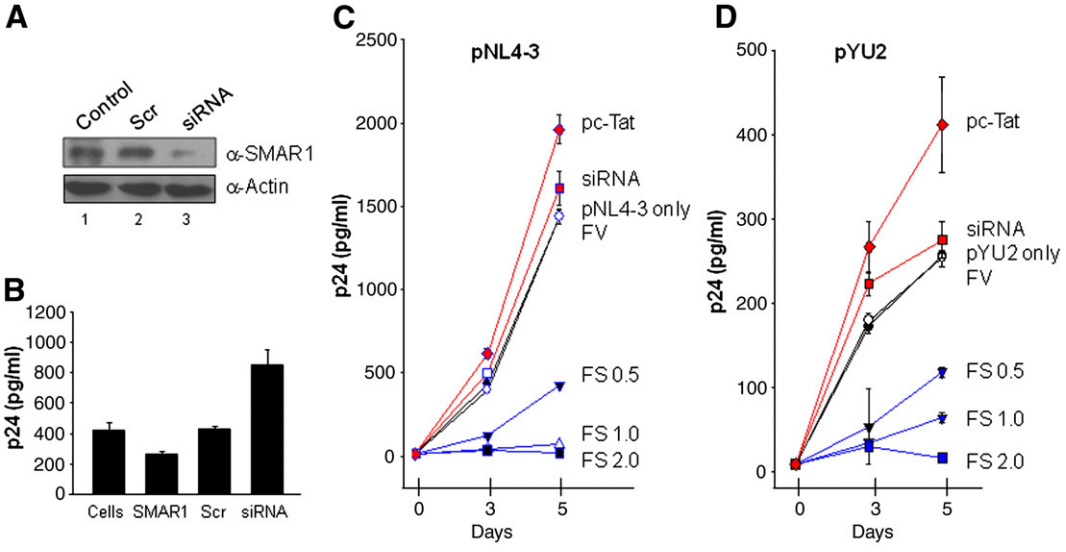

Fig. 6. SMAR1 inhibits basal LTR-mediated transcription. (A) Immunoblot showing knockdown of SMAR1 in ACH-2 cells. ACH-2 cells were transfected twice with SiRNA specific for SMAR1, Flag-SMAR1 or scrambled SiRNA within a span of 42 h and p24 levels analyzed 24 h second Transfection. Control cells were treated with equal amounts of lipofectamine as other samples. (B) Basal virion production upon SMAR1 overexpression/ silencing by SiRNA. (C, D) Single-round virion production assays showing a dose dependent suppression of virion production by SMAR1. HIV-1 Molecular clones pNL4-3 or pYU-2 were cotransfected with increasing concentrations of Flag-SMAR1 (FS), SiRNA specific for SMAR1, vector alone (FV) or pcTat and p24 counts were analyzed 48 h post infection. Transfection of SMAR1 siRNA or Tat enhances virus production whereas overexpression of SMAR1 reduced virus production.

mediated transcription. Further analysis showed that SMAR1 binds specifically to a hexanucleotide sequence present in the MAR consensus element. Our database of MARs present in LTRs of all the HIV isolates consisted of 24,512 bases. Of the 4096 all arithmetically possible hexanucleotides, any one hexanucleotide can occur only six times randomly in a given database of 24,512 bases. The hexanucleotide sequence to which SMAR1 binds CAAAGA occurs 74 times in the compiled database containing 219 sequences which is more than 12-fold higher than that of expected random frequency which shows that occurrence of these sequences in not random but highly specific and conserved. There is a large amount of degeneracy within this consensus which is expected in all the MAR sequences. This is because, degeneracy allows two similar sequences to be regulated differentially based on the factor recruitment or affinity to bind to nuclear matrix. Hence, degeneracy in MAR sequences is biologically very relevant and important. Additionally, SMAR1 mediated recruitment of HDAC1 to the LTR, leading to deacetylation of local histones. The resultant change in chromatin environment decreases the association of RNA Pol II with LTR thereby enforcing transcriptional repression of the HIV provirus. We also found that PMA stimulation abrogated the SMAR1 mediated repression of HIV promoter. It is conceivable that the nuclear translocation of NF-κB and its binding to the HIV promoter might alter local chromatin remodeling, thus eliminating SMAR1 and its repressor complex from LTR. SMAR1 appears to exert transcriptional repression of HIV-1 promoter through bimodal mechanisms: (1) directly by recruiting HDAC1/Sin3 repressor complex and (2) by promoting the binding of nuclear matrix to LTR. These bimodal actions of SMAR1 make it a strong negative regulator of HIV transcription. However, further studies are needed to determine the factors in nuclear matrix that bind to SMAR1 and how they coordinate in causing transcriptional repression. Our studies presented here strongly suggest SMAR1 as the likely candidate that regulates the viral transcription dependent on the cue provided by the cellular microenvironment. A follow up of molecular events that occur at LTR upon activation with PMA or TNF-α show that SMAR1 binds to LTR which is transcriptionally silent and upon its activation, SMAR1 rapidly falls off LTR. This gives rise to a cascade of events like dissociation of HDAC1/Sin3A from LTR ultimately leading to transactivation of LTR promoter. The dissociation of HDAC1 coincides with the time of acetylation of histones and recruitment of p300 which suggests that dissociation of SMAR1 from LTR is necessary for histone acetylation to take place. There was also a decrease in trimethylation at histone H4K20 upon activation which follows the same pattern as association of SMAR1 with LTR. Thus it will be interesting to study if SMAR1 can recruit any histone methyl transferases to LTR and causes tri-methylation of histones. Our findings support an earlier model in which the association of RNA Pol II can initiate transcription, but cannot efficiently support elongation in the absence of Tat or NF-κB (Bisgrove et al., 2005; Copeland, 2005). Thus, HIV transcription is essentially multifactorial and blocks occur at different levels in different cells.

The results presented here suggest that recruitment of SMAR1/HDAC1 complex anchors LTR to nuclear matrix maintaining a repressive state. The ablation of this binding upon activation by PMA leads to binding of NF-κB, p300 and other transcriptional activators that resulting in activation of proviral transcription (Fig. 7). This is strengthened by the observation that knockdown of SMAR1 in latent ACH-2 cells lead to significant increase in LTR transcription. However, we believe that the observed residual HDAC1 binding might be a result of LSF/YY-1, c-Myc, AP-4 or p50 mediated recruitment to regions downstream of NRE. Thus, binding of SMAR1 and thus nuclear matrix to LTR in line with the fact that recruitment of HDAC1 is a general mode in which HIV transcription is regulated and the repressor binding sites are spread all along the LTR to ensure proper and coordinated regulation of gene expression. Nuclear matrix thus forms a microenvironment to recruit co-regulatory factors for the combinatorial regulation/modulation of gene expression in the three-dimensional context of nuclear organization. Since HIV, HTLV and HPV viral promoters contain MARs, it is tempting to speculate that viruses have an inbuilt strategy of using host transcriptional regulatory machinery through association with nuclear matrix which confers transcriptional latency or activation, depending on the context. Therefore, a detailed study of cellular factors that regulate transcription of HIV may further enhance our understanding of HIV replication in T-cells. This may lead to specific targeted therapies against quiescent reservoir of HIV provirus in combination with other antiretroviral drugs.

Conclusions

Cellular pathogens and their hosts have co-evolved with mechanisms to counter and survive with each other. HIV-1 has cunningly acquired tools to regulate and utilize many host factors for its own propagation. So far we know that HIV integrates into the human genome and uses cellular machinery to either be active in transcription or be latent. However, the choice to be active or latent is made by the virus or host is not known. We have identified for the first time that HIV itself harbors DNA sequences mimicking human genome. This leads to binding of various critical host transcription factors like SMAR1 to viral DNA and recruit other factors which decide the transcriptionally active or latent configuration of HIV genome. Such mimicking also aids HIV in differentially partitioning itself between active chromatin territories (ACTs) or associate with nuclear matrix fraction to be transcriptionally latent. The present study also expands our understanding of how SMAR1 represses HIV transcription. Further, the anti-HIV activity of prostaglandins is shown to be mediated by SMAR1. The binding site for SMAR1 is the same in all subtypes of HIV-1, HIV-2 and even HTLV. Thus enhancing SMAR1

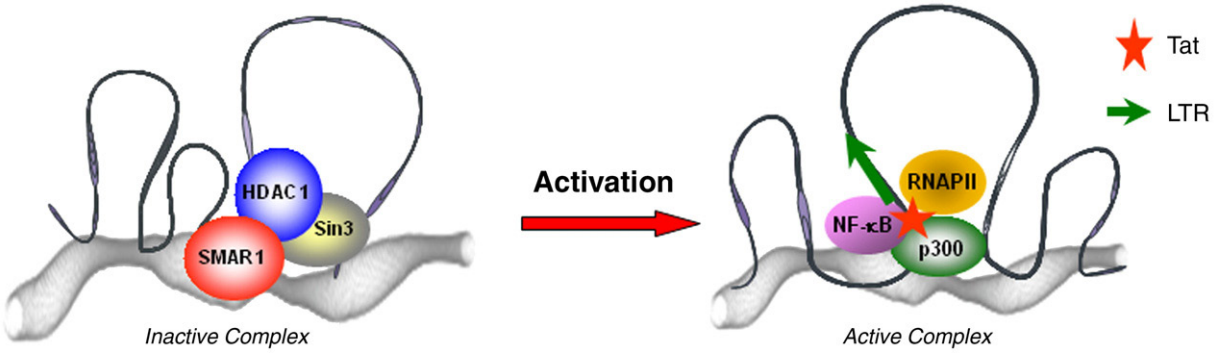

Fig. 7. SMAR1 tethers LTR to nuclear matrix. Schematic representation of molecular events before and after activation. SMAR1 tethers LTR to nuclear matrix. SMAR1 recruits HDAC1/Sin3A complex to HIV LTR and condenses chromatin at the LTR thereby downregulating LTR-mediated transcription upon activation.

protein expression provides a potential means of inhibiting transcription of many types of HIV.

Materials and methods

Plasmids and constructs

Cloning and expression of LTR-Luciferase construct, pcDNA-Tat and plasmids expressing full length SMAR1 and various truncations have been previously described (Pavithra et al., 2007). L1 (5′ CAAGATATCCTTGATCTGTGG) and L2 (5′ AAGGGGAACCAAGAGA) primers were used for amplification of the 1.5 kb region comprising the 5′ LTR and its downstream sequence (1.5 kb LTR) from the HXB3 genomic clone of HIV-1 (NIH AIDS reagent program). The amplified product was purified and cloned into pGEM-T easy which was then cut out using *Eco*RI, Klenowed and further subcloned into the *Sma*I site of SK+ (Stratagene), producing NC-1. NC-1 was digested with *Xho*I and *Bam*HI, and the resultant LTR fragment was cloned into pGL3-Basic vector pre-digested with *Xho*I and *Bam*HI (Promega), and named LTR-Luc. pcDNA-Tat, the expression vector for Tat, was constructed by cloning HXB3 Tat in pcDNA 3.1 using *Eco*RI and *Not*I sites. pNL4-3 and pYU2 molecular clones were obtained from NIH reagent and reference program.

Cell culture and transfections

293T, HeLa and TZM-bl cell lines were grown in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% FCS (Invitrogen), 100 U/ml penicillin (Invitrogen), 100 μg/ml Streptomycin (Invitrogen) and 2 mM L-glutamine in 5% CO_2 humidified atmosphere at 37°C and manipulated following treatment with 0.1% Trypsin-EDTA (Invitrogen). CEM-GFP, Jurkat and ACH-2 cells were grown in RPMI 1640 (Invitrogen) under similar conditions. Cells were seeded at a density of 1–3 $\times 10^6$ cells per 35 mm dish. Transfections were done with indicated plasmids using Lipofectamine 2000 as per manufacturer's instructions (Invitrogen). SiRNA specific for SMAR1 and control scrambled siRNA were synthesized from Ambion (SMAR1 siRNA [RS] sense, 5 GCAGAGCAUUGACUCCAAGTT; antisense, 5 CUUGGAGUCAAUGCUCUGCTT; scrambled siRNA sense, 5′ UACCGUAGGCAUGCAAAGCTT; antisense, 5′ AUGGCAUCCGUACGUUUCGTT ; SMAR1 siRNA [NS] sense, 5′ GAGAAGCUAGACCUGGUCATT; antisense, 5 UGACCAGGUCUAGCUUCUCTT. For transfections in ACH-2 cells, cells were grown overnight in serum-free media and transfections were done twice with 24 h time between transfections to ensure better efficiency using Lipofectamine 2000. Ten percent FCS was added 5 h post transfection. Cells were activated with either 20 ng/ml of TNF-α or 50 ng/ml PMA (Sigma) for 2 h unless otherwise indicated.

p24 ELISA

ELISA for p24 antigen was performed using standard ELISA kits from Perkin Elmer according to the manufacturer's protocol. Briefly, all the samples were diluted in and 200 μl of each diluted sample was used for the assay. Culture medium from uninfected cells was taken as negative control. Quantitation was done by plotting absorbance values against standard graph. All the assays were done in triplicate.

Viral production and cell Infection

For virus production, 5×10^6 HEK293T cells were transfected with pNL4-3 molecular clone using Lipofectamine 2000 in 90-mm culture dishes. After 16 h, the medium was replaced with complete media and supernatants containing viral particles were harvested 24 h later. The number of infective viral particles per ml was established by infecting 1×10^4 HeLa based MAGI $CD4^+/CXCR4^+/CCR5^+$ cells with different amounts of viral suspension. The titer of the virus stock was measured as $TCID_{50}$/ml by β-galactoside assay 72 h after infection and subjecting the values to Spearmann–Karber formula.

Human PBMCs were isolated from fresh human blood using Ficoll (Sigma) gradient. For infection in Jurkat cells and human PBMCs, the indicated amounts of viral supernatant was incubated with 5×10^6 cells/ml in serum-free RPMI containing 0.4 μg/ml polybrene (Sequabrene, Sigma) with intermittent tapping at 30 min intervals for 6 h. The cells were then washed three times with RPMI and grown in RPMI containing 10% FCS.

Immunoblotting and antibodies

Cells were scraped in 1× PBS and collected at indicated time points and lysed using TNN buffer Equal amounts of proteins were taken for immunoblotting following estimation by Bradford's reagent (BioRad). Proteins resolved by SDS-PAGE, were electroblotted onto PVDF membrane (Amersham). The membrane was blocked overnight in Tris-buffered saline containing 0.1% Tween-20 (TBST) and 10% bovine serum albumin (BSA). The membrane was then probed with primary antibody in TBST for 2 h, followed by three 10-min TBST washes at room temperature. Incubation with the secondary antibody was done for 1 h, and three 10-min TBST washes were given prior to detection. Proteins were detected using enhanced chemiluminescence substrate (Amersham).

Electrophoretic mobility shift assay (EMSA)

For EMSA, oligonucleotide labeling was done by a Klenow reaction using [α^{-32}P] dCTP in a 20 μl reaction containing 1 mM dATG mix, Klenow buffer, and 0.5 U of Klenow DNA polymerase (Invitrogen). The sequences of all the oligos used are given in Table 1. Probe purification was done using Probequant G-50 column (Amersham Biosciences). Binding reactions were performed in a 10 μl total volume containing 10 mM HEPES (pH 7.9), 1 mM dithiothreitol, 50 mM KCl, 2.5 mM $MgCl_2$, 10% glycerol, 0.5 to 1 μg double-stranded poly (dI-dC), 10 μg BSA, and 1 μg of recombinant protein. GST-SMAR1 as well as GST (160–350) and GST (350–548) truncation clones were purified as described in Pavithra et al. (2007). Samples were incubated for 5 min at room temperature prior to addition of radiolabeled probe. The samples were then incubated for 15 min at room temperature, and the products of binding reactions were resolved by 8% native polyacrylamide gel electrophoresis. The gels were dried under vacuum and processed for autoradiography.

Luciferase reporter assays

Forty hours after transfection, the culture medium was removed, cells were washed with phosphate buffered saline (PBS), resuspended in 100 μl of cell lysis buffer. After two freeze–thaw cycles, cells were centrifuged at 9500 rpm for 15 min and, protein concentration estimated. Equal amounts of protein were used for the assays. Luciferase activity assays were performed using Luclite substrate (Perkin Elmer, USA) in Top-Count luminometer (Packard Life sciences, USA). Graphs were plotted from data obtained as a mean of three independent experiments along with computed standard deviations as error bars.

ChIP analysis

Assays were performed using Chromatin Immunoprecipitation (ChIP) assay kit (Upstate Biotechnology) following manufacturer's instructions. 1×10^6 cells were plated per 30 mm dish and treated with TNF-α or PMA or left untreated. After treatment, DNA–protein interactions were fixed by adding formaldehyde directly to the media to a final concentration of 1%, incubated at 37 °C for 10 min. Cell were washed with PBS , pelletted and cells were lysed in SDS-lysis buffer by

sonication. The samples were then centrifuged at 15,000 rpm in order to clear the debris and chromatin extracts were incubated with 2 μg of the indicated antibody or the isotype control at 4 °C overnight. The antibody-chromatin complex was precipitated by adding protein A-Sepharose bead, incubated for 4 h by rotating at 4 °C and centrifuged at 3000 rpm for 5 min. ChIP assays were carried out using anti-SMAR1, anti-HDAC1, anti- acetyl Histone 3 Lysine-9 (H3K9), anti-Histone 4 Lysine 16 (H4K16), anti-RNA polymerase II, anti-phospho Serine 2 RNA Polymerase II, anti-phospho Serine 5 RNA Polymerase II, anti-phospho Histone 3 Serine 10, anti-Histone 4 Lysine 20 tri-methyl antibodies (Cell Signaling). Input DNA, Rabbit IgG (r-IgG), and Mouse IgG (m-IgG) pulled DNA served as controls for all the experiments. Immunoprecipitated DNA was then subjected to 30 cycles of semi quantitative PCR using the primers mentioned below. The forward and reverse primers used to amplify MAR respectively are 5′ GAAGTTTGACAGCCTCCTA and 5′ CTCCCCAGTCCCGCCCAGG. To amplify region downstream of LTR, primers used are 5 TGACTCAGATTGGCTGCAC and 5′ AATTTCTACTAATGCTTTA. The primers used to amplify β-actin promoter were 5 GCCAGCAGCAAGCCTTGG and 5′ GCCACTGGGCCTCCATTC.

Preparation of nuclear matrix

Nuclear matrices from uninfected and HIV-1 infected cells lines were prepared according to a well established protocol Cockerill and Garrard, 1986a, 1986b). Cultured cells were washed once in PBS, resuspended in RSB (10 mM NaCl; 3 mM $MgCl_2$; 10 mM Tris–HCl; 0.5 mM PMSF, pH 7.4), incubated on ice for 10 min and then homogenized with a Dounce homogenizer. Nuclei were pelletted by centrifuging the samples at 6000 rpm for 10 min. Nuclei in RSB, 0.25 M sucrose, and 1 mM $CaCl_2$ were digested with 100 mg/ml DNAse I (Sigma) for 2 h at 23 °C. After centrifugation for 10 min at 750 rpm at 4 °C, pellets were suspended in RSB-0.25 M sucrose, and an equal volume of cold solution containing 4 M NaCl, 20 mM EDTA, and 20 mM Tris–HCl, (pH 7.4) was added. After centrifugation at 10,000 rpm for 10 min, pellets were extracted twice by suspension in cold solution of 2 M NaCl, 10 mM EDTA, 10 mM Tris–HCl, 0.5 mM PMSF, and 0.25 mg/ml BSA and centrifuged at 4 °C at 4500 rpm for 15 min. The resulting Nuclear Matrices were washed with RSB at 4 °C, suspended in the same solution and stored at -80 °C.

Matrix binding assay

The assay was performed as previously described [28]. Nuclear matrix isolated from 1×10^7 cells was suspended in 90 μl MAR-binding buffer (20 mM Tris–HCl pH 7.4, 50 mM NaCl, 2 mM EDTA, 0.25 M Sucrose, and 0.25 mg/ml BSA). Sheared salmon sperm DNA (100 μg/ml) and 5 ng/ml (50,000 cpm) ^{32}p-labeled DNA fragments from MAR region of HIV-1 LTR were mixed and incubated with nuclear matrix fraction at 25 °C for 4 h with constant gentle shaking. Reaction mixture was diluted with 1 ml binding buffer, centrifuged and the matrix-bound fragments were solubilized in 0.5% SDS. The soluble mixture was treated with 0.5 mg/ml Proteinase K for 5 h, phenol–chloroform extracted, ethanol precipitated and finally resolved on an 8% polyacrylamide–0.1% SDS gel. For *in vivo* matrix binding assays, nuclear matrix and chromatin fractions were isolated from activated or unactivated 5×10^7 exponentially growing TZM-bl or CEM-GFP cells and bound DNA fragments isolated by phenol-chloroform-isoamylalcohol method. DNA isolated from these fractions were subjected to PCR for quantifying the amount of LTR present in matrix or chromatin fractions.

South-Western blotting

South-Western blotting as per protocols established by Raziuddin et al. with minor modifications. Protein from nuclear matrix (10 and 20 μg) was resolved by 10% SDS PAGE, electrotransferred onto PVDF membrane. The membrane was prehybridized for 2 h at room temperature with buffer containing 10 mM HEPES and 5% BSA (Amersham) and then hybridized with buffer containing 10 mM HEPES (pH 7.9), 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.5% BSA, 5 μg/ml heat denatured sheared salmon sperm DNA (Sigma) and 1×10^5 cpm of ^{32}p-labeled LTR probes for 1 h at room temperature with constant agitation. The blot was then washed three times for 20 min each at room temperature with the same buffer with 300 mM NaCl, air-dried and exposed for autoradiography.

Competing interest statement

The authors have declared that no competing interests exist.

Authors' contributions

KS designed and performed most of the experiments and wrote the manuscript. LP, SS and SS performed Western blotting and gel shift experiments. PD and NS participated in viral infection experiments. UR and DM designed and analyzed the data. SC directed and supervised the experiments, analyzed data and wrote the manuscript. All the authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.virol.2010.01.017.

References

Bailey, T.L., Williams, N., Misleh, C., Li, W.W, 2006. MEME: discovering and analyzing DNA and protein sequence motifs. Nucleic Acids Res. (34), W369–W373.
Barre-Sinoussi, F., Chermann, F.R., Nugeyre, M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W., Montagnier, L, 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science (220), 868–871.
Bisgrove, D., Lewinski, M., Bushman, F., Verdin, E, 2005. Molecular mechanisms of HIV-1 proviral latency. Expert. Rev. Anti. Infect. Ther. (3), 805–814.
Bode, J., Kohwi, Y., Dickinson, L., Joh, T., Klehr, D., Mielke, C., Kohwi-Shigematsu, T, 1992. Biological significance of unwinding capability of nuclear matrix-associating DNAs. Science (255), 195–197.
Breyne, P., Van Montagu, M., Depicker, N., Gheysen, G, 1992. Characterization of a plant scaffold attachment region in a DNA fragment that normalizes transgene expression in tobacco. Plant Cell (4), 463–471.
Cockerill, P.N., Garrard, W.T, 1986a. Chromosomal loop anchorage sites appear to be evolutionarily conserved. FEBS Lett. (204), 5–7.
Cockerill, P.N., Garrard, W.T, 1986b. Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. Cell (44), 273–282.
Copeland, K.F, 2005. Modulation of HIV-1 transcription by cytokines and chemokines. Mini. Rev. Med. Chem. (5), 1093–1101.
Dijkwel, P.A., Hamlin, J.L, 1988. Matrix attachment regions are positioned near replication initiation sites, genes, and an interamplicon junction in the amplified dihydrofolate reductase domain of Chinese hamster ovary cells. Mol. Cell Biol. (8), 5398–5409.
Du, C., Basyuk, I.E., Lin, Y.L., Triboulet, R., Knezevich, A., Chable-Bessia, C., Mettling, C., Baillat, V., Reynes, J., Corbeau, P., Bertrand, E., Marcello, A., Emiliani, A., Kiernan, R., Benkirane, M, 2007. Suv39H1 and HP1gamma are responsible for chromatin-mediated HIV-1 transcriptional silencing and post-integration latency. EMBO J. (26), 424–435.
Gaynor, R, 1992. Cellular transcription factors involved in the regulation of HIV-1 gene expression. AIDS (6), 347–363.
Guelen, L., Pagie, L., Brasset, E., Meuleman, W., Faza, M.B., Talhout, W., Eussen, B.H., de Klein, A., Wessels, L., de Laat, W., van Steensel, B, 2008. Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature (453), 948–951.
Heessen, S., Fornerod, M, 2007. The inner nuclear envelope as a transcription factor resting place. EMBO Rep. (8), 914–919.

Imai, K., Okamoto, T, 2006. Transcriptional repression of human immunodeficiency virus type 1 by AP-4. J. Biol. Chem. (281), 12495–12505.
Jiang, G., Espeseth, A., Hazuda, D.J., Margolis, D.M., 2007. c-Myc and Sp1 contribute to proviral latency by recruiting histone deacetylase 1 to the human immunodeficiency virus type 1 promoter. J. Virol. (81), 10914–10923.
Korner, M., Bellan, A.H., Brini, A.T., Farrar, A.L, 1990. Characterization of the human immunodeficiency virus type 1 enhancer-binding proteins from the human T-cell line Jurkat. Biochem. J. (265), 47–554.
Kulkarni, A., Pavithra, L., Rampalli, S., Mogare, D., Babu, K., Shiekh, G., Ghosh, S., Chattopadhyay, S, 2004. HIV-1 integration sites are flanked by potential MARs that alone can act as promoters. Biochem. Biophys. Res. Commun. (322), 672–677.
Lassen, K., Han, Y., Zhou, Y., Siliciano, J., Siliciano, R.F, 2004. The multifactorial nature of HIV-1 latency. Trends Mol. Med. (10), 525–531.
Marban, C., Suzanne, S., Dequiedt, F., de Walque, S., Redel, L., Van Lint, C., Aunis, D., Rohr, O, 2007. Recruitment of chromatin-modifying enzymes by CTIP2 promotes HIV-1 transcriptional silencing. EMBO J. (26), 412–423.
Naghavi, M.H., Schwartz, S., Sonnerborg, A., Vahlne, A, 1999. Long terminal repeat promoter/enhancer activity of different subtypes of HIV type 1. AIDS Res. Hum. Retroviruses (15), 1293–1303.
Pavithra, L., Rampalli, S., Sinha, S., Sreenath, K., Pestell, R.G., Chattopadhyay, S, 2007. Stabilization of SMAR1 mRNA by PGA2 involves a stem loop structure in the 5′ UTR. Nucleic Acids Res. (35), 6004–6016.
Pavithra, L., Singh, S., Sreenath, K., Chattopadhyay, S, 2009. Tumor suppressor SMAR1 downregulates Cytokeratin 8 expression by displacing p53 from its cognate site. Int. J. Biochem. Cell Biol. (41), 862–871.
Pearson, R., Kim, Y.K, Hokello, J., Lassen, K., Friedman, J., Tyagi, M., Karn, J, 2008. Epigenetic silencing of human immunodeficiency virus (HIV) transcription by formation of restrictive chromatin structures at the viral long terminal repeat drives the progressive entry of HIV into latency. J. Virol. (82), 12291–12303.
Rampalli, S., Pavithra, L., Bhatt, A., Kundu, T.K., Chattopadhyay, S, 2005. Tumor suppressor SMAR1 mediates cyclin D1 repression by recruitment of the SIN3/histone deacetylase 1 complex. Mol. Cell Biol. (25), 8415–8429.
Reddy, K.L., Zullo, J.M., Bertolino, E., Singh, H, 2008. Transcriptional repression mediated by repositioning of genes to the nuclear lamina. Nature (452), 243–247.
Schubeler, D., Mielke, C., Maass, K., Bode, J, 1996. Scaffold/matrix-attached regions act upon transcription in a context-dependent manner. Biochemistry (35), 11160–11169.
Seo, J., Lozano, M.M., Dudley, J.P, 2005. Nuclear matrix binding regulates SATB1-mediated transcriptional repression. J. Biol. Chem. (280), 24600–24609.
Sims III, R.J., Reinberg, D., 2008. Is there a code embedded in proteins that is based on post-translational modifications? Nat. Rev. Mol. Cell Biol. (9), 815–820.
Tyagi, M., Karn, J, 2007. CBF-1 promotes transcriptional silencing during the establishment of HIV-1 latency. EMBO J. (26), 4985–4995.
Williams, S.A., Kwon, H., Chen, L.F., Greene, W.C, 2007. Sustained induction of NF-kappa B is required for efficient expression of latent human immunodeficiency virus type 1. J. Virol. (81), 6043–6056.
Zhang, C., Jenkins, H., Goldberg, M.W., Allen, T.D., Hutchison, C.J, 1996. Nuclear lamina and nuclear matrix organization in sperm pronuclei assembled in Xenopus egg extract. J. Cell Sci. (109), 2275–2286.