Integrase interactor 1 (Ini1/hSNF5) is a repressor of basal human immunodeficiency virus type 1 promoter activity

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INTRODUCTION

Integration of a DNA copy of the human immunodeficiency virus type 1 (HIV-1) RNA genome into the host-cell chromatin is a central step in the virus life cycle catalysed by the virus integrase (IN) enzyme. Although integration can occur throughout the whole genome, the target site of integration is not random, and different retroviruses show distinct preferences (Bushman, 2003; Bushman et al., 2005). Recent work has demonstrated a strong preference for transcribed regions and an avoidance of transcriptionally silent heterochromatin in the case of HIV-1, whereas the gammaretrovirus murine leukemia virus shows an integration preference near the transcription start sites of actively transcribed genes. The differences observed between the integration profiles of these two viruses strongly suggest that cellular cofactors actively tether proviral DNA to specific regions of the genome. Active selection of integration target sites is likely to be a physiologically relevant step in the retroviral life cycle and the recruitment of distinct cellular factors assisting in this process may reflect an adaptation of different viruses to their replication strategy, a point of view supported by studies of yeast retrotransposons (Bushman, 2003; Bushman et al., 2005). Intriguingly, all cellular proteins that have been identified through an association with the HIV-1 integration process (e.g. HMGa1, BAF, LEDGF/p75, EED and Ini1/hSNF5) have normal cellular functions in chromatin remodelling and organization, making them attractive candidates for guiding the HIV-1 pre-integration complex to favoured sites for integration (Van Maele et al., 2006). However, to date, only LEDGF/p75 has been shown to play a role in targeting HIV-1 DNA integration (Ciuffi et al., 2005, 2006).

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Integrase interactor 1 (Ini1/hSNF5/BAF47/SMARCB1), the core subunit of ATP-dependent SWI/SNF chromatin remodelling complexes, which regulate the expression of numerous eukaryotic genes by altering DNA–histone interactions (Kingston & Narlikar, 1999; Kingston et al., 1996; Wang et al., 1996), was the first host protein identified as an IN-interacting factor by two-hybrid screenings (Kalpana et al., 1994). Ini1/hSNF5 has been reported to interact with other virus proteins, including the Epstein–Barr virus nuclear antigen 2 (Kwiatkowski et al., 2004; Wu et al., 1996, 2000), human papillomavirus E1 (Lee et al., 1999) and Kaposi’s sarcoma-associated herpesvirus (human herpesvirus-8) K8 (Hwang et al., 2003), as well as the cellular factors c-MYC (Cheng et al., 1999), ALL1 (MLL) (Rozenblatt-Rosen et al., 2003), as well as the cellular factors c-MYC (Cheng et al., 1999), ALL1 (MLL) (Rozenblatt-Rosen et al., 1998), GADD34 (Adler et al., 1999), and p53 (Lee et al., 2002). Moreover, Ini1/hSNF5 has been found to act as a tumour suppressor, as mutations in the ini1 gene lead to aggressive paediatric atypical teratoid and malignant rhabdoid tumours (Versteege et al., 1998), suggesting a role for the SWI/SNF complex in control of the cell cycle. Further studies have shown that Ini1/hSNF5 regulates cell proliferation by inhibiting activation of E2F-dependent genes through the p16ink4a–CDK4/cyclin D–Rb pathway (Imbalzano & Jones, 2005).

The exact role of Ini1/hSNF5 in HIV-1 replication remains unclear. The finding that Ini1/hSNF5 stimulates the DNA-joining activity of HIV-1 IN in vitro (Kalpana et al., 1994) and is recruited from the nucleus to incoming pre-integration complexes of HIV-1 (Turelli et al., 2001) raise the possibility that Ini1/hSNF5 may contribute to preferential selection of transcriptionally active genes as integration sites of HIV-1 (Schroder et al., 2002). More recent evidence has indicated, however, that Ini1/hSNF5 is dispensable for HIV-1 transduction per se (Ariumi et al., 2006; Boese et al., 2004) and may have an inhibitory effect on the early steps of HIV-1 replication (Maroun et al., 2006). Moreover, it has been demonstrated that SWI/SNF complexes interact with the integrated HIV promoter in a Tat-dependent manner and that Tat-mediated activation of the HIV promoter requires the SWI/SNF complex in synergy with the co-activator p300 (Aghbollah et al., 2006; Ariumi et al., 2006; Mahmoudi et al., 2006; Treand et al., 2006). This suggests that Ini1/hSNF5-containing complexes are instead implicated in transcriptional regulation of the HIV-1 promoter.

As a consequence of integration, HIV-1 gene expression occurs in the context of host-cell chromatin and requires cellular factors. Emerging data suggest that dynamic regulation of chromatin structure in the vicinity of the long terminal repeat (LTR) promoter adds an additional level of complexity to the regulation of HIV-1 expression, and SWI/SNF complexes have been implicated in these processes (Aghbollah et al., 2006; Angelov et al., 2000; Ariumi et al., 2006; Brigati et al., 2003; Bukrinsky, 2006; He et al., 2002; Henderson et al., 2004; Lusic et al., 2003; Mahmoudi et al., 2006; Marcello et al., 2004; Pumfery et al., 2003; Treand et al., 2006). Nucleosome remodelling can alter the accessibility of DNA to specific activators or repressors, general transcription factors and RNA polymerase. Besides being necessary for activation, SWI/SNF complexes are required for transcriptional repression of some genes (Katsani et al., 2003; Martens & Winston, 2003). It has been hypothesized that a dynamic equilibrium between chromatin remodelling activator and repressor factors at the positioned nucleosomes of the HIV-1 LTR could be involved in the switch of different transcriptional states (He et al., 2002). This implies that SWI/SNF complexes could exert differential effects on basal or Tat-dependent transcription. To probe this issue, we investigated the immediate and long-term effects of Ini1/hSNF5 on the basal transcriptional potential of the HIV-1 promoter.

**METHODS**

**Cell culture, immunofluorescence analysis, Western blotting and fluorescence-activated cell sorting (FACS) analysis.** 293T, HeLa and HeLa-derived cell lines were cultured as described previously (Boese et al., 2004). For immunofluorescence, cells were fixed in 4 % paraformaldehyde in PBS for 10 min, rinsed three times in 100 mM glycine in PBS, permeabilized in 0.1 % Triton X-100 in PBS for 2 min and blocked with 1 % BSA in PBS for 10 min. Cells were incubated with primary antibody (anti-Ini1/hSNF5; Sigma) diluted 1:500 in 1 % BSA for 2 h, followed by secondary antibody (Alexa Fluor 588-conjugated anti-rabbit Ig; Invitrogen) diluted 1:400 in 1 % BSA for 1 h. All steps were performed at room temperature and samples were washed thoroughly in PBS between all steps (except for the washes in glycine/PBS mentioned above). Samples were mounted in Vectashield hard mounting medium containing DAPI (Vector Laboratories). Western blotting was carried out following standard protocols and using specific antisera as described previously (Boese et al., 2004). For FACS, cells were harvested, washed and fixed in 2 % (w/v) paraformaldehyde in PBS. Enhanced green fluorescent protein (EGFP) expression was analysed on a FACScalibur cytometer using CellQuest software (both from Becton Dickinson).

**RNA interference.** Small interfering RNAs (siRNAs) corresponding to conserved sequences were selected to target all published transcripts of human Ini1/hSNF5. The siRNA duplexes used were Ini1-II (annealed oligonucleotides 5’-GGAGAACUCCAGCAAGAG- AAaGdTdT-3’ and 5’-CUUCUCUGUGAGAGUCGdTdT-3’ corresponding to nt 927–945) and Ini1-III (5’-GGAGAACUCCAGCAAGAG- AAaGdTdT-3’ and 5’-CUUCUCUGUGAGAGUCGdTdT-3’ corresponding to nt 702–720) (both under GenBank accession no. U04847). Pre-designed non-targeting siRNA controls were purchased from Dharmacon. As a further control, we used siRNAs against the human Tpr protein (5’-GGUGGAGAGCGCAACAGdTdT-3’ and 5’-CAGAAAGCGAAGGGGdTdT-3’) that were selected and tested in our laboratory. The conditions of the RNA interference (RNAi) protocol were as described previously (Boese et al., 2004).

**Virus preparation and infections.** Vesicular stomatitis virus G protein (VSV-G)-psuedotyped HIV-1 vector particles were produced in 293T cells as described previously using pTRIP-LTR-EGFP, pTRIP-ΔU3-EF1α-EGFP (Sirven et al., 2001), pTRIP-CMV-EGFP (Zennou et al., 2000) or pLTR-Tat-IRES-EGFP (Jordan et al., 2001) as the genomic vector by calcium phosphate transfection (Boese et al., 2004; Zennou et al., 2000). The infectious titre of the stocks was determined by limiting dilution analysis of transfected cells using the vector-
encoded EGFP protein as a marker and measuring HIV-1 Gag p24 with an antigen-capture ELISA (NEN Life Science Products). Titrated stocks of an envelope-defective HIV-1 mutant pseudotyped with the HIV-1 envelope protein (HIV-1_Lai Env/SRIII) and HIV-1_Lai were kindly provided by Olivier Schwartz (Pasteur Institute, Paris, France). Infections were performed as described previously (Boese et al., 2004; Sommer et al., 2004) and HIV-1 p24 antigen production was monitored as described above.

**Cell viability assay.** At the indicated time points after transfection, cell viability was evaluated with 100 μg resazurin (Sigma) ml⁻¹. After 2 h of incubation at 37 °C with 5 % CO₂, resorufin fluorescence was measured at 544 nm (excitation)/595 nm (emission) on a SpectraMax M5 microplate reader ( Molecular Devices). Cell viability was expressed relative to control-transfected cells.

**Chromatin immunoprecipitation (ChIP) analysis and quantitative PCR.** ChIP analyses were performed using a commercially available kit (Upstate) following the manufacturer’s instructions. The antibodies used were against: Ini1 (rabbit antiserum against Ini1), HDAC-1 (mouse antibody against HDAC1), HDAC-4 (rabbit antiserum against HDAC4), HP1α (mouse antibody against heterochromatin protein 1α), H3K9Ac (rabbit antiserum against histone H3 acetylated on lysine 9), H4Ac5 [rabbit antiserum against hyperacetylated (penta-)histone H4], H3K9Me2 (rabbit antiserum against histone H3 dimethylated on lysine 9), H3K4Me2 (rabbit antiserum against histone H3 dimethylated on lysine 4) and H4K20Me3 (rabbit antiserum against trimethyl-histone H4 lysine 20). All antibodies were purchased from Upstate except for anti-Ini1 ( Sigma).

Quantitative PCR was carried out on an ABI Prism 7000 sequence detection system using SYBR Green ( Applied Biosystems). All primers were designed using PrimerExpress 2.0 software (Applied Biosystems). The primer pair chosen for detection of co-precipitated proteins was specific for Ini1/hSNF5, as expression of several unrelated proteins was not affected. Moreover, Ini1/hSNF5 expression levels remained unchanged after transfection of siRNAs targeting unrelated proteins (Boese et al., 2004). Due to the transient effect of siRNAs, depletion lasted only for about 3 days and normal levels of Ini1/hSNF5 protein were present by day 6 after transfection (Fig. 1b).

We first monitored the effect of Ini1/hSNF5 depletion on EGFP expression in HeLa cells that had previously (2 weeks before) been infected with HIV-1 particles pseudotyped with VSV-G and containing the TRIP–LTR–EGFP vector genome, which directs the expression of EGFP under the control of the authentic HIV-1 promoter (Boese et al., 2004). Surprisingly, expression levels were increased at early time points after siRNA treatment and returned to levels comparable to the controls at day 8 (Fig. 1c and data not shown). The effect was specific for the HIV-1 promoter, as depletion of Ini1/hSNF5 in HeLa cells that had been transduced with lentiviral vectors expressing EGFP under the control of the cellular EF1α or cytomegalovirus (CMV) promoter, respectively, did not significantly affect reporter gene expression (Fig. 1c). Furthermore, cell viability was not impaired in the absence of Ini1/hSNF5 in these cells (Fig. 1d). This indicated that Ini1/hSNF5-containing SWI/SNF complexes exert a repressive effect on basal transcription from the integrated HIV-1 promoter, which is in striking contrast to the recently reported synergy in Tat-dependent transactivation of the HIV-1 promoter (Agbottah et al., 2006; Ariumi et al., 2006; Bukrinsky, 2006; Mahmoudi et al., 2006; Treand et al., 2006).

**RESULTS**

Ini1/hSNF5 is involved in repression of basal HIV-1 transcription

Recent evidence suggests that the core subunit of the ATP-dependent chromatin remodelling complex SWI/SNF, Ini1/hSNF5, a cellular interaction partner of the HIV-1 integrase, is implicated in HIV-1 transcription rather than in the integration process itself (Bukrinsky, 2006). In particular, it has become evident that Ini1/hSNF5-containing SWI/SNF complexes facilitate Tat-mediated transactivation of the HIV-1 LTR promoter (Agbottah et al., 2006; Ariumi et al., 2006; Bukrinsky, 2006; Mahmoudi et al., 2006; Treand et al., 2006). However, the HIV-1 genome associates with Ini1/hSNF5 before nuclear migration (Turelli et al., 2001), suggesting a possible role in Tat-independent basal expression. To probe this issue, we used a recently described and validated RNAi protocol that specifically targets all published variants of Ini1/hSNF5 (Boese et al., 2004). The application of siRNAs against Ini1/hSNF5 led to a reduction in endogenous protein to levels undetectable by immunofluorescence analysis or Western blotting at 48 h after siRNA transfection (Fig. 1a, b; Boese et al., 2004). As shown previously, the silencing effect was specific for Ini1/hSNF5, as expression of several unrelated proteins was not affected. Moreover, Ini1/hSNF5 expression levels remained unchanged after transfection of siRNAs targeting unrelated proteins (Boese et al., 2004). Due to the transient effect of siRNAs, depletion lasted only for about 3 days and normal levels of Ini1/hSNF5 protein were present by day 6 after transfection (Fig. 1b).

Depletion of Ini1/hSNF5 during integration enhances early, Tat-independent expression levels resulting in an early boost of HIV-1 replication

As Ini1/hSNF5 is recruited early to the pre-integration complex (Turelli et al., 2001), we also monitored the effect of Ini1/hSNF5 depletion during integration on basal expression levels of the HIV-1 promoter. This was facilitated by the fact that RNAi-mediated knockdown of Ini1/hSNF5 resulted in a substantial decrease in endogenous protein for at least 48 h, a time window sufficient for the completion of retroviral integration after infection (Fig. 1b). HeLa cells were infected with TRIP–LTR–EGFP particles 2 days after siRNA treatment and expression levels were determined by FACS analysis. EGFP expression was significantly increased compared with the controls 2 days after infection of cells depleted of Ini1/hSNF5, further supporting a repressive effect of SWI/SNF complexes on basal HIV-1 transcription (Fig. 2a). Importantly, however, elevated expression levels persisted only for about
Fig. 1. Ini1/hSNF5 represses basal expression from the HIV-1 promoter. (a) Specific RNAi-mediated depletion of Ini1/hSNF5 in Hela cells 48 h after siRNA transfection, shown by indirect immunofluorescence analysis using an Ini1/hSNF5-specific antibody (left panels). The mock-transfected control sample is shown in the upper panels. Nuclei were stained by DAPI (right panels). (b) Western blot analysis (using anti-Ini1 antiseraum; kindly provided by C. Muchardt, Pasteur Institute, Paris, France) 2 and 6 days after RNAi treatment as indicated. Staining of α-tubulin served as a loading control (lower panel). (c) EGFP expression in cell lines generated by infection with VSV-G-pseudotyped vectors expressing EGFP under the control of the retroviral LTR (LTR–EGFP), the cellular EF1α promoter (ΔU3–EF1α–EGFP) or the CMV promoter (CMV–EGFP) at 2 (shaded bars) or 4 (filled bars) days afterIni1/hSNF5 depletion. The mean fluorescence intensity as measured by FACS analyses is indicated relative to control transfections (non-targeting RNAi) of the same cell lines. Error bars represent the SD of two independent experiments performed in duplicate. (d) Cell viability assays measuring the metabolic activity via resazurin reduction to the highly fluorescent resorufin. Fluorescence intensity is indicated relative to control-transfected cells (non-targeting) of the same cell line and was measured at 2 (shaded bars) or 4 (filled bars) days after RNAi. Error bars indicate the SD of experiments performed in duplicate.

6 days and continuously declined, reaching levels comparable to the controls at day 9 after infection, and remained stable thereafter for a prolonged time in culture (Fig. 2a and data not shown). The results indicated that the early recruitment of Ini1/hSNF5 establishes a repressive environment for basal transcription at the freshly integrated HIV-1 promoter, probably as a result of the assembly of SWI/SNF chromatin remodelling complexes. To corroborate these findings, CD4+/CXCR4+/CCR5+ HeLa P4P cells depleted of Ini1/hSNF5 were infected with an envelope-defective HIV-1 mutant pseudotyped with the HIV-1 envelope protein (HIV-1LAI Δenv/SRIII) or with the wild-type virus (HIV-1LAI). Under these conditions, HIV-1 gene expression will initially depend on basal, Tat-dependent transcription resulting in increased virus particle production and faster replication kinetics (Fig. 2b, c). We next monitored the effect of Ini1/hSNF5 depletion on HIV-1 gene expression in a system in which Tat-dependent transactivation was already established. To this end, we employed HeLa cells that had been infected with HIV-1 particles pseudotyped with VSV-G and containing a TRIP–LTR–Tat–IRES–EGFP vector genome. As shown in Fig. 2(d), the relative levels of reporter gene expression were also increased after depletion of Ini1/hSNF5 in these cells, albeit less pronounced than with HeLa LTR–EGFP cells, indicating that the positive effect of Ini1/hSNF5 depletion on basal transcription is attenuated by the negative effect of Ini1/hSNF5 depletion on Tat-dependent transcription. Taken together, these observations are in agreement with recent evidence implicating Ini1/hSNF5 in the transcriptional regulation of HIV-1 (Agbottah et al., 2006; Ariumi et al., 2006; Bukrinsky, 2006; Mahmoudi et al., 2006; Treand et al., 2006), but intriguingly reveal opposing effects on basal and Tat-dependent gene expression. Hence, SWI/SNF complexes may enable HIV-1 to adapt its replication strategy to the cellular environment by initiating transcriptional latency or facilitating productive infection.
Depletion of Ini1/hSNF5 during integration affects the responsiveness of the integrated LTR to histone deacetylases in the long term

SWI/SNF chromatin remodelling complexes are involved in the formation of stable epigenetic marks on promoters and have been implicated in retroviral gene silencing (Felsenfeld & Groudine, 2003; Iba et al., 2003; Khorasanizadeh, 2004; Martens & Winston, 2003). To explore further the effects of Ini1/hSNF5 on the fate of integrated HIV-1, we compared the transcriptional potential of polyclonal stable cell lines harbouring proviruses established in the presence or absence of Ini1/hSNF5, termed HMLG (infected in the presence of Ini1/hSNF5) and HILG (infected in the absence of Ini1/hSNF5), respectively. In accordance with a general repressive effect of Ini1/hSNF5 on basal HIV-1 gene expression, elevated levels of EGFP expression could be observed after a second depletion of Ini1/hSNF5 in HILG and HMLG cells (data not shown). Importantly, this effect was similar in both cell lines, ruling out the possibility that general effects of Ini1/hSNF5 depletion on the chromatin architecture resulting in an increased accessibility of normally disfavoured chromatin domains for HIV-1 integration are responsible for the enhanced expression in the absence of Ini1/hSNF5.
To determine the responsiveness of the integrated LTR to virus and cellular signals known to stimulate HIV-1 transcription via distinct pathways, we ectopically expressed the virus transactivator Tat and used phorbol esters [tetradecanoyl phorbol acetate (TPA)] and a histone deacetylase (HDAC) inhibitor [trichostatin A (TSA)]. Tat binds to the transactivation response element at the 5′ end of all virus transcripts and serves as a scaffold for the assembly of a switch to efficient virus gene expression by recruitment of positive transcription elongation factors such as the P-TEFb complex, resulting in a tremendous increase in proviral transcription rate (Brigati et al., 2003). Phorbol esters mimic T-cell mitogen activation and activate NF-κB, a transcription factor involved in HIV-1 transcription initiation (Lassen et al., 2004). HDAC inhibitors activate HIV-1 transcription in latently infected cell lines or in transiently transfected HIV-1 LTR reporter constructs, presumably by causing histone hyperacetylation (Van Lint et al., 1996; Verdin et al., 1993). Ectopic expression of Tat and treatment with TPA resulted in virtually identical increased expression levels in HMLG as well as in HILG cells (Fig. 3a, b), suggesting that the potential to respond to signals affecting transcriptional initiation and elongation was comparable for proviruses established in the presence or absence of Ini1/hSNF5. In contrast, significant differences were observed after treatment with TSA, with higher and more sustained expression levels in HILG cells (Fig. 3c), indicating substantial differences in the chromatin environment of the proviruses present in the two cell lines. To support this notion, we sought to explore the effect of HDAC-1 and HDAC-4 on HIV-1 expression in the two cell lines. Whilst overexpression of both HDACs had only moderate repressive effects on HIV-1 transcription in our hands, there were nevertheless notable differences. In particular, HDAC-4 clearly repressed HIV-1 expression to higher levels in HILG cells than in HMLG cells (Fig. 3d).

![Graphs](https://example.com/graphs.png)

**Fig. 3.** Transcriptional response of the integrated HIV-1 LTR in HMLG and HILG cells to various stimuli. EGFP mean fluorescence intensity was measured in HMLG (shaded bars) and HILG (filled bars) cells by FACS analysis. Error bars represent the SD from at least two independent experiments. (a–c) FACS analysis 24 h (day 1) and 48 h (day 2) after treatment with TPA (10 ng ml⁻¹) (a), after ectopic expression of HIV Tat (b) and after treatment with 60 nM TSA (c). (d) FACS analysis of HMLG and HILG cells 24 h after transfection of the pSG5-HDAC-1 and pSG5-HDAC-4 expression plasmids.
Depletion of Ini1/hSNF5 during integration is associated with increased levels of histone methylation at the virus promoter

Taken together, these results indicated differences in the chromatin environment of proviruses present in HMLG and HILG cells and prompted us to investigate the presence of known regulators of chromatin structure and various histone modifications of nucleosomes positioned in the virus LTR by ChIP analysis. In agreement with a general avoidance of heterochromatin for HIV-1 integration, HP1z, which is primarily associated with centromeric heterochromatin (Maison & Almouzni, 2004), was minimally enriched at the virus LTR in both cell lines. Similarly, Ini1/hSNF5, HDAC1 and HDAC4 were not enriched at the HIV-1 LTR, indicating that these proteins are not stably associated with the virus promoter (Fig. 4a–d). Acetylation of histone tails (in particular histones H3 and H4) is frequently considered as a mark of active promoters. In agreement with the comparable EGFP transcription levels in HMLG and HILG cells, levels of H3K9 acetylation and penta-acetylated H4 were not significantly different in the two cell lines (Fig. 4e, f). In contrast, striking differences in histone methylation associated with a repressive chromatin structure (Khorasanizadeh, 2004; Peterson & Laniel, 2004) of nucleosomes positioned in the LTR were observed in HILG cells compared with HMLG cells (Fig. 4g–i). In particular, dimethylation at H3K9 and, to a lesser extent, dimethylation at H3K4 was enriched in HILG cells, whilst trimethylation at H4K20 was again comparable in both cell lines (Fig. 4g–i). This pattern of histone tail modifications correlated well with the declining expression levels observed early after infection with Ini1/hSNF5 depletion.

DISCUSSION

Our results not only demonstrate that the SWI/SNF component Ini1/hSNF5 is involved in the repression of basal HIV-1 transcription but also importantly provide evidence that the SWI/SNF-dependent repression of basal HIV-1 expression affects the epigenetic regulation of the integrated virus promoter. We have provided clear evidence that the short-term, repressive effect of Ini1/hSNF5 is specific for the HIV-1 promoter, as expression from the cellular EF1a or the CMV promoter was not affected by Ini1/hSNF5 depletion (Fig. 1c). Whilst this was not directly related to the long-term effects of Ini1/hSNF5 depletion during integration shown in Fig. 4, we feel that it is unlikely that Ini1/hSNF5 depletion will have long-term effects on these promoters, as they are not affected by Ini1/hSNF5 per se. Nevertheless, it remains possible that virus sequences, including native retroviral LTR sequences, which were retained in all of the virus constructs, may provide a common determinant.

Cellular proteins that have been identified through an association with the HIV-1 integrase have been commonly considered to assist in the integration process or to guide the HIV-1 pre-integration complex to favoured sites for integration (Bushman, 2003; Bushman et al., 2005; Van Maele et al., 2006). However, many of these factors (e.g. HMGa1, BAF, LEDGF/p75, EED and Ini1/hSNF5) have normal cellular functions in chromatin remodelling and organization and some may in fact be recruited to the site of integration to ensure a beneficial chromatin environment for virus gene expression shortly after integration. In support of this assumption, it has recently been demonstrated that the cellular protein Daxx interacts with the avian sarcoma virus integrase early after infection and may mediate the repression of virus gene expression via the recruitment of HDACs (Greger et al., 2005), which appears to parallel the results described here. More recently, several host factors interacting with the Moloney murine leukemia virus integrase were identified in a yeast two-hybrid screen and a number of the proteins identified also interacted with the HIV-1 integrase both in yeast and in vitro (Studamire & Goff, 2008). One category of proteins identified in this study includes DNA-binding and chromatin modification factors such as transcriptional repressor polycomb proteins, suggesting the intriguing possibility of an early, integrase-mediated recruitment of chromatin-binding proteins and transcriptional regulators to the site of integration to generate an advantageous chromatin environment for virus gene expression immediately after integration (Studamire & Goff, 2008).

We have clearly shown that depletion of the SWI/SNF component Ini1/hSNF5 releases a repressive effect on basal expression of the HIV-1 LTR. Recent observations indicate that Ini1/hSNF5-deficient cells are equally susceptible to lentiviral vector-mediated transduction compared with Ini1/hSNF5-expressing cells (Ariumi et al., 2006; Boese et al., 2004). We have also clearly demonstrated by quantitative PCR that the number of successful integration events per cell is not significantly different in cells that were infected during Ini1/hSNF5 or mock knockdown (HMLG and HILG) after prolonged culture (Boese et al., 2004), indicating that the effect we have described here is independent of integration itself. This is also supported by the demonstration of the specific effect of Ini1/hSNF5 knockdown on basal LTR-driven gene expression in cells, in which integration occurred under identical conditions (namely in the presence of Ini1/hSNF5; Fig. 1). This not only concerns the number of integration sites but may also indicate that integration site targeting is not significantly affected, although we did not perform an in-depth study to test whether integration site selection was modified in Ini1/hSNF5-knockdown cells. Whilst this is certainly an interesting question, it may not easy to provide definite answers and will require further studies.

Taking into account the demonstration that Ini1/hSNF5 is recruited from the nucleus to incoming pre-integration complexes of HIV-1 before nuclear migration and integration (Turelli et al., 2001), it appears likely that Ini1/hSNF5 knockdown has a direct effect on the LTR,
although we cannot formally rule out the possibility that depletion of Ini1/hSNF5 results in the loss of expression of a repressor protein that regulates the HIV-1 LTR. Nevertheless, Ini1/hSNF5-containing SWI/SNF complexes have been shown to be directly involved in the regulation of HIV-1 transcription by the demonstration that they act as positive co-factors in Tat-dependent transactivation of the HIV-1 LTR (Agbottah et al., 2006; Ariumi et al., 2006; Bukrinsky, 2006; Mahmoudi et al., 2006; Treand et al., 2006). We observed increased particle production after infection of Ini1/hSNF5-depleted cells (Fig. 2), probably due to increased basal LTR activity driving the expression of Tat. These results are in agreement with data published by Maroun et al. (2006), which also showed increased particle production in Jurkat cells transiently depleted for Ini1/hSNF5 at early time points (1–3 days post-infection).

On the other hand, Ariumi et al. (2006) reported reduced HIV-1 replication at day 6 after infection in HeLa P4.2 cells constitutively expressing a small hairpin RNA to stably knockdown Ini1/hSNF5. Unfortunately, these authors did not assess the effect of the stable Ini1/hSNF5 knockdown at earlier time points in their system, but overall our data together with published data suggest that (transient) Ini1/hSNF5 depletion may boost virus particle production at early time points due to increased basal LTR activity driving the expression of Tat, whereas, at later time points after infection of a culture, the negative effect that (constitutive) Ini1/hSNF5 depletion exerts on Tat-dependent transcription becomes more pronounced.

Proteins of the SWI/SNF family cooperate with other enzymes to activate (e.g. histone acetyl transferases) or
repres (e.g. histone deacetylases) transcription, and HIV-1 may exploit this ability to adjust its own transcriptional programme to the cellular environment. It has recently been demonstrated that latent virus genomes reside within introns of active host genes, suggesting that latency is not simply due to an inaccessibility of the integrated proviruses to the transcriptional machinery (Han et al., 2004). The finding that HIV-1 integration in the absence of Ini1/hSNF5 results in an altered epigenetic regulation of virus gene expression thus provides a molecular mechanism for the establishment of post-integration latency within actively transcribed genes. Ini1/hSNF5-containing SWI/SNF complexes may serve as a platform to recruit factors such as mSIN3A/HDAC complexes (Pal et al., 2003; Sif et al., 2001) to establish a repressive chromatin environment at the LTR promoter in cells (e.g. resting CD4 T cells) expressing insufficient nuclear levels of the crucial activation-dependent host transcription factors (e.g. NFkB and P-TEFb) required for expression of sufficient levels of HIV-1 Tat (Lassen et al., 2004). On the other hand, in activated cells expressing high levels of transcription factors required to overcome the transcription initiation block that is an automatic consequence of the nature of the HIV-1 promoter, Ini1/hSNF5 could represent the scaffold for the assembly of activating SWI/SNF complexes that synergize with the p300 acetyltransferase to activate the HIV promoter in a Tat-dependent manner (Agbottah et al., 2006; Ariumi et al., 2006; Bukrinsky, 2006; Mahmoudi et al., 2006; Treand et al., 2006). Whilst follow-up studies will be required to determine whether the molecular mechanism(s) involved in the effects of Ini1/hSNF5 on already integrated proviruses differ from those that can be seen when Ini1/hSNF5 is depleted during integration, Ini1/hSNF5 appears to be recruited early to the integrated proviruses to initiate one of two mutually exclusive transcription programmes, namely post-integration latency or high-level, Tat-dependent gene expression. This is in line with emerging evidence that certain cellular factors are recruited via retroviral integrase proteins, not to assist in the integration process itself but rather to modulate the virus transcription programme immediately after integration (Greger et al., 2005; Studamire & Goff, 2008). The prompt sensing of the cellular activation state and adjustment of the virus transcription programme will impact on the ability of HIV-1 to evade host immune responses either by hiding in a transcriptionally silent state within resting CD4 T cells or by speeding up the generation of progeny, thereby decreasing the time window in which infected cells are visible to the host immune system.

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