Two-long terminal repeat (LTR) DNA circles are a substrate for HIV-1 integrase.

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ABSTRACT

Integration of the HIV-1 DNA into the host genome is essential for viral replication and is catalyzed by the retroviral integrase. To date, the only substrate described to be involved in this critical reaction is the linear viral DNA produced in reverse transcription. However, during HIV-1 infection, two-long terminal repeat DNA circles (2-LTRcs) are also generated through the ligation of the viral DNA ends by the host cell’s non-homologous DNA end-joining pathway. These DNAs contain all the genetic information required for viral replication, but their role in HIV-1’s life-cycle remains unknown. We previously showed that both linear and circular DNA fragments containing the 2-LTR palindrome junction can be efficiently cleaved in vitro by recombinant integrases, leading to the formation of linear 3'-processed-like DNA. In this report, using in vitro experiments with purified proteins and DNAs along with DNA endonuclease and in vivo integration assays, we show that this circularized genome can also be efficiently used as a substrate in HIV-1 integrase-mediated integration both in vitro and in eukaryotic cells. Notably, we demonstrate that the palindrome cleavage occurs via a two-step mechanism leading to blunt-ended DNA product, followed by a classical 3'-processing reaction; this cleavage leads to integrase-dependent integration, highlighted by a 5-bp duplication of the host genome. Our results suggest that 2-LTRc may constitute a reserve supply of HIV-1 genomes for proviral integration.

Introduction

Establishment of a stable infection by HIV-1 requires the insertion of the retroviral DNA into the host genome. This step is catalyzed by the viral integrase (IN) using the linear viral DNA issued from the reverse transcription. HIV-1 IN catalyzes the 3'-processing of the viral U3 and U5 ends and then the integration of the newly 3'-processed ends into the target DNA during a strand-transfer reaction (1-3). In addition to integrated DNA,
several forms of unintegrated DNA can be detected in infected cells. Unintegrated viral genomes included linear form generated from the reverse transcription process, circular forms resulting from autointegration and circular genomes harboring one or two long terminal repeats (LTRs) (1-LTR circles: 1-LTRc and 2-LTR circles: 2-LTRc; respectively) (4). If no integration occurs, linear viral DNA is rapidly degraded or circularized into 1- or 2-LTRc (4-7). 2-LTRc are generated by ligation of the cDNA ends by the host cell non-homologous end-joining (NHEJ) pathway (8). Additionally recombination between the 2 LTRs of linear DNA can lead to 1-LTR DNA circles (1-LTRc). These circular genomes are detected in the nucleus of infected cells, even if a portion of 1-LTRc originates from reverse transcription (4). Previous studies have shown that, by inhibiting the integration process with strand-transfer inhibitors such as Raltegravir (RAL), the amount of 2-LTRc substantially increases both in cell cultures and in patients (9,10). To date, the role of the 2-LTRc remains misunderstood. They are usually described as by-products of the reverse transcription, without any significant role in the HIV-1 replication. However, they could be involved, with 1-LTRc, in a weak transcription of HIV-1 accessory genes such as Nef (11) proving their potential role during infection. Recently, it has been reported that viral production could be detected from unintegrated DNA forms following infection of resting CD4 T cells, supporting their role in the overall replication process (12,13).

Interestingly, the HIV-1 2-LTR junction, formed by the ligation of the LTR extremities in infected cells is palindromic. Furthermore, several studies have shown that both oligonucleotides and circular DNA, containing the palindromic junctions, can be cleaved in vitro by recombinant HIV-1 and PFV-1 INs, leading to the formation of linear 3'-processed-like DNA (14-16). Consistently, our recent study highlighted that unintegrated viral DNA, more particularly 2-LTRc, could be used as a reserve supply of genomes for proviral integration (15). These results suggest that the 2-LTRc could serve as functional intermediates in the retrovirus replication cycle, which implies that IN can cleave the palindromic junction of the 2-LTRc, leading to linear DNA that can be subsequently integrated into the target DNA. This hypothesis was addressed in the present work both at the biochemical level and in a eukaryote cellular context. We particularly addressed the question of whether the viral DNA ends formed upon 2-LTR junction cleavage could be compatible with strand-transfer and full-integration reactions and, whether the integration reaction occurs via an IN-dependent mechanism, i.e. leading to the 5-bp duplication of the host genome.

Biochemical analyses led us to show that HIV-1 IN catalyzes the formation of integrated products from the LTR-LTR junction oligonucleotides using in vitro concerted integration assay. The physiological relevance of this process was further addressed in the previously established yeast integration eukaryotic model (17) as well as in the virological context, showing that IN also catalyzes the integration of a DNA substrate containing the 2-LTRc junction into genomic DNA. Our data highly suggest that 2-LTRc can serve as substrates for HIV-1 IN and, thus, may serve as a new source for the retroviral genome integration. This leads us to reconsider the different ways for the HIV-1 integration and, also, to re-analyze the impact of the 2-LTRc accumulation particularly during anti-IN treatments.

Results and discussion

**In vitro** cleavage of the 2-LTR palindromic junction by IN is improved by LEDGF/p75 and inhibited by RAL

An oligonucleotide (ODN) mimicking the palindromic sequence found at the LTR-LTR junction was previously shown to be efficiently cleaved by HIV-1 IN in vitro, leading to the formation of viral ends required for integration (13). Moreover, the cellular IN cofactor LEDGF/p75 has been involved in the enhancement of IN activity (18). Therefore, we have first analysed whether LEDGF/p75 could also promote this cleavage. Using the 38bp DNA mimicking the 2-LTR junction (referred as U5•U3, Figure 1A, left) in the palindrome
cleavage assay, we showed that LEDGF/p75 increased cleavage efficiency mediated by IN by a 3-fold factor (Figure 1B). The resulting product of IN-mediated cleavage is 5'-TGTGGAAATCTCTTAGCA indicated 5'-AGCA on the left of the gel.

Then, in order to better mimic a two-LTR circle, the 2-LTR junction fragment was inserted in a plasmid containing a constitutive yeast TEF1 promoter-dependent zeocine resistance gene (named U5•U3 circle, Figure 1A, right) and used as substrate for HIV-1 IN cleavage (Figure 1C, upper panel). Incubation of the U5•U3 circle substrate with IN led to a protein concentration dependent linearization of this DNA with an optimal activity reached at 1 µM IN (Figure 1C, upper panel). In this condition, 50% of the plasmid was linearized (indicated “L” in Figure 1C, corresponding to the signal obtained by linearization of U5•U3 circle with SalI (lane 4)). The specificity of the cleavage was assessed using the catalytically inactivated D116A IN as well as the plasmid deleted of the palindromic junction (replaced by a random sequence: Random circle, Figure 1C, right panel). In these conditions, less than 5% of the plasmid DNA was linearized (Figure 1C, right panel) highlighting a very poor contaminating non-specific endonuclease activity in IN preparations. Taken together, these data indicate that the linearization of the DNA substrate by the wt IN was due to specific IN catalytical properties and was dependent of the presence of the palindromic junction. Moreover, incubation of U5•U3 circle substrate with IN in the presence of LEDGF showed that this IN cofactor can increase the palindrome cleavage efficiency not only when located on a short DNA sequence as mentioned above, but also of a circular substrate (Figure 1D, left). The cleavage quality was then checked by sequencing the viral ends starting from the LTR-LTR junction, compatible with the strand-transfer reaction, but it also indicates that interaction of IN with LEDGF can generate viral DNA ends compatible with the 3'-processing reaction.

To better assess the specificity of the cleavage, we next analysed the inhibition of the 2-LTR junction cleavage by IN using RAL treatment. Results showed in Figure 2A revealed that the cleavage of the DNA (38 bp) containing the palindromic sequence, in the presence or absence of LEDGF/p75, is inhibited by RAL in a dose-dependent manner. Similarly, RAL efficiently inhibited the cleavage of the U5•U3 circle in a dose-dependent manner independently of the presence of LEDGF (Figure 2B). Remarkably, the IC₅₀ found for the inhibition of the palindrome cleavage (500 to 900 nM) is closer to the IC₅₀ found for the inhibition of the 3’-processing reaction (micromolar range; (19)) than the IC₅₀ characterizing the strand-transfer reaction (around 5 nM) (19) suggesting that the endonucleolytic cleavage of the palindromic junction requires a DNA/IN complex more comparable to the one involved in the 3’-processing than to the one involved in the strand-transfer reaction.

**In vitro integration of the U5•U3 junction directs reaction toward full site integration**

To determine whether this cleaved two-LTR DNA can further involved as integration substrate, concerted integration assays were performed with the U5•U3 substrate. Integration efficiency was then compared with the one obtained with the DNA (38 bp) mimicking the viral DNA end found in infected cells (U5) (Figure 3A). Integration products were detected after incubation of the U5•U3 DNA, pBSK acceptor vector and IN under standard conditions previously established (20). The formation of all the expected half site (HSI) and full site integration (FSI) products were catalyzed by IN (Figure 3A). Comparison of the integration profiles obtained with U5•U3 or U5 substrates indicates substantial variation in terms of enzyme concentration optimum. Indeed, the maximum integration efficiency using the U5•U3 or U5 DNA was obtained with 1 µM and
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500 nM IN, respectively (Figure 3A, right). This difference is in accordance with the one previously described in cleavage assays (13) and suggests that different IN oligomers or structures are involved in the integration depending on the U5•U3 or U5 substrates. Furthermore, no integration product was obtained using a random DNA (Random), indicating that the integration reaction strictly requires the presence of the viral sequences (Figure 3A, right).

We further analyzed the quality of the integration products obtained, by comparing the integration profiles observed with U5•U3 or U5 DNA using optimal IN concentration for both substrates, i.e. 1 µM and 500 nM, respectively. As reported in Figure 3B, the proportion of FSI versus HSI products obtained with either the U5•U3 or the U5 DNA was different. Indeed, using the one viral U5 ended DNA led to a major proportion of HSI integration (80% of integration products), whereas only a small proportion of FSI integration was observed (20% of integration products). In contrast, when the U5•U3 was used as substrate for concerted integration, the proportion of FSI was clearly improved (50% of integration products), indicating that the pre-cleavage of the 2-LTR junction by IN could drive the reaction toward a FSI reaction with higher efficiency than when using the U5 end DNA (Figure 3B, right panel). The reaction specificity was checked using RAL that specifically inhibits integration reaction (21). As reported in Figure 3C, RAL induced a strong inhibition of the integration in a dose-dependent manner from both U5•U3 and U5 DNA indicating that the reaction was catalyzed by IN with a strand transfer intermediary mechanism similar to the typical integration of the linear viral sequence ended substrate. Taken together, our data demonstrate that a DNA mimicking the palindrome found at the HIV-1 2-LTR junction can serve as integration substrate in vitro.

Previous analyses of the palindrome cleavage by HIV-1 IN led us to demonstrate that the reaction was optimally performed by tetrameric enzyme (13). The IN tetramerization on the palindromic junction could improve the further concerted integration step since this oligomeric state has been shown to be involved in this specific activity (22-25). Since the cellular IN cofactor LEDGF/p75 has been previously shown to stabilize IN tetramers (18), we performed concerted integration assays using the U5•U3 DNA substrate in order to study the influence of LEDGF/p75 on integration efficiency. As reported in Figure 3D, LEDGF/p75 stimulates in a dose-dependent manner the integration reaction using the U5•U3 substrate, and more particularly the HSI products as already reported using the U5 substrate (26).

However, this system did not allow the analysis of the structure of the integration products, i.e. viral and genomic DNA sequences found at the integration sites. To better ascertain this point and to address the question of whether integration can take place in an eukaryote cell starting from a 2-LTR junction-containing substrate, we first used the previously characterized yeast integration model (17).

A plasmid encompassing the 2-LTR junction can be integrated in the genome of Saccharomyces cerevisiae expressing HIV-1 IN

The U5•U3 circle was used as substrate for HIV-1 IN-dependent integration in yeast (Figure 1A). After transformation of the JSC310 zeocine sensitive yeast strains expressing HIV-1 IN with this circular putative integration substrate, zeocine resistant clones were selected from 2 to 5 hours post-transformation (Figure 4A). Importantly, the processed product of the Figure 1C, i.e. the product resulting from cleavage of the plasmid with the palindromic sequence by IN (named U5•U3 processed circle, Figure 1A), was purified and used as a substrate. As shown in Figure 4A, both U5•U3 circle and U5•U3 processed circle substrates were able to be integrated into yeast genomic DNA after transformation of IN expressing cells. Moreover, U5•U3 processed circle substrates were able to be integrated into yeast genomic DNA after transformation of IN expressing cells. Moreover, U5•U3 processed circle showed a higher integration efficiency suggesting that the limiting step of the process was the DNA cleavage. Comparison of integration efficiency was also performed with the linear U5•U3 substrate, the Random circle substrate, and by using the D116A IN expressed in yeast cells (Figure 4B). Very few clones were selected in the cases of the Random circle and the D116A IN.
IN catalytic mutant, highly suggesting that the integration was mediated by the catalytic activity of IN (Figure 4B). Even if a higher number of zeoR clones was obtained using the classical linear substrate highlighting a better integration efficiency with this DNA, the number of zeoR clones obtained with the palindrome-containing plasmid was significantly higher than in control experiments. In order to confirm that the zeoR clones selection was actually due to HIV-1 IN-dependent integration events, the selected clones were further analysed by sequencing.

**Sequences analyses of the pseudo-viral integrated DNA ends and the cellular integration loci**

The integrated products were then further analyzed by sequencing the viral extremities fused to the cellular DNA (Figure 4C-D), as reported in materials and methods section. Both viral DNA ends and flanking cellular DNA sequences of pseudo-provirus derived from U5•U3 circles and U5•U3 processed circles substrates were compared with the integrants selected using the linear U5•U3 substrate. As reported in Figure 4C, similar cellular sequences were found using the linear U5•U3 substrate and the U5•U3 processed circles since, in both cases, about 50% of the integrants displayed the typical 5-bp duplication at both plasmid-genomic DNAs junctions. Interestingly, when the U5•U3 circle was used in the yeast integration experiment, we observed a clear improvement of the reaction fidelity since in that case the percentage of accurate 5-bp duplication was increased from 50 to 80-90% (Figure 4C). Then, the analysis of the viral DNA ends sequences indicated that when the linear U5•U3 substrate was used, a large percentage of integrants displayed the typical AGCA viral extremity, as expected from the correct 3’-processing step (Figure 4D). Interestingly, all integrated events displaying this sequence (AGCA) are obtained from events highlighting the 5-bp duplication suggesting a better quality of integration using the palindromic substrate. Some defective ends were also selected, but when the U5•U3 circle substrates (processed or not) were assayed, we observed a higher number of defective viral ends structures in addition to the expected correct structure, indicating a defect in terms of fidelity. Interestingly, the profile observed for the viral DNA ends sequences of integrants from the linear U5•U3 substrate was similar to the one found after *in vitro* cleavage of the U5•U3 DNA substrate by IN alone (Figure 1B, bottom panel). Moreover, as in the case of *in vitro* cleavage of U5•U3 DNA substrate by IN/LEDGF, the number of blunt viral DNA ends was increased with the circular U5•U3 substrates (processed or not) (Figure 4D).

The LTR-LTR junction cleavage leads to correct integration events involving 5-bp duplication of the host cell genome in infected cells.

To better ascertain whether integration can take place from the 2-LTR junction in the context of viral infection, we introduced in the genomic sequence of a lentiviral vector (pHR'•CMV-GFP) either a 175-bp palindromic sequence mimicking the canonical LTR-LTR junction (PAL+, 5’-CAGTACTG-3’) or a 175-bp sequence for which the central palindrome has been modified (PAL-, 5’-CACATGTG-3’) between the CMV promoter and the cDNA encoding the eGFP. Among the different integration events (leading to neomycin resistance except for form 3 which is coupled to the formation of form 2), any event that originates from PAL cleavage would lead to GFP negative cells as the CMV promoter will be separated from the eGFP cDNA (Figure 5A). Note that IN-independent insertion events, could also lead to GFP negative cells (Figure 5A, form 6). However, our experiments using IN defective (D116N) vectors did not allow the selection of neomycin resistant cells (data not shown) highly suggesting that IN independent integration events were negligible in these conditions.

The two constructions (PAL+ and PAL-) have been used for MT4 cells infection and neomycin was added at d4 post-infection at 1 mg/ml. For the PAL- vectors, 90-95% of the neomycin resistant cells expressed the GFP due to a standard integration process (Figure 5A, form 1). For the PAL+ vectors, the part of the neomycin resistant cells also positive for GFP expression significantly decreased (70-77%) (Figure 5B).
The 20% of the neomycin resistant cells that did not express GFP could reasonably result from the cleavage of the PAL sequence and a subsequent integration into the host genome. 30 individual neomycin resistant clones and negative for GFP expression have been isolated. To ensure that the neomycin resistance is actually due to integration using PAL and/or LTR sequences, we first checked that these GFP negative clones did not harbour the PAL sequence. As expected, none of the 30 clones analysed harboured a complete PAL sequence. We then designed a set of PCR experiments to characterize these clones (a to d in Figure 5A). The first PCR (a) allowed (i) to detect the presence of Neo cDNA and the 5’ moiety of the PAL sequence and (ii) to detect all integration forms originating from the use of PAL and/or LTR sequences. Among 30 clones, 25 were negative for the PCR “a” and were not further analysed. The 5 other clones were tested in PCR b (detecting forms 3, 4 and 5), c (detecting forms 2, 4 and 5) and d (detecting forms 4 and 5) (Figure 5A). As expected, all the 5 clones were positive in PCR c, 3 clones were positive in PCR b, and 1 clone was positive in PCR d. The sequences of the insertion sites showed clearly a 5-bp duplication for the 5 clones (Figure 5C) that is typical of HIV-1 IN-dependent integration. We can note that one clone seems to be due to the integration of 1-LTR circle using PAL sequence as substrate for cleavage and strand transfer (clone 9 corresponding to the form 4). For the other 4 clones, the integration is due to the usage of both PAL and LTR sequences.

Altogether, our results demonstrate that IN can cleave specifically the internal palindromic sequence (at the PAL level) in the viral context, leading to proper integration events. In this setting, efficiency of integration mediated by internal cleavage of the PAL junction is estimated at 17% (5 clones displaying integration from PAL among 30 clones analyzed) but this percentage is probably underestimated since recombination from the PAL sequence with the LTR could occur.

Our results suggest that integration from a 2-LTR substrate could process through a three-step mechanism, a proper two-steps mechanism for the LTR-LTR junction cleavage including the blunt cleavage of the palindromic LTR-LTR junction and the subsequent 3’-processing, and finally strand-transfer. Brown and colleagues reported that, in the case of MLV, viral intermediates involved in the integration process displayed a 2-mer overhang instead of 4-mer overhang. Therefore, they excluded the roles of the palindromic junction and 2-LTR substrates since they assume that the cleavage of this junction should lead to a 4-mer overhang (a reasonable hypothesis based on a one-step cleavage mechanism) (27). Here, we addressed the possibility that this could be due to a weak sensitivity in the detection since 2-LTR circles fraction is very weak in a WT infection. We then also characterized these viral intermediates by an independent approach based on LM-PCR under optimal conditions regarding the number of 2-LTR circles (i.e. presence of a well-known anti-integrase drug: Raltegravir which leads to a strong 2-LTR circles accumulation). In order to characterize the viral ends originating from the 2-LTR circles, MT4 cells were infected in the presence of 500 nM Raltegravir. 3 days post-infection, only 2-LTR and 1-LTR circles were detected by quantitative PCR (Figure 5D). Importantly, in these conditions, no linear DNA can be detected. When Raltegravir is removed from the cell medium, de novo linear DNA can be detected at 84 and 96 hours post-infection concomitant to a decrease in 2-LTR circles. These results clearly indicate that de novo linear DNA originates from 2-LTR circles confirming our previous report (15). In the present study, the newly formed linear DNA was sequenced (at 84 and 96 hours post-infection) by a LM-PCR approach (4) (Figure 5D). We found that all linear viral DNA harbor a 2-mer overhang with the canonical 5’-CAGT end and not a 4-mer overhang according to the results found by Brown and colleagues. Our results regarding the 2-mer overhang are in accordance with those described in Figure 1D showing that the cleavage of the palindrome DNA substrate by IN is compatible with the formation of blunt-ended DNA product. All together, our results reconcile the apparent discrepancy concerning the role of the palindromic junction in the integration process, suggesting that the cleavage of the
palindromic sequence occurs by a two-steps mechanism: the formation of the blunt product followed by a standard 3’-processing reaction leading to the canonical 2-mer overhang.

Taken together our data show that circular DNA containing a 2-LTR junction can be integrated into chromosomes in both a yeast and infected cells context. Biochemical experiments indicated that the 2-LTR junction can be cleaved and subsequently integrated in a concerted manner by HIV-1 IN in vitro. It is important to note that 2-LTR circles only represent a small fraction of viral genomes in infected cells with a WT virus (28). Therefore, during a WT infection, when linear DNA is abundant, such a linear DNA directly issued from reverse transcription corresponds to the main substrate for integration. Our results highly suggest that a circular form of DNA containing the palindromic LTR-LTR junction can serve as an alternative substrate for integration and that this role is enhanced in conditions where 2-LTR circles have been pre-accumulated representing nearly half of the viral genome, for example using strand-transfer inhibitors such as Raltegravir (15). In conditions where the IN inhibitor is destabilized from the pre-integration complex, the palindromic junction can be cleaved leading to de novo linear viral DNA that can be further integrated in the host cell genome.

Taking into account that (i) in average 50% of the LTR-LTR junction display canonical palindromic features studied in this report (from 20% to 76% depending of the study (29,30)), (ii) the efficiency of LTR-LTR cleavage is about 50% and (iii) 50% of the resulting linear DNA is integrated, we estimate that approximately 10% of the 2-LTR circles can be used as a substrate for viral DNA integration. This leads to a new interpretation for the putative function of the 2-LTR circles found in HIV-1 infected cells. Indeed, our data highly suggest that these structures can be integrated in further integration steps and, thus, could serve as stock for multiple integration events.

Experimental procedures

Proteins- HIV-1 IN was purified using previously described protocols with some modification (20). HIV-1 IN was expressed in E.coli (Rosetta) and the cells were lysed in buffer containing 50 mM Hepes pH 7.5, 5mMEDTA, 1 mM DTT, 1 mM PMSF. The lysate is centrifuged and IN extracted from the pellet in buffer containing 1 M NaCl, 50 mM Hepes pH 7.5, 1mMEDTA, 1 mM DTT, 7 mM CHAPS. The protein is then purified on butyl column equilibrated with 50 mM Hepes pH7.5, 200 mM NaCl, 1 M ammonium sulfate, 100mMEDTA, 1mM DTT, 7 mMCHAPS, 10% glycerol. The protein is further purified on heparin column equilibrated with 50mM Hepes pH7.5, 200 mM NaCl, 100 mM EDTA, 1 mM DTT, 7 mM CHAPS, glycerol (10%)

LEDGF was expressed in PC2 bacteria and the cells were lysed in lysis buffer containing 20mM Tris pH 7.5, 1 M NaCl, 1 mM PMSF added lysozyme and protease inhibitors. The protein was purified by nickel-affinity chromatography and the His-tag was removed with 3C protease, 4°C over night. After dilution down to 150mM NaCl, the protein is further purified on SP column equilibrated with 25mM Tris pH7.5, 150 mM NaCl (gradient from 150 mM to 1M NaCl), then added DTT 2mM final and concentrate for Gel filtration. Gel filtration is performed on a superdex 200 column (GE Healthcare) equilibrated with 25mM Tris pH7.5, 500 mM NaCl. 2mM DTT are added to the eluted protein. The protein is then concentrated to about 10mg/ml. The IN-LEDGF/p75 complex is reconstituted to a IN/LEDGF ratio of 2. A SDSPAGE gel stained with Coomassie is provided (Figure 1B) and show a purity >95% for each protein.

In-vitro concerted integration-Standard concerted integration reactions were performed mainly as described previously (20) using purified HIV-1 IN (0.25 to 3 µM), circular target DNA plasmids pBSK-zeo (100 ng) and the different 38 bp double stranded 5’-end-labeled donor DNA (10 ng) (described in Figure 1A) and mimicking either the 2-LTR junction (U5•U3), or the LTR viral end (U5) or displaying a randomized non viral sequence (Random). Oligonucleotides (ODN) used to form the U5•U3 DNA are: U5 5’-TGTGGAAATCTCTAGCAGTACTGGAAG
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GGCTAATTTG and U3 5’- CAAATTAGCCCTCCAGTACTGCTAGAG ATTTTCCACA. IN was incubated for 20 minutes at 4°C with both the donor and the acceptor DNA before adding the reaction mixture (20 mM HEPES, pH 7.5; 10 mM DTT; 10 mM MgCl₂; 15% DMSO; 8% PEG, 30 mM NaCl) in a final volume of 10 µl. The reaction was further incubated for 90 min at 37°C, and was then stopped by adding a phenol/isoamyl alcohol/ chloroform mix (24/1/25 v/v/v). The aqueous phase was loaded on a vertical 1% agarose gel in the presence of 1% bromophenol blue and 1 mM EDTA. After separation of the products, the gel was treated with 5% TCA for 20 min, dried and autoradiographed. After reaction, three types of products are detected: donor/donor products (d/d) corresponding to the strand transfer of one viral end from one donor molecule to another one, circular half site (HSI) products corresponding to the strand transfer of one viral end from one donor molecule to a circular acceptor plasmid and linear full site (FSI) products corresponding to the strand transfer of two viral ends from two independent donor molecules into a circular acceptor plasmid leading to its linearization.

DNA endonuclease assay-Standard reactions were performed as described previously (31). The pGEM-T-2LTR (U5•U3 circle) or the pGEM-T-Random (Random circle) described in Figure 1A were used as DNA substrate. Purified IN (0.5-1 µM) was incubated with 300 ng of plasmid DNA in a reaction mixture of 10 µl containing 20 mM HEPES pH 7.5, 10 mM DTT, 0.05% NP40, 30 mM NaCl and MnCl₂ or MgCl₂ (7.5 mM). The reaction mixture was incubated for 1 hour at 37°C and stopped by addition of 2 µl of 1% bromophenol blue and 1 mM EDTA (standard conditions). Samples were analyzed on a 1% agarose minigel containing ethidium bromide. Electrophoresis was carried out and DNA was detected by fluorescence upon exposure to UV light. Activity was evaluated by quantification of the bands corresponding to the different topological forms of the plasmid using the Image J software after scanning.

In-vivo yeast integration assay-Yeast integration assays were performed essentially as described in (17). JSC310 yeast cells harboring either pHIV1SF2IN or pHIV1SF2IN-D116A vectors were grown for 72 hours under maximal expression conditions (10 ml YNB containing 0.1% glucose and supplemented with required amino acids) until an OD₆₀₀nm of about 10 was reached (stationary phase). Yeast aliquots were used for transformation with an excess of the DNA substrate under conditions previously described (32). After transformation, 10⁹ viable cells, calculated by counting the number of cells recovered after plating on non-selective medium, were cultured in YCAD liquid medium for 1 to 5 hours and then plated on solid YCAD medium supplemented with zeocin (400 µg/ml) to select the cells expressing the Sh ble gene from the DNA substrate. After 5 days of culture, resistant clones were recovered, stocked and further analyzed. Transformants were selected on medium lacking uracile and histidine.

Sequence analysis of the plasmid cleavage events-Sequencing was performed according to the ligation-mediated PCR (LM-PCR) method, using the 11GTb linker (ODN 5’-GTGAATTCAGATC-3’ hybridized with ODN 5’-GCGGTGACCCGGGAGATCTGAATTC-3’), as previously described by Munir and colleagues (33). Briefly, a ligation reaction mixture was carried out by addition of 11GTb linker (30 nM) to plasmid cleavage products in the presence of 10 units of ligase from the Quick ligation kit (NEB) according to the manufacturer’s instructions (2 h, room temperature, 20 µL of final volume), and submitted to PCR. After amplification of the ligation product and purification on agarose gel, DNA fragments were sequenced.

Sequence analysis of the integration events-Sequencing was performed using chromosomal DNA digested by BamH1, religated with T4 DNA ligase (PROMEGA) and amplified using 5’-U3-junction (5’-GATGCGCGGAGTCCGA-3’) and 3’-U5-junction (5’-AGACCGGTGTACGATGTA-3’) primers. The amplification products were used in PCR-based sequencing (ABI Prism big dye terminator cycle sequencing ready reaction kit, Applied Biosystems) using the same primers.
Cells and viruses—HIV-1 stocks were prepared by transfecting 293T cells with the various HIV-1 molecular clones derived from HpGK and the VSV-G plasmid. The viral construction encompasses the CMV promotor with eGFP and the 175-bp of the LTR-LTR junction (PAL +). A second construction was obtained by disrupting the palindromic sequence (PAL -). MT4 cells were infected with 60 ng of p24 gag antigen per 10^6 cells, corresponding to a multiplicity of infection (m.o.i.) of 0.3. Cells were analyzed during the course of the experiment and Neomycin resistant GFP – cells were sorted at 8 days post-infection using a FACS Aria SORP (Institut Gustave Roussy platform).

Integration sites analysis—After cells sorting, cells were grown and DNA was extracted using QiaAmp blood kit (Qiagen). Integration sites were determined using MfeI restriction and LM-PCR as previously described (34). The primers sets for analysis were (Figure 5A): PCR PAL used PAL for (5’-GACACCGACTCTAGCTAGAG-3’) and PAL rev (5’-CACCATGAATTCCTCGAGT-3’); PCR a used NEO129 (5’-ACAACAGACAATCGGCTGCT-3’) and AA55M (5’-GCTAGAGATTTCACACTGACTAA-3’); PCR b used HIVR1 (5’-ACTGCACTAGTTGTAGACCATCA-3’) and revPALsal1 (5’-CAAGGCTACTTCCCTGGTGAC-3’); PCR c used MH531 (5’-TGTGTCCGGCTCTGTTGTGT-3’) and NEO130 (5’-TCGTCCAGATCATCCTGATC-3’) and PCR d used GFPfor (5’-ACCACCTACGAGCAACACC-3’) and PHR’-LTRfor (5’-CCCTTCGCTTCACAAGTCCC-3’).

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AUTHOR CONTRIBUTIONS: CR, ST, ET, PL, FS, SM, DL and HL conducted most of the experiments and analysed the results. VP conducted the biochemical experiments using IN-LEDGF complex. OD, ED and VP conceived the idea for the project and wrote the article.

FOOTNOTES

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The abbreviations used are: IN, Integrase; wt, wild-type; ODN, oligonucleotide.

References

1. Delelis, O., Carayon, K., Saib, A., Deprez, E., and Mouscadet, J. F. (2008) Integrase and integration: biochemical activities of HIV-1 integrase. Retrovirology 5, -
2. Engelman, A., Mizuuchi, K., and Craigie, R. (1991) HIV-1 DNA integration: mechanism of viral DNA cleavage and DNA strand transfer. Cell 67, 1211-1221
3. Sinha, S., and Grandgenett, D. P. (2005) Recombinant human immunodeficiency virus type 1 integrase exhibits a capacity for full-site integration in vitro that is comparable to that of purified preintegration complexes from virus-infecte cells. J Virol 79, 8208-8216
4. Munir, S., Thierry, S., Subra, F., Deprez, E., and Delelis, O. (2013) Quantitative analysis of the time-course of viral DNA forms during the HIV-1 life cycle. Retrovirology 10, 87
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5. Fischer, M., Trkola, A., Joos, B., Hafner, R., Joller, H., Muesing, M. A., Kaufman, D. R., Berli, E., Hirschel, B., Weber, R., and Gunthard, H. F. (2003) Shifts in cell-associated HIV-1 RNA but not in episomal HIV-1 DNA correlate with new cycles of HIV-1 infection in vivo. *Antivir Ther* 8, 97-104

6. Wu, Y. T., and Marsh, J. W. (2003) Early transcription from nonintegrated DNA in human immunodeficiency virus infection. *J Virol* 77, 10376-10382

7. Yoder, K., Sarasin, A., Kraemer, K., McIlhatton, M., Bushman, F., and Fishel, R. (2006) The DNA repair genes XPB and XPD defend cells from retroviral infection. *Proc Natl Acad Sci U S A* 103, 4622-4627

8. Kilzer, J. M., Stracker, T., Beitzel, B., Meek, K., Weitzman, M., and Bushman, F. D. (2003) Roles of host cell factors in circularization of retroviral DNA. *Virology* 314, 460-467

9. Hazuda, D. J., Felock, P., Witmer, M., Wolfe, A., Stillmock, K., Grobler, J. A., Espeseth, A., Gabryelski, L., Schleif, W., Blau, C., and Miller, M. D. (2000) Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science* 287, 646-650

10. Reigadas, S., Andreola, M. L., Wittkop, L., Cosnefroy, O., Anies, G., Recordon-Pinson, P., Thiebaut, R., Masqueller, B., and Fleury, H. (2010) Evolution of 2-long terminal repeat (2-LTR) episomal HIV-1 DNA in raltegravir-treated patients and in vitro infected cells. *J Antimicrob Chemother* 65, 434-437

11. Sloan, R. D., and Wainberg, M. A. (2011) The role of unintegrated DNA in HIV infection. *Retrovirology* 8, 52

12. Chan, C. N., Trinite, B., Lee, C. S., Mahajan, S., Anand, A., Wodarz, D., Sabbaj, S., Bansal, A., Goepfert, P. A., and Levy, D. N. (2016) HIV-1 latency and virus production from unintegrated genomes following direct infection of resting CD4+ T cells. *Retrovirology* 13, 1

13. Delelis, O., Parissi, V., Leh, H., Mbemba, G., Petit, C., Sonigo, P., Deprez, E., and Mouscadet, J. F. (2007) Efficient and specific internal cleavage of a retroviral palindromic DNA sequence by tetrameric HIV-1 integrase. *Plos One* 2, e608

14. Delelis, O., Petit, C., Leh, H., Mbemba, G., Mouscadet, J. F., and Sonigo, P. (2005) A novel function for spumaretrovirus integrase: an early requirement for integrase-mediated cleavage of 2 LTR circles. *Retrovirology* 2, 31

15. Thierry, S., Munir, S., Thierry, E., Subra, F., Leh, H., Zamborlini, A., Saenz, D., Levy, D. N., Lesbats, P., Saib, A., Parissi, V., Poeschla, E., Deprez, E., and Delelis, O. (2015) Integrase inhibitor reversal dynamics indicate unintegrated HIV-1 dna initiate de novo integration. *Retrovirology* 12, 24

16. Zhang, D. W., He, H. Q., and Guo, S. X. (2014) Hairpin DNA probe-based fluorescence assay for detecting palindrome cleavage activity of HIV-1 integrase. *Anal Biochem* 460C, 36-38

17. Desfarges, S., San Filippo, J., Fournier, M., Calmels, C., Caumont-Sarcos, A., Litvak, S., Sung, P., and Parissi, V. (2006) Chromosomal integration of LTR-flanked DNA in yeast expressing HIV-1 integrase: down regulation by RAD51. *Nucleic Acids Res* 34, 6215-6224

18. Cherepanov, P., Maertens, G., Proost, P., Devreese, B., Van Beeumen, J., Engelborghs, Y., De Clercq, E., and Debyser, Z. (2003) HIV-1 integrase forms stable tetramers and associates with LEDGF/p75 protein in human cells. *J Biol Chem* 278, 372-381

19. Reigadas, S., Anies, G., Masqueller, B., Calmels, C., Stuyver, L. J., Parissi, V., Fleury, H., and Andreola, M. L. (2010) The HIV-1 Integrase Mutations Y143C/R Are an Alternative Pathway for Resistance to Raltegravir and Impact the Enzyme Functions. *Plos One* 5, -

20. Lesbats, P., Metfiot, M., Calmels, C., Baranova, S., Nevinsky, G., Andreola, M. L., and Parissi, V. (2008) In vitro initial attachment of HIV-1 integrase to viral ends: control of the DNA specific interaction by the oligomerization state. *Nucleic Acids Res* 36, 7043-7058

21. Hazuda, D., Iwamoto, M., and Wenning, L. (2009) Emerging Pharmacology: Inhibitors of Human Immunodeficiency Virus Integration. *Annu Rev Pharmacol* 49, 377-394
Integrase and 2-LTR circles

22. Faure, A., Calmels, C., Desjober, C., Castroviejo, M., Caumont-Sarco, A., Tarrago-Litvak, L., Litvak, S., and Parissi, V. (2005) HIV-1 integrase crosslinked oligomers are active in vitro. *Nucleic Acids Res* **33**, 977-986

23. Krishnan, L., Li, X., Naraharisetty, H. L., Hare, S., Cherepanov, P., and Engelmann, A. (2010) Structure-based modeling of the functional HIV-1 intasome and its inhibition. *Proc Natl Acad Sci U S A* **107**, 15910-15915

24. Li, M., and Craigie, R. (2009) Nucleoprotein complex intermediates in HIV-1 integration. *Methods* **47**, 237-242

25. Li, M., Mizuuchi, M., Burke, T. R., and Craigie, R. (2006) Retroviral DNA integration: reaction pathway and critical intermediates. *Embo J* **25**, 1295-1304

26. Maillot, B., Levy, N., Eiler, S., Crucifix, C., Granger, F., Didier, P., Godet, J., Pradeau-Aubreton, K., Emiliani, S., Nazabal, A., Lesbats, P., Parissi, V., Mely, Y., Moras, D., Schultz, P., and Ruff, M. (2013) Structural and functional role of INI1 and LEDGF in the HIV-1 preintegration complex. *Plos One* **8**, e60734

27. Brown, P. O., Bowerman, B., Varmus, H. E., and Bishop, J. M. (1989) Retroviral integration: structure of the initial covalent product and its precursor, and a role for the viral IN protein. *Proc Natl Acad Sci U S A* **86**, 2525-2529

28. Butler, S. L., Hansen, M. S. T., and Bushman, F. D. (2001) A quantitative assay for HIV DNA integration in vivo. *Nat Med* **7**, 631-634

29. Bona, R., Baroncelli, S., D’Ettorre, G., Andreotti, M., Ceccarelli, G., Filati, P., Leone, P., Blasi, M., Michelini, Z., Galluzzo, C. M., Mallano, A., Vullo, V., and Cara, A. (2013) Effects of raltegravir on 2-long terminal repeat circle junctions in HIV type 1 viremic and aviremic patients. *AIDS Res Hum Retroviruses* **29**, 1365-1369

30. Svarovskaia, E. S., Barr, R., Zhang, X., Pais, G. C., Marchand, C., Pommier, Y., Burke, T. R., Jr., and Pathak, V. K. (2004) Azido-containing diketo acid derivatives inhibit human immunodeficiency virus type 1 integrase in vivo and influence the frequency of deletions at two-long-terminal-repeat-circle junctions. *J Virol* **78**, 3210-3222

31. Parissi, V., Caumont, A., de Soultrait, V. R., Desjober, C., Calmels, C., Fournier, M., Gourgue, G., Bonneu, M., Tarrago-Litvak, L., and Litvak, S. (2003) The lethal phenotype observed after HIV-1 integrase expression in yeast cells is related to DNA repair and recombination events. *Gene* **322**, 157-168

32. Chen, J. W., Evans, B. R., Yang, S. H., Araki, H., Oshima, Y., and Jayaram, M. (1992) Functional Analysis of Box-I Mutations in Yeast Site-Specific Recombinase-Flp and Recombinase-R - Pairwise Complementation with Recombinase Variants Lacking the Active-Site Tyrosine. *Mol Cell Biol* **12**, 3757-3765

33. Munir, S., Thierry, E., Malet, I., Subra, F., Calvez, V., Marcelin, A. G., Deprez, E., and Delelis, O. (2014) G118R and F121Y mutations identified in patients failing raltegravir treatment confer dolutegravir resistance. *J Antimicrob Chemother*

34. Wu, X., Li, Y., Crise, B., and Burgess, S. M. (2003) Transcription start regions in the human genome are favored targets for MLV integration. *Science* **300**, 1749-1751
Figure 1: IN-mediated cleavage of linear or circular DNA substrates containing the 2-LTR junction is improved by LEDGF. (A) Linear and circular DNA substrates. The double stranded DNA (38 bp) mimicking the palindromic junction (U5•U3), the HIV-1 U5 end (U5) or carrying a non-viral random sequence (Random) were used in in vitro cleavage assays. The U5•U3 DNA was formed by hybridization of U5 5’- TGTGAAAATCTCAGTACTGGGAAGGGCTAATTTG and U3 5’- CAAATTAGCCCTTCCAGTACTGCTAGAGATTTTCCAC. For the U5•U3 DNA, the U5 sequence (in bold) and the U3 sequence are indicated. For the U5 DNA, the CAGT end of the U5 end is indicated in bold. The unprocessed 1200 bp linear yeast integration substrate (linearized U5•U3) containing the two viral U3 and U5 ends was obtained by PCR amplification of the P TEF1ShBleCYC1tt fragment from the pTEF1/Zeo plasmid (Invitrogen) as described in (17). The 4237 bp two HIV-1 LTR junction circle (U5•U3 circle) was generated by pGEM-T cloning of the DNA fragment 2LTRPTEF1ShBleCYC1tt obtained by PCR amplification of the P TEF1ShBleCYC1tt cassette from the pTEF1/Zeo plasmid (Invitrogen) using the 3’-Cont-Zeo (5’- TTGCAAAATTAAAGCCTTCGAGGCTCC-3’) and the 5’-2LTR junction-Zeo (5’- TGTGAAAATCTCAGTACTGGGAAGGGCTAATTTGCCACCACCATAGCTTCCCAAATGT TTTCCTACTCC-3’) primers. The 4237 processed 2-LTR junction (U5•U3 processed circle) was generated by IN-mediated cleavage as described in Figure 1C. P_{TEF1}: yeast TEF1 promoter, Sh Ble: zeocine resistance encoding gene. (B) Left panel: 1 μM of IN +/- 3 μM of LEDGF were incubated for 1 hour at 37°C with 10 nM of the U5•U3 DNA (38bp). In this experiment U5 ODN was radiolabelled at the 5’ end. Reaction products were loaded on an 18% acrylamide gel. Products were quantified using the Image J software and the percentage of cleavage was reported (middle panel). The arrow shows the location of the cleavage mediated by IN on the radiolabelled U5 ODN. The resulting cleavage product is
5'-TGTGAAAATCTCTAGCA indicated as 5': -AGCA on the left of the denaturing gel. Right panel: Coomassie-stained gel image of the purified protein. Lane 1: Integrase (IN). Lane 2: LEDGF/p75. Lane 3: IN/LEDGF complex. The molecular weight of the protein ladder is indicated in kD (PageRuler, Thermofisher). (C) Cleavage assay of the U5•U3 circle (300 ng) performed in presence of increasing concentrations of WT (lanes 2 (500 nM) and 3 (1 μM)) or inactivated D116A IN (lanes 6 (500 nM), 7 (750 nM) and 8 (1μM)). Lane 4: positive control, U5•U3 linearized by SalI digestion. Control experiment was performed using the Random circle obtained by cloning the random sequence (38bp) into the pGEM-T vector, with increasing concentrations of WT IN (right panel). The positions of the Close circular native (CC), Open circular single strand cut (OC) and Linearized double strand cut (L) plasmid DNA are reported. The percentage of linearized substrate was quantified by densitometry estimation using the Image J software and reported (right panel). All the values correspond to the mean ± standard deviation (error bars) of three independent sets of experiments. (D) The same assay was performed with or without LEDGF (3 μM), IN WT (500 nM) and U5•U3 circle (300 ng) or Random circle (300 ng) substrate. The percentage of linearized substrate was quantified as mentioned above and reported (middle panel). Sequences of the linearized products were obtained as previously described in the Materials and Methods section.
Figure 2: IN-mediated cleavage of U5•U3 junction is inhibited by RAL. (A) Left panel, cleavage of the palindrome was performed using 12 nM of U5•U3 DNA in the presence of 1 µM IN and 500 nM EDTA (1), 0 nM (2), 500 nM (3), 1000 nM (4) or 1500 nM (5) RAL. Same experiment was performed using 1 µM IN and 3 µM of LEDGF in the absence (6) or presence (7) of 500 nM RAL. The resulting cleavage product are indicated 5':-AGCA, 5':-AGCAG and 5':-AGCAGT on the left of the denaturing gel. The products of the reaction were quantified using the Image J software and reported as a percentage of cleavage from the substrate, right panel. (B) 2-LTR junction cleavage assay was performed with the U5•U3 circle substrate (300 ng), in the presence of 500 nM IN and 0 (1), 50 (2), 100 (3), 250 (4) or 500 (5) nM RAL. Same experiment was performed in the presence of 3 µM LEDGF (6→10) using 0 nM (7), 500 nM (8), 1000 nM (9) or 1500 nM (10) RAL. The gel from one representative experiment is shown. The cleavage products from three independent experiments were quantified as described above and the percentage of linearized substrate for IN alone or in the presence of LEDGF with increasing concentration of RAL, was reported below the gels.
Figure 3: Concerted integration by IN of a substrate containing the 2-LTR junction. (A) Concerted integration assay was performed with 100 ng of pBSK-zeo acceptor plasmid, 10 ng of the 5'-P32 U5•U3 donor DNA and 0.25 (1), 0.50 (2) or 1.00 (3) µM IN. The reaction products were loaded on a 1 % agarose gel. The position and structures of the donor substrate and different products obtained after half-site (HSI), full-site (FSI) and donor/donor integration (d/d) are explicitly shown. M: Markers (base pairs). The heterointegration products (FSI + HSI) were quantified by densitometry estimation using the Image J software and reported (right panel). (B) Integration reactions were performed using 1µM IN, 100 ng of pBSK-zeo acceptor plasmid and 10 ng of the 38 bp 5-P32 2LTR junction (U5•U3), the viral end U5 fragment or Random donor DNA. The different integration products obtained were quantified by densitometry estimation using the Image J software. The proportion of FSI and HSI was evaluated for each integration conditions and reported in right panel. (C) The concerted integration assay was performed in the same conditions as described in panel A, in the presence of increasing RAL concentrations. The heterointegration products (FSI + HSI) detected on agarose gel were quantified by densitometry estimation using the Image J software. (D) Concerted integration assay was performed with 1 µM IN, 100 ng of pBSK-zeo acceptor plasmid, 10 ng of the 38 bp 5-P32 U5•U3 DNA, and in the presence of increasing LEDGF concentration (0 (1), 2.5 (2), 5 (3), or 10 µM (4)). The heterointegration products (FSI + HSI) obtained under these conditions and detected on agarose gel were quantified by densitometry estimation using the Image J software and reported in right panel. All values obtained from the described experiments correspond to the mean ± standard deviation (error bars) of at least three independent sets of experiments.
Figure 4: Integration of 2-LTR junction DNA into yeast chromosome. Yeast integration assay was performed as described in (17) and in the material and methods section using either the 2-LTR junction circle or the processed 2-LTR junction obtained and purified as indicated in the legend of Figure 1C. (A) Number of zeocine resistant clones selected after 1-5 hours post-transformation with U5•U3 circle or U5•U3 processed circle. (B) Same experiments performed with the U5•U3, U5•U3 circle or Random circle substrates after expression of the wild type IN, or with the U5•U3 circle after expression of the inactivated D116A enzyme. (C-D) 100 clones obtained in the yeast integration assays performed using the linear U5•U3, processed U5•U3 circle or U5•U3 circle were sequenced as reported in the materials and methods section. (C) Number of correct 5-bp duplications and other repeats found at each extremities of the integrated DNA. (D) The viral end sequence was also analysed by sequencing and the number of clones harbouring the correct AGCA viral ends or other structures is reported.
Figure 5: The palindromic sequence is involved in IN-dependent integration in the virological context. (A) Panel A shows the PAL sequence construction (175 bp of the U5-U3 sequence) and the possible integration events using only LTR extremities (form 1) or only PAL extremities (forms 4 and 5), or a combination of both (forms 2 and 3). Form 6 represents unwanted integration events possibly due to recombination occurring during reverse transcription. The designed PCR experiments are represented in red. (B) Flow cytometry analysis during PAL + and PAL – infections. Neomycin was added 4 days post-infection. NeoR/GFP – cells were sorted 8 days post-infection. (C) Sequences of the integration sites. Results are the mean from three representative independent experiments ± standard deviation (error bars). (D) Sequences of linear viral DNA after Raltegravir removal. Cells were infected in the same conditions as already described (15). Briefly, MT4 cells were infected with NLENG1-ES-IRES WT pseudotyped with VSV-g envelope in the presence of Raltegravir (500 nM). 72 hours post-infection, cells were washed and fresh medium, without Raltegravir, was added. 2-LTR circles (2-LTRc, black column); 1-LTR circles (1-LTRc, grey column) and linear viral DNA (light grey column) were quantified by quantitative PCR as already described (15). Linear viral DNA was sequenced by a LM-PCR approach using a blunt ended linker at 84 and 96 hours post-infection (4). All sequences displayed the same pattern highlighting the canonical 5’-end of the 3’ LTR (5’-CAGT end). In italic, sequence of the viral DNA; in bold, sequence of the linker.
Two-long terminal repeat (LTR) DNA circles are a substrate for HIV-1 integrase
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