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Nucleoprotein complex intermediates in HIV-1 integration

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Abstract

Integration of retroviral DNA into the host genome is an essential step in the viral replication cycle. The viral DNA, made by reverse transcription in the cytoplasm, forms part of a large nucleoprotein complex called the preintegration complex (PIC). The viral integrase protein is the enzyme within the PIC that is responsible for integrating the viral DNA into the host genome. Integrase is tightly associated with the viral DNA within the PIC as demonstrated by functional assays. Integrase protein catalyzes the key DNA cutting and joining steps of integration *in vitro* with DNA substrates that mimic the ends of the viral DNA. Under most *in vitro* assay conditions the stringency of the reaction is relaxed; most products result from “half-site” integration in which only one viral DNA end is integrated into one strand of target DNA rather than concerted integration of pairs of DNA as occurs with PICs and *in vivo*. Under these relaxed conditions catalysis appears to occur without formation of the highly stable nucleoprotein complexes that is characteristic of the association of integrase with viral DNA in the PIC. Here we describe methods for the assembly of nucleoprotein complex intermediates in HIV-1 DNA integration from purified HIV-1 integrase and substrates that mimic the viral DNA ends.

Keywords

Site-specific recombination; Retrovirus; Integrase; Integration; Nucleoprotein complex

1. Introduction

Integration of a DNA copy of the viral genome into chromosomal DNA of the host cell is an essential step in the replication cycle of HIV-1 and other retroviruses [1,2]. The chemical steps of DNA cutting and joining, which occur in two separate reactions, are catalyzed by the virally encoded integrase protein. In the first step of the integration process, two nucleotides are removed from each 3'-end of HIV-1 DNA in a reaction termed 3'-end processing. Cleavage occurs to the 3'-side of a CA dinucleotide that is conserved among many DNA transposons as well as retroviruses. This reaction exposes the terminal 3'-hydroxyl group that is to be joined to target DNA upon integration. In the second step, DNA strand transfer, the hydroxyl groups at the 3'-ends of the processed viral DNA attack a pair of phosphodiester bonds in the target DNA, to generate an integration intermediate in which the 3'-ends of the viral DNA are covalently joined to the 5'-ends of the target DNA at the site of integration. The integration intermediate then undergoes DNA repair to complete the integration process; the repair steps are likely to be carried out by cellular enzymes [3,4].

Both the 3'-end processing and DNA strand transfer activities of HIV-1 integrase can be recapitulated *in vitro* with short DNA substrates that mimic the viral DNA ends [5–7]. However, under those reaction conditions the strand transfer products mostly result from a “half-site” reaction in which only one viral DNA end is joined to one strand of target DNA, rather than concerted integration of a pair of viral DNA ends as occurs *in vivo*. Furthermore, highly stable complexes between integrase and viral DNA that resemble the association of integrase with the viral DNA in preintegration complexes (PICs) isolated from infected cells have not been observed with this reaction system.

Many enzymes that catalyze reactions at specific sites in DNA molecules have a high affinity for that DNA sequence. In some cases assembling nucleoprotein complexes of the enzyme and DNA is as simple as mixing the enzyme with DNA containing the appropriate recognition sequence. However, assembly of functional complexes of HIV-1 integrase with viral DNA substrate is a more complex process. The bacteriophage Mu transposase is an extensively studied model system that is useful in considering some of key features of complex assembly with HIV-1 integrase. Mu transposase binds specifically to three binding sites at each end of the Mu genome. Although specific, binding is quite weak and the transposase can be dissociated with 200 mM NaCl. However, in the presence of a divalent metal ion, and cofactors that we will not discuss here, a very stable complex called a transpososome (reviewed in [8]) is assembled. The heart of the transpososome is a tetramer of transposase that stably bridges a pair of Mu DNA ends. Once assembled, the transpososome is stable to challenge with high ionic strength, detergents, and heat. The assembly process is a downhill energetic pathway, but an energy barrier must first be climbed before the transpososome can be assembled. Assembly of fully functional complexes between HIV-1 integrase and viral DNA is a similar process. The primary binding of HIV-1 integrase to viral DNA ends is weak and exhibits little sequence specificity, but a pair viral DNA ends subsequently “lock” in with a tetramer of integrase to form a highly stable complex that is an intermediate on the integration pathway.

Improved *in vitro* reaction systems [9–12] have enabled concerted DNA integration to be studied *in vitro*. Under conditions that promote concerted DNA integration, highly stable complexes are formed between integrase and DNA substrate [13,14]. The first stable complex is the stable synaptic complex (SSC) in which integrase is stably associated with a pair of viral DNA ends. The SSC is a transient intermediate that integrates the pair of viral DNA ends into a target DNA. The integration product remains stably bound by integrase in a second stable complex, the strand transfer complex (STC) (Fig. 1). We describe protocols for generating these stable complexes between integrase and DNA substrate that appear to closely mimic the complexes formed *in vivo* as judged by functional assays.

2. Procedures

2.1. Preparation of viral DNA substrates

Efficient assembly of stable complexes between HIV-1 integrase and viral DNA substrate occurs under reaction conditions that promote concerted DNA integration [11,13] and requires viral DNA substrate longer than several hundred base pairs. Sequence specificity does not extend beyond the terminal 20 bp and there are no sequence-specific requirements for the flanking DNA. We constructed a plasmid pSca355 [11] (Fig. 2A) that when digested with ScaI and HincII generates a 1.5 kb linear fragment terminating with 32 bp of the blunt-ended U5 terminal DNA sequence (Fig. 2B). Additional restriction sites within this fragment allow substrates of different lengths to be made. Our typical reactions use a 1 kb fragment made by further cleavage with BanI (Fig. 2B). For some purposes it is necessary to manipulate the usually blunt terminal viral DNA sequence, for example to make a pre-processed viral DNA substrate with a 3'-dideoxyadenosine to trap the SSC. Such sequence

modifications are conveniently made in oligonucleotides that are subsequently ligated to a longer linear DNA fragment to make the final DNA substrate (Fig. 2C). Although a U5 end normally pairs with a U3 end *in vivo*, a pair of U5 ends can support near wild type levels of HIV-1 infectivity [15] and are equally efficient at promoting concerted DNA integration and SSC and STC formation *in vitro* (see supplementary material in [13]). Note that viral DNA sequence is present at only one end of the substrate and pairing occurs between two separate DNA molecules.

2.1.1. Preparation of blunt-end viral DNA substrates

Preparation of pSca355 DNA: Because the linear ~1 kb viral DNA substrate must be excised from plasmid pSca355 and purified by gel electrophoresis, it is advisable to start with a 500 µg or larger scale of plasmid preparation. Many standard protocols and commercial kits are available for this purpose. We use the QIAfilter Plasmid Maxi Kit (QIAGEN). The blunt-end viral DNA substrate is prepared from pSca355 as follows:

1. Dissolve the DNA in a suitable volume of buffer (10 mM Tris pH 8.5) and determine the concentration by measuring the absorbance at 260 nm. If using a quartz cuvette with 1 cm diameter, the concentration c of total DNA (in µg/µl) is roughly $c = A_{260} * f * 0.05$ µg/µl; where f is the dilution factor. The yield of plasmid from a 200 ml culture should be approximately 500 µg.
2. Restriction digestion. Digest 500 µg of the plasmid DNA with 500 U each of ScaI and HincII (New England Biolabs) at a DNA concentration of 0.3 mg/ml. Incubate the reaction mixture at 37 °C for 2 h.
3. *Purification of the 1513 bp DNA fragment.* Pour a preparative 1% agarose gel (SeaKem GTG) in TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0) buffer. Add DNA loading buffer (containing Na dodecyl sulfate [SDS]) to the restriction digestion mixture, and electrophorese at 5 V/cm for 1 h. Stain the gel with ethidium bromide, visualize under UV light, and excise the 1513 bp band. Purify the DNA from the agarose by means of a QIAEX II Gel Extraction Kit following the recommended protocol (QIAGEN, see the QIAEX II Handbook). Scale up volumes proportionally according to the amount of DNA loaded onto the gel: briefly, excise the DNA band from the agarose gel with a clean sharp scalpel and weigh the gel slice. Add 3 volumes of solubilization buffer to 1 volume of gel. Estimate the amount of DNA (e.g. approximately 150 µg of 1513 bp DNA fragment is expected from 500 µg plasmid DNA). Add 450 µl of silica gel suspension to 150 µg of the DNA gel solution and mix. Incubate at 50 °C for 10 min or until the agarose is completely solubilized, vortexing every 2 min to keep the agarose in suspension. Centrifuge the sample for 60 s at 10,000g and carefully remove the supernatant with a pipette. Wash the pellet once with solubilization buffer. Wash the pellet with washing buffer twice. Air-dry the pellet for 15 min or until the pellet becomes white. Elute the DNA twice with 200 µl of 10 mM Tris pH 8.5. Combine the eluates and extract the DNA further by adding an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1), vortexing, and centrifugation for 2 min at full speed in a microcentrifuge. Add 10% final volume of 3 M sodium acetate and 2 volumes of ethanol to precipitate the DNA. Centrifuge for 10 min at full speed in a microcentrifuge to pellet the DNA. Carefully wash the DNA pellet with cold 70% ethanol, centrifuge at maximum speed for a minute, remove the supernatant, dry the pellet, and dissolve in a suitable volume of water or 10 mM Tris pH 7.5, 0.1 mM EDTA (TE buffer). The recovery of DNA is typically about 50%, but the yield may vary between preparations.

4. Preparation of the 984 bp viral DNA substrate. Repeat steps 2 and 3, this time digesting the 1513 bp DNA fragment purified in step 3 with BanI.
5. *Labeling the viral DNA substrate with ^{32}P .* Measure the DNA concentration by UV absorbance. Mix 7.0 μg of the purified 984 bp viral DNA substrate, 10 μl of 3000 Ci/mmol [$\gamma\text{-}^{32}\text{P}$] ATP (PerkinElmer), 2 μl (20 U) T4 polynucleotide kinase (PNK) (New England Biolabs), 4 μl of x10 PNK buffer, and water to make up to 40 μl reaction volume. Incubate at 37 °C for 1 h. Stop the reaction by adding EDTA to the final concentration of 10 mM and incubate at 65 °C for 15 min to inactivate the PNK. Remove unincorporated ^{32}P -ATP using a spin column (Bio-Rad, Micro Bio-Spin 6 Chromatography Column). Briefly: resuspend the gel by inverting the column sharply several times. Drain the excess buffer by gravity. Centrifuge at 1000g for 2 min to remove remaining buffer. Carefully apply the sample directly to the center of the column and centrifuge for 4 min at 1000g. Measure the concentration of the eluted DNA by UV absorbance. The final concentration is typically 140–160 ng/ μl . Store the labeled DNA at –20 °C.

2.1.2. Preparation of pre-processed viral DNA substrate with 3'-

dideoxyadenosine—Ligation of oligonucleotides to non-specific flanking DNA is a convenient method to prepare viral DNA substrates with chemically modified ends because oligonucleotides are readily amenable to modification both during synthesis and post-synthesis.

1. Synthesize and HPLC purify the following oligonucleotides (this step may be contracted to a commercial source. We have used Integrated DNA Technologies):

E6 5'-pAATTCTTTTAGTCAGTGTGGAAAATCTCTAGC

E3 5'-ACTGCTAGAGATTTTCCCACTGACTAAAAG
2. Prepare an approximately 1 kb DNA fragment containing an EcoRI sticky end. We use a 970 bp EcoRI to PstI fragment of pML10 (derived from pCR2.1 (Invitrogen) by deleting the sequence between positions 283 and 505, which removes additional EcoRI and PstI sites). Purify as described in Section 2.2.1 for the standard viral DNA integration substrate.
3. Assemble the terminal deoxynucleotidyl transferase (TdT) reaction mixture on ice by mixing:

2 μl x10 reaction buffer 4 (New England Biolabs).

2 μl x10 CoCl_2 (New England Biolabs).

0.4 μl 10 mM ddATP (Roche).

2 μl 1 $\mu\text{g}/\mu\text{l}$ oligonucleotide E6.

1 μl (20 U) TdT (New England Biolabs).

12.6 μl H_2O .

Incubate at 37 °C for 30 min.

Stop the reaction by heating at 70 °C for 20 min.
4. Anneal with oligonucleotide E3 by adding:

2 μl 1 $\mu\text{g}/\mu\text{l}$ oligonucleotide E3.

1 μl 1 M NaCl.

Slowly cool to room temperature.

5. Ligation. Mix:

23 μ l annealed DNA from step 2.

24 μ l 0.5 μ g/ μ l 1 kb EcoRI–PstI DNA fragment.

6 μ l x10 T4 ligase buffer (New England Biolabs).

4 μ l (800 U) T4 Ligase (New England Biolabs).

3 μ l H₂O.

Incubate at 16 °C overnight.

6. Purify the ligated DNA by Qiagen MinElute Reaction Cleanup Kit according to manufacturer's instructions. Digest with PstI and purify the 1 kb fragment by gel electrophoresis as described in Section 2.2.1.

2.2. Expression and purification of HIV-1 integrase

The following purification method is essentially as previously described for purification of wild type His-tagged HIV-1 integrase [16]. Briefly, integrase is expressed in *Escherichia coli* BL21 (DE3) and the cells are lysed in buffer containing 0.1 M NaCl. The lysate is centrifuged and integrase is extracted from the pellet in buffer containing 1 M NaCl. The protein is then purified by nickel-affinity chromatography. Following removal of the His-tag by thrombin digestion, the protein is further purified by gel filtration on a Superdex-75 column (GE Healthcare).

2.2.1. Buffers

Lysis buffer: 20 mM Hepes pH 7.5, 100 mM NaCl, 2 mM 2-mercaptoethanol.

Extraction buffer: 20 mM Hepes pH 7.5, 1 M NaCl, 2 mM 2-mercaptoethanol.

Equilibration buffer A: 20 mM Hepes pH 7.5, 1 M NaCl, 2 mM 2-mercaptoethanol, 5 mM imidazole.

1st Washing buffer: 20 mM Hepes pH 7.5, 1 M NaCl, 2 mM 2-mercaptoethanol, 10% glycerol.

2nd Washing buffer: 20 mM Hepes pH 7.5, 1 M NaCl, 2 mM 2-mercaptoethanol, 20 mM imidazole, 10% glycerol.

Elution buffer A: 20 mM Hepes pH 7.5, 1 M NaCl, 2 mM 2-mercaptoethanol, 10% glycerol.

Elution buffer B: 20 mM Hepes pH 7.5, 1 M NaCl, 2 mM 2-mercaptoethanol, 0.8 M imidazole, 10% glycerol.

Dialysis buffer: 20 mM Hepes pH 7.5, 0.75 M NaCl, 2 mM DTT, 0.3 M imidazole, 10% glycerol.

Equilibration buffer B: 20 mM Hepes pH 7.5, 0.5 M NaCl, 5 mM DTT, 1 mM EDTA, 10% glycerol.

Gel filtration buffer: 20 mM Hepes pH 7.5, 1 M NaCl, 5 mM DTT, 10% glycerol.

2.2.2. Purification of HIV-1 integrase

1. Thaw and suspend cells from a 2 l culture in 100 ml *Lysis buffer* on ice. Add lysozyme to 0.4 mg/ml (stock solution 100 mg/ml). Incubate on ice with occasional stirring until the suspension becomes viscous (about 30 min).
2. Sonicate in short bursts until the lysate is no longer viscous. It is important to sonicate on ice and to carefully monitor the temperature of the lysate between bursts of sonication. Do not let it rise above 8 °C.
3. Extract and solubilize the integrase. Centrifuge the sonicated lysate at 40,000g for 45 min. Discard the supernatant and homogenize the pellet in 100 ml *Extraction buffer*. A short sonication burst may help solubilization. Stir for 1 h at 4 °C. Centrifuge at 40,000g for 45 min. Retain the supernatant which contains the solubilized integrase. The yield of integrase may be improved by repeating the 1 M NaCl extraction with another 50 ml *Extraction buffer* and combining the supernatants.
4. Prepare a 5 ml Ni-charged Chelating Sepharose column (GE Healthcare) by washing the column sequentially with water and *Equilibration buffer A*.
5. Filter the supernatant from step 3 through a 0.45 µm filter (optional) and load it on to the Chelating Sepharose column.
6. Wash the column with *1st Washing buffer* and then *2nd Washing buffer* until A_{260} reaches baseline.
7. Elute the integrase using an imidazole gradient (*Elution buffer A* to *B*) over 15 column volumes.
8. Pool fractions containing integrase, add EDTA to 1 mM, and concentrate if necessary with an Amicon Ultra 15 centrifugal filter (Millipore).
9. Dialyze the integrase against *Dialysis buffer* overnight at 4 °C.
10. Add thrombin to the integrase protein to a final concentration of 40 NIH U/mg of integrase. Incubate at 25 °C for 1 h. Place on ice and check the completeness of thrombin digestion by SDS–polyacrylamide gel electrophoresis (PAGE). Continue the incubation if necessary until the His-tag is completely removed (His-tag removal can also be accomplished by adding thrombin to the integrase during the dialysis step at 4 °C overnight. In this case the thrombin concentration should be fourfold lower).
11. Remove thrombin from the preparation by adsorption to a column of benzamidine-Sepharose 6B (GE Healthcare) equilibrated with *Equilibration buffer B*.
12. Concentrate the integrase with an Amicon Ultra 15 centrifugal filter (Millipore) and load onto a Superdex 75 column (GE Healthcare) equilibrated with *Gel filtration buffer*.
13. Pool the integrase-containing fractions, measure the concentration, and aliquot. Freeze in liquid nitrogen and store at –70 °C.

2.3. Assembly of HIV-1 integrase SSC and STC

Efficient complex assembly occurs under reaction conditions that favor concerted integration [13]. The SSC is a transient intermediate and is chased to the STC unless the DNA strand transfer step is blocked (Fig. 3).

2.3.1. Generation of the STC—Reaction conditions and electrophoretic analysis products are essentially as described [13]. Thaw frozen integrase and DNA stocks on ice and keep all freshly made buffer stocks, except dimethyl sulfoxide (DMSO), on ice.

- 1 Mix the following components in a 1.5 ml eppendorf tube:
 - 0.5 μ l of 1 M Hepes pH 7.5,
 - 3.0 μ l of 100% DMSO (Sigma),
 - 2.5 μ l of 100 mM DTT,
 - 12.5 μ l of 20% polyethylene glycol (PEG)-6000 (Hampton Research),
 - 1.7 μ l of 0.3 mM ZnCl_2 ,
 - 0.8 μ l of 2.5 M NaCl,
 - 1.0 μ l of 250 mM MgCl_2 ,
 - 1.5 μ l H_2O ,
 - 0.45 μ l of 0.16 mg/ml integrase,
 - 0.5 μ l 160 ng/ μ l ^{32}P -labeled 1 kb viral DNA substrate.

The final concentrations of individual components are: 20 mM Hepes pH 7.5, 12% DMSO, 10 mM DTT, 10% PEG-6000, 10 mM MgCl_2 , 20 μ M ZnCl_2 , and 100 mM NaCl in a volume of 24.5 μ l.

- 2 Mix and incubate on ice for 30 min.
- 3 Add: 0.5 μ l 500 ng/ μ l pBR322 DNA (New England Biolabs) as the target DNA.
- 4 Incubate on ice for 30 min.
- 5 Transfer the reaction mixture to 37 °C and incubate for 2 h.
- 6 Stop the reaction by adding EDTA to the final concentration of 10 mM. Add bovine serum albumin (BSA) to a final concentration of 1 mg/ml. The BSA helps to prevent non-specific interaction of the complexes with agarose during gel electrophoresis. Add SDS and proteinase K to the final concentrations of 0.1% and 0.4 mg/ml, respectively, and incubate at 37 °C for 1 h before loading the gel.
- 7 Complexes are separated from unreacted DNA substrate by fractionation through an 0.8% agarose (12 $\times$ 24 cm)–TBE–1 M urea gel in TBE buffer containing 1 M urea at 7.7 V/cm for 3.5 h at 4 °C. The reaction mixture can be loaded directly onto the gel without addition of loading buffer. The 1 M urea in the gel and running buffer reduces interaction of complexes with the agarose and improves resolution.
- 8 Dry the gel (e.g. SpeedGel Drier (Thermo Scientific) at 60 °C for 2 h) and image by PhosphorImager (e.g. Fuji BAS-2500 PhosphorImager) or expose to X-ray film.

2.3.2. Modifications to trap the SSC—The SSC is a transient intermediate on the integration reaction pathway. The stable complexes formed in the above protocol are mainly STCs, which are product complexes containing a pair of viral DNA ends and target DNA stably associated with integrase (Figs. 1 and 3A). Several strategies are available to trap and accumulate the intermediate SSC complex by preventing it from forming the STC and completing integration.

- a. Substitute the blunt-end viral DNA substrate with a pre-cleaved DNA substrate containing a terminal 3'-deoxyadenosine (see protocol in Section 2.1.2). This substrate is blocked at the DNA strand transfer step because it lacks the 3'-hydroxyl of the adenosine that serves as the nucleophile for strand transfer (Fig. 3B).
- b. Substitute an active site mutant integrase for wild-type integrase and use a viral DNA substrate with a pre-cleaved substrate. Integrase containing the substitution of glutamine for glutamate-152 (E152Q) [17] is suitable and can be purified using the same procedure as for wild type integrase.
- c. Block the DNA strand transfer step with a selective inhibitor of DNA strand transfer, such as Raltegravir [18].

2.3.3. Selection of viral DNA substrates and integrase—A major viral DNA substrate requirement for assembly of the SSC and STC, and for concerted integration of pairs of DNA ends, is that it be longer than about 300 bp; as the length is further reduced the efficiency of product formation drops off precipitously [11]. The viral DNA sequence requirements do not extend beyond 10–20 bp and there appears to be no sequence specificity for the flanking DNA. The mechanistic basis of this requirement for “long” DNA substrate is unknown and is a question we are attempting to address. Recent results demonstrate that increasing the concentration of “short” viral substrates to that of integrase in the reaction significantly affects the efficiency of concerted foamy viral DNA integration *in vitro* [19].

SSCs can be efficiently assembled with pre-cleaved viral DNA ends and these complexes go on to form STCs. Therefore processing of the viral DNA ends by integrase does not appear to play a role in SSC assembly. However, *in vitro*, integrase can “by-pass” normal complex assembly and catalyze one-end integration without the formation of stable complexes. This more promiscuous pathway tends to be favored with pre-cleaved substrates [11]. SSCs and STCs can be assembled with wild type, F185K, F185H, or W235F integrase. The efficiency differs by only a few-fold among these proteins but is generally higher with W235F and wild type integrase than with the F185K and F185H mutants.

3. Concluding remarks

Integrase stably pairs the viral DNA ends within the PIC and it is this complex that is the target of inhibitors such as Raltegravir, rather than free integrase protein. Ultimately high-resolution structural information on the SSC will be required to understand the detailed mechanism of action of this and other classes of integrase inhibitors and mechanisms of drug resistance. Unfortunately, the low abundance of PICs in infected cell extracts does not permit even low-resolution biophysical studies of complexes of integrase and viral DNA isolated from this source. *In vitro* assembly of nucleoprotein complexes of integrase and viral DNA that mimics the association of integrase and viral DNA in the PIC can be expected to facilitate future biophysical and structural studies of HIV-1 integrase and its interaction with inhibitors.

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References

1. Brown, PO. Retroviruses. Coffin, JM.; Hughes, SH.; Varmus, HE., editors. Cold Spring Harbor Laboratory Press; 1997. p. 161-203.

2. Craigie, R. Mobile DNA II. Craig, NL.; Craigie, R.; Gellert, M.; Lambowitz, AM., editors. ASM Press; Washington, DC: 2002. p. 613-630.
3. Brin E, Yi JZ, Skalka AM, Leis J. J Biol Chem. 2000; 275:39287–39295. [PubMed: 11006285]
4. Yoder KE, Bushman FD. J Virol. 2000; 74:11191–11200. [PubMed: 11070016]
5. Bushman FD, Fujiwara T, Craigie R. Science. 1990; 249:1555–1558. [PubMed: 2171144]
6. Sherman PA, Fyfe JA. Proc Natl Acad Sci USA. 1990; 87:5119–5123. [PubMed: 2164223]
7. Engelman A, Mizuuchi K, Craigie R. Cell. 1991; 67:1211–1221. [PubMed: 1760846]
8. Chaconas G. Biochem Cell Biol. 1999; 77:487–492. [PubMed: 10668626]
9. Hindmarsh, P.; Leis, J. Advances in Virus Research. Vol. 52. 1999. p. 397-410.
10. Sinha S, Pursley MH, Grandgenett DP. J Virol. 2002; 76:3105–3113. [PubMed: 11884535]
11. Li M, Craigie R. J Biol Chem. 2005; 280:29334–29339. [PubMed: 15958388]
12. Sinha S, Grandgenett DP. J Virol. 2005; 79:8208–8216. [PubMed: 15956566]
13. Li M, Mizuuchi M, Burke TR, Craigie R. EMBO J. 2006; 25:1295–1304. [PubMed: 16482214]
14. Pandey KK, Bera S, Zahm J, Vora A, Stillmock K, Hazuda D, Grandgenett DP. J Virol. 2007; 81:12189–12199. [PubMed: 17804497]
15. Masuda T, Kuroda MJ, Harada S. J Virol. 1998; 72:8396–8402. [PubMed: 9733892]
16. Craigie, R.; Hickman, AB.; Engelman, A. HIV: A Practical Approach. Karn, J., editor. Vol. 2. Oxford University Press; New York: 1997. p. 53-71.
17. Engelman A, Craigie R. J Virol. 1992; 66:6361–6369. [PubMed: 1404595]
18. Cocohoba J, Dong BJ. Clin Ther. 2008; 30:1747–1765. [PubMed: 19014832]
19. Valkov E, Gupta SS, Hare S, Helander A, Roversi P, McClure M, Cherepanov P. Nucleic Acids Res. 2009; 37:243–255. [PubMed: 19036793]

Fig. 1.

Stable nucleoprotein complexes on the HIV-1 DNA integration reaction pathway. Under appropriate reaction conditions a tetramer of integrase and a pair of viral DNA ends form a highly stable nucleoprotein complex, the stable synaptic complex (SSC), that is an intermediate on the reaction pathway. Integrase then catalyzes the concerted integration of the pair of viral DNA ends in the SSC to form the strand transfer complex (STC) in which the integration product remains stably associated with integrase. Deproteinization of the STC yields the integration product.

Fig. 2. Generation of viral DNA substrates. (A) 32 bp of HIV-1 U5 terminal DNA sequence was cloned into a derivative of pCR 2.1 (Invitrogen) to generate pSca355. The HIV-1 U5 sequence is depicted by the arrowhead. Cleavage with ScaI and HincII liberates a 1513 bp fragment terminating with the blunt-end U5 terminal sequence. (B) Cleavage of the 1513 bp fragment by BanI generates the 984 bp fragment (1 kb blunt-end viral DNA substrate). (C) Generation of pre-cleaved viral DNA substrate terminating with 3'-dideoxyadenosine. Dideoxyadenosine is incorporated on the "pre-cleaved" strand oligonucleotide using terminal deoxynucleotidyl transferase (TdT). This strand is then annealed with its complement to make a duplex oligonucleotide terminating with precleaved viral DNA sequence with a dideoxyadenosine at one end and an EcoRI site at the other end. Finally the oligonucleotide duplex is ligated to a 970 bp EcoRI to PstI fragment of pML10 (derived from pCR2.1 (Invitrogen)).

Fig. 3.

Detection of the SSC and STC by agarose gel electrophoresis. (A) Electrophoresis of stable complexes of HIV-1 integrase and DNA. The identity of the complexes is depicted on the right of the gel. At the end of the time course, most of the stable complexes are STCs. The SSC appears at earlier time points but is chased to the STC by later time points. In addition to the complexes shown in Fig. 1, there is an STC species that migrates at approximately 4.5 kb resulting from an SSC utilizing another viral DNA end molecule, rather than the circular DNA, as the target for integration. (B) Trapping the SSC by using a pre-cleaved viral DNA substrate with a 3'-dideoxyadenosine. Same as panel A, except a pre-cleaved viral DNA substrate with a 3'-dideoxyadenosine was used. DNA strand transfer is blocked and the SSC accumulates. The additional bands that form a ladder at later time points result from self-association of SSCs into higher order entities.