Cooperation between Reverse Transcriptase and Integrase during Reverse Transcription and Formation of the Preintegrand Complex of Ty1

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Reverse transcriptase (RT) and integrase (IN) play a central role in the replication and transposition of retroelements. Increasing evidence suggests that the interaction between these two enzymes is functional and plays an important role in replication. In the yeast Saccharomyces cerevisiae retrotransposon Ty1, the interaction of IN with RT is critical for the formation of an active conformation of RT. We show here that the RT associated with VLPs is active only if it is in close interaction with IN. To probe the IN-RT cis-trans relationship, we have used a complementation assay based on coexpressing two transposons. We show that IN acts in cis to activate RT and that a functional integrase provided in trans is not able to complement replication and transposition defects of IN deletion or IN active-site mutant elements. Our data support a model in which IN not only interacts closely with RT during reverse transcription but also remains associated with RT during the formation of the preintegrative complex.

Two key catalytic enzymes, integrase (IN) and reverse transcriptase (RT), play a central role in the replication and transposition of retroviral and retrotransposon cDNA. Increasing evidence suggests that functional and physical interactions between these two enzymes occur during replication (15, 28, 40, 43). In some retroviruses IN is an integral part of the active enzyme; it is an α/β heterodimer composed of a short RT (α) polypeptide and an incompletely processed RT-IN (β) intermediate in the avian leukemia viruses (18, 36) and an oligomer of the α/β type in human T-cell leukemia virus type 1 (34). It has also been proposed that the active form of the yeast Saccharomyces cerevisiae retrotransposon Ty3 RT is an α/β heterodimer (27). In other retroviruses, RT and IN are fully separated by proteolytic processing during virion maturation, but mutations or deletions in the nonconserved domain of IN affect several stages of viral replication other than integration, including the initiation of reverse transcription, the level of cDNA, 3' end DNA processing, or nuclear entry (19, 28, 40, 43). Coimmunoprecipitation experiments with antibodies to RT or IN have demonstrated that the human immunodeficiency virus type 1 (HIV-1) or murine leukemia virus proteins interact physically in vitro (16, 17, 32). Recently, Hehl et al. (15) have mapped the domains of interaction of HIV-1 IN and RT in the C-terminal domain of IN and in the finger-palm domain and carboxyl-terminal half of the connection subdomain of RT. Furthermore, Zhu et al. (43) have shown that mutation of an N-terminal Cys residue of HIV-1 IN (C130S) that disrupts the interaction between IN and RT abolishes the ability of the virus to initiate endogenous reverse transcription.

In the yeast Saccharomyces cerevisiae retrotransposon Ty1, interactions between IN and RT are also important for the function of RT (37, 39). In vitro, an active Ty1 recombinant protein can be obtained only if amino acid residues encoded by the C-terminal region of IN are fused to the N-terminal domain of RT (37). In vivo, the IN and RT proteins of Ty1 are expressed and assembled in the virus-like particles (VLPs) as part of a large Gag-Pol-p199 precursor protein (1, 14, 22, 23, 42). After assembly of the VLPs, the Gag-Pol precursor is processed by the pol-encoded protease to liberate the mature Gag-p45, PR-p20, IN-p71, and RT-RH-p63 proteins. The main RT species identified in Ty1 VLPs is a mature p63 protein (63 kDa), but Ty1 RT retains its activity when it is fused to the IN domain in PR-IN-RT and IN-RT fusion proteins (22) or in the Gag-Pol-p199 precursor (42). In a recent study we used IN deletion mutants to investigate the role of IN on RT activity in Ty1 VLPs (39). We identified two domains in the C-terminal region of the Ty1 integrase that affect RT activity in vivo. Deletion of a domain spanning amino acid residues 233 to 520 increases the exogenous specific activity of RT up to 20-fold, whereas removal of a domain rich in acidic amino acid residues between residues 521 and 607 decreases its activity.

We have suggested that interaction between the acidic residues of IN and a basic domain in the N-terminal region of RT could be critical for its correct folding and for the formation of an active conformation of the enzyme. Folding of a protein is a hierarchical process, and the secondary structure of a protein is determined primarily by interaction among amino acid residues that are close in sequence (30). The order of the domains encoded within the pol gene of Ty1 is protease-integrase-RT (PR-IN-RT); thus, it is possible that during synthesis of the Gag-Pol-p199 precursor, the presence of the C-terminal region of IN which is synthesized before RT is necessary in cis to induce proper folding of the RT by facilitating interaction among amino acid residues that are close in sequence (30). The order of the domains encoded within the pol gene of Ty1 is protease-integrase-RT (PR-IN-RT); thus, it is possible that during synthesis of the Gag-Pol-p199 precursor, the presence of the C-terminal region of IN which is synthesized before RT is necessary in cis to induce proper folding of the RT by facilitating interaction between the acidic domain of IN and the basic domain of RT. To probe the IN-RT cis-trans relationship, we used a complementation assay based on coexpressing two transposons. We show here that the C-terminal domain of IN acts in cis to activate RT. A functional integrase provided in trans is not able to rescue the replication and transposition defects of IN deletion or IN active-site mutant elements. Our data support a model in which IN not only interacts closely with RT during
reverse transcription but also remains associated with the RT during formation of the preintegrative complex (PIC).

MATERIALS AND METHODS

Yeast strains and plasmid DNA. Yeast strain LV47 (MATa ura3ΔΔ51 trp1ΔΔ63 his3ΔΔ200 spo3-10 GAL+) was kindly provided by P. Lesage (33). Yeast strain YH50 (MATa ho ura3-167 trp1Δ1 leu2-3 his3ΔΔ200 spo3-202) and the isogenic rad52Δ:LEU2 strain AGY49 were kindly provided by A. Gabriel. All strains are spt3Δ; they lack a transcription factor required for normal expression of genomic Ty1 elements. This eliminates endogenous Ty1 transcription and thus potential trans-complementation of plasmid-born elements.

pGTy1-H3mHIS3AI and pJEFl105 plasmids were kindly provided by D. Garfinkel and J. Boeke. The pGTy1-H3mHIS3AI plasmid contains the Ty1-H3 element, the expression of which is under the control of the inducible GAL1 promoter. It is marked with a HIS3 reporter gene in the antisense orientation. An artificial intron (AI) in the sense orientation has been inserted in the HIS3 gene. The pJEFl105 plasmid contains the Ty1-H3 element, the expression of which is under the control of the inducible GAL1 promoter. It is marked with a neo gene.

Ty1 VLP purification. Ty1 VLPs were isolated from yeast strain LV47 cotransformed with a TRP1-based pGPo(−)-hisAI (an RT active-site mutant of pGTy1-H3mHIS3AI) and the URA3-based wild-type plasmid pJEFl105 (pJEFlneo) (8) or IN mutant versions of this plasmid (pJEFlINneo or pJEFlMutINneo).
Proteins are protected from proteolytic degradation, probably because proteases do not gain access to the interior of the VLPs (5). In this case, the RT and Gag proteins cofractionate with the RT activity.  

DNA oligonucleotides and labeling. The DNA oligonucleotides were purchased from Thermo Electron (Ulm, Germany) and labeled at their 5' termini with [32P] using [γ-labeled]ATP and T4 polynucleotide kinase.  

Plasmid construction and mutagenesis. Deletion mutants of pJE1105 were made as described previously (39). The QuickChange multi-site-directed mutagenesis kit from Stratagene was used to mutagenize the active site of IN. The oligonucleotides used for generating the mutations were as follows (mutations that also created new restriction sites are indicated in boldface, and new restriction sites are underlined): 5′-CAAGACCCTTTTCATATCTACACATATTTGAGCATGTTTCACATCATACCTACACC-3′, 5′-CAAGCGCCAGTGTCTTTGTTATCTGAAATGGGTCGAGTTGCTGTTGAAAGATTCATACCTACAC-3′, and 5′-CGCGGTTCGAGTTGCTGTTGAAAGATTCATACCTACAC-3′. Mutants were identified by digestion with Spol, Xhol, or BsmHI. The TRP1-marked RT active-site mutant reporter plasmid, pGTY1-H3mHIS3AI, was constructed by subcloning a PvuII-AflII-digested fragment from AGH64 (35) bearing the RT active-site mutation into similarly digested pGTY1-H3mHIS3AI plasmid. Since PvuII-AflII digestion removed a 3.47-kb PvuII-PvuII fragment, the resulting plasmid was digested with PvuII and ligated to the PvuII-PvuII fragment. The resulting plasmid with the correct orientation of the PvuII fragment was digested with Eagl-Apal and ligated to a similarly digested fragment containing the TRP1 gene.  

Iterative primer extension. To detect minus-strand and plus-strand strong-stop DNA replication intermediates, iterative primer extension was carried out with 5’-labeled strand-specific oligonucleotides (38). Nucleic acids extracted from VLPs were incubated with the labeled primer in a 20-µl volume using a PCR buffer (10 mM Tris-HCl [pH 8.8], 50 mM KCl, 0.1% Triton X-100, 2 mM MgCl2), along with 2 U of Taug polymerase. Primer extension products were generated by 30 cycles of denaturation (30 s at 95°C), annealing (30 s at 46°C), and extension (60 s at 72°C). At the end of the reaction, 10% formamide dye was added to the reaction mixture. Products were denatured by heating at 90°C for 2 min prior to loading them on an 8% polyacrylamide–8 M urea denaturing gel and then analyzed by phosphorimaging. The primers used to detect ssDNA and dsDNA were 5′-CAATCCTTGCGTTTCAGCTTCCCAATCCTTGCGTTTCAGCTTCCCAATCCTTGCGTTTCAGCTTCCCAATCCTTGCGTTTCAGCTTCCCAATCCTTGCGTTTCAGCTTCCCAATG-3′ (complementary to Ty1-H3 positions 5716 to 5697) and 5′-GGAGAACCTTCTTAGTATATCTGCATACC-3′ (Ty1-H3 positions 243 to 270 or 5827 to 5854).  

Southern blot analysis. Ty1 VLPs were prepared from 600-ml cultures and purified on sucrose step gradients as described by Eichinger and Boeke (8). The VLPs were concentrated by centrifugation at 40,000×g of VLP DNA was digested with EcoRI and the DNA fragments were separated by agarose gel electrophoresis. The resulting DNA fragments were isolated using a GeneClean kit from StrataGene, ligated to a similarly digested fragment containing the TRP1 gene, and resuspended in 75 µl of TE. DNA was quantitated by spectrophotometry (NanoDrop ND-1000). A total of 10 µg of VLP DNA was digested with EcoRI or EcoHII (BstEI) and separated on 1% agarose gel. The nucleic acids were blotted onto a Hybond N+ membrane (Amerham) and probed either with a Xhol-HindIII fragment of the Tn903 neo gene or a SacI-Nhel HIS fragment of the pGTY1-H3mHIS3AI plasmid. The probes were labeled with [α-32P]dGTP by random priming with the Megaprime DNA labeling system of Amerham. Hybridization of the probe was detected with a phosphorimager screen.  

Transposition assay. Yeast cells were grown at 30°C overnight in yeast nitrogen base medium without amino acids (YNB) supplemented with 2% glucose (wt/vol) and the required amino acids. Cells were washed in water and diluted in YNB medium supplemented with 2% galactose (wt/vol) and the required amino acids and then grown for 2 days at 22°C to induce transposition. Aliquots were plated onto YNB glucose plates without histidine at 30°C to end the transposition induction and to determine the fraction of His+ prototrophs. The cultures were titrated on YPD plates. For the qualitative transposition assay, cells with an optical density at 600 nm of 1 to 1.5 were diluted 10-fold. Then, 10 µl of each dilution was spotted onto YNB glucose plates without histidine and incubated for 2 days at 30°C.  

RESULTS

An active Ty1 RT can be obtained from VLPs provided the interaction between RT and IN is maintained. Although RT activity can be detected associated with VLPs in yeast cells overexpressing the Ty1 element, it has not been possible to use...
VLP preparations as a source of active purified RT. The main RT species identified in Ty1 VLPs is a mature 63-kDa protein generated by proteolytic processing of the Gag-Pol-p199 precursor at the IN/RT cleavage site. The fact that Ty1 RT is active within the VLPs, where it is in close contact with other components of the VLPs, but is inactivated after dissociation of the VLPs suggests that interactions of RT with other VLP components play a role in the activity of RT. As discussed in the introduction, IN is one of the VLP components critical for reverse transcription, and it probably interacts physically with RT (39). To test whether the close association between the two proteins is necessary for the activity of RT, we have blocked the cleavage site between IN and RT by substituting the wild-type IN/RT protease cleavage site (PCS) LIAAVK by AAGSAA (22) so that the two proteins are maintained together after dissociation of the VLPs. It was shown previously by Merkulov et al. (22) that mutations blocking the IN/RT PCS do not affect cleavage at the other sites (Gag/PR or PR/IN). We introduced the IN/RT cleavage site mutation in a wild-type element carrying the full-length IN (pJEFPMutPCS) and in an IN deletion mutant pJEFD2173-3600 (pJEFD MutPCS) that retains the 115-amino-acid residue C-terminal domain of IN that is important for RT activity (39) but lacks residues 45 to 520 of IN spanning part of the N-terminal region of IN containing the conserved zinc-binding sequence HHCC and the central core domain with the conserved active-site motif DDE (Fig. 3A). VLPs were purified from cells expressing these elements and fractionated on sucrose step gradients. The exogenous RT activity on fractions from the density gradient was determined in an oligo(dG)/poly(rC) primer-template assay by measuring the incorporation of 32P-radiolabeled dGTP into high-molecular-weight poly(dG) (Fig. 3B). A high level of RT activity was observed with the wild type and the pJEFD MutPCS element, whereas it was lower for the pJEFP MutPCS element containing a full-length IN protein (Fig. 3B). Immunoblotting was performed on the peak fractions of wild type (WT), MutPCS, and ΔMutPCS VLPs using anti-RT antibodies. The volumes of VLPs that were found to incorporate the same amount of dGTP into high-molecular-mass poly(dG) by the oligo(dG)/poly (rC) primer-template assay were loaded onto the gel and probed with Ty1 RT antibodies. The peak fractions of WT, MutPCS, or ΔMutPCS VLPs using anti-RT antibodies were loaded onto the gel and probed with Ty1 RT antibodies. The peak fractions of WT, MutPCS, or ΔMutPCS VLPs using anti-RT antibodies were loaded onto the gel and probed with Ty1 RT antibodies. The peak fractions of WT, MutPCS, or ΔMutPCS VLPs using anti-RT antibodies were loaded onto the gel and probed with Ty1 RT antibodies. The peak fractions of WT, MutPCS, or ΔMutPCS VLPs using anti-RT antibodies were loaded onto the gel and probed with Ty1 RT antibodies.

FIG. 3. (A) Schematic representation of the Gag-Pol polyprotein of the WT element, the IN/RT protease cleavage site (PCS), mutant element and the IN deletion mutant element bearing an IN/RT PCS mutation. The sequences of the Gag/IN, PR/IN, IN/RT, and mutant IN/RT PCS are indicated. The endpoints of the deletion are indicated by vertical dotted lines at positions 2173 and 3600. These numbers are the Ty1-H3 positions (2) and correspond to amino acid residues 45 and 521 of the IN protein, which contains 635 amino acids. (B) VLPs were prepared from cells expressing these elements and purified on a sucrose step gradient. RT activity was assayed using an oligo(dG)/poly(rC) primer template. 100% incorporation is the label in the peak fraction (fraction 5) of WT VLPs. It is equal to 3 to 7 pmol of dGTP incorporated in 30 min for 4 μl of sucrose gradient-purified VLPs. (C) Immunoblot analysis. The volumes of VLPs that were found to incorporate the same amount of dGTP into high-molecular-mass poly(dG) by the oligo(dG)/poly(rC) primer template assay were loaded onto the gel and probed with Ty1 RT antibodies. (D) The RT activity of the native or salt-treated VLPs was tested on an exogenous primer-template. The percent activities of salt-treated VLPs relative to native VLPs are indicated.
ing the pJEFMutPCS element was not as good (29%). Since we have observed (unpublished results) that a recombinant 145-kDa IN/RT fusion protein was not very soluble, it is possible that the low recovery of RT activity of salt-treated Mut-PCS VLPs is due to a poor solubility of the protein in the experimental conditions used to test the RT activity.

Overall, we can conclude from these results that RT associated with VLPs remains active if it is maintained in close association with IN. This is in line with the observation that an active recombinant protein can be expressed in Escherichia coli only as a fusion protein with amino acid residues encoded by the C-terminal domain of IN fused to the N-terminal domain of RT (37) and confirms that interaction between RT and IN is functional and critical for the polymerase activity of RT (39).

A full-length IN does not trans complement the reverse transcription defect of IN deletion or active site mutant in vivo. In a previous study we used IN deletion mutants to investigate the role of IN on RT activity in Ty1 VLPs (39). The deletion mutants retained exogenous polymerase activity in a homopolymer primer-template assay and were able to synthesize minus-strand strong-stop DNA (−ssDNA) in vivo, but they failed to synthesize plus-strand strong-stop DNA (+ssDNA). The lack of +ssDNA synthesis was not due to the inhibition of RNase H activity, which generates the polyuridine tract primer for plus-strand synthesis, but was attributed to a defect in minus-strand strong-stop DNA transfer from the 5′ end to the 3′ end of the Ty1 RNA (39). To test whether a full-length IN provided in trans can rescue the strand transfer defect of the IN deletion mutants, we used a complementation assay based on coexpressing two transposons (Fig. 1). A TRP1-based reporter plasmid, pGPol(−)hisAI (a derivative of pGTy1-H3mHIS3AI [7]; see Materials and Methods) bearing a Ty1 element with the D211E active-site mutant of RT (35) was transformed with a Ty1 plasmid. VLPs purified from these cells had the same RT activity shown in Fig. 4, His+ prototrophs were generated in this control experiment. To control that the His+ prototrophs were generated by inte-grase activity independently of homologous recombination (31), we have repeated the transposition assay in a rad52 strain (AGY49) incapable of homologous recombination. His+ prototrophs were also generated in this strain (Fig. 4B), indicating that transposition in the cells transformed by the pJEFWTneo and the pGPol(−)hisAI plasmids indeed occurred by integrase activity independently of homologous recombination. Furthermore, this result indicates that the active site mutation of RT is recessive and does not inhibit reverse transcription of the Ty1 element by the active RT encoded by the pJEFWTneo element. This is in agreement with previous studies showing that GAL-Ty1 elements with mutations in IN or RT are complemented at a low level by genomic Ty1 elements in SPT3+ strains (6). The production of HIS3+ cells was decreased 30- to 40-fold in cells cotransformed with pGPol(−)hisAI and pJEFWTneo plasmids compared to a yeast strain expressing a wild-type Ty1-HIS3 element that has a transposition frequency of 1.2 × 10−3 (Fig. 4A). This is in part due to the fact that HIS3 cDNA can be synthesized only in VLPs containing one or two molecules of the Ty1-HIS3 transcripts and also because the VLPs contain an inactive RT derived from the pGPol(−)hisAI plasmid which probably competes with the active enzyme for binding to the primer that initiates reverse transcription. We next checked whether yeast cells transformed with the reporter pGPol(−)hisAI plasmid and pJEFPneo plasmids containing different kinds of IN deletions were able to generate His+ prototrophs (Fig. 4). In contrast to the control experiment, none of these combinations were able to generate His+ prototrophs. Since recombination between plasmids is a concern in these experiments, this result shows that wild-type Ty1 elements were not generated by recombination between the two mutant plasmids. To determine whether the mutant VLPs containing the IN deletions were able to synthesize Ty1 replication intermediates, an iterative primer extension assay (Materials and Methods) was carried out to detect minus- and plus-strand strong-stop DNA. As shown in Fig. 5A and B, the two strong-stop DNAs were detected in control VLPs. In VLPs containing the Δ2173-3600 deletion, only minus-strand strong-stop DNA was detected. A cDNA synthesis assay developed for the fission yeast Schizosaccharomyces pombe (21) and adapted for Ty1 by Merkulov et al. (22) confirmed that double-stranded cDNA was synthesized in the control VLPs and that VLPs containing the IN deletion mutant pJEFA2173-3600 failed to make cDNA (Fig. 5C). Therefore, the full-length IN protein supplied in trans was unable to complement the strand transfer defect of IN deletion mutants. This suggests that the N-terminal domain and/or the central domain containing the active site of IN are involved in strand transfer.

We next checked whether the active site of IN plays a role in strand transfer. The conserved aspartate and glutamate residues of the active site (DD35E) of IN were replaced with alanine, and yeast cells were cotransformed with this IN active-site mutant element (pJEFMutINneo) and with the reporter pGPol(−)hisAI plasmid. VLPs purified from these cells had the same RT activity
FIG. 4. (A) Schematic representation of the Gag-Pol polyproteins expressed in yeast cells cotransformed with the pGPOL(-) plasmid and the WT pJEFl0105 plasmid (pJEFlWneo), the IN deletion mutant derivatives of pJEFl0105 (pJEFl2173-3600HRB, pJEFl2173-3928HRB, and pJEFl2173-3855HRB), or the IN active-site mutant of pJEFl0105 (pJEFlMutIN). The deletions were filled in by inserting the yeast gene Hrb1 (gray rectangle) in order to avoid steric hindrance between the PR/IN and IN/RT cleavage sites and allow complete processing of the Gag-Pol polyprotein (37). In a previous study we showed that the deletion mutants 2173-3600 and 2173-3855 retained RT activity. The level of RT activity in 2173-3855 VLPs was low compared to that of WT or 2173-3600 VLPs. Transposition was induced at 22°C in a medium supplemented with 2% galactose and ended at 30°C in a medium containing 2% glucose. The fraction of His—prototrophs was determined to calculate the frequency of transposition. The transposition frequency of a positive control with yeast cells containing the WT pGTy1-H3mHIS3AI plasmid was 1.2 $10^3$. Transposition of yeast cells cotransformed with pGPOL(-) and WT pJEFl0105 plasmids is decreased 30- to 40-fold (see text). Synthesis of -sssDNA and +sssDNA was determined by iterative primer extension. (B) Qualitative transposition assay in yeast strains LV47, YH50, or AGY49. A total of 10 μl of induced cells with an optical density at 600 nm of 1 to 1.5 and 10 μl of a 10-fold dilution were spotted onto glucose plates without histidine and incubated for 2 days at 30°C. A positive control with the WT pGTy1-H3mHIS3AI plasmid and a negative control with the pGPOL(-)hisAI plasmid are shown.
Fig. 5. (A and B) Iterative primer extension analysis of Ty1 replication intermediates in the VLPs. 5'-end-labeled primers complementary to -sssDNA (A) or +sssDNA (B) were annealed to VLP-derived nucleic acids and repeatedly extended with Taq polymerase. The extension products were loaded on an 8% denaturing polyacrylamide gel and mapped with sequence ladders as size markers. The asterisks mark the positions where the -sssDNA and +sssDNA should map. PBS, primer binding site; PPT, polypurine tract. (C) Southern blot analysis of cDNA produced in yeast cells transformed with mutant elements. VLP-extracted nucleic acids were digested with EcoRI and RNase A, separated on a 1% agarose gel, and transferred onto a Hybond N+ membrane (Amersham). The membrane was probed with a XhoI-HindIII fragment of the Tn903 neo gene 32P labeled by random priming. Equal amounts of DNA were loaded in all lanes. The EcoRI VLP cDNA fragment containing the neo gene migrates at 2.9 kbp. M, marker obtained by digesting the pJEFl105 plasmid with SmaI. The SmaI fragment containing the neo gene migrates at 3.3 kbp.

(Fig. 6A) and contained the same amount of RT, IN, and Gag as the VLPs purified from control cells containing the reporter plasmid pGPol(-)hisA1 and the pJEFW7neo plasmid (Fig. 6B). Southern blot analyses showed that equivalent amounts of double-stranded cDNA were synthesized in these cells (Fig. 6C). Therefore, mutation of the active site of IN does not impair DNA synthesis. The surprising observation was that transposition was inhibited in spite of cDNA synthesis. This cannot be attributed to a dominant effect of the active-site mutation in IN since Moore et al. (26), using a similar intragenic complementation test, showed that IN active-site mutations were recessive.

DISCUSSION

Experiments in this report suggest that IN and RT form a stable complex and do not dissociate during the entire Ty1
reverse transcription cycle. To explain our result, we propose a model of Ty1 replication (Fig. 7) in which the IN and RT domains of the Gag-Pol precursor remain tightly associated after maturation of the precursor and during the whole replication cycle. All of the steps of reverse transcription are then performed by an IN/RT complex containing an active RT. In the last step of replication, plus-strand and minus-strand DNA syntheses are completed simultaneously by two IN/RT complexes. After completion of DNA synthesis, the two IN/RT complexes are poised for the formation of the PIC. If the IN/RT complexes that have performed replication carry an inactive IN, as in yeast cells cotransformed with the pJEFMutINneo and the pGPol(−)hisAI plasmid, the PIC that is transported to the nucleus would be incapable of integrating the Ty1 cDNA into the host cell DNA, and this would explain the lack of transposition. Our model is not in contradiction with the intragenic complementation observed by Moore et al. (26) using catalytic and NLS defective IN mutants because in cells where these mutants are coexpressed, the IN/RT complexes both carry an active RT that can participate in replication. Therefore, PICs containing a dimer (or multimer) with NLS(−) and IN(−) proteins can be formed and translocated to the nucleus by the NLS function of the catalytically inactive IN molecule, while the catalytic activity of the NLS mutant molecule performs the integration reaction.

In the scheme presented in Fig. 7, one IN/RT complex is
postulated to bind stably to each end of the cDNA to form the core of the PIC. The intragenic complementation experiments of Moore et al. (26) also suggested that IN proteins could dimerize (or multimerize) and form a PIC on a given cDNA molecule. Tight binding of Ty1 RT to the ends of the primer-template was recently inferred by Pandey et al. (29) from in vitro studies with a recombinant enzyme. These authors have shown that Ty1 RT binds its primer-template much more strongly than either avian myeloblastosis virus or Moloney murine leukemia virus RTs and have speculated that stable dimerization of the nuclear PIC of Ty1 would permit to determine whether RT remains closely associated with IN after entry of the PIC into the nucleus or whether, given that IN does not require other proteins to catalyze correct two-ended integration (25), RT and IN are dissociated at some stage before entry of the PIC in the nucleus.

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