Reversion of the Lethal Phenotype of an HIV-1 Integrase Mutant Virus by Overexpression of the Same Integrase Mutant Protein*

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Stéphane Priet‡, Jean-Marc Navarro, Gilles Querat, and Joséphine Sire§

From the Pathogénie des Infections à Lentivirus, INSERM U372, 163 Avenue de Luminy, BP 178, 13276 Marseille-Cedex 9, France

The human immunodeficiency virus type 1 (HIV-1) integrase (IN) is essential for integration of viral DNA into host cell chromatin. We have reported previously (Priet, S., Navarro, J. M., Gros, N., Querat, G., and Sire, J. (2003) J. Biol. Chem. 278, 4566–4571) that IN also plays a role in the packaging of the host uracil DNA glycosylase UNG2 into viral particles and that the region of IN encompassing residues 170–180 was responsible for the interaction with UNG2 and for its packaging into virions. In this work, we aimed to investigate the replication of HIV-1 viruses rendered deficient in virion-associated UNG2 by single or double point mutations in the region 170–180 of IN. We show that the L172A/K173A IN mutant virus was deficient for UNG2 packaging and was defective for replication because of a blockage at the stage of viral DNA integration in host cell DNA. In vitro assays using long term repeat mimics, however, demonstrate that the L172A/K173A IN mutant was catalytically active. Moreover, trans-complementation experiments show that the viral propagation of L172A/K173A viruses could be rescued by the overexpression of Vpr-L172A/K173A IN fusion protein in a dose-dependent manner and that this rescue is independent of UNG2 packaging. Altogether, our data indicate that L172A/K173A mutations of IN induce a subtle defect in the function of IN, which nevertheless dramatically impairs viral replication. Unexpectedly, this blockage of replication could be overcome by forcing the packaging of higher amounts of this same mutated integrase. This is the first study reporting that blockage of the integration process of HIV-1 provirus carrying a mutation of IN could be alleviated by increasing amounts of IN even carrying the same mutations.

HIV-11 integrase (IN) is an essential component required for the persistence of infection in vivo. IN is incorporated into viral particles as a part of the Gag-Pol precursor polyprotein that contains Gag (matrix, capsid (CA), nucleocapsid NCp7, and p6) and Pol (protease, reverse transcriptase, and IN) domains. Each of these domains is expressed individually upon proteolytic processing of the precursor by the viral protease. The functional role of IN is to catalyze the integration of the viral genome into the cellular genome through a two-step process that includes processing of the viral LTR ends and integration (reviewed by Ref. 1). The processing step removes the terminal TG dinucleotide at each 3′-end of the proviral genome generating recessed 3′-ends. The integration step allows the nucleophilic attack of the host DNA by the recessed 3′-OH group in a trans-esterification reaction.

Mutational analysis of IN identified distinct functional domains within the protein. The N-terminal domain (residues 1 to 50) contains a highly conserved HHCC motif that binds zinc (2, 3, 4). The catalytic core domain spans residues 51–212 and contains the invariant residues Asp-64, Asp-116, and Glu-152 (5, 6, 7, 8, 9). The C-terminal domain (residues 213–288) binds to the viral DNA and also possesses nonspecific DNA binding properties (10, 11, 12, 13, 14). Genetic studies have indicated that mutations in IN affect multiple and distinct steps in the replication cycle, including protein maturation, virion morphology, uncoating, reverse transcription, and integration per se, revealing multiple consequences of altered IN in the viral life cycle (15).

Although in vitro studies have demonstrated that IN alone is sufficient to promote the integration process (1), lines of evidence indicate that other viral and cellular proteins can regulate this process. For example, it has been reported that Ncp7 might influence the coupled joining by promoting DNA distortion (16) and that reverse transcriptase might play a role in blocking the auto-integration process (17). Host proteins, upon association with IN, have also been reported to improve in vitro viral DNA integration. The barrier-to-autointegration factor (BAF) acts as an inhibitor to the undesirable autointegration process (18), and the HMGI(Y) protein stimulates concerted viral DNA integration (19). DNA-dependent protein kinase has been reported to be involved in the completion of the integration process (20). In some cases host proteins, such as the integrase inhibitor 1 (Ini1) factor or the uracil DNA glycosylase (UNG2) enzyme, interact with IN and are specifically incorporated into viral particles (21, 22).

In a previous study, we demonstrated that leucine residue 172 of IN was important for the packaging of the host UNG2 enzyme (23). We have shown that virion-associated UNG2 plays a role similar to its cellular counterpart and participates in the correction of G:U mispairs to G:C pairs. The present study was designed to investigate whether the presence of UNG2 inside viral particles was required for efficient viral replication. We used a series of viruses mutated for each of the residues encompassing the region 170–181 of the α5 helix of IN, which is located outside of the catalytic triad of aspartic acid and glutamic acid residues, the D, D35E sequence motif. This series of mutations enabled us to classify viruses as deficient or proficient for UNG2 packaging (23). Our results show

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1 The abbreviations used are: HIV-1, human immunodeficiency virus; type 1; IN, integrase; ΔIN, IN minus; CA, capsid; LTR, long terminal repeat; UNG2, uracil DNA glycosylase; GST, glutathione S-transferase; TCID₅₀, 50% tissue culture infective dose.

‡ Recipient of a fellowship of the French Ministry of Research.
§ To whom correspondence should be addressed. Tel.: 33-491-82-75-91; Fax: 33-491-82-60-61; E-mail: jsire@inserm-u372.univ-mrs.fr.

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The three-domain structure of HIV-1 IN is depicted at the top. The sequence of the IN region of the HIV-1 AD8 isolate encompassing residues 170–181 is shown at the bottom. The position of wild-type residues substituted for alanine residues is indicated.

**FIG. 1.** Diagram of the domain structure and mutations of HIV-1 IN. The three-domain structure of HIV-1 IN is depicted at the top. The sequence of the IN region of the HIV-1 AD8 isolate encompassing residues 170–181 is shown at the bottom. The position of wild-type residues substituted for alanine residues is indicated.

**FIG. 2.** Replication kinetics of wild-type and IN mutant viruses. Viruses produced in the cell-free supernatant of 293T cells transfected with each of proviral DNAs were harvested, calibrated for similar amounts of CA p24 antigen, and used to infect Sup T1 cells. Virus production was monitored by measuring the reverse transcriptase (RT) activity in the cell-free supernatant twice a week.

The following. i) Viruses with IN mutated at residues 172/173, 174, 178, and 180/181 are impaired for replication. ii) The failure to replicate of viruses with mutations at residues 174, 178, and 180/181, but not of those with mutations at residues 172/173, correlates with a loss of the in vitro catalytic activity of IN. iii) Although IN with mutation at residues 172/173 still retains its enzymatic activity, the integration process of L172A/K173A viruses is abrogated in the context of infection. iv) Finally, overexpression in trans of VPR-L172A/K173A IN fusion protein rescues, in a dose-dependent manner, the replication of L172A/K173A viruses, indicating that the defect of cis IN mutated viruses was compensated by increasing amounts of mutated IN. Altogether, our data point out the importance of leucine residue 172 in the viral integration process and Ung2 packaging.

**MATERIALS AND METHODS**

**Plasmid Construction**—The construction of IN mutated viruses has been described previously (23). DNA fragments each containing IN mutations were also PCR amplified and cloned into pGEX 5X2 (Amersham Biosciences) in order to generate plasmids containing 20 mM Hepes, pH 7.2, 1 mM diethiothreitol, and 10 mM MnCl2. Additions of 20 mM EDTA, and DNA products were recovered by ethanol precipitation in the presence of 10% formamide loading dye. The samples were visualized by autoradiography. Where indicated, increasing amounts (5, 25, and 100 nM) of GST wild-type IN and GST-L172A/K173A IN were used in the assay.

**Plasmid Construction**—The construction of IN mutated viruses has been described previously (23). DNA fragments each containing IN mutations were also PCR amplified and cloned into pGEX 5X2 (Amersham Biosciences) in order to generate plasmids containing IN fusion proteins. For trans-complementation experiments, the pLR2P-Vpr-IN expression vector kindly provided by J. Kappes (24) was used. Each of the IN mutations was introduced into this plasmid by PCR procedures in order to generate pLR2P-Vpr-IN mutants. The entire sequence of each mutated IN was checked by DNA sequencing. In the pLR2P-Vpr-IN vector, the Vpr gene was also mutated (W54G) to prevent any interaction between Vpr and Ung2, and the vector was used to construct Vpr-W54G-IN(L172A/K173A) double mutant fusion. As a control, a Vpr-only expression vector was constructed by introducing a stop codon at the end of the Vpr reading frame in the pLR2P-Vpr-IN vector.

**Cell Lines, Transfection, and Infection**—Human 293T cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with antibiotics and 10% fetal calf serum. Viral stocks were produced by transfection of each of the proviral DNAs into sub-confluent 293T cells with the FuGENE 6 transfectant reagent (Roche Applied Science) according to the manufacturer’s protocol. For viral replication studies, SupT1 cells were infected with viral stocks obtained from the cell-free supernatant of transfected 293T cells and calibrated for equivalent amounts of CA p24 antigen by HIV-1 p24 antigen capture assay kit (Coulter). Propagation of viral infection was followed by measuring the reverse transcriptase activity in cell-free supernatants twice a week. For trans-complementation studies, provirus expression plasmids were cotransfected in 293T cells at a ratio of 2:1 with pLR2P-Vpr-IN expression vectors. Viral supernatants were then calibrated for similar amounts of CA p24 antigen by an HIV-1 p24 antigen capture assay kit. Serial 5-fold dilutions were used to infect the C8166 indicator cell line. The infectivity of trans-complemented viruses was calculated by the Reed-Muench method (25) to determine the 50% tissue culture infective dose (TCID50). Expression of Vpr-IN fusion proteins in trans-complemented viruses was analyzed by Western blot with rabbit polyclonal anti-IN antibody (a gift from D. Trono). Similar amounts of trans-complemented viruses were used as judged by the level of CA p24 antigen in the viral lysate revealed by Western blot with sheep polyclonal anti-p24 antibody (Aalto Bio Reagents, Rathfarimd-Dublin, Ireland). The presence of Ung2 was revealed by Western blot using anti-Ung2 antibody kindly provided by G. Slupphaug.

**Purification of Viruses**—Virion particles released in the cell-free supernatant of transfected cells were collected by ultracentrifugation and highly purified through 8–18% Optiprep density gradient as described (26). Gradient fractions coinciding with the peak of the reverse transcriptase activity were pooled and normalized for equivalent amounts of the CA p24 antigen. Viral lysate was obtained by lysis of purified virions in TNE buffer (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 1 mM EDTA) in the presence of 0.2% of Triton X-100.

**In Vitro Catalytic Activity of IN—GST-IN fusion proteins were expressed in E. coli (29). GST derivatives were eluted from agarose beads in the presence of 20 mM glutathione, and the purity of proteins was estimated by Coomassie Blue staining. The amounts of GST derivatives were quantified by comparison with the bovine serum albumin (BSA) standard. The oligonucleotide pair used for processing activity measurement was 71 (5'-GGTGTTGAAAATCTTACAGGATGTC-3') and 72 (5'-ACTGCTAGAGATTTTGTTGAAAATCTCTAGCAGT-3'). The oligonucleotide pair used for strand transfer activity measurement was 70 (5'-GGTGTTGAAAATCTTACAGGATGTTC-3') and 72. Oligonucleotides 70 and 71 were 5'-end labeled with T4 polynucleotide kinase in the presence of [γ-32P]ATP and purified through Microspin G50 columns (Amersham Biosciences) before annealing to oligonucleotide 72. IN catalytic activity was assayed essentially as described (27).

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Briefly, 200 nM of each GST derivative were incubated with 10 nM of the appropriate set of oligonucleotides in a final volume of 20 μl of a buffer containing 20 mM Hepes, pH 7.2, 1 mM diethiothreitol, and 10 mM MnCl2. After an incubation of 1 h at 37 °C, the reactions were stopped by the addition of 20 μl EDTA, and DNA products were recovered by ethanol precipitation in the presence of 10 μg of tRNA carrier. DNA products of the 3′-processing and DNA strand transfer reactions were dried and dissolved in 10 μl of 95% formamide loading dye. The samples were separated on 8% urea, 15% polyacrylamide denaturing gels in 1X TBE. Gels were fixed in 10% acetic acid, and radiolabeled products were visualized by autoradiography. Where indicated, increasing amounts (5, 25, and 100 nM) of GST wild-type IN and GST-L172A/K173A IN were used in the assay.
Determination of Viral DNA Integration—Viral stocks from transfected 293T cells were filtered through 0.45-μm pore size filters and treated with DNase I (20 μg/ml) for 30 min at 37 °C in the presence of 10 mM MgCl₂. H9 cells (10⁶) were infected for 2 h by spinoculation (28) with equal amounts of IN mutant viruses; then cells were washed extensively with phosphate-buffered saline and cultured for 18 additional hours. The total DNA was isolated from the infected cells by the QIAamp DNA Mini Kit protocol (Qiagen) and eluted with 200 μl of elution buffer. Viral DNA products corresponding to the different steps of retrotranscription were assessed by quantitative real-time PCR as described in literature (29). Real-time PCR was performed with an ABI PRISM 7000 apparatus (Applied Biosystems) using PCR primers and TaqMan probes described previously (29). The integrated forms of proviral DNA were assessed using a two-step PCR amplification with 5'-Alu primer (5'-TCCCAGCTACTGGGGAGGCTGAGG-3') and L1 primer (5'-AGGCCAGCTTTATGAGGCTTAGGC-3') followed by nested PCR with L2 primer (5'-CTGTGGATCTCACACACACAAAGGC-3') and L3 primer (5'-GCTGCTTATATGTAGCATCTGAGGGC-3') as described (30).

RESULTS

Propagation of IN Mutant Viruses in Infected Cell Culture—In a previous report (23) we showed that residues encompassing the region 170–181 of IN were critical for host UNG2 packaging. We performed alanine-scanning mutagenesis and delineated the residues responsible for UNG2 packaging (Fig. 1). In this study we investigated the replication of UNG2-deficient or UNG2-proficient viruses. Each of the IN mutated viruses was examined for replication in infected cells. Viral stocks were produced from 293T cells transfected with each IN mutant proviral DNA. Transfected cells yielded mutated viruses at a level corresponding to 80–100% of the wild-type level, suggesting that mutations in IN did not seem to affect virion assembly and release (data not shown). Similar amounts of viruses, as estimated by CA p24 antigen measurement, were used to infect Sup T1 cells, and propagation of infection was monitored by measuring the reverse transcriptase activity in the cell-free supernatant of infected cells twice a week. As shown in Fig. 2, viruses carrying mutations at residues 170/171 or 176/177 displayed wild-type kinetics of replication. In contrast, viruses with mutations 172/173, 174, 178, or 180/181 did not replicate at all. This complete failure to replicate is reminiscent of viruses with a defect at the integration stage of the proviral genome and suggests that the catalytic properties of mutated IN could be altered.

Catalytic Activity of IN Mutants—We therefore investigated whether disrupting some residues of the region 170–181 of IN affects its catalytic function. We measured processing and integration steps by using in vitro assays with similar amounts of purified GST-IN proteins. For these assays, IN mutant proteins fused in N terminus to GST were purified from bacterial lysate and incubated with appropriate oligonucleotides. The presence of faster migrating DNA species is the hallmark of the processing process (Fig. 3, top panel), and the presence of slower-migrating DNA species is the hallmark of the integration proc.

![Fig. 3. Catalytic activity of IN point mutants in vitro.](image-url)

![Fig. 4. Integration process of L172A/K173A IN viruses.](image-url)
Impaired Integration Process of L172A/K173A Viruses—We next examined which step(s) of the viral life cycle, including reverse transcription, nuclear import, and integration processes, was altered in cells infected with L172A/K173A viruses. H9 cells were infected with similar amounts of viruses from the H9004, L172A, K173A, and D116A viruses (Fig. 3, bottom panel). As a negative control, we used the GST-IN protein with a missense mutation (D116A) in the catalytic core domain of IN, impairing the integration process (6). Results showed that IN proteins with mutations at residues 170/171, 172/173, and 176/177 behaved as wild-type (Fig. 3). In contrast, IN with mutations at residues 174, 178, or 180/181 was enzymatically inactive, explaining why viruses carrying these mutations failed to replicate. Intriguingly, the L172A/K173A IN protein exhibited wild-type catalytic activity, although this mutation in the context of HIV-1 virus infection was shown to impair replication. A provirus bearing the single point mutation L172A was constructed, and results of the infectivity study showed that residue L172A alone was responsible for both UNG2 packaging (23) and the defect of replication (not shown).

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We then measured the integration process by evaluating proviral integration events using an Alu LTR PCR assay with primers corresponding to the viral LTR and the repetitive Alu sequences present in the cellular DNA. Results in Fig. 4 show the complete absence of integrated DNA products for D116A and L172A/K173A viruses in contrast to wild-type viruses, indicating that the L172A/K173A virus was defective at the integration step, although its integrase was demonstrated to be functional in the LTR mimic assays in vitro.

Rescue of the Replication of L172A/K173A Viruses—To address whether replication of L172A/K173A viruses could be restored, we used a previously reported experimental system (24, 31–34) in which IN protein was incorporated in trans into viral particles via a fusion with HIV-1 Vpr (Fig. 5A). It has been reported that proviruses mutated in one domain of IN can be rescued by trans-expressed IN containing mutations in another domain (14). This successful trans-complementation was indeed due to the ability of INs to oligomerize each subunit, providing distinct functions to obtain a wild-type IN phenotype. Viral stocks were generated by cotransfection of D116A, IN minus (ΔIN), or L172A/K173A proviral DNA together with expression vectors encoding VPR wild-type IN, VPR-L172A/K173A IN, or VPR-D116A IN fusion proteins. The C8166 indicator cell line was infected with serial 5-fold dilutions of viral stocks. The infectivity of trans-complemented viruses was measured by the number of cell-forming syncytia and expressed as the TCID50. Expression of Vpr-IN fusion proteins in trans-complemented viral particles was demonstrated by Western blotting (Fig. 5B). The apparently higher levels of IN in trans-complemented viruses, compared with non-trans-complemented viruses, is likely due to proteolytic cleavage of the Vpr-IN protein by the viral protease.

As expected, the trans-expression of wild-type IN rescued the replication of the ΔIN, L172A/K173A, and D116A viruses (Fig. 5C). The trans-expression of D116A IN, which is catalytically incompetent, restored the replication of L172A/K173A viruses but not that of ΔIN and D116A viruses. Conversely, trans-
expression of L172A/K173A IN has the capacity to rescue the replication of D116A and H9004 IN viruses up to 50% of that shown with trans wild-type IN. Unexpectedly, trans-complementation experiments showed that trans L172A/K173A IN can rescue the propagation of L172A/K173A viruses, although both cis- and trans-expressed IN proteins carried the same mutation. This restoration is approximately twice less efficient than that observed with trans wild-type IN, whatever the cis-expressed viruses. Altogether, these results suggest that mutations of residues 172 and 173 of IN fused to Vpr did not alter the catalytic property of IN.

The Rescue of Replication Is Independent of UNG2 Packaging—Previous studies have reported that Vpr associates with UNG2 (35) and that Vpr can promote the packaging of overexpressed HA-tagged UNG2 into viral particles (36). To investigate whether the rescue of L172A/K173A viruses might be due to the packaging of host UNG2 via the Vpr portion within the Vpr-IN fusion protein, we analyzed the presence of packaged UNG2 into highly purified IN viruses trans-complemented with either Vpr-IN or VPR-L172A/K173A IN. Results presented in Fig. 6 show that IN virus was devoid of detectable endogenous UNG2 and that neither Vpr fusions with wild-type IN nor those with mutated IN were able to direct a substantial packaging of UNG2. As a control, similar amounts of the HIV-1 wild-type virus were analyzed and showed the presence of packaged UNG2. These data indicate that IN promoted UNG2 packaging in the context of the Gag-Pol polyprotein but not in the context of a Vpr-IN fusion. To ascertain that no UNG2 packaging was responsible for the rescue, we performed trans-complementation assays with a Vpr-IN fusion protein, containing Vpr mutated at tryptophan 54, fused to L172A/K173A IN. The W54R and W54G mutations have been shown to abolish the interaction with UNG2 (36). Our results indicate that the W54G-VPR-L172A/K173A IN fusion protein rescued the viral propagation of L172A/K173A viruses to the same extent as the wild-type VPR-L172A/K173A IN fusion protein, demonstrating that the Vpr-UNG2 association was not involved in the successful rescue of L172A/K173A viruses (data not shown).

Trans-complementation of cis L172A/K173A IN is Dose-dependent—To understand how the overexpression of L172A/K173A IN can rescue the propagation of L172A/K173A viruses, we carried out trans-complementation experiments with increasing amounts of wild-type and L172A/K173A IN fusion proteins. The expression level of fused proteins in both cell lysate and viral particles was analyzed by Western blotting with anti-IN antibody. Amounts of virions analyzed were revealed by Western blotting with anti-CA p24 antibody. B, the infectivity of trans-complemented viruses was expressed as the TCID_{50} per milliliter of viral stock. Values represent the mean of two independent experiments.

To demonstrate that IN alone, and not IN-associated proteins, was involved in the rescue of L172A/K173A viruses, we performed trans-complementation experiments with increasing amounts of wild-type and L172A/K173A IN fusion proteins. The expression level of fused proteins in both cell lysate and viral particles was analyzed by Western blot (Fig. 7A). Trans-complementation of L172A/K173A IN viruses with increased doses of wild-type or L172A/K173A IN proteins led to a dose-dependent rescue of viral propagation (Fig. 7B). These data indicate that the failure to replicate of L172A/K173A viruses could be compensated by increasing amounts of trans L172A/K173A IN protein delivered into viral particles.

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formed in vitro experiments wherein the catalytic activity of increasing amounts of recombinant L172A/K173A IN was compared with that of wild-type IN. As shown in Fig. 8, both the processing and DNA strand transfer of L172A/K173A IN fusion protein, although this latter was catalytically active, were nevertheless ~4 times less efficient than those of wild-type IN. These in vitro results are consistent with in vivo results showing that trans L172A/K173A IN in infected cells rescues the replication of cis L172A/K173A viruses in a dose-dependent manner.

DISCUSSION

In a previous report (23), we analyzed a series of HIV-1 viruses with point mutations in the 170–181 region of IN to determine which residues of IN were important for host UNG2 packaging. We demonstrated that leucine residue 172, and not adjacent residues, was required for the incorporation of UNG2 into viral particles. In this study, we have investigated the replicative properties of HIV-1 viruses deficient or proficient for the presence of UNG2 in viral particles. We showed that UNG2-proficient viruses (E170A/H171A and V176A/Q177A viruses) replicated as wild-type viruses, except those whose mutations induced severe alterations of the catalytic activity of IN (T174A, M178A, and V180A/F181A viruses). In contrast, the UNG2-deficient virus (L172A/K173A IN mutant virus) failed to replicate and was impaired at the stage of integration of proviral DNA into host cell chromatin.

In trans-complementation assays, proviruses mutated in one domain of IN can be rescued by trans-expressed IN containing a mutation in another domain (14). This trans-complementation was due to the ability of INs to oligomerize, each subunit providing distinct functions to obtain a wild-type IN phenotype. The replication defect of viruses carrying the L172A/K173A IN mutation can be rescued by the coexpression of trans of the catalytically incompetent D116A IN protein, indicating that the two mutated IN proteins retain their ability to oligomerize and that the defect of the L172A/K173A IN viruses was compensated by the presence of the wild-type leucine 172 residue in D116A. Therefore, the replication blockage of L172A/K173A viruses was not due to alteration of the catalytic properties of the mutated IN per se.

Remarkably, these L172A/K173A viruses were also rescued by dose-dependent trans-expression of the same mutated IN. These data correlate well with the integration assays using LTR mimics in vitro, which show that four times more L172A/K173A IN than wild-type IN was required to achieve a similar integration pattern. Because the first step of the integration reaction, namely the processing of the 3′-ends of the LTR mimics, is affected, we propose that mutated IN is less efficient in binding to the LTR ends. This weakness in LTR binding was alleviated by allowing a higher concentration of mutated IN. We propose that the integration process is a rate-limiting process in the natural course of HIV-1 infection. It is possible that the integration activity of viruses with the L172A/K173A IN mutation is just below the threshold. A slight enhancement of the expression of IN in infected cells, even carrying the same mutation, might surpass the threshold and lead to the restoration of the integration process.

In trans-complementation assays, we observed that host UNG2 could not be packaged by the Vpr-IN fusion despite being clearly dependent on the presence of IN in the context of the Gag-Pol precursor. We can speculate on two hypotheses, which are not mutually exclusive. First, the folding of IN processed from the Gag-Pol precursor may not accurately reflect the folding of IN processed from the Gag-Pol precursor, leading to differential bindings of UNG2. Such a differential in binding between INs deriving from either the Vpr-IN fusion or the Gag-Pol precursor has been reported in the case of the HA-S6 trans dominant mutant of the integrase interactor 1 (Ini 1) (21). Second, although the IN domain of the Gag-Pol precursor is required for UNG2 packaging, it may not be sufficient. Indeed, we have reported previously that UNG2 can also bind viral reverse transcriptase (23). It may be that a triple interaction between UNG2 and both the reverse transcriptase and IN domains of the Gag-Pol precursor is necessary to allow the efficient packaging of UNG2.

Because the L172A/K173A mutation led to a severe blockage in provirus integration in vitro, we were unable to follow the replication of the sole UNG2-deficient virus over many replication cycles. The integration defect could be rescued by trans over expression of IN, either wild-type or mutated, but it allowed only one replicative cycle. However, we have previously reported (23) that wild-type but not UNG2-deficient viruses have the ability to repair G:U mispairs, suggesting a role of UNG2 in the control of the accuracy of reverse transcription. If the rescued proviral DNA is riddled with G to A mutations due to unprocessed uracils in the absence of packaged UNG2, that would lead to impairment replication in subsequent passages of the virus. The role of the virion-associated UNG2 enzyme in the viral life cycle remains an open question and will require studies of viral replication in UNG2-deficient T cells.

In conclusion, we have investigated the replicative properties of UNG2-deficient or -proficient IN mutant viruses in order to study the role of packaged UNG2 in the viral life cycle. We have not been able to draw definite conclusions about the importance of UNG2 for HIV-1 replication, but we have found an interesting mutant of IN that could be rescued by allowing a higher concentration of the very same mutant integrase in the viral particle. Whether the mutation L172A/K173K leads to a reduced affinity of IN for its LTR substrate or to another undefined defect remains to be investigated.

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