

Targeting Human Immunodeficiency Virus (HIV) Type 2 Integrase Protein into HIV Type 1

HONGMEI LIU,¹ XIAOYUN WU,¹ HONGLING XIAO,¹ AND JOHN C. KAPPES^{1,2,3*}

Departments of Medicine¹ and Microbiology,² University of Alabama at Birmingham, Birmingham, Alabama 35294, and Birmingham Veterans Affairs Medical Center, Research Service, Birmingham, Alabama 35233³

Received 17 March 1999/Accepted 2 July 1999

Integrase (IN) is the only retroviral enzyme necessary for the integration of retroviral cDNA into the host cell's chromosomes. The structure and function of IN is highly conserved. The human immunodeficiency virus type 2 (HIV-2) IN has been shown to efficiently support 3' processing and strand transfer of HIV-1 DNA substrate in vitro. To determine whether HIV-2 IN protein (IN²) could substitute for HIV-1 IN function in vivo, we used HIV-1 Vpr to deliver the IN² into IN mutant HIV-1 virions by expression in *trans* as a Vpr-IN fusion protein. *Trans*-complementation with IN² markedly increased the infectivity of IN-minus HIV-1. Compared with the homologous *trans*-IN protein, infectivity was increased to a level of 16%. Since IN has been found to play a role in reverse transcription (Wu et al., J. Virol. 73:2126–2135, 1999), cells infected with IN²-complemented HIV-1 were analyzed for DNA products of reverse transcription. DNA levels of approximately 18% of that of wild type were detected. The homologous *trans*-IN protein restored the synthesis of viral cDNA to approximately 86% of that of wild-type virus. By complementing integration-defective HIV-1 IN mutant viruses, which were not impaired in cDNA synthesis, the *trans*-IN² protein was shown to support integration up to a level of 55% compared with that of the homologous *trans*-IN protein. The delivery of heterologous IN protein into HIV-1 particles in *trans* offers a novel approach to understand IN protein function in vivo.

Like all other retroviruses, human immunodeficiency virus type 1 (HIV-1) and HIV-2 integrate a DNA copy of their RNA genome into the host cell's chromosomes. Integration of the viral cDNA is catalyzed by the integrase (IN) protein (3). Sequence analysis has shown that many features of the primary structure of IN are highly conserved among retroviruses and retrotransposons. The amino-terminal domain contains an array of histidine and cysteine residues that form a zinc finger motif (5, 6, 18). The central domain contains the catalytic core, which is defined by three acidic residues with stereotyped spacing. This D,D-35-E motif is a universal feature of integrase proteins and is essential for catalytic activity (9, 10, 22, 25, 36). The carboxy-terminal domain is the least conserved region of IN. It binds to the viral DNA ends and also exhibits nonspecific DNA binding properties (19, 24, 30, 31, 37). The sequence conservation of integrase suggests strong structure-function relationships. IN function has been studied extensively in vitro by using purified enzyme and short oligonucleotide substrate that mimics the ends of the viral DNA molecule (8, 21, 32). Such analysis has shown that IN proteins can utilize different retroviral DNA substrates in the in vitro integration reaction. The HIV-2 IN (IN²) has been shown to efficiently support 3' processing and strand transfer of HIV-1 substrate in vitro (37). Similarly, the feline immunodeficiency virus (FIV) IN can cleave and integrate HIV and Moloney murine leukemia virus (MoMLV) DNA ends (38). HIV-1 IN is active on FIV substrate but is barely active on MoMLV substrate (35, 38). While the analysis of the integration reaction in vitro has provided a great deal of information on IN function and has helped to elucidate the molecular mechanism of viral DNA integration, the conditions used to study IN in vitro do not fully duplicate those in vivo.

In the context of a replicating virus, HIV-1 IN is expressed and assembled into virions as the C-terminal component of a larger 160-kDa Gag-Pol polyprotein (Pr160^{Gag-Pol}). After proteolytic processing of Pr160^{Gag-Pol} and entry of the virus core into the host cell, IN exists as a 32-kDa protein together with other viral and cellular proteins that make up the viral nucleoprotein complex (34). Several in vivo studies have suggested that the IN protein may be involved in other step of the virus life cycle. Host cell proteins that bind to IN or the HIV-1 preintegration complex (PIC) and promote integration have been identified (13, 20). Through analysis in nondividing cells, mutations in the C terminus of IN have been shown to disrupt the movement of the viral PIC into the nucleus (15), suggesting a role of IN in the nuclear import machinery pathway (15, 16). In other studies, some IN mutations, including those in highly conserved amino acids residues, were found to have no apparent effect on IN activity in vitro while dramatically reducing the formation of the provirus in infected cells (11, 12, 26, 29). These results were initially explained in part by changes in the structure of the Pr160^{Gag-Pol} precursor protein, resulting in aberrant virus assembly and maturation. By incorporating IN protein into virions in *trans*, independently of Pr160^{Gag-Pol}, we recently demonstrated that the mature IN protein itself promotes the initiation of viral DNA synthesis (40). These results indicate that IN may play important roles in the virus life cycle at several different levels.

To examine whether IN² could function in place of HIV-1 IN during virus infection, two experimental approaches were undertaken. In the first, we replaced the HIV-1 IN coding region with that of the IN², generating a virus that contained an HIV-1-HIV-2 chimeric *pol* gene (Fig. 1). In the second approach, the IN² protein was incorporated into HIV-1 virions in *trans* by expression as a fusion partner of Vpr (Vpr-IN). This strategy is based on our previously findings and those of others, which have shown that Vpr can be used as a vehicle to deliver enzymatically active IN into HIV-1 virions in *trans*, independently of Pr160^{Gag-Pol} (14, 28, 40, 41).

* Corresponding author. Mailing address: University of Alabama at Birmingham, Department of Medicine, 1900 University Blvd., THH 513H, Birmingham, AL 35294. Phone: (205) 934-0051. Fax: (205) 975-7300. E-mail: KappesJC@uab.edu.

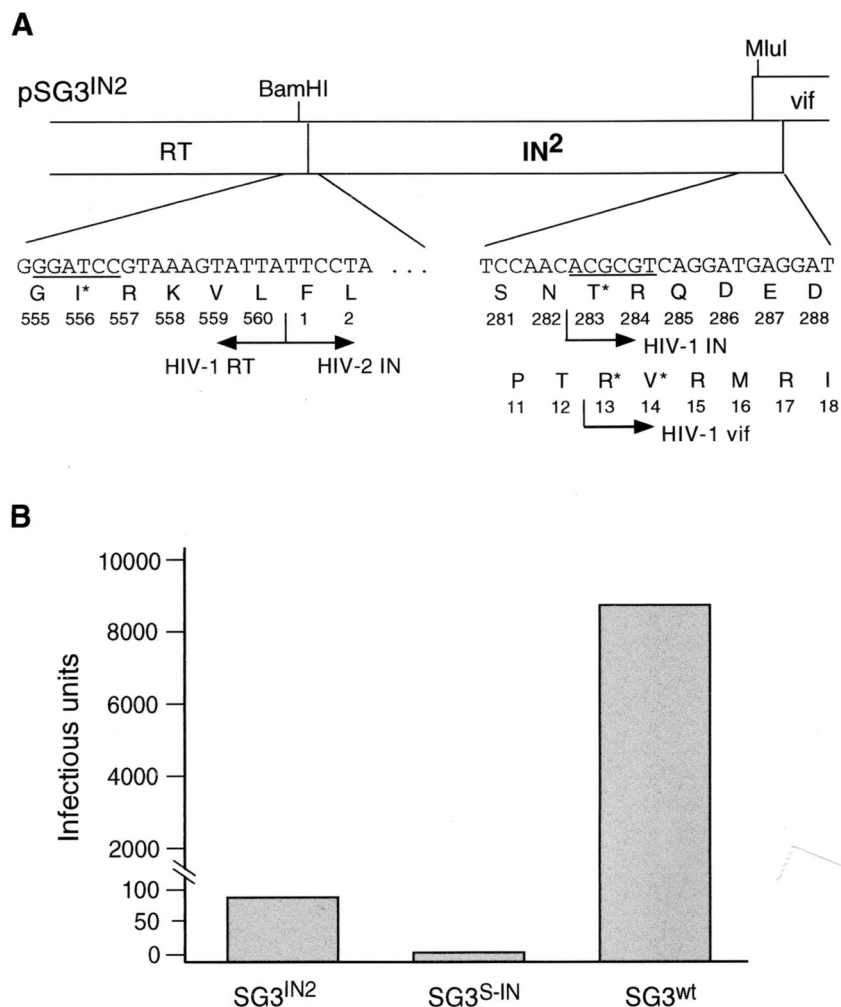


FIG. 1. Analysis of IN² protein function when expressed in *cis* with the HIV-1 genome. (A) Insertion of IN² into the *pol* gene of HIV-1. The HIV-1 SG3^{wt} DNA (41) was cut with the *Bam*HI and *Sal*I endonucleases to remove *IN*, *vif*, and the 5' half of *vpr* from the DNA genome. A fragment of pSG3^{wt} DNA encompassing 16 bp of 3' *IN* sequence, *vif* and the 5' half of *vpr* was amplified by PCR, wherein the sense primer contained a *Mlu*I restriction site. In a separate reaction, an IN-containing DNA fragment was PCR amplified from the HIV-2 ST clone, wherein the sense and antisense primers contained *Bam*HI and *Mlu*I restriction sites, respectively. The two PCR-amplified DNA fragments were ligated into the *Bam*HI-*Sal*I-cut pSG3^{wt} DNA, generating the chimeric virus pSG3^{IN2}. Sequence analysis confirmed an open chimeric *pol* reading frame (PR-RT-IN²). The 3' 18 nucleotides of the HIV-1 IN are retained in the chimeric protein. (B) Infectivity of SG3^{IN2} virions. 293T cells were transfected by calcium phosphate DNA precipitation methods with pSG3^{IN2} DNA. Forty-eight hours later, the culture supernatants were collected, filtered through 0.45- μ m-pore-size filters, and analyzed for HIV-1 p24 antigen by enzyme-linked immunosorbent assay (Coulter Inc.). Next, 25, 5, and 1 ng of each virus (p24 antigen equivalents) was used to infect monolayer cultures of P4 indicator cells (7). Two days later, the cells were stained, and infection-positive cells were counted as described earlier (41). The virus infectivity results represent infectious units per 25-ng equivalent of p24 antigen. These results were highly reproducible in three independent experiments. The data shown are from a single representative experiment.

Characterization of HIV-1 that encodes an HIV-1-HIV-2 chimeric Pol protein. An IN-containing DNA fragment was PCR amplified from the HIV-2 ST proviral clone (23) and ligated into the *Bam*HI and *Mlu*I restriction sites of the HIV-1 pSG3^{wt} molecular clone, generating pSG3^{IN2} (Fig. 1A). To determine whether the SG3^{IN2} virus was infectious, 293T cell monolayers were transfected with pSG3^{IN2} DNA. For controls, HIV-1 wild-type (SG3^{wt}) and IN-minus (pSG3^{S-IN}; see references 28 and 41) viruses were also transfected. The supernatants of the transfected cultures were collected 72 h later and divided into three aliquots. One aliquot was ultracentrifuged to pellet virus for immunoblot analysis, the second aliquot was analyzed for reverse transcriptase (RT) activity and p24 antigen concentration (Coulter Inc.), and the third was used to infect CD4-LTR- β -galactosidase indicator cells (P4) (7). No differences between the wild-type and SG3^{IN2} virions with respect to RT activity (relative to p24 antigen concentration) and

proteolytic processing of the Gag and Pol proteins were detected (data not shown). However, the SG3^{IN2} virions exhibited a marked decrease in infectivity (Fig. 1B). Compared with the IN-minus SG3^{IN2} virions, the SG3^{IN2} virions were reproducibly more infectious.

The HIV Gag-Pol precursor protein not only serves to incorporate the viral enzymes in the virus particle but also plays an important role in virion assembly. Several studies have shown that mutations within IN can cause defects in virus particle production, and virion composition and morphology (1, 2, 4, 11, 33). While our results show that the chimeric SG3^{IN2} virus was impaired in infectivity, they do not precisely show at what step(s) in the life cycle the virus was defective. Therefore, it was not possible to understand whether virus infection was blocked because the chimeric Gag-Pol protein was not properly folded and caused a defect in the late stages of the virus life cycle or whether the heterologous IN protein

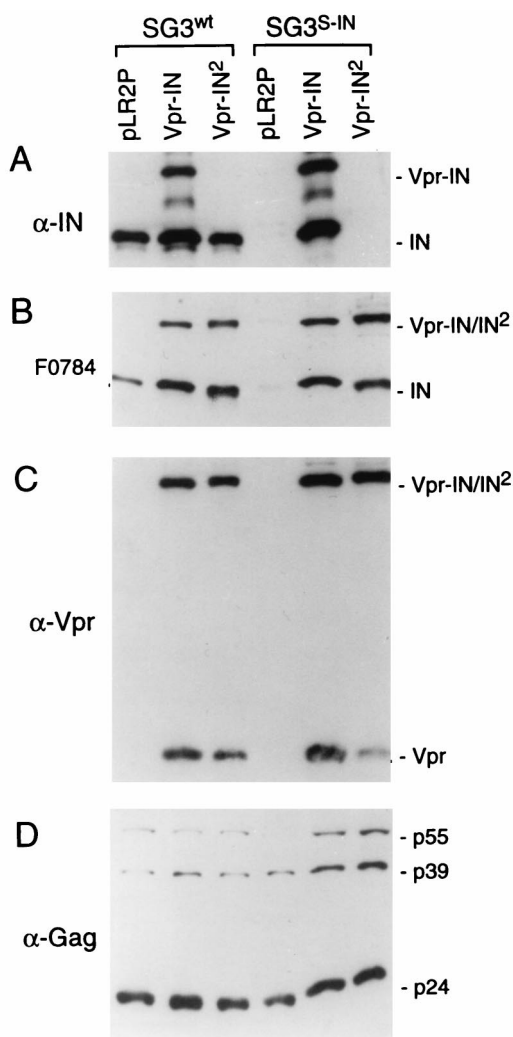


FIG. 2. Incorporation of the Vpr-IN fusion protein into IN-minus virions. (A) pSG3^{S-IN} and pSG3^{wt} were separately cotransfected into 293T cells with pLR2P-vprIN², pLR2P-vprIN, and pLR2P (vector alone), respectively. As described earlier (41), the extracellular progeny virions were concentrated from the supernatants of each culture by ultracentrifugation, lysed, and analyzed by immunoblot analysis using anti HIV-1 IN peptide antibody (A), human anti-HIV-2 antiserum (B), anti-Vpr (C), and anti-Gag (D) antibodies as probes.

itself was unable to mimic the function of the HIV-1 IN protein during the early stages of the virus life cycle.

Incorporation of IN² protein into HIV-1 virions by expression in trans. To avoid the possible dominant-negative effects of the chimeric Gag-Pol precursor protein, we fused the HIV-1 *vpr* gene with the HIV-2 *IN* gene (IN²) and placed the *vpr-IN*² gene fusion into the pLR2P expression plasmid (pLR2P-vprIN²) for complementing HIV-1 *IN* mutant viruses in *trans*. The pLR2P-vprIN² plasmid was cotransfected into 293T cells with the pSG3^{S-IN} IN-minus clone. As controls, pSG3^{S-IN} was cotransfected with the Vpr HIV-1 IN expression vector (pLR2P-vprIN; references 28, 40, 41) and the pLR2P vector. Immunoblot analysis of the progeny virions detected two species of anti-HIV-1 IN-reactive proteins (Fig. 2A), a 47-kDa protein, which is consistent with the combined masses of IN (32 kDa) and Vpr (15 kDa), and a 32-kDa protein. The detection of the 32-kDa protein in SG3^{S-IN} virions complemented with Vpr-IN indicated proteolytic processing of the fusion protein and liberation of IN. The Vpr-IN² fusion protein was not detected

with the HIV-1 anti-IN antiserum. However, using antiserum (F0784) obtained from an HIV-2-infected individual as a probe, the 47- and 32-kDa proteins were detected in both SG3^{wt} and SG3^{S-IN} virions that were complemented with either the Vpr-IN or Vpr-IN² fusion proteins (Fig. 2B). Using anti-Vpr antibody, the 47-kDa Vpr-IN and Vpr-IN² fusion proteins and their respective Vpr cleavage products were detected (Fig. 2C). While SG3 contains an open *vpr* reading frame, the virally encoded Vpr protein was not detected under the conditions used. Anti-Gag antibody detected approximately similar amounts of viral Gag proteins (Fig. 2D). These results confirmed that the IN² protein can be incorporated into HIV-1 virions when expressed in *trans* as a fusion partner of Vpr, and subsequently liberated during proteolytic maturation of the virus particle.

The IN² protein restores the infectivity of HIV-1 IN-minus virus. To analyze whether the IN² protein was functional, SG3^{S-IN} virions complemented with the *trans*-IN² protein were analyzed on P4 indicator cells. Our results indicated that virus infectivity was rescued to a level of 16% compared with virions complemented with the homologous HIV-1 *trans*-IN protein (Table 1). This result represents an increase of approximately 100-fold over noncomplemented SG3^{S-IN} virions. Immunoblot analysis performed on the viral stocks that were used for infection confirmed that the *trans*-IN- and *trans*-IN²-complemented virions contained approximately equal amounts of the respective fusion protein (data not shown). These results suggested that the IN² protein can function in place of the HIV-1 IN in vivo, albeit with significantly reduced efficiency.

IN² supports integration of the HIV-1 provirus. To directly examine whether the heterologous *trans*-IN² protein could complement the defect in integration of IN-minus HIV-1, a single-cycle integration assay was used. This assay utilized VSV-G-pseudotyped virus, which contains the hygromycin resistance gene within the HIV-1 genome in place of *env*. In cells infected with pseudotyped virus, where viral DNA is synthesized, integrated, and expressed, the hygromycin resistance gene allows for the outgrowth of cells in the presence of selection medium. VSV-G-pseudotyped, hygromycin-resistant, IN-minus virus (hy-SG3^{S-IN}) was complemented by DNA cotransfection with Vpr-IN² and Vpr-IN, respectively. Transfection-derived viral stocks were filtered through 0.45- μ m-pore-size filters, and divided into two aliquot sets. One aliquot set was subjected to ultracentrifugation to pellet virions and was examined by immunoblot analysis. Similar levels (relative to CA protein) of virion associated Vpr-IN and Vpr-IN² were detected (data not shown). The second aliquot set was normalized for p24 antigen concentration and was used to infect HeLa CD4 cells in hygromycin selection medium as described earlier (40). Complementation with the IN² protein produced

TABLE 1. Infectivity of IN-minus HIV-1 complemented with HIV-2 IN protein in *trans*

| Virus | Result of cotransfection with ^a : | | |
|---------------------|--|-----------------------------|-----------------------------|
| | Control | Vpr-IN ² | Vpr-IN |
| SG3 ^{wt} | 28.7 × 10 ³ | 22.4 × 10 ³ | 23.1 × 10 ³ |
| SG3 ^{S-IN} | 3 | 0.75 × 10 ³ (16) | 4.8 × 10 ³ (100) |

^a Four micrograms each of the SG3^{wt} and SG3^{S-IN} clones was separately cotransfected into 293T cells with 2 μ g of the pLR2P (control), Vpr-IN², or Vpr-IN expression plasmids. Numbers indicate the number of infectious units per 50-ng equivalent (p24 antigen) of input virus, as determined using P4 indicator cells. The numbers in parentheses indicate infectious units relative to the number of Vpr-IN-complemented SG3^{S-IN} virions, which was arbitrarily set to 100. These results are representative of three independent experiments.

TABLE 2. Integration of IN mutant HIV-1 complemented with HIV-2 IN protein in *trans*

| Hygromycin-resistant virus | Result of cotransfection with ^a : | | |
|----------------------------|--|-----------------------------|-----------------------------|
| | Control | Vpr-IN ² | Vpr-IN |
| hy-SG3 ^{wt} | 24.3 × 10 ⁴ | 18.9 × 10 ⁴ | 20.6 × 10 ⁴ |
| hy-SG3 ^{S-IN} | 17 | 0.65 × 10 ⁴ (22) | 3.0 × 10 ⁴ (100) |
| hy-SG3 ^{D116A} | 53 | 1.0 × 10 ⁴ (28) | 3.6 × 10 ⁴ (100) |
| hy-SG3 ^{AA35A} | 23 | 2.1 × 10 ⁴ (55) | 3.8 × 10 ⁴ (100) |

^a Four micrograms of each of the hy-SG3^{wt}, hy-SG3^{S-IN}, hy-SG3^{D116A}, and hy-SG3^{AA35A} clones (40) were separately cotransfected into 293T cells with 2 μg of the VSV-G *env* plasmid and 2 μg of either the pLR2P (control), Vpr-IN², or Vpr-IN expression plasmids. Numbers indicate the mean number of hygromycin-resistant colonies per 100-ng equivalent (p24 antigen) of input virus. The numbers in parentheses indicate the number of hygromycin-resistant colonies relative to the number produced by Vpr-IN-complemented SG3^{S-IN} virions, which was arbitrarily set to 100. These results are representative of three independent experiments.

22% of the number of resistant colonies produced by complementation with the homologous *trans*-IN protein (Table 2). Complementation of wild-type virions (hy-SG3^{wt}) with either Vpr-IN or Vpr-IN² resulted in a slight reduction (approximately twofold) in CFU (data not shown). These results are consistent with the infectivity results (Table 1).

We have recently reported that in addition to catalyzing integration, the HIV-1 IN protein plays a nonenzymatic role in the initiation of RT (40). Therefore, to directly analyze the ability of the heterologous IN to catalyze proviral DNA integration, independently of its role in viral DNA synthesis, the Vpr-IN and Vpr-IN² fusion proteins were used to complement the hy-SG3^{D116A} and the hy-SG3^{AA35A} mutant viruses (40). The hy-SG3^{AA35A} mutant contains alanine substitutions in each of the three amino acid residues that make up the catalytic triad of IN. While defective in integration, these mutants produce near wild-type levels of viral DNA following infection. Transfection-derived virions were normalized for p24 antigen and used to infect HeLa-CD4 cells. The Vpr-IN²-complemented hy-SG3^{D116A} and hy-SG3^{AA35A} viruses produced resistant colonies at levels of 28 and 55%, respectively, compared with those complemented with the homologous *trans*-IN protein (Table 2). Taken together, these results clearly show that heterologous IN² protein can catalyze integration of the HIV-1 provirus. Moreover, these data confirm reports that the IN² protein can efficiently support the integration of HIV-1 DNA substrate *in vitro* (36–38).

IN² protein does not efficiently promote HIV-1 DNA synthesis. Several reports have shown that viruses containing certain mutations in IN, including IN deletion mutants, are defective in the synthesis of their viral DNA. We reported that this is predominately due to a role that the IN protein plays in the initiation step of RT. Our published data demonstrated that IN-minus virions (SG3^{S-IN}) synthesize reduced levels of viral cDNA (5 to 10% of that of wild-type virus) and that this defect can be overcome by providing IN in *trans* (40). Therefore, the above results (Tables 1 and 2) would suggest that the defect in the infectivity of IN²-complemented SG3^{S-IN} virus was largely due to a block in viral DNA synthesis after infection. To test this directly, we analyzed the synthesis of the nascent viral cDNA in infected cells. SG3^{S-IN} virions containing Vpr-IN² were derived by DNA cotransfection and used to infect HeLa-CD4 cells. Eighteen hours later, the cell monolayers were trypsinized, washed extensively, and divided into two aliquot. One aliquot set was lysed and analyzed for intracellular p24 antigen concentration as described earlier (28, 39). DNA was extracted from the other aliquot set, normalized for intercellular p24 antigen concentration, and analyzed for early (R-U5)

and late (R-Gag) viral DNA products as described earlier (40). Serial fivefold dilutions of the SG3^{wt} DNA were prepared and analyzed in parallel as a reference to assess the relative amounts of DNA synthesized by the mutant viruses. The IN-minus SG3^{S-IN} virus (lane 7) produced significantly less R-U5 DNA compared with wild-type SG3^{wt} virions (Fig. 3A). When complemented with the *trans*-IN² protein the SG3^{S-IN} virions produced 18% of the wild-type levels of R-U5 DNA (lane 5). This represents an increase in DNA synthesis of approximately four- to fivefold. Complementation of SG3^{S-IN} with the homologous *trans*-IN proteins generated 86% the levels of R-U5 DNA compared with SG3^{wt}. For control, an equivalent (p24 antigen) amount of the SG3^{D116A} mutant virus was analyzed and found to contain near wild-type levels (89%) of R-U5 DNA. No R-U5 DNA was detected in cells infected with the RT-IN-minus SG3^{S-RT} virions (40), confirming that the R-U5 DNA detected by PCR was the product of reverse transcription. The absence of this DNA product in cells infected with SG3^{S-RT} also indicates that the band migrating slightly slower

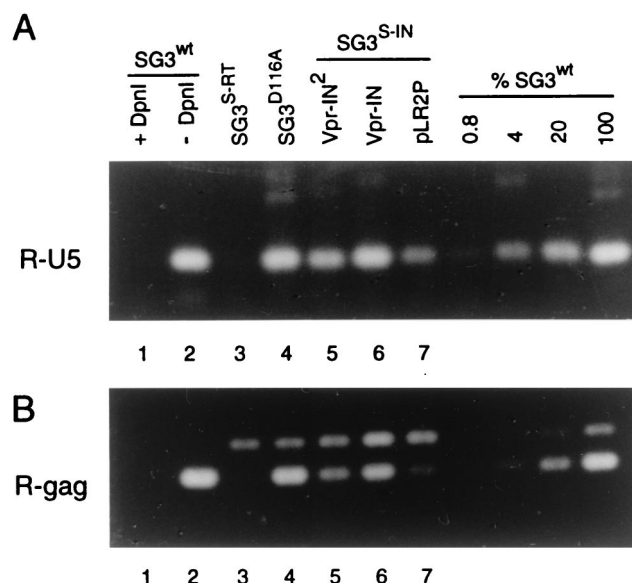


FIG. 3. Reverse transcription of Vpr-IN²-complemented viruses. pSG3^{S-IN} was cotransfected into 293T cells by calcium phosphate DNA transfection methods with pLR2P-vpr-IN², pLR2P-vpr-IN, and pLR2P, respectively. As controls, pSG3^{wt}, pSG3^{S-RT}, and pSG3^{D116A} were also transfected. Forty-eight hours later, culture supernatants were filtered through 0.45-μm-pore-size filters and analyzed by HIV-1 p24 antigen enzyme-linked immunosorbent assay (ELISA) (Coulter Inc.). The virus-containing culture supernatants were normalized to 500 ng of p24 antigen (CA), treated with RNase-free DNase H (20 U/ml for 2 h) (Promega Corporation) and placed on cultures of HeLa-CD4 cells at 37°C. After 4 h, the cell monolayers were washed, trypsinized, resuspended in fetal bovine serum, and divided into two aliquot sets. One aliquot set (which contained one-tenth of the total number of cells) was lysed in phosphate-buffered saline containing 1% Triton X-100 and analyzed by p24 antigen ELISA to quantify intracellular CA protein. The other aliquot set was placed back in culture medium at 37°C for an additional 14 h. The cells were then washed and total DNA was extracted by organic methods. Next, 250-pg equivalents (p24 antigen) of each DNA extract was analyzed by PCR methods for early (R-U5) (A) and late (R-gag) (B) viral DNA products of RT. The amplified products were resolved on 1.5% agarose gels and stained with ethidium bromide. To assess the relative amount of each of the amplified DNA products, four serial fivefold dilutions of the wild-type (SG3^{wt}) DNA were analyzed in parallel. The undiluted 250-pg sample was arbitrarily set to 100. As a control for the efficiency of the *DpnI* cleavage of potential carryover plasmid DNA, 6,250 copies of pSG3^{wt} DNA were analyzed after digestion with *DpnI* as described previously (17, 40). The ethidium bromide staining intensity of each amplified DNA product was measured with a Lynx 5000 molecular biology workstation (Applied Imaging) as described previously (27). The data shown is from a representative experiment that was repeated three times, each time with independent transfection-derived virus preparations.

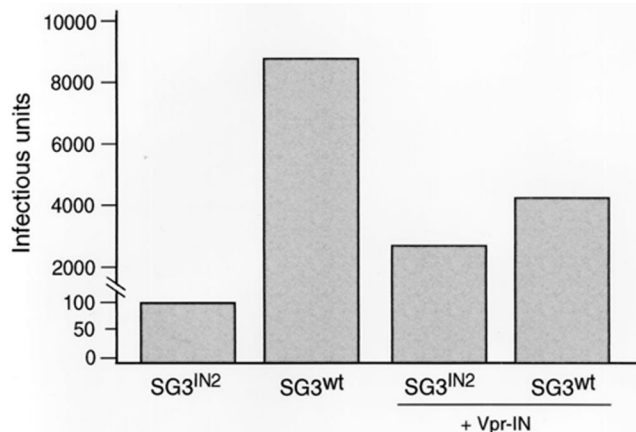


FIG. 4. Complementation of SG3^{IN2} virus with homologous *trans*-IN protein. pSG3^{IN2} and pSG3^{wt} were transfected alone and separately cotransfected into 293T cells with pLR2P-vprIN. The culture supernatants were collected 48 h later, filtered through 0.45- μ m-pore-size filters, and analyzed for HIV-1 p24 antigen by enzyme-linked immunosorbent assay (Coulter Inc.). Next, 25, 5, and 1 ng of each virus (p24 antigen equivalents) was used to infect monolayer cultures of P4 indicator cells. Two days later, the cells were stained and infection-positive cells were counted. The virus infectivity results represent infectious units per 25-ng equivalent of p24 antigen. The data shown are the means from three independent experiments.

than the R-Gag band is not a product of viral DNA contamination. Nearly identical results were obtained using the R-Gag primer pair to detect late products of viral DNA synthesis (Fig. 3B). These results show that the IN² protein can support the synthesis of HIV-1 DNA, but with significantly reduced efficiency compared with HIV-1 IN. They are also in strong agreement with our results on virus infectivity (Table 1) and together suggest that the factor limiting infectivity was primarily the defect in the synthesis of viral cDNA.

Complementation of SG3^{IN2} virus with *trans*-IN. To better understand the defect in the infectivity of the SG3^{IN2} virus (Fig. 1), pSG3^{IN2} was cotransfected with the HIV-1 *trans*-IN protein (Vpr-IN) and analyzed for infectivity. Figure 4 shows a 27-fold increase in infectivity. This represented an increase that was near that of wild-type virus when complemented with Vpr-IN. This result suggested that the expression of a chimeric Gag-Pol precursor protein did not cause a severe defect in the assembly or maturation of the SG3^{IN2} virions. Rather, these data may support our earlier findings for a role of an RT-IN intermediate in the formation of infectious HIV-1 particles (41).

In this report, we examined whether the IN² protein could mimic the DNA synthesis and integration activities of the HIV-1 IN at the virus replication level. Our data show that while the heterologous IN protein can support HIV-1 DNA synthesis, it is significantly less efficient than the homologous IN protein. The *trans*-IN² protein causes an increase in viral DNA synthesis of IN-minus virus of four- to fivefold. However, the HIV-2 *trans*-IN protein appeared to support integration of the provirus with greater efficiency, the integration frequency of the integration-defective D116A mutant virus was increased by almost 200-fold. It was noteworthy that the *trans*-IN² protein was consistently less efficient in rescuing the DNA synthesis defect of the SG3^{D116A} mutant virus compared with that of the SG3^{AA35A} mutant. It is possible that the D116A mutant IN protein has a dominant-negative effect, perhaps through the formation of nonfunctional heterodimers with the IN² protein. The ability of the IN² protein to support integration with relatively high efficiency indicates that it associates with the

reverse transcription and preintegration complexes. Moreover, these results suggest that the mere association of the heterologous IN protein with the RT complex is not sufficient to efficiently promote viral DNA synthesis, but rather that specific interactions between the IN protein and other viral components are required. The delivery of heterologous IN protein into HIV-1 particles in *trans* offers a novel approach to understand IN protein function in vivo.

This research was supported by National Institutes of Health grant CA73470 and by the facilities of the AIDS Central Virus and Protein Expression Cores of the Birmingham Center for AIDS Research (P30-AI-27767). This research was also supported by a Merit Review Award funded by the Office of Research and Development, Medical Research Service, U.S. Department of Veterans Affairs.

REFERENCES

1. Ansari-Lari, M. A., L. A. Donehower, and R. A. Gibbs. 1995. Analysis of human immunodeficiency virus type 1 integrase mutants. *Virology* 211:332-335.
2. Ansari-Lari, M. A., and R. A. Gibbs. 1996. Expression of human immunodeficiency virus type 1 reverse transcriptase in *trans* during virion release and after infection. *J. Virol.* 70:3870-3875.
3. Brown, P. 1997. Integration, p. 161-204. In J. M. Coffin, S. H. Hughes, and H. E. Varmus (ed.), *Retroviruses*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. Bukovsky, A., and H. Göttinger. 1996. Lack of integrase can markedly affect human immunodeficiency virus type 1 particle production in the presence of an active viral protease. *J. Virol.* 70:6820-6825.
5. Burke, C. J., G. Sanyal, M. W. Bruner, J. A. Ryan, R. L. LaFemina, H. L. Robbins, A. S. Zeff, C. R. Middaugh, and M. G. Cordingley. 1992. Structural implication of spectroscopic characterization of a putative zinc-finger peptide from HIV-1 integrase. *J. Biol. Chem.* 267:9639-9644.
6. Bushman, F. D., A. Engelman, I. Palmer, P. Wingfield, and R. Craigie. 1993. Domains of the integrase protein of human immunodeficiency virus type 1 responsible for polynucleotidyl transfer and zinc binding. *Proc. Natl. Acad. Sci. USA* 90:3428-3432.
7. Clavel, F., and P. Charneau. 1994. Fusion from without directed by human immunodeficiency virus particles. *J. Virol.* 68:1179-1185.
8. Craigie, R., T. Fujiwara, and F. Bushman. 1990. The IN protein of Moloney murine leukemia virus processes the viral DNA ends and accomplishes their integration in vitro. *Cell* 62:829-837.
9. Drelich, M., R. Wilhelm, and J. Mous. 1992. Identification of amino acid residues critical for endonuclease and integration activities of HIV-1 IN in vitro. *Virology* 188:459-468.
10. Engelman, A., and R. Craigie. 1992. Identification of conserved amino acid residues critical for human immunodeficiency virus type 1 integrase function in vitro. *J. Virol.* 66:6361-6369.
11. Engelman, A., G. Englund, J. M. Orenstein, M. A. Martin, and R. Craigie. 1995. Multiple effects of mutations in human immunodeficiency virus type 1 integrase on viral replication. *J. Virol.* 69:2729-2736.
12. Engelman, A., Y. Liu, H. Chen, M. Farzan, and F. Dyda. 1997. Structure-based mutagenesis of the catalytic domain of human immunodeficiency virus type 1 integrase. *J. Virol.* 71:3507-3514.
13. Farnet, C. M., and F. D. Bushman. 1997. HIV-1 cDNA integration: requirement of HMGI(Y) protein for function of preintegration complexes in vitro. *Cell* 88:483-492.
14. Fletcher, T. M., III, M. A. Soares, S. McPhearson, H. Hui, M. Wiskerchen, M. A. Muesing, G. M. Shaw, A. D. Leavitt, J. D. Boeke, and B. H. Hahn. 1997. Complementation of integrase function in HIV-1 virions. *EMBO J.* 16:5123-5138.
15. Gallay, P., T. Hope, D. Chin, and D. Trono. 1997. HIV-1 infection of nondividing cells through the recognition of integrase by the importin/karyopherin pathway. *Proc. Natl. Acad. Sci. USA* 94:9825-9830.
16. Gallay, P., S. Swingler, J. Song, F. Bushman, and D. Trono. 1995. HIV nuclear import is governed by the phosphotyrosine-mediated binding of matrix to the core domain of integrase. *Cell* 83:569-576.
17. Heininger, N. K., M. I. Bukrinsky, S. A. Haggerty, A. M. Ragland, V. Kewalramani, M.-A. Lee, H. E. Gendelman, L. Ratner, M. Stevenson, and M. Emerman. 1994. The Vpr protein of human immunodeficiency virus type 1 influences nuclear localization of viral nucleic acids in nondividing host cells. *Proc. Natl. Acad. Sci. USA* 91:7311-7315.
18. Johnson, M. S., M. A. McClure, D.-F. Feng, J. Gray, and R. F. Doolittle. 1986. Computer analysis of retroviral *pol* genes: assignment of enzymatic functions to specific sequences and homologies with nonviral enzymes. *Proc. Natl. Acad. Sci. USA* 83:7648-7652.
19. Kahn, E., J. P. G. Mack, R. A. Katz, J. Kulkosky, and A. M. Skalka. 1991. Retroviral integrase domains: DNA binding and the recognition of LTR sequences. *Nucleic Acids Res.* 19:851-860.

20. Kalpana, G. V., S. Marmon, W. Wang, G. R. Crabtree, and S. P. Goff. 1994. Binding and stimulation of HIV-1 integrase by a human homolog of yeast transcription factor SNF5. *Science* **266**:2002–2006.
21. Katzman, M., R. A. Katz, A. M. Skalka, and J. Leis. 1989. The avian retroviral integration protein cleaves the terminal sequences of linear viral DNA at the in vivo site of integration. *J. Virol.* **63**:5319–5327.
22. Kulkosky, J., K. S. Jones, R. A. Katz, J. P. G. Mack, and A. M. Skalka. 1992. Residues critical for retroviral integrative recombination in a region that is highly conserved among retroviral/retrotransposon integrases and bacterial insertion sequences transposases. *Mol. Cell. Biol.* **12**:2331–2338.
23. Kumar, P., H. Hui, J. C. Kappes, B. S. Haggarty, J. A. Hoxie, S. K. Arya, G. M. Shaw, and B. H. Hahn. 1990. Molecular characterization of an attenuated human immunodeficiency virus type 2 isolate. *J. Virol.* **64**:890–891.
24. LaFemina, R. L., P. L. Callahan, and M. G. Cordingley. 1991. Substrate specificity of recombinant human immunodeficiency virus integrase protein. *J. Virol.* **65**:5624–5630.
25. LaFemina, R. L., C. L. Schneider, H. L. Robbins, P. L. Callahan, K. LeGrow, E. Roth, W. A. Schlieff, and E. A. Emini. 1992. Requirement of active human immunodeficiency virus type 1 integrase enzyme for productive infection of human T-lymphoid cells. *J. Virol.* **66**:7414–7419.
26. Leavitt, A. D., G. Robles, N. Alesandro, and H. E. Varmus. 1996. Human immunodeficiency virus type 1 integrase mutants retain in vitro integrase activity yet fail to integrate viral DNA efficiently during infection. *J. Virol.* **70**:721–728.
27. Liu, H., X. Wu, M. Newman, G. M. Shaw, B. H. Hahn, and J. C. Kappes. 1995. The Vif protein of human and simian immunodeficiency viruses is packaged into virions and associates with viral core structures. *J. Virol.* **69**:7630–7638.
28. Liu, H., X. Wu, H. Xiao, J. A. Conway, and J. C. Kappes. 1997. Incorporation of functional human immunodeficiency virus type 1 integrase into virions independent of the Gag-Pol precursor protein. *J. Virol.* **71**:7701–7710.
29. Masuda, T., V. Planelles, P. Krogstad, and I. S. Y. Chen. 1995. Genetic analysis of human immunodeficiency virus type 1 integrase and the U3 *att* site: unusual phenotype of mutants in the zinc finger-like domain. *J. Virol.* **69**:6687–6696.
30. Miller, M. D., C. M. Farnet, and F. D. Bushman. 1997. Human immunodeficiency virus type 1 preintegration complexes: studies of organization and composition. *J. Virol.* **71**:5382–5390.
31. Mumm, S. R., and D. P. Grandgenett. 1991. Defining nucleic acid-binding properties of avian retroviruses integrase by deletion analysis. *J. Virol.* **65**:1160–1167.
32. Sherman, P. A., and J. A. Fyfe. 1990. Human immunodeficiency virus integration protein expressed in *Escherichia coli* processes selective DNA cleavage activity. *Proc. Natl. Acad. Sci. USA* **87**:5119–5123.
33. Shin, C.-G., B. Taddeo, W. A. Haseltine, and C. M. Farnet. 1994. Genetic analysis of the human immunodeficiency virus type 1 integrase protein. *J. Virol.* **68**:1633–1642.
34. Swanstrom, R., and J. W. Wills. 1997. Synthesis, assembly, and processing of viral proteins, p. 263–334. *In* J. M. Coffin, S. H. Hughes, and H. E. Varmus (ed.), *Retroviruses*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
35. van Gent, D. C., Y. Elgersma, M. W. J. Bolk, C. Vink, and R. H. A. Plasterk. 1991. DNA binding properties of the integrase protein of human immunodeficiency viruses types 1 and 2. *Nucleic Acids Res.* **19**:3821–3837.
36. van Gent, D. C., A. A. M. Oude Groeneger, and R. H. A. Plasterk. 1992. Mutational analysis of the integrase protein of human immunodeficiency virus type 2. *Proc. Natl. Acad. Sci. USA* **89**:9598–9601.
37. van Gent, D. C., C. Vink, A. A. M. Oude Groeneger, and R. H. A. Plasterk. 1993. Complementation between HIV integrase proteins mutated in different domains. *EMBO J.* **12**:3261–3267.
38. Vink, C., K. H. van der Linden, and R. H. A. Plasterk. 1994. Activities of the feline immunodeficiency virus integrase protein produced in *Escherichia coli*. *J. Virol.* **68**:1468–1474.
39. von Schwedler, U., J. Song, C. Aiken, and D. Trono. 1993. Vif is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. *J. Virol.* **67**:4945–4955.
40. Wu, W., H. Liu, H. Xiao, J. A. Conway, E. Hehl, G. V. Kalpana, V. Prasad, and J. C. Kappes. 1999. Human immunodeficiency virus type 1 integrase protein promotes reverse transcription through specific interactions with the nucleoprotein reverse transcription complex. *J. Virol.* **73**:2126–2135.
41. Wu, X., H. Liu, H. Xiao, J. A. Conway, E. Hunter, and J. C. Kappes. 1997. Functional RT and IN incorporated into HIV-1 particles independently of the Gag/Pol precursor protein. *EMBO J.* **16**:5113–5122.