Simian Virus 40-Based Replication of Catalytically Inactive Human Immunodeficiency Virus Type 1 Integrase Mutants in Nonpermissive T Cells and Monocyte-Derived Macrophages

Citation

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:41482903

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

Accessibility
Simian Virus 40-Based Replication of Catalytically Inactive Human Immunodeficiency Virus Type 1 Integrase Mutants in Nonpermissive T Cells and Monocyte-Derived Macrophages

Richard Lu,† Noriko Nakajima,‡ Wolfgang Hofmann,† Monsef Benkirane,§ Kuan Teh-Jeang,¶ Joseph Sodroski,* and Alan Engelman†

Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Department of Pathology, Harvard Medical School, and Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts 02115, and Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

Received 13 August 2003/Accepted 7 October 2003

Integrase function is required for retroviral replication in most instances. Although certain permissive T-cell lines support human immunodeficiency virus type 1 (HIV-1) replication in the absence of functional integrase, most cell lines and primary human cells are nonpermissive for integrase mutant growth. Since unintegrated retroviral DNA is lost from cells following cell division, we investigated whether incorporating a functional origin of DNA replication into integrase mutant HIV-1 might overcome the block to efficient gene expression and replication in nonpermissive T-cell lines and primary cells. Whereas the Epstein-Barr virus (EBV) origin (oriP) did little to augment expression from an integrase mutant reporter virus in EBV nuclear antigen 1-expressing cells, simian virus 40 (SV40) oriT dramatically enhanced integrase mutant infectivity in T-antigen (Tag)-expressing cells. Incorporating oriT into the nef position of a full-length, integrase-defective virus strain yielded efficient replication in Tag-expressing nonpermissive Jurkat T cells without reversion to an integration-competent genotype. Adding Tag to integrase mutant-oriT viruses yielded 11.3-kb SV40-HIV chimeras that replicated in Jurkat cells and primary monocyte-derived macrophages. Real-time quantitative PCR analyses of Jurkat cell infections revealed that amplified copies of unintegrated DNA likely contributed to SV40-HIV integrase mutant replication. SV40-based HIV-1 integrase mutant replication in otherwise nonpermissive cells suggests alternative approaches to standard integrase-mediated retroviral gene transfer strategies.

Integration of retroviral cDNA into a host cell chromosome is a key step in the viral life cycle. Integration inextricably links the virus to the host, such that it persists as cellular DNA for the remainder of the infected cell’s life. Integration is mediated by the viral integrase (IN) protein, the proteolytic cleavage product of the carboxyl-terminal portion of the Gag-Pol polyprotein. IN enters the cell as a component of the infecting virion.

After entry and uncoating, the virion enzyme reverse transcriptase (RT) copies genomic RNA into linear double-stranded cDNA containing a copy of the viral long terminal repeat (LTR) at each end, and this DNA form is the substrate for IN-mediated DNA recombination (see reference 5 for a review). A variety of circular DNA products also form during the early phase of retroviral infection. Whereas some of these result from IN-mediated autointegration of linear viral cDNA into itself, host recombination enzymes form two other types of circles (5). Homologous DNA recombination yields circles containing one copy of the LTR (1-LTR), and circles containing two tandem copies of the LTR (2-LTR) form by nonhomologous DNA end joining (32).

Functional IN protein is required for productive retroviral replication in most instances. Certain highly permissive transformed T-cell lines, however, supported replication of catalytically inactive class I human immunodeficiency virus type 1 (HIV-1) IN mutant viruses under conditions that restricted growth of pleiotropic class II IN mutants (38). This finding led to the suggestion that, under certain conditions, HIV-1 might productively replicate from unintegrated DNA templates. However, since host cell DNA recombination machinery nonspecifically integrates exogenous DNA at a low frequency (11, 21), it is important to consider the potential role of host-mediated or illegitimate DNA recombination (17, 29) in IN mutant viral replication. For example, since the recombination frequency of a class I IN mutant virus was greater than mutant viral titers (relative to that of the wild type [WT]) across cell lines, it seemed likely that illegitimately integrated proviruses contributed to mutant viral replication in permissive T-cell lines (38). In this study, a novel strategy was developed to investigate productive HIV-1 replication from unintegrated DNA.

Unintegrated HIV-1 DNA is lost from cells through one of two different means. Nascent linear cDNA as part of the reverse transcription-preamplification nucleoprotein complex is degraded via a proteosome-dependent process (7, 45). In con-
trast, 1-LTR and 2-LTR circles are relatively stable, their numbers diminishing only following cell division (7, 43). Unintegrated DNA circles may therefore fail as viral replication templates simply because they lack a functional origin of DNA replication. To address this, the Epstein-Barr virus (EBV) origin of replication (oriP) was inserted into a class I IN-activating mutant vector and gene expression was measured in cells expressing the EBV nuclear antigen 1 (EBNA-1) protein. Similarly, by incorporating the simian virus 40 (SV40) origin of replication (oriT) and infecting T-antigen (Tag)-expressing cells, we assayed whether amplifying the level of unintegrated DNA overcame the block to IN mutant replication in nonpermissive cells. Whereas oriP did little to enhance IN mutant gene expression in EBNA-1-expressing cells, oriT greatly enhanced mutant infectivity in Tag-expressing cells. Moreover, full-length IN-defective strains carrying both oriT and Tag replicated in nonpermissive Jurkat T cells and primary monococyte-derived macrophages (MDM). Our results demonstrate HIV-1 replication in primary human cells in the absence of functional IN enzyme, indicating a potential strategy for attenuated viral gene expression in the absence of efficient integration.

MATERIALS AND METHODS

Plasmids. HIV-1 vector pHL.GFP.oriT, which carried oriT downstream of green fluorescent protein (GFP), was built by amplifying oriT (345 bp) from pMAMneo (Clontech Laboratories, Inc., Palo Alto, Calif.) with NorI-tailed primers and Phi DNA polymerase (Stratagene, La Jolla, Calif.), followed by enzyme digestion and ligation to NorI-digested pHLib.GFP (38). To construct a minimal oriT, the 20 randomized EBNA-1 family-repeat binding sites in oriP were amplified with 5′-CGATGTCTGGTGATGAAAGATCAGCTCCCG (XhoI site highlighted in bold) and 5′-CCTGCGGGCGCTCTGGAGAGCAGCGCTGGGCC (NorI site in bold), and the four EBNA-1 dyad symmetry binding sites were separately amplified with 5′-ACGGGCGGCGCTACGCTGGCCTTGTGAC (Nor site in bold) and 5′-CGATGTCTGGTGATGAAAGATCAGCTCCCG (Xho site in bold). The two PCR products were digested with NorI, ligated, and then amplified with the XhoI-tailed primers. Plasmid pNLX-NorI was built by digesting this product with XhoI and ligating the resulting 223-bp family-of-repeat-dyad symmetry-containing fragment to XhoI-digested pNL4.3 (1, 24). Plasmid pHL.GFP.oriP was built by amplifying oriP from pHL.GFP with BsrGI-tailed primers, digesting it with BsrGI, and ligating it to BsrGI-digested pHLib.GFP.

Plasmids pCMV-Tag (8) and pSG5-K1 (56), encoding wild-type and E107K Tag, respectively, were generous gifts from James DeCaprio (Dana-Farber Cancer Institute). Plasmids encoding replication-defective U19 (26, 41) and transforming vectors required the transfection of three or four different plasmids, depending on the identity of the viral envelope (Env), as previously described (38). Cells (5 × 10^5) were plated in six-well plates 24 h prior to infection.

Viruses and infections. Virus stocks were generated by transfecting 293T or HeLa cells with calcium phosphate as previously described (15, 38); HeLa cells were used for Tag-oriT containing viruses to circumvent Tag-expressing 293T cells. Virus stocks for RQ-PCR assays generated with FuGENE 6 (Roche Molecular Biochemicals, Indianapolis, Ind.) were treated with DNase I prior to infection essentially as previously described (10, 33).

Whereas full-length HIV-1 NL4.3 was derived from one plasmid, two plasmids were required for em- deletion-containing HIV-1NL4.3, and GFP- and pac-expressing vectors required the transfection of three or four different plasmids, depending on the identity of the viral envelope (Env), as previously described (38). Transfected cell supernatants were assayed for Mgt°-dependent T-labeling reaction activity (15, 38) filtered through 0.45-μm-pore-size filters prior to infection. CV-I, CVI/EBNA, and COS-1 cells (2 × 10^5) plated in six-well plates 24 h prior to infection were infected with 3 × 10^6 RT cpm of VSV-G-pseudotyped, GFP-expressing virus for 1.5 h at 37°C, washed three times with serum-free DMEM, and cultured in 2 ml of DMEM. Cells were trypsinized, fixed with 2% formaldehyde, and analyzed by FACS. Anion exchange purification by HiTrap Q and Jurkat TagC15 cells (2 × 10^6) were infected with 10^6 RT cpm of T-labeling activity (15, 38) and GFP-expressing viruses in 0.5 ml for 60 min, washed once, and plated in 5 ml of RPMI. Raji T4/R5 cells were infected with 3 μg of pac- and GFP-expressing viruses by transfecting 293T or HeLa cells with calcium phosphate as previously described (15, 38); HeLa cells were used for Tag-oriT containing viruses to circumvent Tag-expressing 293T cells. Virus stocks for RQ-PCR assays generated with FuGENE 6 (Roche Molecular Biochemicals, Indianapolis, Ind.) were treated with DNase I prior to infection essentially as previously described (10, 33).

Single-round viruses containing deletions in env were used to initiate infections for real-time quantitative PCR (RQ-PCR) assays. Whereas env deletion-containing variants of pNL4.3/NorI and IN mutant pNL4/N(N) were previously described (33), pNL4/Tag-oriT was made by swapping the 2.3-kb env deletion-containing EcoRI/R3BanHI fragment of pNL4.3/NorI into the corresponding fragment in pNL4/N.Tag-oriT. Expression vectors for vesicular stomatitis virus glycoprotein G (VSV-G) and WT and D116A IN mutant Gag-Pol, Rev, and HIV-1_q3.4, gp120-gp41 were previously described (34, 38).

Single-round expression vectors carrying the gene for puromycin acetyltransferase (pac) were constructed as follows. WT and GKH Cld/XhoI Tag-oriT fragments isolated from plasmid pSG5-K1 (56) were digested with Cld/XhoI and ligated to Cld/XhoI-digested pBluescript II SK + (Stratagene). The NorI site in the resulting plasmids was mutated to an Msci site by QuikChange mutagenesis. Tag was amplified with XhoI- and NorI-tailed primers, digested, and ligated to XhoI/NorI-digested pHPluro (Clontech Laboratories, Inc.). The resulting IRES-Tag-oriT fragments were amplified with NorI-tailed primers, and digested DNAAs were ligated to NorI-digested pHPluro (38), generating pHPluro.Tag-oriT and pHPluro.GKH-orT.

Cells. 293T (42), HeLa (18), CV-1 (25), CVI/EBNA (36), and COS-1 (20) cells were grown in Dulbecco's modified Eagle medium containing 10% fetal calf serum, 100 μg of penicillin per ml, and 100 μg of streptomycin per ml (DMEM). Jurkat (51), Jurkat TagC15 (39), 1D Jurkat, and Raji B lymphoblast cells (44) were used for the expression of C105G and E107K/D402H/CCR5 (Raji T4/R5; a generous gift from Hyeryun Choe, Children's Hospital, Boston, Mass.) were grown in RPMI 1640 medium containing 10% fetal calf serum, 100 μg of penicillin per ml, and 100 μg of streptomycin per ml (RPFI). MDM were derived from peripheral blood mononuclear cells as previously described (38).

Derivation of Tag-expressing 1D Jurkat cells. Jurkat cells (3 × 10^5) were infected with 3 μg of pCMV-Tag by using Lipofectamine (Invitrogen Corp., Carlsbad, Calif.) for 20 min at 4°C. Whereafter, cells were seeded in 96-well plates and individual G418-resistant colonies were expanded and assayed for Tag expression by Western blotting and cell surface CD4 by fluorescence-activated cell sorting (FACS).

Western blotting was performed essentially as previously described (8). Cells (5 × 10^5) washed in phosphate-buffered saline (PBS) were resuspended in 1 ml of Nonidet P-40 (NP-40) lysis buffer [1% [vol/vol] NP-40, 10% [wt/vol] glycerol, 137 mM NaCl, 20 mM Tris-HCl [pH 8.0]], and once with NP-40 lysis buffer. Following fractionation through 7.5% polyacrylamide gels and electrophoresis to Hybrid-Bond C extra (Amersham Pharmacia Biotech Inc., San Leandro, Calif.) were treated with G418 sulfate (0.8 mg/ml) 2 days posttransfection. After 2 weeks, cells were seeded in 96-well plates and individual G418-resistant colonies were expanded and assayed for Tag expression by Western blotting and cell surface CD4 by fluorescence-activated cell sorting (FACS).

For cell surface CD4 detection, PBS-washed cells were incubated with phycoerythrin-conjugated OKT4 antibody (Ortho-McNeil Pharmaceuticals, Raritan, N.J.) for 20 min at 4°C. In the dark, following two washes with PBS, cells were fixed with 2% formaldehyde and analyzed by FACS.
2 × 10^6) were similarly infected with 10^7 RT cpm of VSV-G pseudotypes. GFP values from cells mock infected with Env^- virus were subtracted from VSV-G-mediated infections.

For assays of viral spread, T cells (2 × 10^6) were infected with 10^7 RT cpm of WT (approximately 1.5 μg of p24, equating to an approximate multiplicity of infection of 0.04) (34, 38) or IN mutant HIV-1Δ14-5, for 18 h at 37°C, washed three times with serum-free RPMI, and resuspended in 5 ml of RPMI unless otherwise noted. MDM infected with 18 × 10^7 RT cpm for 18 h at 37°C were washed two times with serum-free RPMI and resuspended in 1.5 ml of macrophage-drawing medium (30% [vol/vol] RPMI 1640 medium containing 25 mM HEPES, 45% [vol/vol] serum-free RPMI, 15% [vol/vol] L-929 cell conditioned medium, 10% [vol/vol] human serum type AB [BioWhittaker Inc., Walkersville, Md.], 0.055 mM β-mercaptoethanol, 2.5 U of macrophage colony-stimulating factor [PeproTech Inc., Rocky Hill, N.J.] per ml.

Southern blotting and PCR. Infected 1D Jurkat cells were lysed and fractionated, and DNA samples were analyzed by Southern blotting and inverse PCR as previously described (38). Levels of unintegrated HIV-1 DNA were quantified by PhosphorImager with ImageQuant version 1.11 (Molecular Dynamics, Sunnyvale, Calif.). For sequencing of passaged viruses, infected cells were lysed by the Hirt method as previously described (38). Whereas the pol region of N/N/oriT was molecularly cloned and sequenced as described for mutant N/N in reference 38, N/N/Tag or T/Tag pol and oriT PCR products were sequenced directly following purification from polyacylamide gels (38).

For RQ-PCR, Jurkat cells (6 × 10^6) infected with 6 × 10^6 RT cpm of VSV-G-pseudotyped viruses by spinoculation (40) at 530 °C for 2 h at 25°C were washed and cultured in 5 ml of RPMI. Approximately 1.2 × 10^6 cells were removed at each time point. When the culture was reduced to 2 ml, 2 ml of fresh RPMI was added, followed by the addition of fresh RPMI (2 ml) every 24 h. DNA was extracted from cells with the Qiagen DNeasy Tissue kit (Qiagen, Valencia, Calif.), and 10 μl of DNA-containing eluate was analyzed by RQ-PCR.

Three different HIV-1 amplicons, late reverse transcription (LRT) products, 2-LTR circles, and integrated proviruses, were analyzed by RQ-PCR. Primers, PCR conditions, and plasmids for generating HIV-1Δ14-5/R/LRT and 2-LTR standard curves were as described previously (33). Provirus content was quantified with HIV-1 and Alu primers as described previously (6, 28). The standard curve for Alu Roo PCR was prepared essentially as previously described (6), with genomic DNA from cells stably transduced with HIV-1 expression vector pHii6.GFP. Jurkat cells (6 × 10^6) infected with VSV-G-pseudotyped pHii6.GFP (1.8 × 10^7 RT cpm) were cultured for 40 days. GFP expression was stimulated with tumor necrosis factor alpha at 10 ng/ml, and stimulated cells were sorted to 97 to 98% GFP positivity by FACS. Genomic DNA prepared from expanded cells with the Qiagen DNeasy tissue kit was adjusted to 50 ng/μl, and a 1:2 dilution series was used to generate the Alu standard curve by normalizing the resulting genomic LRT and Alu Roo PCR signals to a plasmid-generated LRT standard curve.

Levels of LRT, 2-LTR, and Alu Roo PCR products were normalized to either cellular mitochondrial cytochrome oxidase (plasmid DNA containing a cloned copy of human cytochrome oxidase was a generous gift from M. D. Miller, Merck Research Laboratories) or endogenous retrovirus 3 (ERV-3) (55). Normalized values were expressed in arbitrary units.

Isolation of puromycin-resistant colonies. Puromycin-resistant colonies were isolated following infection with single-round pac-expressing viruses essentially as previously described (38), with the exception that cells were infected by spinoculation at 530 × g for 2 h at 25°C prior to incubation at 37°C.

RESULTS

SV40-based amplification of HIV-1 IN mutant gene expression in infected cells. Although certain highly permissive T-cell lines supported the continual replication of HIV-1 in the absence of IN catalytic function, the majority of cell lines, as well as primary human CD4^- cells, failed to support IN mutant growth (38). Since unintegrated HIV-1 DNA is significantly less stable than integrated proviruses (7, 43), we incorporated different origins of DNA replication into IN mutant HIV-1 to investigate the relationship between unintegrated DNA replication and mutant virus growth in nonpermissive cell types.

The replication mechanisms of two different DNA viruses, EBV and SV40, were analyzed. Whereas EBV uses its EBNA-1 protein to stably maintain the copy number of oriP-containing episomes (27), SV40 uses Tag protein to replicate and amplify oriT-containing amplicons (12).

Our preliminary approach was to insert oriP or oriT into a class I IN mutant GFP expression vector and assay infectivity in cells expressing the various viral proteins. Three related African green monkey cell lines, CV-1 (25), CV1/EBNA (36), and COS-1 (20), were initially analyzed. Whereas CV1/EBNA cells stably express EBNA-1, COS-1 is a CV-1 derivative line that stably expresses Tag. Cells infected with equal RT activity of WT or IN mutant VSV-G-pseudotyped virus were assayed for GFP expression at various times postinfection.

In repeated experiments, 10 to 20% of the CV-1 cells infected with the WT expression vector were GFP positive 3 days postinfection (dpi) (Fig. 1A and data not shown). At this time, 3 to 5% of the cells infected with IN active-site mutant D116A (2, 38, 52) were GFP positive (Fig. 1A). Neither oriT (Fig. 1A) nor oriP (not shown) altered the pattern of D116A mutant gene expression in CV-1 cells.

About 45% of the WT-infected CV1/EBNA1 cells were GFP positive at 3 dpi (Fig. 1B). In this case, about 8% of the D116A-infected cells were positive (Fig. 1B) and oriP did not appear to alter this expression pattern. In repeated experiments, D116A.oriP and D116A supported similar levels of GFP expression and the rate of GFP loss was, for the most part, unaffected by oriP (Fig. 1B).

In contrast, oriT greatly enhanced IN mutant infectivity in Tag-expressing cells. Whereas 20 to 30% of the WT-infected COS-1 cells were GFP positive at 3 to 6 dpi, about 3% of the D116A-infected cells were positive at 6 dpi (Fig. 1C). Notably, almost 60% of the D116A.oriT-infected cells were positive at 3 dpi (Fig. 1C). In contrast to the WT infection, the D116A.oriT infection was transient, as only a small number of cells remained GFP positive at 9 dpi (Fig. 1C). Enhanced GFP ex-
expression required oriT as part of the D116A virus, as COS-1 cells infected with virus made from a D116A construct carrying oriT in the plasmid backbone instead of the viral genome supported GFP expression levels indistinguishable from those of the parental D116A mutant (not shown). We noted that COS-1 cells infected with D116A.oriT supported high gene expression levels regardless of whether the virus was produced in 293T cells, which express Tag protein, or HeLa cells, which do not express Tag (data not shown). We also noted that cells infected with the WT or the D116A or D116A.oriT mutant virus displayed doubling times and percent viabilities indistinguishable from those of mock-infected COS-1 cells (data not shown).

Although the results in Fig. 1B revealed that oriP did not noticeably affect D116A gene expression in CV1/EBNA cells, we next assayed D116A.oriP in a second EBNA-1-expressing cell line, Raji T4/R5, that was derived from B-lymphoblast Raji cells (44). In repeat experiments, 8 to 11% of the WT virus-infected Raji T4/R5 cells were GFP positive at 5 dpi (Fig. 1D). The D116A mutant was weakly active in these cells, as only 0.2 to 0.3% were GFP positive at 2 to 5 dpi in repeated experiments (Fig. 1D and data not shown). Since approximately 1% of the D116A.oriP-infected cells were positive at 3 to 5 dpi (Fig. 1D), oriP stimulated the level of D116A gene expression three- to fourfold in Raji T4/R5 cells. However, since oriP did not enhance D116A expression in CV1/EBNA1 cells (Fig. 1B) and D116A.oriP expression was only 10 to 15% of the WT level in Raji T4/R5 cells (Fig. 1D) under conditions in which D116A.oriT expressed more GFP than the WT in COS-1 cells (Fig. 1C), we focused our attention on SV40-based vectors for the remainder of this study.

D116A.oriT expression was next assayed in nonpermissive human T cells. Infections were conducted with CD4$^+$ Jurkat T cells (51), which were nonpermissive for class I IN mutant replication (38), and their Tag-expressing derivative Jurkat TagC15 cells (39). The results closely paralleled the results obtained with CV1 and COS-1 cells: whereas D116A and D116A.oriT supported similar low levels of GFP expression in Jurkat cells (Fig. 2A), D116A.oriT was significantly more active than D116A in Jurkat TagC15 cells (Fig. 2B). The D116A.oriT infection also subsided much faster than the WT infection in Jurkat TagC15 cells (Fig. 2B).

Class I IN mutant replication in Tag-positive Jurkat cells requires oriT. We next analyzed the effect of SV40 sequences on the spreading replication of a class I IN mutant. Full-length mutant N/N carrying amino acid substitutions D64N and D116N in the active site of the IN enzyme (38) was modified to contain oriT in the viral nef region. N/N.oriT replication was compared to WT and N/N replication in Jurkat and Jurkat TagC15 cells.

Results of preliminary experiments revealed that Jurkat TagC15 cells failed to support detectable levels of WT HIV-1NL4-3 replication under conditions that yielded efficient growth in parental Jurkat cells (data not shown). This prompted a comparison of CD4 levels on the Jurkat and Jurkat TagC15 cell surface, which revealed the majority of Jurkat TagC15 cells as CD4$^-$ (data not shown). Parental Jurkat cells were therefore transfected with a Tag expression vector, and single-cell subclones were screened for cell surface CD4 expression by FACS and Tag protein content by Western blotting. The 1D Jurkat cell subclone, which was approximately 97% CD4 positive, expressed Tag at a level similar to that of Jurkat TagC15 cells (data not shown).

1D Jurkat cells infected with HIV-1NL4-3 supported peak virus replication at 5 to 6 dpi (Fig. 3). In contrast, N/N failed to replicate in 1D Jurkat cells over 2 months of observation (Fig. 3 and data not shown). Thus, 1D Jurkat cells, like parental Jurkat cells (38), were nonpermissive for class I IN mutant viral replication. In contrast, N/N.oriT replicated to high levels, peaking at approximately 10 dpi, proving that oriT drove N/N mutant replication in 1D Jurkat cells (Fig. 3). N/N.oriT replication also required Tag protein, as parental Jurkat cells infected with as much as $5 \times 10^7$ RT cpm of N/N.oriT (fivefold more virus than in Fig. 3) failed to support detectable HIV-1 growth (data not shown). We concluded that class I IN mutant HIV-1 can replicate in nonpermissive Jurkat T cells if oriT is present in the viral genome and Tag is provided in the cell. We noted an apparent requirement for a class I IN mutation in this system, as 1D Jurkat cells infected with $10^7$ RT cpm of the pleiotropic class II IN C-terminal deletion mutant 1-212 (33, 38) carrying oriT failed to support detectable levels of HIV-1 replication (not shown).

To investigate the mechanism of N/N.oriT replication, virus harvested from 1D Jurkat cells was passed onto fresh 1D Jurkat and Jurkat cells. Whereas parental Jurkat cells infected...
with 10^6 RT cpm did not support detectable virus growth, 1D Jurkat cells infected with either 10^6 or 10^8 RT cpm supported efficient HIV-1 replication (data not shown). Thus, infectious 1D Jurkat cell-derived N/N.oriT maintained its starting cell-type-dependent phenotype upon passage. 1D Jurkat cells were lysed by Hirt extraction at the peak of the second round of infection, and the resulting Hirt supernatant was amplified by PCR with pol-specific primers. Sequencing revealed that six independent molecular clones each retained the D64N and D116N codon changes in IN. Thus, N/N.oriT replicated in 1D Jurkat cells without reverting to an integration-competent phenotype or genotype.

**Spreading N/N.oriT replication in 1D Jurkat cells.** The results of the previous section showed that the supernatant of N/N.oriT-infected 1D Jurkat cells contained high levels of RT activity (Fig. 3). Although this was interpreted as evidence for spreading HIV-1 replication, we considered the alternative model in which 1D Jurkat cells might yield high levels of supernatant N/N.oriT in the absence of viral spread. Since cells infected with SV40 can produce very high levels of unintegrated DNA (12), N/N.oriT DNA replication could, in theory, yield high levels of viral gene expression in the absence of virus spread. We therefore devised the coculture experiment to investigate whether virus spread was required for high levels of N/N.oriT expression in 1D Jurkat cells.

At 40 h postinfection (hpi), 1D Jurkat cells infected with either WT or N/N.oriT virus were extensively washed and cocultured at a ratio of 1:10 with three different types of uninfected cells: Tag^- CD4^- Jurkat TagC15 cells, Tag^- CD4^+ parental Jurkat cells, and Tag^+ CD4^+ 1D Jurkat cells (Fig. 4). This experimental strategy investigated the CD4 and Tag requirements for efficient WT versus N/N.oriT gene expression after coculture. As expected, high levels of WT expression required virus spread: coculturing of WT-infected cells with 10^8 RT cpm did not support detectable virus growth, 1D Jurkat cells infected with either 10^6 or 10^8 RT cpm supported efficient HIV-1 replication (data not shown). Thus, infectious 1D Jurkat cell-derived N/N.oriT maintained its starting cell-type-dependent phenotype upon passage. 1D Jurkat cells were lysed by Hirt extraction at the peak of the second round of infection, and the resulting Hirt supernatant was amplified by PCR with pol-specific primers. Sequencing revealed that six independent molecular clones each retained the D64N and D116N codon changes in IN. Thus, N/N.oriT replicated in 1D Jurkat cells without reverting to an integration-competent phenotype or genotype.

**FIG. 4. Spreading N/N.oriT replication in 1D Jurkat cells.** (A) 1D Jurkat cells (2 × 10^5) infected with 10^6 RT cpm of WT (Δ), N/N.oriT (▲), or mock-treated supernatant (+) were assayed for RT activity at the indicated times. (B) RT assays of cell supernatants following coculture of 2 × 10^5 cells from panel A with 1.8 × 10^6 1D Jurkat TagC15 cells. (C and D) RT assays following coculture of 2 × 10^5 cells from the experiment whose results are shown in panel A with 1.8 × 10^6 Jurkat and 1D Jurkat cells, respectively.

CD4^- Jurkat TagC15 cells failed to yield detectable HIV-1 levels under conditions in which CD4^+ Jurkat and 1D Jurkat cells supported efficient WT virus expression (Fig. 4B to D). If N/N.oriT replicated in 1D Jurkat cells in the absence of viral spread (Fig. 3 and 4A), then each coculture condition would, in theory, yield equivalent levels of N/N.oriT in cell supernatants. However, akin to the WT virus, high levels of N/N.oriT were only detected after coculture with cells that supported replication of cell-free virus, namely, CD4^- Tag^- 1D Jurkat cells (Fig. 4). On the basis of this, we concluded that 1D Jurkat cells support the spreading replication of IN mutant N/N.oriT virus.

**FIG. 5. N/N.Tag-oriT replication in Jurkat cells requires functional Tag protein.** (A) Jurkat cells infected with WT (□), N/N.Tag-oriT (△), N/N.U19-oriT (○), or mock-treated (+) supernatant were assayed for RT activity at the indicated times. (B and C) Jurkat cells infected with the indicated levels of virus derived from peak days of WT and N/N.Tag-oriT replication in panel A. Arrows in panels A and C indicate cultures lysed by Hirt extraction. Similar replication curves were obtained following a second set of primary and secondary infections of Jurkat cells. pssg, passage.

N/N.Tag-oriT replication in Jurkat cells requires functional Tag protein. In the previous section, N/N.oriT replication required trans expression of Tag protein in 1D Jurkat cells. We next tested whether expression of Tag in cis from the incoming viral genome would support N/N.oriT replication in parental Tag^- Jurkat cells. For this, the gene for Tag was inserted into the viral nef position upstream of oriT. A second virus expressing the U19 Tag mutant, which is unable to replicate oriT-containing DNA substrates (26, 41), was also made. We noted that incorporating Tag increased the normal size of the HIV-1 RNA genome from 9.1 to 11.3 kb.

Jurkat cells infected with 10^6 RT cpm of WT HIV-1 NL4-3 supported peak virus replication at 8 dpi (Fig. 5A), and in agreement with our previous report (38), N/N failed to detectably replicate under these conditions (data not shown). In contrast, N/N.Tag-oriT replication was detected in Jurkat cells. In repeated experiments, N/N.Tag-oriT grew with a delay of about 4 days compared with the WT and yielded about fourfold less virus (Fig. 5A). Importantly, N/N.U19-oriT failed to replicate under these conditions, proving that the DNA replication function of Tag was necessary for N/N.Tag-oriT virus replication.

To investigate the mechanism of N/N.Tag-oriT replication, viruses harvested from cells infected as described in the legend to Fig. 5A were passed onto fresh Jurkat cells at two different multiplicities of infection (Fig. 5B and C). Infections initiated with 10^6 RT cpm yielded viral replication profiles similar to first-round profiles (compare Fig. 5B and A), and infections...
initiated with 10-fold less virus did not significantly alter this result (Fig. 5C). At 16 dpi, first (Fig. 5A, arrow)- and second (Fig. 5C, arrow)-round N/N.Tag-oriT-infected cells were lysed by Hirt extraction and Hirt supernatants were subjected to PCR with pol-, Tag-, and oriT-specific primers. Whereas the overall size of each PCR product was monitored by gel electrophoresis (Fig. 6), the pol and oriT products were additionally analyzed by DNA sequencing. Although Tag and oriT sequences were both maintained following two rounds of N/N.Tag-oriT replication (Fig. 6A and B), an internal deletion in oriT arose during virus passage (Fig. 6B and data not shown). Substituting this deletion-containing oriT for the full-length element in N/N.Tag-oriT revealed that each origin supported a similar profile of N/N.Tag-oriT replication in Jurkat cells (not shown). Since the sequence of the second-round (Fig. 5C) pol PCR product (Fig. 6A, lane 3) revealed maintenance of both substitutions in the IN active site, we concluded that N/N.Tag-oriT replicated through two rounds in nonpermissive Jurkat cells without acquiring an integration-competent genotype.

**Southern blotting and inverse PCR analyses of unintegrated and integrated HIV-1 DNA.** Our results demonstrate that Tag can drive the replication of oriT-containing class I IN mutant HIV-1 in nonpermissive Jurkat T cells. We next investigated the mechanism of SV40-dependent IN mutant replication, considering two alternative models. Since SV40 replicates from amplified copies of unintegrated DNA (12), it seemed plausible that SV40-based HIV-1 IN mutants might likewise replicate from amplified copies of unintegrated DNA. Alternatively, this predicted increase in unintegrated DNA might yield a parallel increase in the level of illegitimate DNA recombination, which might also contribute to mutant viral replication (38). To begin to distinguish between these two possibilities, nuclei purified from WT-, N/N-, and N/N.oriT-infected 1D Jurkat cells were lysed and DNA in the supernatant and pellet fractions was assayed by Southern blotting and inverse PCR, respectively, as previously described (38). WT and N/N.oriT each reached their replication peak at 3 dpi in this experiment.

Consistent with results of previous studies (2, 15, 23, 29, 52), cells infected with class I IN mutant N/N displayed a transient increase in the level of unintegrated DNA: whereas cells infected with N/N contained more 1-LTR and 2-LTR circles than did WT-infected cells at 1 dpi, the N/N circles decreased 2.5-fold to below the WT levels by 3 dpi (Fig. 7A). In stark contrast, the level of N/N.oriT circles greatly increased during the course of the experiment. Whereas the level of N/N.oriT circles was similar to that of the WT at 1 dpi, cells infected with N/N.oriT contained approximately 10-fold more 1-LTR and 2-LTR circles than did cells infected with the WT by 3 dpi (Fig. 7A).

Genomic DNA isolated at 3 dpi was next analyzed for evidence of HIV-1 integration. Inverse PCR detects the frequency and distribution of genomic HindIII sites flanking a population of integrated proviruses (30, 38; Fig. 7C). In addition to these variably sized products indicative of provirus distribution (labeled X in Fig. 7C), all forms of WT HIV-1-NL4-3 yield an internal 1,102-bp inverse PCR product (Fig. 7C and reference 38). 2-LTR circles, which are recovered at a low level in genomic DNA preparations, yield a novel internal fragment of 261 bp (Fig. 7C). Because of the presence of a HindIII site in oriT, we note that the internal 1,102-bp WT inverse PCR product was, instead, 358 bp for N/N.oriT (Fig. 7C).

As predicted, genomic DNA recovered from WT-infected cells yielded a heterogeneous ladder of inverse PCR products in addition to the 1,102- and 261-bp products (Fig. 7B, lane 3). Although genomic DNA from N/N.oriT-infected cells revealed both internal products, a ladder representing the normal frequency and distribution of provirus integration was not detected (Fig. 7B, lane 4). We did note the presence of a heterogeneous background smear that extended upward from the 261-bp 2-LTR circle product (lane 4). Owing to the difficulty of quantifying this signal, we next used RQ-PCR to get a better handle on the extent of SV40-based IN mutant integration.

**HIV-1 DNA quantitation by RQ-PCR.** RQ-PCR was used to quantify levels of WT and IN mutant DNAs in acutely infected cells. PCR primers and TaqMan probes were chosen to detect the following HIV-1 species: (i) LRT products, which represent the total level of intracellular HIV-1 DNA after the second template switch of reverse transcription, (ii) 2-LTR circles, which represent a fraction of the total unintegrated DNA population, and (iii) Alu RQ-PCR, which represents the fraction of integrated provirus DNA. Infections for RQ-PCR assays were conducted with single-round env deletion-containing viruses to restrict cDNA synthesis and integration to single infectious cycles. Viral RQ-PCR signals were normalized to ERV-3, an endogenous retrovirus-like element present at two copies per normal diploid human cell (55).

In repeated experiments, Jurkat cells infected with the WT and N/N viruses supported peak LRT values at 6 to 12 hpi (Fig. 8A and B). As expected from the results in Fig. 7A and numerous previous reports (2, 15, 23, 29, 33, 52), N/N converted four to fivefold more of its total DNA into 2-LTR circles than did the WT in repeated experiments (Fig. 8A and B and Table 1). Whereas the WT converted between 15% (Fig. 8A) and 69% (Table 1) of its total DNA into integrated proviruses in
repeated experiments, the level of N/N provirus formation was below the detection limit of the RQ-PCR assay (0.2% of the total N/N DNA integrated). We noted the requirement for the VSV-G Env glycoprotein in this system (6), as Jurkat cells infected with single-round HIV-1NL4-3 carrying WT IN and the HIV-1 Env glycoprotein failed to yield a detectable Alu RQ-PCR signal (data not shown). Consistent with this observation, we previously demonstrated that Jurkat cells infected with VSV-G pseudotypes supported approximately 30-fold more cDNA synthesis than did cells infected via the HIV-1 Env (34).

**FIG. 7.** N/N.oriT replication in the absence of normal provirus formation. (A) 1D Jurkat cells (1.6 x 10^8) infected with 8.0 x 10^8 RT cpm of WT (W), N/N (N), N/N.oriT (T), or mock-treated (m) supernatant for 4 h at 37°C were lysed at the indicated times, and unintegrated nuclear DNA was detected by Southern blotting. Whereas the first four lanes were exposed to X-ray film overnight, the remaining lanes were exposed to film for 3 h. Linear DNA was not detected in this analysis, likely because of its loss during alkaline lysis (3). (B) Inverse PCR. Lanes: 1, genomic DNA isolated from mock-infected cells; 2, 1 ng of plasmid pNL4-3 (1) mixed with 10 μg of mock-infected genomic DNA prior to digestion with HindIII; 3 and 4, genomic DNA isolated at 3 dpi with the indicated viruses; 5, mock-infected DNA mixed with Hirt supernatant from approximately 10^4 N/N.oriT-infected 1D Jurkat cells prior to HindIII digestion; 6, mock-infected DNA plus 1 ng of pN/N.oriT. Whereas the gel in lane 3 was exposed to X-ray film overnight, the gel in lanes 1, 2, and 4 to 6 was exposed to film for 5 h. The migration positions of the 1,102-, 358-, and 261-bp internal PCR products are indicated on the right; migration positions of molecular mass standards (sizes in base pairs are shown) are indicated on the left. (C) Inverse PCR strategy. Solid lines, HIV-1 DNA; open boxes, HIV-1 LTRs; vertical arrows, relevant HindIII sites; dashed lines, cellular DNA; black box, oriT; P, location of PCR primers (not drawn to scale); X, variably sized products indicative of provirus distribution. Whereas the HindIII sites at positions 8,131 and 9,606 in WT HIV-1NL4-3 yielded a 1,102-bp internal PCR product (38), oriT introduced an additional HindIII site, thereby reducing the size of the WT product to 358 bp. The WT and N/N.oriT viruses each yielded a 2-LTR-specific 261-bp product (38).

**FIG. 8.** Real-time profiles of HIV-1 LRT, 2-LTR, and Alu RQ-PCR DNAs in WT-, N/N-, and N/N.Tag-oriT-infected cells. (A) Total cellular DNA was isolated from WT-infected Jurkat cells at the indicated times. Levels of LRT (○), 2-LTR (□), and Alu (△) RQ-PCR products were normalized to ERV-3. (B and C) RQ-PCR values for N/N- and N/N.Tag-oriT-infected cells, respectively, were determined as described for panel A. Error bars indicate variation between duplicate RQ-PCR assays. AU, arbitrary units.
expression from N/N.Tag-oriT in these single-round infection assays, the WT and N/N.Tag-oriT gene expression profiles were compared to their RQ-PCR profiles. Although these viruses carried env deletions, efficient gene expression was predicted to yield viruslike particles containing readily detectable RT activity in cell supernatants (46). The WT and N/N.Tag-oriT RT activities each peaked at 60 hpi under these conditions (Fig. 9). Since the WT Alu RQ-PCR signal peaked at 12 hpi (Fig. 8A and 9A), there was an approximately 48-h delay between the peaks of WT integration and gene expression (Fig. 9A). In contrast, the N/N.Tag-oriT Alu RQ-PCR signal, which was undetectable until 36 hpi, did not peak until 72 hpi (Fig. 9C). Since the peak of N/N.Tag-oriT integration was delayed approximately 60 h from that of the WT under conditions in which the two viruses displayed similar gene expression profiles, we noted that the level of N/N.Tag-oriT expression was approximately 50% of that of the WT. We infer that this reduced level of mutant gene expression was in large part responsible for the delay in N/N.Tag-oriT replication observed in spreading infection assays (Fig. 5). AU, arbitrary units.

TABLE 1. 2-LTR and Alu RQ-PCR levels in WT and IN mutant infections

<table>
<thead>
<tr>
<th>Virus</th>
<th>Expt 1</th>
<th>Expt 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-LTR</td>
<td>Alu</td>
</tr>
<tr>
<td>WT</td>
<td>0.9 (0.2)</td>
<td>15.3 (8.5)</td>
</tr>
<tr>
<td>N/N</td>
<td>4.6 (2.3)</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>N/N.Tag-oriT</td>
<td>57.0 (7.2)</td>
<td>0.8 (0.1)</td>
</tr>
</tbody>
</table>

* Percentage of peak LRT, calculated by using peak 2-LTR and Alu RQ-PCR values.
* Data from Fig. 8.
* Values in parentheses indicate variations between duplicate RQ-PCR assays.
supported N/N.Tag-oriT replication at about 10% of the WT level, cells derived from different blood donors supported 4 to 24% of the WT replication in repeated experiments (data not shown). Since N/N.U19-oriT failed to support a detectable level of HIV-1 replication (Fig. 10B), the DNA replication function of Tag was essential for N/N.Tag-oriT virus replication in MDM.

Tag is an oncoprotein that transforms cells through its ability to bind and sequester the cellular tumor suppressor retinoblastoma binding (pRB) and p53 proteins (reviewed in references 12 and 49). Whereas the Tag sequence 106LFCE (residues conserved in other viral oncoproteins in bold) mediates binding to pRB, p53 interacts with a larger C-terminal region of Tag (12, 49). Since IN mutant viral replication assays were until now conducted with oncogenic Tag protein, we next investigated the replication capacity of td variants of Tag. Whereas Tag changes C105G and E107K each disrupt pRB binding (13, 56), D402H interferes with p53 binding (31, 35). The following Tag mutants were tested: single mutants E107K and D402H, double mutant C105G/E107K, and triple mutant C105G/E107K/D402H (GKH). Jurkat cells infected with each td variant supported HIV-1 replication at a level that was similar to that of parental N/N.Tag-oriT (data not shown). Although N/N.D402H-oriT reproducibly replicated to a higher level than did either N/N or N/N.U19-oriT, none of the td variants replicated to the same level as parental N/N.Tag-oriT in MDM (Fig. 10B and data not shown).

**DISCUSSION**

IN-mediated integration of retroviral cDNA into a cell chromosome is required for productive HIV-1 replication in most instances (38). Although IN-defective HIV-1 expressed relatively high levels of the capsid (9, 47), Tat (2, 15, 52), and Nef (53) proteins in a variety of cell types, only a limited number of highly permissive T-cell lines supported HIV-1 replication in the absence of IN function (38). These observations suggested that integrated proviruses were more efficient transcription templates than unintegrated HIV-1 DNA. Whereas the reason(s) behind differential gene expression from integrated versus unintegrated DNA was unclear, one possibility was the relative instability of unintegrated DNA templates. Whereas integrated proviruses are expressed and replicated as if they were host genes, unintegrated HIV-1 DNA is lost from cells via a proteosome-dependent process (7, 45) and/or following division (7, 43). In this study, we investigated whether incorporating a functional origin of replication might overcome the block to class I IN mutant viral replication in nonpermissive cell types.

**SV40-based amplification of class I IN mutant gene expression.** Our initial approach was to insert oriP or oriT into a class I IN mutant viral expression vector and assay infectivity in cells expressing either the EBNA-1 or the Tag protein (Fig. 1). This experimental design is reminiscent of a previous report wherein the polyomavirus origin of replication conferred extrachromosomal DNA replication on a murine retroviral vector in cells expressing polyomavirus large T (4). Apart from the different viruses, the main difference between that study and ours was that the murine vector carried functional IN whereas functionally inactive IN was studied here.

Since oriP did not substantially alter the pattern of IN mutant gene expression in two different cell types under conditions in which oriT greatly stimulated expression (Fig. 1), our results indicate that simply maintaining the copy number of unintegrated DNA does not yield a functional HIV-1 replicon and instead copy number amplification was required for efficient expression from unintegrated DNA templates. This conclusion is consistent with the notion that unintegrated DNAs formed by retroviral infection are significantly poorer transcriptional templates than are integrated proviruses.

**Mechanism of SV40-based HIV-1 IN mutant replication in nonpermissive cells.** Our results demonstrate that the combined action of oriT in cis and Tag protein in trans conferred a spreading replication phenotype on class I IN mutant N/N in nonpermissive 1D Jurkat T cells (Fig. 3 and 4). Interestingly, the pleiotropic C-terminal IN deletion mutant 1-212 (38) failed to function under these conditions. Since these so-called class II mutants accumulate significantly less viral DNA in cell nuclei than either WT HIV-1 or class I IN mutants (33), we speculate that prenuclear defects in HIV-1 cDNA metabolism precluded class II mutant function under these novel infection conditions. Furthermore, by incorporating the gene for Tag into the oriT-containing class I IN mutant, we developed 11.3-kb SV40–HIV-1 chimeras that replicated in nonpermissive Jurkat T cells (Fig. 5) and primary MDM (Fig. 10). Since the replication-defective Tag mutant U19 (26, 41) failed to function in these assays, the DNA replication function of Tag was essential for class I IN mutant viral replication under these infection conditions.

We performed a variety of experiments to address the mechanism of SV40-based IN mutant replication. Results of Southern blotting (Fig. 7A) and RQ-PCR (Fig. 8) assays indicated that cells infected with class I IN mutant-SV40 replicons contained 10- to 50-fold more unintegrated DNA than their WT-infected counterparts. Although these increased levels of unintegrated DNA were anticipated (4), an important issue was to determine levels of IN mutant DNA recombination and assess to what extent integration played a role in IN mutant gene expression and virus replication.

Both inactivating changes in the IN active site were maintained following N/N.oriT replication in 1D Jurkat cells and N/N.Tag-oriT replication in Jurkat cells. Consistent with these...
observations, genomic DNA prepared from N/N.Tag-oriT-infected 1D Jurkat cells failed to reveal the normal pattern of HIV-1 integration by inverse PCR (Fig. 7B). However, since this DNA revealed a heterogeneous smear of inverse PCR products, we used an RQ-PCR assay to quantify the extent of N/N.Tag-oriT integration in Jurkat T cells.

Results of Alu RQ-PCR assays revealed 0.8 to 2.0% N/N.Tag-oriT integration under conditions in which the WT accomplished 15 to 69% provirus formation (Fig. 8 and Table 1). Since N/N.Tag-oriT-infected Jurkat cells contained approximately 50-fold more total HIV-1 DNA than WT-infected cells, N/N.Tag-oriT yielded two- to fourfold more proviruses than the WT in repeat experiments (Fig. 8 and data not shown). Since the peak of N/N.Tag-oriT integration was delayed approximately 60 h from the peak of WT provirus formation under conditions in which both viruses yielded similar kinetics of gene expression, it seems likely that the large pool of unintegrated DNA contributed to N/N.Tag-oriT expression (Fig. 9). However, this analysis did not rule out the possibility that integrated proviruses also contributed to N/N.Tag-oriT gene expression.

Similar to our previous approach (38), we attempted to determine precise frequencies of IN mutant DNA recombination by comparing the number of D116A.Tag-oriT or D116A.GKH-oriT expression vector. These results indicate that stable integration of both Tag and oriT was highly cytopathic to Jurkat cells, an interpretation that is consistent with the observation that cellular transformation by SV40 is usually accompanied by mutations that inactivate the DNA replication functions of either Tag or oriT (12).

**Chimeric SV40–HIV-1 expression vectors.** Retroviral vectors are avidly studied for their utility in human gene therapy. Although the WT IN in these vectors confers the highly desirable trait of stable transduction, this is accompanied by the usual observation that stable integration of both Tag and oriT was highly cytopathic to Jurkat cells, an interpretation that is consistent with the observation that cellular transformation by SV40 is usually accompanied by mutations that inactivate the DNA replication functions of either Tag or oriT (12).

Chimeric SV40–HIV-1 expression vectors. Retroviral vectors are avidly studied for their utility in human gene therapy. Although the WT IN in these vectors confers the highly desirable trait of stable transduction, this is accompanied by the usual observation that stable integration of both Tag and oriT was highly cytopathic to Jurkat cells, an interpretation that is consistent with the observation that cellular transformation by SV40 is usually accompanied by mutations that inactivate the DNA replication functions of either Tag or oriT (12).

Although certain applications use gutted SV40 vectors that no longer encode Tag (reviewed in reference 48), other approaches rely on the DNA replication function of Tag (13). The oncogenic potential of Tag protein is an obvious limitation in the latter case. Since transfected SV40 vectors expressing td variants of Tag replicated to higher levels than WT Tag vectors in numerous cell lines, it was suggested that td Tag might confer a safety advantage on replication-based SV40 gene therapy vectors (13). On the basis of these findings, we engineered different td variants of Tag into N/N.Tag-oriT and tested the replication of the resulting viruses in nonpermissive Jurkat cells and MDM. Our results confirmed the earlier observation of efficient td Tag function in an established cell line (13). In contrast, macrophage-tropic derivatives of these viruses failed to yield substantial levels of gene expression in infected MDM (Fig. 10). On this basis, we speculate that binding of both pRB and p53 by Tag was required for maximum N/N.Tag-oriT gene expression in MDM. By extension, SV40 gene therapy vectors carrying td Tag may have limited applications in primary human cells, because sequestering pRB and p53 is likely important to drive cells into S phase for efficient expression from DNA replication-based vectors. Although this may limit the utility of chimeric SV40–HIV gene delivery vectors in primary human cells, such constructs may be useful in suicidal gene therapy approaches in which destruction of cycling tumorigenic cells is the desired result (reviewed in reference 54).

**ACKNOWLEDGMENTS**

We thank J. DeCaprio and M. D. Miller for plasmid DNAs. J. DeCaprio for monoclonal antibody PAb101, R. Bram and G. Crabtree for Jurkat TagC15 cells, H. Choe for Raji T4/R5 cells, and J. DeCaprio for valuable discussion. Plasmid p83-10 was obtained from Ronald Desrosiers through the AIDS Research and Reference Reagent Program.

This work was supported by NIH grants AI45313 (A.E.) and AI128691 (Dana-Farber Cancer Institute Center for AIDS Research), the G. Harold and Leila Y. Mathers Foundation, the Friends 10, the Bristol-Myers Squibb Foundation, the International AIDS Vaccine Initiative, and the Japanese Foundation for AIDS Prevention (N.N.).

**REFERENCES**


ERRATUM

Simian Virus 40-Based Replication of Catalytically Inactive Human Immunodeficiency Virus Type 1 Integrase Mutants in Nonpermissive T Cells and Monocyte-Derived Macrophages

Richard Lu, Noriko Nakajima, Wolfgang Hofmann, Monsef Benkirane, Kuan-Teh Jeang, Joseph Sodroski, and Alan Engelman

Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Department of Pathology, Harvard Medical School, and Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts 02115, and Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892