Gene Ther Mol Biol Vol 4, 133-141. December 1999.

# HIV-1 DNA integration: advancing anti-HIV-1 gene therapy approaches by blocking and modulating the process

**Review Article**

**Aaron Geist, Mohamad BouHamdan, Giuseppe Nunnari, Roger J. Pomerantz* and Joseph Kulkosky**

The Dorrance H. Hamilton Laboratories, Center for Human Virology, Division of Infectious Diseases, Department of Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

***Correspondence**: Roger J. Pomerantz, M.D., Professor of Medicine, Biochemistry and Molecular Pharmacology, Chief, Division of Infectious Diseases, Director, Center for Human Virology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107. Tel: (215) 503-8575; Fax: (215) 923-1956; E-mail: roger.j.pomerantz@mail.tju.edu

**Abbreviations: HIV-1**, human immunodeficiency virus type 1; **IN**, integrase; **RT**, reverse transcriptase; **PR**, protease; **PIC**, pre-integration complex; **ASV**, avian sarcoma virus; **LTRs**, long terminal repeats; **Ig**, immunoglobulin; **PBMCs**, peripheral blood mononuclear cells; **SFv**, single-chain variable fragment; **CAT**, chloramphenicol acetyltransferase

**Key Words**: Gene Therapy, Retroviruses, Integration

**Received: 27 July 1999; accepted: 10 August 1999**

## Summary

**The efficient replication of retroviruses requires integration of the double-stranded DNA copy of viral RNA into chromosomal DNA of the infected host cell. Integration for all retroviruses, including human immunodeficiency virus type 1 (HIV-1), represents the stable incorporation of viral DNA into that of the host and depends upon the function of the viral encoded enzyme, integrase (IN). Among various classes of animal viruses, integration of viral DNA is a unique and defining step in the life cycle of retroviruses and results in the permanent establishment of the viral genome in the infected host cell. The practical consequences of this activity is the presence of a viral DNA template capable of directing the production of progeny virus for the life span of the infected cell. This template persists even despite the imposition of therapeutic regimens available currently that involve the combinatorial use of inhibitors against HIV-1 reverse transcriptase (RT) and protease (PR) that are effective in reducing circulating virus in most HIV-1 seropositive patients. Efficient blockade of integration, or the steps preceding integration, would be far preferable than attempts to suppress the production of viral products from cells that already harbor an HIV-1 provirus.**

**The actual mechanistic details for integration of all retroviruses is remarkably similar and such an understanding is vital in a new era of rational drug design that not only relies upon an intimate knowledge of the structure and function of individual viral products, but alternatively utilizes those details to manipulate these products with the goal of halting viral replication completely or ablating the presence of infected host cells in the seropositive patient. Several small molecule inhibitors of HIV-1 integrase are in development with the eventual hope of including such compounds in combination therapy with PR and RT inhibitors. However, there are anti-IN gene therapy strategies being pursued that are highlighted in this review and which can be employed to inhibit function of the protein with a similar goal of blocking HIV-1 infection within host cells.**

## I. HIV-1 DNA integration

HIV-1 is a recently emerged human pathogen (Barrie-Sinoussi et al., 1983; Gallo et al., 1983). For most individuals, infection by this complex retrovirus results in the gradual loss of host immune surveillance and protective immune defense functions leading ultimately to death. It is anticipated that shortly beyond the year 2000, cumulative deaths as a consequence of HIV-1 infection, particularly in developing countries, will reach levels approaching those observed previously for the most severe of human plagues.

As illustrated in **Figure 1**, the early stages of the life-cycle of HIV-1 bears remarkable similarities with other retroviruses. All retroviruses encode a bank of enzymes that are absolutely required for their efficient replication (Katz and Skalka, 1994). These polypeptides, protease (PR), reverse transcriptase (RT), RT-associated RNase H activity, and integrase (IN) are attractive antiviral targets as they manifest discrete enzymatic functions that are conveniently assayed *in vitro* and also typically *in vivo*. All are relatively small proteins that are also amenable to a variety of sophisticated structural analyses. On this basis, it is not surprising that the most effective therapeutic agents currently in use, and developed to inhibit or block HIV-1 replication, are those that effectively neutralize two proteins from this panel of retroviral enzymes, RT and PR (Mellors, 1996).

While combination therapy using RT and PR inhibitors can be highly effective in suppressing viral replication in most individuals, the regimen suffers drawbacks. Therapy may not be well-tolerated by some patients, emergence of resistant virus can occur, and perhaps most importantly, HIV-1 proviral DNA remains detectable in certain reservoirs within the body in most patients even after reasonably prolonged treatment (Richman, 1994). These factors prompt the need to develop more effective inhibitors particularly those against additional HIV-1 viral products. The development of novel strategies is also warranted, including judicious gene therapeutic approaches that will either cripple the virus prior to cell infection, prevent HIV-1 replication during *de novo* infection of target cells or, finally, eliminate HIV-1 infected cells that are sequestered within tissue reservoirs of patients refractive to anti-HIV-1 compounds in use currently.

Integrase (IN) has become an increasingly attractive candidate for the development of antivirals for reasons, which relate to the success in the discovery or design PR and RT inhibitors. For instance, like reverse transcription and PR maturation of viral proteins, integration of retroviral DNA is an obligatory step in the life cycle of retroviruses, including HIV (La Femina et al, 1992), and is required for the efficient replication of all retroviruses in their respective host cells. The process of integration is generally regarded to occur in two important biochemical and temporally discrete steps (Kulkosky and Skalka, 1994). The first step, as illustrated in **Figure 2**, results in the removal of two nucleotides from the 3' ends of the double-stranded viral DNA which is generated by reverse transcription of genomic viral RNA. This reaction, referred to as *processing*, appears to occur primarily, if not exclusively, in the cytoplasmic compartment of the host cell during a natural viral infection (Brown et al, 1989). The second step occurs in the cell nucleus and involves insertion of the processed viral DNA into host chromosomal DNA. As depicted in **Figure 2**, this reaction is referred to as *joining*. Both processing and joining activities are mediated by IN and this protein is necessary and sufficient to catalyze these reactions *in vitro* (Craigie et al., 1990; Katz et al., 1990).

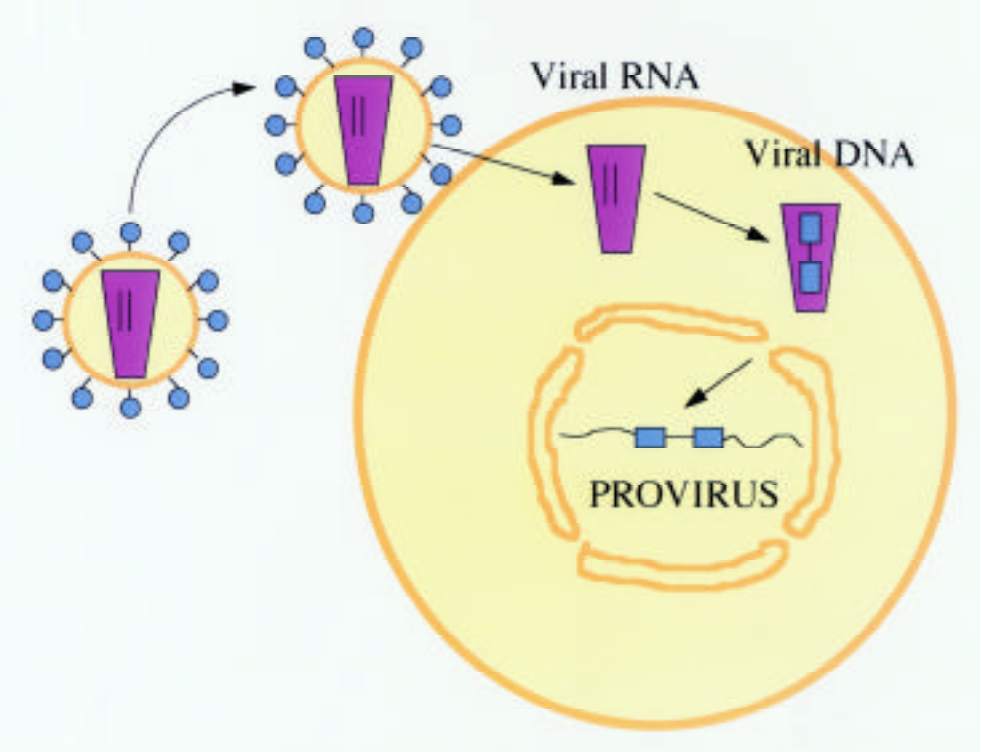


**Figure 1.** Early events of retroviral replication leading to the establishment to integrated proviral DNA. The viral core enters the cell after attachment of the virus particle to the cell surface. The viral RNA is converted to double-stranded DNA by the process of reverse transcription. This DNA copy enters the cell nucleus where it is integrated into the host genomic DNA.

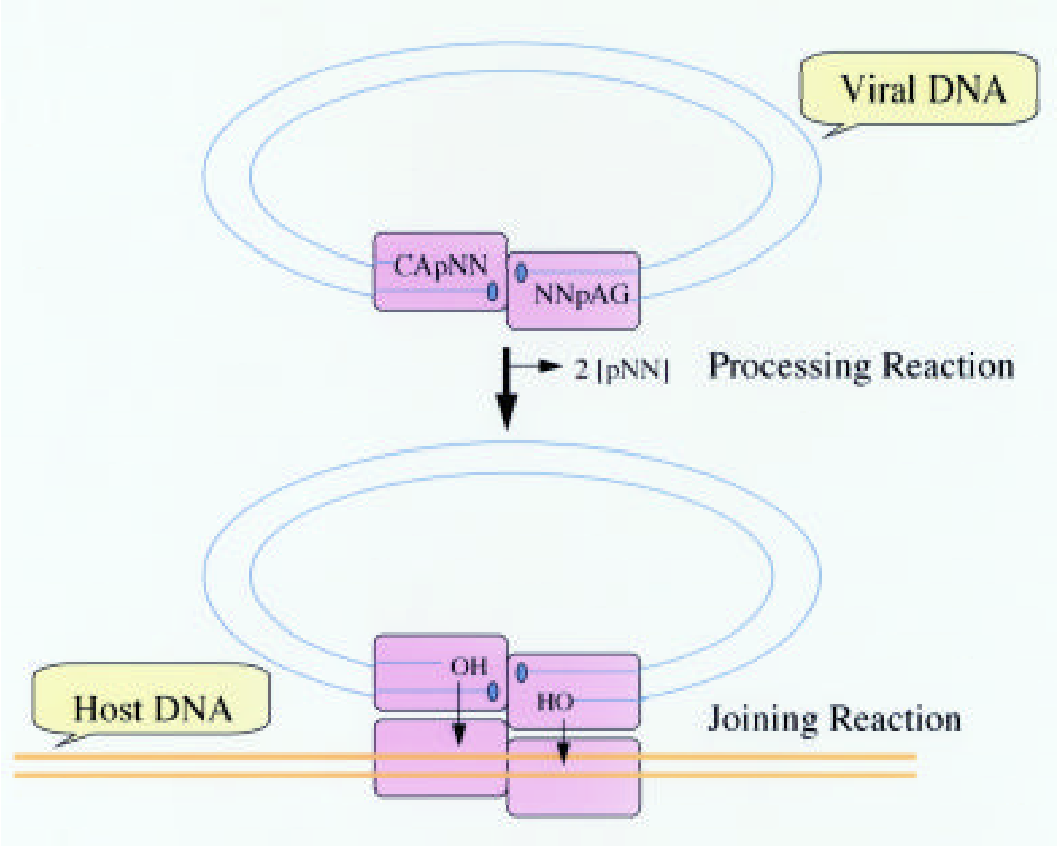


**Figure 2.** The activities of integrase on viral DNA in the cell cytoplasm (**top**) and nucleus (**bottom**). First, linear viral DNA is cleaved to produce recessed ends in the cell cytoplasm resulting in the release of the two 3' terminal nucleotides. Next, viral DNA is joined to host cell DNA by a concerted cleavage-ligation reaction referred to as joining.

The biochemical mechanism involved in processing and joining is a well-understood nucleic acid metabolic activity referred to as a phosphoryltransfer reaction (Mizuuchi, 1992). The removal of nucleotides from the ends of viral DNA by cleavage during processing involves nucleophilic attack by an oxygen atom at a specific phosphorus bond in the viral DNA backbone which is immediately 3' of a strictly conserved **CA** dinucleotide present in all retroviral DNAs as illustrated in **Figure 2**. *In vivo*, the oxygen of a water molecule likely serves as the primary nucleophile involved in this attack, although analyses of the processing reaction as performed *in vitro*, have shown that a variety of nucleophilic molecules can mediate the processing reaction (Vink et al., 1992). *In vitro*, these nucleophiles can include glycerol since the enzyme is typically stored in this stabilizing solvent, but other alcohols and even free amino acids are able to participate as agents of attack. The mechanism for joining is analogous to that of the processing reaction. In this case the oxygen of the 3' hydroxyl groups generated at the ends of viral DNA serve as the nucleophiles which attack the phosphorous atoms in the backbone of the enzyme-bound host DNA. Thus, IN-mediated joining represents a DNA transesterification reaction where phophoryltransfer from viral DNA to host DNA results in covalent linkage of one DNA to the other (Engleman et al., 1991).

IN contains a highly conserved region, referred to as the D, D(35)E domain (see **Figure 3**), that represents the active site of the protein (Kulkosky et al., 1992). The nomenclature for this domain is based on the presence of a triad of invariant acidic residues in all retroviral INs, the latter two of which are separated by 35 intervening amino acids. Mutations within the D, D(35)E domain have parallel effects on both processing and joining assayed *in vitro* and these studies provided the first clue that there was likely to be a single catalytic site which mediated both IN functions (Kulkosky et al., 1992). Deletion mutagenesis coupled with specialized activity assays subsequently confirmed this hypothesis (Bushman et al., 1993).

The acidic residues of the D, D(35)E domain were originally proposed to coordinate binding of the divalent metal cofactor based on the mechanism of action of enzymes with similar metabolic functions (Kulkosky et al., 1992). A stabilized interaction of the metal cofactor with DNA promoted by these residues serves to facilitate attack on a phosphorous atom in the DNA backbone of the substrate and

subsequently the target DNA within an IN/DNA/metal complex. High resolution X-ray crystallographic structural data has verified metal binding by at least two of the triad of acidic residues of the D, D(35)E domain (Goldgur et al., 1998).

In a natural viral infection, IN is associated with the newly reverse-transcribed DNA initially in the cytoplasm of the infected cell. This association occurs within higher order structures referred to as pre-integration complexes (PICs). PICs can be considered to be a derivative of the viral core, which is released into the cell cytoplasm after fusion of both viral and cellular membranes. These high molecular weight structures, isolated easily from virus infected cells, are comprised of viral DNA, IN as well as other proteins derived from both virus and host (Bowerman et al., 1989; Ellison et al, 1990). Unlike the PICs of oncoretroviruses, lentiviral PICs, including those of HIV-1, appear to have the unique ability of traversing the nuclear membrane of non-dividing cells (Bukrinsky et al., 1992). Several studies have suggested a mechanism of active transport for lentiviral PICs though the nuclear pores of non-cycling cells. This process of active transport may be facilitated by three viral proteins, MA, Vpr and IN (Stevenson and Gendelman, 1995). However the role that MA, Vpr and/or IN play in the nuclear import of PICs, either alone or in combination, is controversial (Reil et al., 1998). Nevertheless, understanding the actual mechanism whereby lentiviruses are able to infect non-dividing cells is important, particularly as it relates to the inability of oncoretroviruses to do so. Retroviral delivery vectors, derived from both simple oncoretroviruses, and more recently from lentiviruses, are in wide-spread use and each possess desirable as well as undesirable characteristics. Merging the attractive features of each vector system and purging those which are less desirable is the focus of investigation by several laboratories, in particular, identifying a means to permit infection of non-dividing cells using vectors based upon simple retroviral templates in order to facilitate gene therapeutic approaches.

## II. Gene therapy approaches to block integration in order to ameliorate retroviral-associated diseases

Currently, anti-viral therapies based on RT inhibitors, protease inhibitors, cytokines, and receptor blocking agents are not completely successful. This is due to the ability of HIV-1 to mutate rapidly, which results in development of numerous viral variants, some of which can evade host defenses or become resistant to various anti-viral agents (Erice and Balfour, 1994; St Clair et al., 1991). However, an alternative approach of "anti-viral gene therapy" where host cells may be genetically altered or engineered to confer long-lasting protection against viral infection, or replication, appears to be an attractive and convincing technology (Dropulic and Jeang, 1994; Pomerantz and Trono, 1994). Such strategies directed against several targets are currently being applied towards the inhibition of HIV-1 replication within cells or toward the direct elimination of HIV-1 infected cells. They include exploitation of *trans*-dominant negative mutant HIV-1 protein expression, viral antisense oligonucleotide sequences, specific ribozymes, and HIV-1 *trans*-activated "suicide" genes (Chen et al., 1994; Lisziewicz et al., 1995; Liu et al., 1994; Sun et al., 1995; Yu et al., 1993).

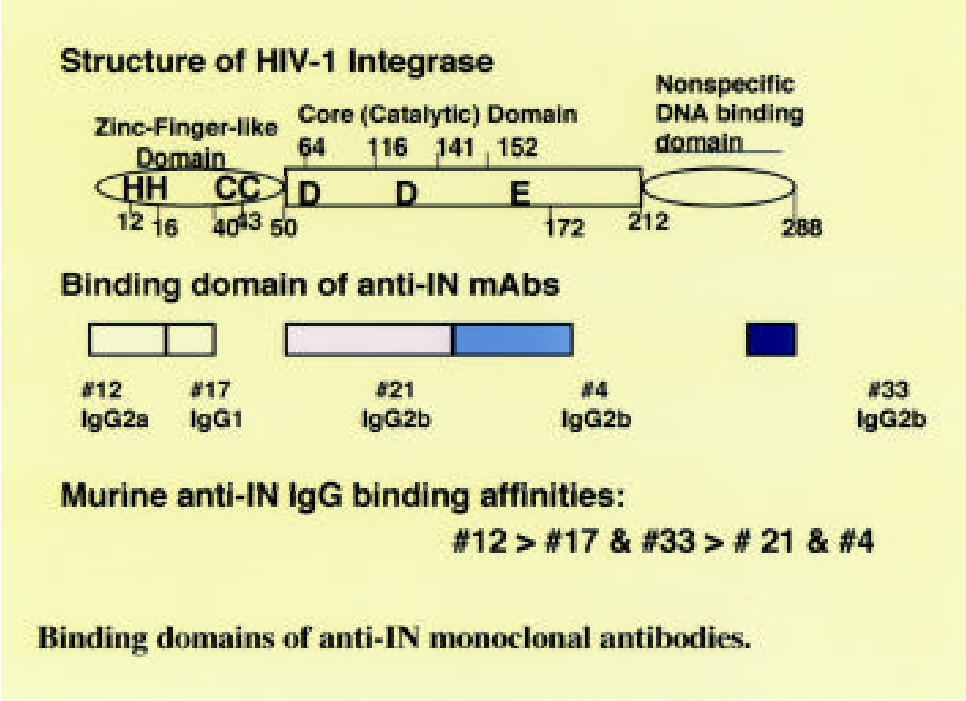


**Figure 3.** Functional domains of HIV-1 IN and binding sites of anti-IN monoclonal antibodies. IN is comprised of three discrete domains (**top**), the N-terminal HHCC region, the catalytic core or the D, D(35)E domain in the mid-portion of IN and the C-terminal DNA binding region. The approximate locations of mAb binding are shown at the **bottom**.

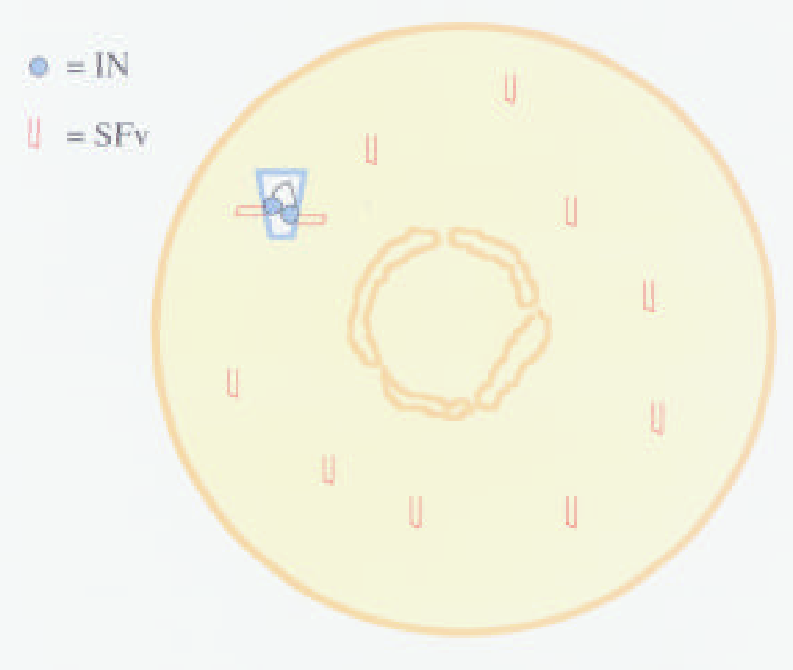


**Figure 4.** Schematic of intracellular expression of anti-IN SFv molecules and their putative binding to IN within the viral core or pre-integration complex (PIC). IN associates with the ends of viral DNA in the cell cytoplasm. The anti-IN SFvs block *de novo* infection of target cells presumably by binding to IN within the viral core structure as shown or its derivative referred to as the pre-integration complex.

Here we present an overview of the efficacy of two independent, but related, gene therapeutic approaches to inhibit HIV-1 replication that target IN function and involve the use of anti-HIV-1 IN SFvs, which are cloned and constructed from their parental murine IgG templates (Bizub-Bender et al., 1994). In the first approach, the inhibitory SFv species are over-expressed within T cell lines or primary PBMCs. Those that specifically target the HIV-1 IN catalytic or carboxy-terminal DNA binding domains, and are localized to either nuclear or cytoplasmic compartments, markedly decrease HIV-1 replication in both types of cells. The second approach involves delivery of the SFv moieties into whole HIV-1 virus particles by fusion to the HIV-1 accessory protein, Vpr, or alternatively to a 23 amino acid Vpr interactor, referred to as the dWF motif (BouHamdan et al 1998). The presence of the SFvs within virions also results in the inhibition of HIV-1 infection of host target cells. The significance of these collective findings is that they provide an opportunity to block HIV-1 replication before integration of viral DNA into the host genome occurs.

## A. Inhibiting integrase (IN) function by the over-expression of anti-IN SFvs in target cells

As multiple HIV-1 proteins have been targeted via specific SFv strategies, there is now some indication that successful inhibition of HIV-1 replication depends upon neutralizing relevant functional domains within the target protein (Mhashilkar et al., 1995; Shaheen et al., 1996). The isolation of a panel of murine hybridomas synthesizing anti-HIV-1 IN IgGs (see **Figure 3**), permitted the subsequent construction of anti-IN SFvs which target a variety of epitopes within functional domains of IN (Kulkosky et al., 1992; Engelman and Craigie, 1992; Bushman et al., 1993). The recombinant SFv sequences were first inserted into a murine retroviral delivery vector in order to transduce cells susceptible to HIV-1 infection. Intracellular expression of certain anti-IN SFvs within cells susceptible to HIV-1, resulted in cells resistant to highly cytopathic strains of HIV-1 and these experiments complement well our additional studies using anti-HIV-1 RT SFvs (Shaheen et al., 1996). SupT1 cells alone, or those expressing the CAT gene product intracellularly as well as the anti-IN SFvs #17 and #21 do not block HIV-1 replication after infection as noted by the release of HIV-1 p24 antigen in the culture supernatants beginning at day 6 and peaking at day 18. In contrast, intracellular expression of anti-IN SFv #12 and #4 provide moderate protection, while SFv #33 results in complete blockade of virus replication even at day 18 following infection by HIV-1 (Levy-Mintz et al, 1996; Levy-Mintz et al., 1998).

Our attempts to propagate HIV-1 following direct infection of anti-IN SFv expressing cells suggested there was a block at early stages of viral replication, after the entry of virions into the cellular cytoplasm (see **Figure 4**) rather than at later stages of the HIV-1 life-cycle during which the assembly and production of virions occur. This is based upon a significant early increase in 2-LTR-containing viral DNA within four to eight hours of infection in cells expressing anti-IN SFv#33 at amounts similar to that observed with IN-mutant virus (Levy-Mintz et al., 1996). Interestingly, the 2-LTR DNA appeared consistently at least two hours earlier in anti-IN SFv#33/NU-transduced cells, in which the SFv is located in the nucleus, than in IN-SFv#33-transduced cells, where the SFv is located in the cytoplasmic compartment (Levy-Mintz et al., 1996; Levy-Mintz et al., 1998). The time difference suggests that HIV-1 IN may be initially

sequestered by the cytoplasmic SFv, delaying nuclear import of the pre-integration complex, where viral DNA ends are joined via nuclear ligase activity. Alternatively, SFv#33/NU may facilitate its transport into the nuclear compartment by virtue of binding to the pre-integration complex.

Although the anti-HIV-1 IN IgGs which were used in these studies are not finely mapped to precise peptide domains, the general binding pattern of the SFvs to IN may provide some clues to how anti-HIV-1 IN SFv moieties might function. Binding of SFvs to regions in the highly conserved central catalytic domain (IN-SFv#4) and to the carboxy-terminal domain may indicate that the relevant portions of those domains may be exposed on the surface of the pre-integration complex. Data from high resolution crystal structure analysis of the core domains of the HIV-1 IN (Goldgur et al., 1998) and ASV IN (Bujacz et al., 1995) proteins indicate that the conserved region, to which IN-SFv#4 binds, is flexible in solution. The binding of a SFv to this domain may interfere with the flexibility required for this domain's enzymatic activity. The carboxy-terminus of IN, to which SFv#33 binds, is a region of least sequence homology among different retroviruses although it has been shown to promote nonspecific DNA binding which may be involved in target DNA interactions (Engelman and Cragie, 1992). When a specific residue in the carboxy-terminus of IN, Trp-235 was substituted with Ala the ability of the HIV-1 provirus to replicate was abolished (Cannon et al., 1996). It has been speculated, therefore, that this region may be required for correct positioning of processed retroviral LTRs to interact with the target host cell DNA, either by virtue of an inherent affinity for DNA or by interactions with cellular proteins that may associate with chromatin.

IN mutations, which eliminate *in vitro* enzymatic activity, frequently inhibit viral replication and block the cytopathic effects of HIV-1 in cell culture. Blockade of the integration process in anti-IN SFv-expressing cells suggests that the HIV-1 IN enzyme, is functionally neutralized in the pre-integration complex upon binding of certain SFvs, prior to completing the integration process (**Fig 4**). Hence, this binding must interrupt some steps before DNA integration into the host genome and establishment of a provirus.

In summary, the results of our experiments extend previous findings that single-chain antibodies can be stably expressed in cells, function in the cell cytoplasm as well as the nucleus, and are non-toxic to human cells. Choosing carefully from a relevant panel of anti-HIV-1 IN murine hybridomas also indicates that the SFvs, derived from these hybridomas, can be precisely manipulated for appropriate intracellular binding to specific epitopes on the target molecules. Thus, these murine SFvs provide the means to: (**i**) control intracellular infections and other diseases; (**ii**) understand the biological mechanisms in cells leading to a disease state and, in particular; (**iii**) aid in choosing the most effective human anti-IN SFvs for development and their ultimate applied use in gene therapeutic protocols to benefit human patients. Moreover, the multiple targets for anti-HIV-1 SFvs also permits the combination of different SFvs, which may result in longer term and more effective protection against HIV-1 replication. This is important since the emergence of mutations conferring resistance to antivirals in HIV-1 proteins, especially RT, may also require such combinations of various SFvs against multiple HIV-1 targets.

## B. Use of Vpr and the dWF motif to deliver anti-IN SFvs directly into HIV-1 virus particles to block function

The highly conserved HIV-1 Vpr protein, expressed in the late-stage of viral production, and incorporated into virions, is an ideal target for testing the "intravirion protein" delivery model for anti-HIV-1 gene therapy. Vpr can be packaged into virions in reasonable quantities, and many investigators have shown that this accessory protein can be fused to proteins for their delivery into progeny virus despite their in trans expression relative to other virion components (Wu et al., 1997; Liu et al., 1997; Fletcher et al 1997). For instance, a chimeric protein, utilizing the conserved protease cleavage site sequences from Gag and Gag-Pol precursor polyproteins as fusion partners to Vpr have been shown to inhibit HIV-1 infectivity (Serio et al., 1997). Other investigators have generated chimeric proteins based on fusion of HIV-1 Vpr and human immunodeficiency virus type II (HIV-2) Vpx, to CAT, staphylococcal nuclease (SN), wild-type and mutated HIV-1 PR, IN, and RT and these have been shown to reduce virion infectivity (Wu et al., 1996; Wu et al., 1995).

As has been discussed previously, intracellular expression of anti-IN SFv#33 in the host target cell has a significant inhibitory effect upon HIV-1 replication following virus challenge of these cells. Our most current studies indicate that the Vpr protein can be used to deliver the anti-IN SFv#33 directly into HIV-1 virions in an attempt to bind IN resident within virus particles. Such a “pre-binding” strategy of IN within virus particles could result in interference with the enzyme’s subsequent functions within the host cell after infection. In order to deliver SFvs into HIV-1 virions, expression plasmids bearing the SFv as a fusion to Vpr were constructed. First, immunoprecipitation indicated that the SFvs fused to Vpr were imported into HIV-1 virions. Additional studies further demonstrated that intravirion encapsidation of an anti-HIV-1 IN-Vpr-SFv decreases HIV-1 infectivity/replication in T-lymphocytic cells about 10 fold, relative to infection by HIV-1 virions not containing the imported anti-IN SFv. This decrease in virion infectivity was monitored by infection of MAGI reporter cells that stain deeply blue if infection were successful. The significance of this finding is that the delivery of SFvs directly into HIV-1 virions provides a new and novel opportunity to neutralize HIV-1 IN within the virus particle prior to binding of the virion to cell surface receptors and clearly before integration of viral DNA into the host genome.

Our laboratory has identified an additional strategy to shuttle polypeptides into virus particles that may have advantages over the import of Vpr chimeras. The putative interactions that occur to mediate such import are outlined in **Figure 5**. Based on these details, the approach employs an

in-frame fusion of a 23 amino acid peptide, referred to as the dWxxF or dWF domain, which was discovered to be a Vpr interactor using phage display technology (BouHamdan et al., 1998). In previous reports we have shown that both the bacterial CAT gene product, as well as HIV-1 IN, are incorporated into virions, as fusions to the dWF motif, likely by docking to the viral accessory protein, Vpr. In fact, the efficiency of import Vpr versus dWF can be comparable depending upon the virus into which these proteins are delivered (BouHamdan et al. 1998; Kulkosky et al., 1999). Furthermore, the import of SFv inhibitors into HIV-1 virions by dWF may have certain advantages over import as a fusion to Vpr. First, the dWF motif is smaller than Vpr, which is 96 amino acids in length. As has been reported previously, direct fusion to Vpr may hamper the function the fusion partner. This can be overcome by including an intervening HIV-1 protease cleavage site between Vpr and the fusion partner (Liu et al., 1997; Fletcher et al., 1997) though there may be situations where inclusion of such a site may be undesirable. Finally, Vpr is known to alter cellular functions therefore the use of dWF would presumably not result in the incorporation of additional Vpr molecules into HIV-1 particles.

It is important to note that most, if not all anti-HIV-1 gene therapeutics, can be overwhelmed by utilizing very high levels of incoming virus with challenge experiments *in vitro*. As such, this may be problematic for inhibiting HIV-1 replication in the interstices of lymphatic tissues of infected-individuals (Embretson et al., 1993). Therefore, "intravirion" gene therapeutics may address this concern by "immunizing" virions which "escape" from the initially infected cells.

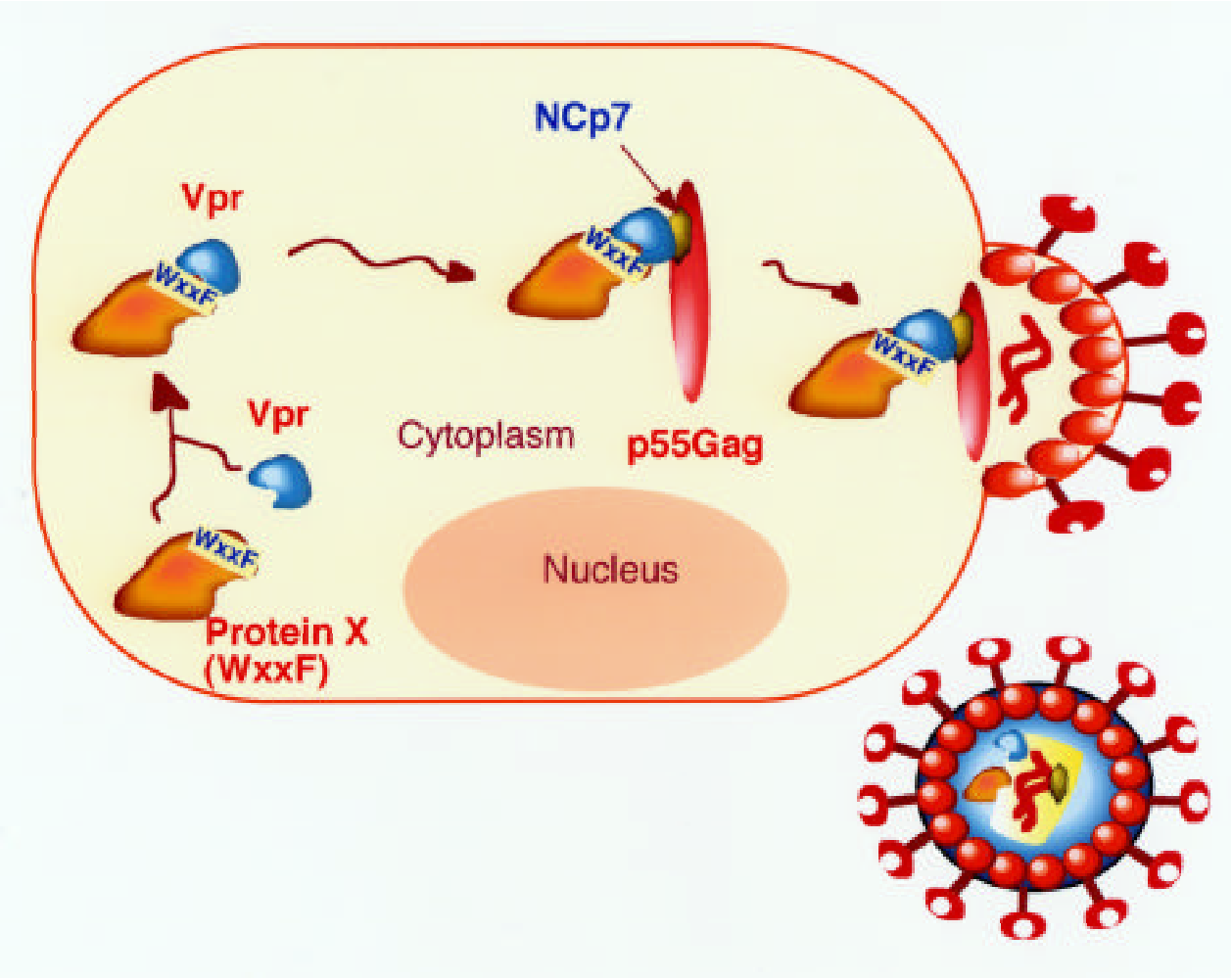


**Figure 5.** Model for intravirion incorporation of proteins via the WxxF motif mediated by Vpr. Proteins containing the WxxF motif presumably first dock onto Vpr. As shown in the schematic, the carboxyl terminus or p6 region of p55, the Gag polyprotein precursor, may also play a role in packaging the complex into HIV-1 virus particles. The model further demonstrates a potential interaction of Gag with Ncp7 that may facilitate this virion import process.

In summary, the ability to package functional anti-HIV-1 SFv-IN, via Vpr or the dWF motif, represents a novel and potentially useful technology that could contribute towards the efficient inhibition of HIV-1 replication. This new class of anti-retroviral agents could be propagated, in the patients, from cell to cell through HIV-1 virions that carry the molecular anti-retroviral. This concept could further assist in the development of efficient gene therapy-based anti-retroviral strategies, as well as in dissection of basic molecular processes within the lentiviral life-cycle.

## Acknowledgements

The authors wish to thank Ms. Rita M. Victor and Ms. Brenda O. Gordon for their excellent secretarial assistance.

## References

Barrie-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vezinet-Brun F, Rouzioux C, Rozenbaum W, and Montagnier L **(1983)** Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). **Science** 220, 868-871.

Bizub-Bender D, Kulkosky J, and Skalka AM **(1994)** Monoclonal antibodies against HIV type 1 integrase: clues to molecular structure. **AIDS Res and Hum Retroviruses** 10, 1105-1115.

BouHamdan M, Xue Y, Baudat Y, Hu B, Sire J, Pomerantz RJ and Duan L-X. **(1998)** Diversity of HIV-1 Vpr interactions involves usage of the Wxxf motif of host cell proteins. **J Biol Chem** 273, 8009-8016.

Bowerman B, Brown P, Bishop JM and Varmus H **(1989)** A nucleoprotein complex mediates the integration of retroviral DNA. **Genes Dev** 2, 469-478.

Brown P, Bowerman B, Varmus H, and Bishop JM **(1989)** Retroviral integration: structure of the initial covalent product and its precursor, and a role for the viral IN protein. **Proc Natl Acad Sci USA** 86, 2525-2529.

Bujacz G M, Jaskolski, Alexandrotas J, Wlodawer A, Merkel G, Katz RA, and Skalka AM **(1995)** High resolution structure of the catalytic domain of avian sarcoma virus integrase. **J Mol Biol** 253, 333-346.

Bukrinsky MI, Sharova N, Dempsey MP, Stanwick TL, Bukrinskaya AG, Haggerty S, and Stevenson M **(1992)** Active nuclear import of human immunodeficiency virus type 1 preintegration complexes. **Proc Natl Acad Sci USA** 89, 6580-6584.

Bushman FD, Palmer A, Wingfield P, and Craigie R **(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.

Cannon PM, ED Byles, SM Kingsman, and AJ Kingsman **(1996).** Conserved sequences in the carboxyl terminus of integrase that are essential for human immunodeficiency virus type 1 replication. **J. Virol** 70, 651-657.

Chen SY, Khouri Y, Bagley J, and Marasco WA **(1994)** Combined intra-and extracellular immunization against human immunodeficiency virus type 1 infection with a human anti-gp120. **Proc. Natl. Acad. Sci. USA** 91, 5932-5936.

Craigie R, Fujiwara T, and Bushman F **(1990)** The IN protein of Molone murine leukemia virus processes the viral DNA ends and accomplishes their integration *in vitro* . **Cell** 62, 829-837.

Dropulic B, and Jeang KT **(1994)** Gene therapy for human immunodeficiency virus infection: Genetic antiviral strategies and targets for intervention. **Hum Gene Ther** 5, 927-939.

Ellison V, Abrams H, Roe T, Lifson J and Brown P **(1990)** Human immuno-deficiency virus integration in a cell free system. **J Virol** 64, 2711-2715.

Embretson J M, Zupancic JL, Ribas A, Burke P, Racz K, Tenner-Racz B and Haase A **(1993)** Massive cohort infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. **Nature** 362, 359-362.

Engelman A. and Craigie R **(1992)** Identification of conserved amino acid residues critical for human immunodeficiency virus type 1 integrase function *in vitro.* **J Virol** 66, 6361-6369.

Engleman A, Mizuuchi K, and Craigie R **(1991)** HIV-1 DNA integration: Mechanism of viral DNA cleavage and DNA strand transfer. **Cell** 67, 1211-1221

Erice A, and Balfour HH **(1994)** Resistance of HIV-1 to anti-retroviral agents: A review. **Clin Infect Dis** 18, 149-156.

Fletcher III TM, Soares MA, McPhearson S, Hui M, Wiskerson M, Muesing MA, Shaw GM, Leavitt AD, Boek JD and Hahn BM **(1997)** Complementation of integrase function in HIV-1 virions. **EMBO J** 16, 5123-5138.

Gallo RC, Sarin PS, Gelman EP, Robert-Guroff M, Richardson E., Kalyanaraman VS, Mann D, Sidhu GD, Stahl RE, Zolla-Pazner S, Leibowitch J, and Popovic M **(1983)** Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). **Science** 220, 865-867.

Goldgur Y, Dyda F, Hickman AB, Jenkins T.M, Craigie R, and Davies DR **(1998)** Three new structures of the core domain of HIV-1 integrase: an active site that binds magnesium. **Proc Natl Acad Sci** 95, 9150-9154.

Katz R A, Merkel G, Kulkosky J, Leis J. and Skalka AM **(1990)** The avian retroviral IN protein is both necessary and sufficient for integrative recombination *in vitro.* **Cell** 63, 87-95.

Katz RA and Skalka AM **(1994)** The retroviral enzymes. **Ann Rev Biochem** 63, 133-173.

Kulkosky J, BouHamdan M, Geist A, and RJ Pomerantz **(1999)** A novel Vpr peptide interactor fused to integrase (IN) restores integration activity to IN-defective HIV-1 virions. **Virology** 255, 77-85.

Kulkosky J, Jones KS, Katz RA, Mack JPG, and Skalka AM **(1992)** Residues critical for retroviral integrative recombination in a region that is highly conserved among retroviral/retrotransposon integrases and bacterial insertion sequence transposases. **Mol Cell Biol** 12, 2331-2338.

Kulkosky J. and Skalka AM **(1994)** Molecular mechanism of retroviral DNA integration. **Pharmac Ther** 61, 185-203.

La Femina RL, Schneider CL, Robins HL, Callahan PL, Le Grow K, Roth E, Schleif WA and Emini EA **(1992)** Requirement of active human immunodeficiency virus type 1 integrase enzyme for productive infection of human T-lymphoid cells. **J Virol** 66, 7414-7419.

Levy-Mintz P, Duan L-X, Zhang H, Hu B, Dornadula G, Zhu M, KulkoskyJ, Bizub-Bender D, Skalka AM and Pomerantz RJ

**(1996)** Intracellular expression of single-chain variable fragments (SFv) to inhibit early stages of the viral life-cycle by targeting HIV-1 integrase. **J Virol** 70, 8821-8832.

Levy-Mintz P, Duan L-X, Zhang H, Hu B, Dornadula G, Zhu M, Kulkosky J, Bizub-Bender D, Skalka AM and Pomerantz RJ. **(1998)** Intracellular expression of single-chain variable fragments (SFv) to inhibit early stages of the viral life-cycle by targeting HIV-1 integrase. errata. **J Virol** 72: 3506.

Lisziewicz J, Sund D, Lisziewicz A, and Gallo RC. **(1995)** Anti-tat gene therapy: a candidate for large stage AIDS patients. **Gene Therapy** 2, 218-222.

Liu H, Wu X, Xiao H, Conway JA and Kappes JC **(1997)** Incorporation of functional human immunodeficiency virus type 1 integrase into virions independent of the Gag-Pol precursor protein. **J Virol** 71, ,7704-7710.

Liu J, Woffendin C, Yang Z-Y, and Nabel GJ **(1994)** Regulated expression of a dominant negative form of Rev improves resistance to HIV replication in T cells. **Gene Therapy** 1, 32-37.

Mellors JW **(1996)** Closing in on human immunodeficiency virus-1. **Nat Med** 3, 274-275.

Mhashilkar AB, Bagley J, Chen S-Y, Szilvay AM, Helland DG, and Marasco WA. **(1995)** Inhibition of HIV-1 Tat-mediated LTR transactivation and HIV-1 infection by anti-tat single chain antibodies. **EMBO J** 14, 1542-1551.

Mizuuchi K **(1992)** Polynucleotidyl transfer reactions in transpositional DNA recombination. **J Biol Chem** 267, 21273-21276.

Pomerantz RJ, and D Trono **(1995)** Genetic therapies for HIV infections: promise for the future. **AIDS** 9, 985-993.

Reil H, Bukovsky AA, Gelderblom HR and Gottlinger HG **(1998)** Efficient HIV-1 replication can occur in the absence of the viral matrix protein. **EMBO J** 17, 2699-2708.

Richman DD **(1994)** Viral resistance to antiretoviral therapy. In: S. Broder, T.C. Merigan and D. Bolognesi (Eds.) **Textbook of AIDS Medicine**. Baltimore. Williams

Serio D, Rizvi TA, Cartas M, Kalyanaraman VS, Weber IT, Koprowski H and Srinivasan A. **(1997)** Development of a novel anti-HIV-1 agent from within:effect of chimeic Vpr-containing protease cleavage site residues on virus replication. **Proc Natl Acad Sci USA** 94: 3346-3351.

Shaheen F, Duan L-X, Zhu MH, Bagasra O, and Pomerantz RJ. **(1996)** Targeting human immunodeficiency virus type 1 reverse transcriptase by intracellular expression of single chain variable fragments to inhibit early stages of the viral life-cycle. **J Virol** 70, 3392-3400.

St. Clair MH, Martin JL, Tudor-Williams G, Bach MC, Vavro CL, King DM, Kellam P, Kemp SD, and Larder BA. **(1991).** Resistance to ddI and sensitivity to AZT induced by mutation in HIV-1 reverse transcriptase. **Science** 253, 1557-1559

Stevenson M and Gendelman H **(1995)** Cellular and viral determinants that regulate HIV-1 infection in macrophages. **J Leuk Biol** 56, 278-288.

Sun LQ, Pyaty J, Smythe J, Wang L, MacPherson J, Gerlach W, and Symonds G. **(1995)** Resistance to human immunodeficiency virus type 1 infection conferred by transduction of human peripheral blood lymphocytes with ribozyme, antisense, or polymeric trans-activation response element constructs. **Proc Natl Acad Sci USA** 92, 7272-7276.

Vink C, Yeheskiely E, Van Der Marel GA, Van Bloom J and Plasterk RH **(1992)** Site-specific hydrolysis and alcoholysis of human immmunodeficiency virus DNA terminal sequences mediated by the viral integrase protein. **Nucl Acids Res** 19, 6691-6698.

Wu X, Liu H, Xiao H and Kappes JC **(1996)** Proteolytic activity of human immunodeficiency virus Vpr- and Vpx-protease fusion proteins. **Virology** 219, 307-313.

Wu X , Liu H, Xiao H, Kim J, Seshaiah P, Natsoulis G, Boeke JD, Hahn BH and Kappes JC **(1995)** Targeting foreign proteins to human immunodeficiency virus particles via fusion with Vpr and Vpx. **J Virol** 69, 3389-3398.

Wu X, Xiao H, Conway JA, Hunter E and Kappes JC. **(1997)** Functional RT and IN incorporated into HIV-1 particles independently of the Gag/Pol precursor protein. **EMBO J** 16, 5113-5122.

Yu M, Ojwang J, Yamada O, Hampel A, Rappaport J, Looney D, and Wong-Staal FW **(1993)** A hairpin ribozyme inhibits expression of diverse strains of human immunodeficiencY virus type 1. **Proc Natl Acad Sci USA** 90, 6340-6344.

Roger J. Pomerantz