Published in final edited form as:
Future Med Chem. 2010 July 1; 2(7): 1055–1060. doi:10.4155/fmc.10.205.

Prospective strategies for targeting HIV-1 integrase function

Yang Luo[1] and **Mark A Muesing**[1,†]

[1] Aaron Diamond AIDS Research Center, 455 1st Avenue, New York, NY 10016 USA

Abstract

Integration is a key step in the HIV-1 life cycle in which the ends of linear viral DNA are covalently joined with host chromosomal DNA. Integrase is the highly conserved and essential viral protein that performs two catalytically related reactions that ultimately lead to the insertion of the viral genome into that of the host cell. The only chemotherapeutic agents against integrase currently available for HIV-1 infected individuals are those that interrupt strand transfer, the second step of catalysis. Accordingly, this article outlines possible future strategies targeting the first catalytic step, 3′ processing, as well as other nonenzymatic, yet indispensible, functions thought to be coordinated by integrase. Importantly, the interruption of irremediable recombination between viral and host DNAs represents the last step after viral entry at which an otherwise irreversible infection can be prevented.

Retroviral integration establishes a pro-viral state in which the viral genome (HIV-1: ~10^4 base pairs) is contiguous and indistinguishable from that of the host chromosomal DNA (~3×10^9), the embedded retrovirus eventually commandeering a genome some 300,000 times larger than itself. At once, the provirus becomes a permanent fixture within the cell, its location within host chromosomal DNA providing a sanctuary site for the productive expression of the viral proteome and well as a mechanism for the generational perpetuation of the viral genome (vDNA).

Over the past 20 years, inhibition of two essential viral enzymes, protease (PR) and reverse transcriptase (RT), have been clinically validated. When used in combination, small-molecule inhibitors of PR/RT are potent antiviral agents. These drugs interrupt their respective catalytic activities and, when administered together, provide a robust regimen for greatly extending the longevity and quality of life of infected individuals. Yet, drug toxicity, lack of patient compliance and the emergence of multidrug resistance make resolute the call for further strategic intervention. The third essential viral enzyme, integrase (IN) was recognized early in the epidemic as a valid antiviral target but initially proved recalcitrant to the identification and development of inhibitors of its catalytic functions.

The biochemical reactions that lead to retro-viral integration, extensively studied *in vitro*, are defined by two catalytically related and sequentially dependent steps (3′-processing followed by strand transfer) [1]. Using purified IN protein and surrogate DNA substrates

[†]Author for correspondence: Tel.: +1 212 448 5060, Fax: +1 212 448 5159, mmuesing@adarc.org.

Financial & competing interests disclosure

Mark Muesing has been awarded an Investigator-Initiated Studies Program (IISP) from Merck, Inc. This work explores 3′ processing directly from HIV-1 infected cultures using new ligation-mediated-PCR methodologies. The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

and building on the earlier biochemical characterization of the enzyme, Hazuda and colleagues created appropriate drug-screening assays to identify true strand-transfer inhibitors (STIs) [2,3]. These organic compounds were chemically refined and developed into a prototypic candidate, raltegra-vir (RAL), subsequently approved for salvage combination therapy (October 2007) [4] and more recently approved for use in all patients (July 2009). First-of-class inhibitors such as raltegravir [5] and elvitegravir (EVG) [6,7] have demonstrated very potent antiviral activity clinically. Although virus obtained from STI-naive infected individuals do not contain amino acid substitutions associated with pre-existing STI resistance [8–12], broad resistance against both drugs can arise in patients *in vivo* or when the virus is challenged by multiple passages in the presence of this inhibitor class *in vitro*. In an effort to circumvent inhibitor resistance and to identify compounds that synergize with the available STIs, compounds that block IN function at other points in the viral life cycle are needed. Accordingly, in this commentary, we explore new approaches that have the potential to interrupt IN function not only at catalysis but also for those requisite nonenzymatic function(s) associated with this remarkable viral protein.

Inhibitors of the 3′-processing reaction

In a sequential set of reactions, IN removes two nucleotides from the 3′ end of each vDNA strand (3′-processing), which serves to activate the $CA^{OH\text{-}3'}$ termini for a subsequent and concerted strand-transfer reaction. This two-step catalysis is directed across a defined assembly pathway of IN oligomerization [13,14]. DNA end-processing is thought to be coordinated in the context of dimeric IN, each dimer bound with one of the ends of vDNA left after reverse transcription of the viral genetic material. Processing catalysis leads to vDNA–IN restructuring; the dimeric units now joined forming tetrameric IN in complex with the 3′-processed vDNA (the intasome) [15–17]. Although both enzymatic reactions catalyzed by IN presumably share a spatially related active site, the STIs are specific to the strand-transfer reaction and do not inhibit 3′-processing *in vitro* [3] or *in vivo* [Muesing M, Unpublished data]. Creation of a stable binding site for STIs appears to require passage through a transient vDNA–IN structural intermediate [18,19] or drug recognition may necessitate a pre-assembled, complete IN tetramer. Understanding STI binding has been considerably strengthened by the recent publication of the 3D structure of a retroviral intasome in complex with either the RAL or EVG inhibitor [15–17]. The prototype foamy virus IN (PFV IN) was chosen for this analysis owing to its exquisite biophysical properties (e.g., high solubility and efficient concerted strand transfer *in vitro*) [15–17,20], those not shared with relatively insoluble HIV-1 IN, a protein that exhibits an *in vitro* propensity for nonspecific aggregate formation with itself and DNA. The derived PFV intasome structure indicates that binding of STIs to the active site forms a ternary complex between the drug, vDNA and the two divalent Mg^{2+} cations, the latter acting as co-factors for IN-mediated, strand-transfer catalysis. In effect, the reactive 3′-hydroxyl groups ($CA^{OH\text{-}3'}$) left at the vDNA ends by prior 3′-processing are selectively displaced by inhibitor binding, disarming any further reactivity of the complex [15,17].

Since neither RAL nor EVG inhibit the 3′-processing reaction even at micromolar concentrations, 3′-processing inhibitors (3PIs) would represent a new class of compounds, specific to disruption of 3′-processing catalysis and perhaps capable of synergistic antiviral activity with the existing STIs. Viral outgrowth in the presence of both STIs and 3PIs would be expected to be particularly challenging for the virus; viral fitness perhaps compromised by the number of amino acid substitutions required within the same protein to confer dual drug resistance [21].

The search for 3PIs has been an ongoing endeavor for the discovery of compounds that interrupt HIV replication at the enzymatic step that precedes and activates the viral DNA

termini for the subsequent strand-transfer step. However, to date, inhibitors that specifically interrupt 3′-processing have not been identified. *In vitro*, styrylquinoline (SQL) derivatives have been documented to compete with viral DNA for IN binding acting to block 3′-processing [22]. In contrast to STIs that are specific for one catalytic step, SQL derivatives may inhibit viral replication at a combination of steps in addition to 3′-processing, including reverse transcription [23] and nuclear import [24], both of which are thought to be facilitated in some measure by IN. Amino acid substitution mutations associated with the SQL resistance map to the IN coding sequence; conversely recombinant viruses incorporating these IN mutations restore drug resistance [23]. SQL-resistant viruses [23] are associated with amino acid substitutions that differentially map within IN from those mutations selected for STI resistance [25–27]. This is consistent with the notion that the mechanism of SQL inhibition is mechanistically distinct from that of the STIs and/or bind to IN using discrete sites of interaction. On the other hand, bifunctional derivatives of 5-CITEP [1-(5-chlo-roindol-3-yl)-3-hydroxy-3-(2H-tetrazol-5-yl)-propenone] display characteristics of both 3PIs and STIs [28]. It has been proposed that these bifunctional β-diketo acids inhibit 3′-processing by binding to the donor DNA (e.g., viral LTR) [28]. If proven effective, potent 3PIs could be used in combination with available STIs (and PR/RT inhibitors) to lessen the possibility of drug resistance escape from antiviral chemo-therapeutic regimens that target both catalytic functions of IN.

Targeting IN multimerization & cryptic inhibitor binding sites

Efforts have been made to discover small molecules that can modulate IN catalytic function through allosteric modulation of the dynamic interaction that exists between its monomeric, dimeric and tetrameric forms. Early work with IN-derived synthetic peptides first highlighted the feasibility of this approach [29,30]. Accordingly, external modulation of the equilibrium that exists between the dimeric and tetrameric forms by perturbation of the binding between the cellular protein LEDGF/p75 (see below) and IN has recently been proposed and demonstrated to be a logical approach for inhibition of the enzymatic functions of IN [31,32]. To date, only one low-molecular-weight organic compound (Chiba-3003) has been identified with this property (micromolar inhibition *in vitro*) [33], while the use of small peptides (shiftides) derived from LEDGF/p75 amino acid sequences have had some inhibitory effects against the virus in standard infection assays [31,32]. It is the shifting of the equilibrium of IN between oligomeric states with small peptides that can negatively modulate IN activity. Apparently, shiftide ‘trapping’ of IN to its dimeric form precludes IN tetramerization by inhibiting interactions made between two DNA-bound IN dimers. Although some of these inhibitors bind preferentially to and stabilize the IN tetramer as compared with the IN dimer, a shorter version of an inhibitory peptide has been shown to preferentially bind the IN dimer [34]. Disruption of the IN dimer has also been demonstrated *in vitro* by selective binding of a tetra-acetylated peptide inhibitor to the dimer interface and also made possible the mapping of a previously unknown inhibitor binding site on IN [35]. Restriction of dimer formation does not directly interfere with IN binding to DNA or to LEDGF/p75 but it compromises the formation of fully functional IN [35]. Interestingly, the inhibitor binds near IN lysine residue 173, a region overlapping with an IN-derived synthetic peptide (amino acids 167–187) known to inhibit enzyme activity via disruption of IN multimerization [29]. Residues 170–180 have also been implicated as important for virion packaging of the host uracil DNA glycosylase (UNG2) [35,36], an enzyme essential to the viral life cycle [37]. Another cryptic inhibitor-binding site may be within the IN C-terminal domain (CTD, residues 212–288). The nucleotide analog pyridoxal 5′-phosphate (PLP) impairs IN activity by binding to the CTD of IN at lysine 244, disrupting IN-DNA binding *in vitro* by mimicking the phosphate backbone of DNA [38]. It has been proposed that basic residues, K244, R262, K264 and K266, form a plausible nucleotide inhibitor-binding pocket

[38]. This structural information may be used to facilitate a rational approach for the design of nucleotide inhibitors directed to inhibit viral or host DNA binding.

Inhibitors to block IN & host co-factors

Several host factors interacting with HIV-1 IN have been implicated in multiple steps of viral replication such as reverse transcription, pre-integration complex formation, nuclear localization, integration and assembly. Interactions made between IN and cellular cofactors, if proven to be essential for HIV-1 infection, also present potential targets for antiviral intervention. The best characterized interaction made between IN and a requisite cellular protein factor is that made between IN and a splice variant ofLEDGF/p75, a host protein that is critical for HIV integration and replication [39–43]. It has been shown to tether an assembled integration complex to host chromatin, thereby acting as an intermediate binding factor between IN and host chromatin factors and serving to guide the intasome to sites where integration can occur [44]. X-ray crystallography of the IN-binding domain (IBD) of LEDGF/p75 as well as a structure of the LEDGF/p75 IBD bound to the IN core domain [45] has facilitated structure-based drug design of peptides (LEDGINs) that disrupt the LEDGF/p75–IN interaction. The IBD of LEDGF/p75 has been mapped to residues 347–429 [46,47]. The peptide segment interacts specifically with the amino terminal and core domains of IN [43,45,48]. Peptides derived from residues 361–370 and 401–403 of the IBD can inhibit IN catalytic activity *in vitro* and viral replication in cells [32]. Animal studies are underway for a potent 2-(quinolin-3-yl) acetic acid derivative (CX06387), which specifically disrupts this interaction and inhibits HIV replication *in vitro* [49,50]. It shows no toxicity in cell cultures and was not cross resistant with STIs. This demonstrates the feasibility of the rational design of small molecules inhibiting critical interactions made between IN and requisite host protein factors. However, recent mutagenic analysis within the catalytic core domain of IN indicates that certain IN mutants unable to interact with LEDGF/p75 are still able to bind host chromatin and suggest that IN has a LEDGF/p75-independent determinant for chromatin binding [51]. Mutant viruses incorporating these same mutations are capable of maintaining a state of low level of replication [51]. This result might limit the efficacy of inhibitors targeting the LEDGF/p75–IN interaction. Further understanding of the mechanisms of chromatin targeting manifested by LEDFG/p75 and other host factors [52] is necessary for the further implementation of this class of inhibitors.

HIV-1 IN has been reported to interact with cellular proteins other than LEDGF/p75. These host factors and their associated cellular complexes have a variety of activities in cells that include chromatin remodeling, DNA repair and its mutagenesis, nuclear import, intracellular protein stability and interconversion between protein conformational states: these are INI1 [53], Rad18 [54,55], LEDGF [56], EED [57], importin 7 [58], Gemin2 [59], UNG2 [36,37], N-recognins [60], APOBEC3F/G [61], von Hippel-Lindau binding protein 1 (VBP1) [62], p300 [63], Transportin-SR2 (TNPO3) [64,65], SAP18-HDAC1 [66] and JNK/PIN1 [67]. Identification of some of these factors may provide a scheme to inhibit other, nonenzymatic IN functionalities. A recent report shows direct interaction between the full-length IN and importin α and the nuclear import of IN is promoted by a specific nuclear localization signal domain located between IN residue 161–173 [68]. Indeed, a peptide bearing this sequence has been reported to inhibit viral infection by blocking the nuclear import of PIC [68]. Characterization of the above purported interactions is, however, still at a rudimentary stage. Furthermore, there have been conflicting reports on whether all of these putative interactions are required for HIV-1 replication or whether targeting multifunctional host proteins has a potential for overt cellular toxicity. On the other hand, it should be noted that targeting host factors has the added utility of being relatively inert with respect to development of drug resistance. Future characterization of the specificity of the discovered cellular interactors will need to be confirmed, and their association with IN validated for their indispensability

to viral replication. This would provide the impetus for subsequent investigation of those requisite host factors expropriated by the pathogen – a subset of which may be represent new targets for antiviral intervention.

Inhibitors that target IN post-translational modification

HIV-1 IN is subject to post-translational modification by ubiquitination, acetylation and phosphorylation. Respectively, these alterations regulate the intracellular stability of IN [60,62,69], the efficiency of integration [63,70,71] and the regulation of the permissiveness of resting $CD4^+$ T cells to HIV-1 infection [67]. For example, it has been shown that p300 and GCN5, two cellular histone acetyl-transferases that regulate host chromatin conformation affect IN acetylation, which in turn increases the affinity of IN for DNA, promoting strand-transfer activity [63]. However, only a slight deficiency was found with regard to viral replication in immortalized cell lines when arginine substitutions were created for those lysine residues targeted by p300 acetylation [71]. Unfortunately, components of major pathways of protein stability, chromatin remodeling and intracellular signaling are involved in a panoply of intracellular events and, apart from their potential to curtail viral replication, their hypothetical enzymatic inhibition would be expected to be deleterious to the host cell. Still, hope will remain if small-molecule inhibitors could be identified that specifically interrupt points of contact between IN and the relevant post-translational modification machineries (in similar fashion to small molecule CX06387 abrogation of IN-LEDGF/p75 binding as described previously).

Future perspective

Effective treatment of HIV infection will advance rapidly in the coming years, partly because of the addition and further elaboration of STIs having been added to the existing antiviral armamentarium. However, the emergence of viral resistance to the STIs makes immediate the call for new agents that can act to complement the STIs in treated individuals. Early implementation of unique classes of RT inhibitors can be used as paradigm for such strategies. Nucleoside and non-nucleoside RT inhibitors bind to different sites within RT, each class of RT drug acting to inhibit reverse transcription by a distinct mechanism. Future development of IN inhibition would be best served by the discovery of second- and third-generation STIs that could be used in combination with inhibitors of IN function, acting through nonoverlapping biochemistries or that use distinct classes of pharmacophores acting through different binding pockets within IN or with activities specific to one of the alternative oligomeric forms of IN.

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