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Mechanisms and inhibition of HIV integration

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Abstract

HIV integrase is required for viral replication and a rationale target for antiretroviral therapies. Integrase inhibitors are potentially complementary to current treatments. This review focuses on the mechanisms of HIV integration. The roles of viral and cellular co-factors during pre-integration complex (PIC) formation and integration are reviewed. The biochemical mechanisms of integration, integrase structures and approaches to inhibit integration are described.

Introduction

Acquired immunodeficiency syndrome (AIDS) represents one of the most important modern epidemics with over 40 million people infected worldwide. In 2005, 3 million people died from AIDS-related diseases and 5 million new HIV infections occurred (www.unaids.org). Recent progress has increased the efficacy of the standard HAART treatment (Highly Active Anti-Retroviral Therapy) which, contains a cocktail of reverse transcriptase and protease inhibitors (for review see [1]). But this treatment remains expensive, generates resistance and is often not well-tolerated by patients [2]. Therefore new therapeutic approaches are warranted. One such approach consists of targeting the third viral enzyme integrase, for which there is currently no inhibitor approved for treatment (for review see [3]). Recently two pharmaceutical companies, Merck Research Laboratories and Gilead Sciences Inc. announced progression to phase III and phase I/II trial for their respective integrase inhibitors [4,5]. These major breakthroughs provide proof of principle for targeting retroviral integration and promises for a new component in the HIV/AIDS treatment.

HIV Life cycle

The primary function of HIV-1 integrase (IN) is to catalyze the insertion of the viral cDNA into host chromosomes. Integration is absolutely required for viral replication. *In vivo*, integration occurs within a large nucleoprotein complex referred to as the preintegration complex (PIC) (for review, see [6]) (Fig. 1). Following reverse transcription in the cytoplasm, the viral cDNA is associated with IN into the PIC until nuclear translocation and integration into a host chromosome. Translocation of the PIC toward the nucleus is probably achieved through interactions with the microtubule network. The nuclear import mechanism of the PIC has not been completely elucidated [6].

In the PIC, a tetramer of IN binds the two viral DNA long terminal repeats (LTR) and is associated with viral and cellular co-factors (for a recent review see [7]) (Fig.1). The barrier-to-autointegration factor (BAF) is a host cellular protein probably involved in chromatin organization, which prevents autointegration and stimulates chromosomal integration. BAF bridges and compacts viral cDNA inside the PIC (Fig.1). In the Moloney Murine Leukemia

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Virus, BAF interacts with the lamina-associated polypeptide (LAP2α), a LEM (for LAP2, Emerin and MAN1) nuclear domain protein associated with the nuclear lamina that could play a role in the nucleoprotein organization of the PIC (Table 1).

The High Mobility Group Protein A1 (HMGA1), a host DNA binding protein involved in the chromosomal architecture, is another component of the PIC that stimulates concerted integration by bridging and compacting the viral cDNA (Table 1).

The integrase interactor 1 protein (INI1/hSNF5) is a human homologue of yeast SNF5, a transcriptional activator and component of the chromatin remodeling SWI/SNF complex. INI1/hSNF5 interacts with IN within the PIC and has been shown to interact with other cellular proteins such as p53 in the cell (Table 1). Following viral entry, INI1/hSNF5 is incorporated into the PIC after 1) being imported into the cell by the viral particle and/or 2) being exported out of the nucleus [7].

LEDGF/p75, a member of the hepatoma-derived growth factor family, also interacts with IN (Table 1). This nuclear protein seems involved in nuclear import and chromosome tethering of the PIC but its exact role during lentiviral integration remains under investigation [7]. The crystal structure of the integrase binding domain of LEDGF/p75 bound to the catalytic core domain of an IN dimer was recently reported [8] and will be discussed later (see Fig. 2).

Four other proteins have recently been added to the list of potential cellular co-factors of retroviral integration that could be part of the PIC [7]. The Polycomb group embryonic ectoderm development (EED) protein, HRP2, the heat-shock protein 60 (HSP60) and the p300 acetyltransferase all interact with IN (Table 1).

Several viral proteins are also part of the PIC (for review see [6]). Matrix (MA) interacts with IN and BAF, and is implicated in the nuclear import of the PIC (Table 1). The viral protein R (Vpr) exhibits karyophilic properties, induces apoptosis after cell-cycle arrest and reduces viral mutations through an interaction with the uracil DNA glycosylase (UNG) (Table 1). Nucleocapsid (NC) is a nucleic acid chaperone and a viral co-factor of reverse transcriptase (RT) that stimulates integration (Table 1). Finally, RT remains associated with the PIC where it interacts with NC and IN (Table 1).

Despites many reports describing the importance of each individual co-factor for efficient concerted integration, the potential molecular mechanisms of their interactions with IN are being further investigated.

Integrase structure

The three viral enzymes (protease, reverse transcriptase and integrase) are encoded within the HIV *POL* gene and translated as a polyprotein. IN is released from the polyprotein by protease cleavage during maturation. IN is a 32-kDa protein comprised of three domains: the amino-terminal domain (NTD), the catalytic core domain (CCD) and the carboxy-terminal domain (CTD) (for review see [9]) (Fig. 2A). No 3-D structure is currently available for the full-length IN nor for part of the enzyme in presence of its DNA substrate. Figure 2 illustrates the dimeric atomic structure of the NTD with the CCD [10] (Fig. 2B) and the structure of the CCD in association with the CTD [11] (Fig. 2C). All three IN domains are important for multimerization and required for 3′-processing and strand transfer.

The NTD (residues 1-50) contains a conserved HHCC binding motif that coordinates one zinc atom. The NTD dimerizes differently in crystal and solution structures suggesting multiple arrangements of IN multimers in tetrameric IN complexes [9]. The NTD interacts with two cellular transcription factors: INI1 and LEDGF/p75 (Table 1).

The CCD (residues 51-211) is structurally similar to other retroviral integrases and to RNase H [9]. This family of polynucleotide transferases contains a canonical three-amino acid DDE motif corresponding to D64, D116, and E152 in HIV IN (Fig. 2A, B & C, in red). Mutation of any of these residues abolishes IN enzymatic activities and viral replication. These residues coordinate presumably two divalent metal ions (Mg^{2+} or Mn^{2+}) in complex with the viral and host DNA [9]. Most CCD structures contain a disordered 'flexible loop' (residues 140-149), which is probably stabilized by DNA binding. The IN binding domain of LEDGF/p75 has been co-crystallized with the IN CCD [8] (Fig. 2D & E).

The CTD (residues 212-288) is the least conserved among retroviral integrases but has an overall SH3 fold [9]. The CTD binds to a broad range of DNA sequences beside the viral LTRs. This domain has also been implicated in protein-protein interactions with reverse transcriptase (RT) and cellular embryonic ectoderm development protein (Table 1).

Published results suggest that IN exists as a tetramer in human cells but the arrangement of a co-crystal of IN plus viral and host DNA has not yet been elucidated by crystallography [9]. Molecular modeling offers insights into this complex and we confine our description to four recent models obtained by reconstruction of the full-length protein and docking of DNA substrates. De Luca *et al.* used automatic docking to illustrate that the viral DNA tracks along a path of positively charged residues extending from the core and N-terminal domains of one subunit to the C-terminal domain of the second subunit in trans [12], as has been shown experimentally [13] and modeled by others [14]. Molecular dynamics predicted that only one subunit site of the dimer is catalytically active [15]. Furthermore, the flexible loop of only the 'active' subunit undergoes conformational change upon DNA binding [15]. Moreover, non-specific DNA binding to one dimer of an IN tetramer and specific DNA binding to another, perhaps reflects host and viral DNA binding, respectively [16]. Karki et al. published a detailed model including diketo acid inhibitor docking illustrating keto-enol chelation of one metal ion [14]. Additional interactions with the IN active site and the viral DNA may provide stronger interactions with IN.

Mechanism of integration

The mechanisms of 3′-processing and strand transfer are schematically diagrammed in Figure 3 (for review, see [3]). Following reverse transcription in the cytoplasm, the viral cDNA is primed for integration by IN trimming of the 3′-ends, referred to as 3′-processing (3′-P). In this reaction, IN catalyzes an endonucleolytic cleavage at the 3′ site of the conserved CA, which generally releases a terminal GT dinucleotide (Fig. 3). This reaction generates CA-3′-hydroxyl ends that provide the nucleophile groups required for the second step, strand transfer (ST). Following PIC migration into the nucleus, IN catalyzes insertion of the two viral cDNA ends into a host chromosome. Genomic integration is random but tends to occurs in transcribed genes. ST consists of ligation of the two 3′-hydroxyl ends to the host chromosome with a five-base-pair stagger across the DNA major groove (see Figure 2 in [3]). This reaction results in a two-base overhang on the viral cDNA 5′-end and a 5 base single-stranded gap at each junction. The trimming and gap repair of the duplex DNA structure is probably completed by host cell DNA repair enzymes, although RT has been proposed to be involved in this reaction.

Hence, two DNA binding sites may exist in the IN complex: one for the donor (viral) cDNA and the other for the acceptor (host chromosome) DNA (Fig. 3, in blue and pink, respectively) [3]. Following 3′-processing, it likely that the active site undergoes a conformational change rendering the acceptor-site competent for binding host DNA, leading to strand transfer. The most successful class of IN inhibitors, the diketo-acid-like derivatives, selectively inhibits the strand transfer reaction. Diketo acids may bind at the interface of the IN-DNA-divalent metal complex to the acceptor site [17] (described below).

Approaches to inhibit viral integration

After over ten years of research, two IN inhibitors are currently in clinical development [4,5]. MK-0518 from Merck Research Laboratories and GS-9137 from Gilead Sciences, Inc. are under focus since their initial successes in clinical trials involved experienced-patients with multidrug-resistances [18,19]. GS-9137 has a synergistic effect when administrated in combination with the cocktail of reverse transcriptase inhibitors zidovudine (AZT)/lamivudine (3TC) and has an additive effect with protease inhibitors such as indinavir (IDV) and nelfinavir [20]. Diketo-acid-like inhibitors bind the IN CCD and generate resistance mutations clustered in the vicinity of the catalytic triad residues (DDE, for review, see [3]). It is generally accepted that these inhibitors act by chelating the divalent metal co-factor (Mg^{2+} or Mn^{2+}) inside the IN active site (Fig. 3 E). Metal chelation by the drug prevents the acceptor DNA (host chromosome) from binding to the acceptor site (Fig 3 D). We described this mechanism as interfacial inhibition [17] because the chelating inhibitor binds at the interface created by the donor DNA bound inside the IN catalytic site with divalent metals [3]. Therefore the active site is stabilized and locked in an inactive conformation. Other approaches have been aimed at targeting the catalytic site of IN. In particular, the inhibition of the IN catalytic activity by nucleic acids decoys such as dinucleotides, DNA aptamers or G-quartet oligonucleotides still remain attractive approaches [21].

Other approaches could also be considered. For example, inhibition of lentiviral integration could be achieved by targeting the different IN interfaces resulting from enzyme multimerization (dimerization and tetramerization) and/or PIC formation. Dimerization has been shown to be impaired by polypeptides derived from interfacial regions of IN [21]. Every viral or cellular co-factor present in the PIC also represents a potential target. In particular, inhibition of the interactions between IN and these components (Table 1) could lead to reduced nuclear translocation of the PIC or impaired integration. The co-crystal structure of IN CCD with LEDGF/p75 reveals details of the binding interface. An 86-residues fragment of LEDGF/p75 binds at the interface of an IN CCD dimer towards a hydrophobic pocket [8] (Fig. 2 D & E). This interface may only be part of the total IN-LEDGF/p75 interaction as the IN NTD has been shown to contribute to LEDGF/p75 binding [7]. Targeting IN-LEDGF/p75 interaction by preventing association or by stabilizing and locking the complex by interfacial inhibition [17], may lead to a reduction in HIV replication. Styrylquinoline derivatives (SQLs) have been shown to inhibit nuclear translocation of the PIC and one resistance mutation (V165I) has been identified in the IN CCD at the binding site for LEDGF/p75 (for review, see[21]). Despite the fact that SQLs also inhibit IN catalytic activity, SQLs may represent potential candidates for inhibiting the IN-LEDGF/p75 interaction. Other co-factors interacting with IN in the PIC such as INI1, MA or RT could also be targeted. For example, IN interacts with RT in the PIC (Table 1) and can be inhibited by small peptides derived from RT [22]. Monoclonal antibodies have been shown to inhibit IN by targeting its CTD [23]. Similar approach could be extended to other regions of IN implicated in binding to key co-factors (Table 1).

Interference with the donor (viral) DNA substrate should also inhibit IN activity. DNA binders such as netropsin [24], DNA triple helix [25] or polyamides [26], inhibit IN catalytic activity in biochemical assays. However, this approach has suffered from difficulties to achieve specific and selective recognition of the viral LTR. One possibility might be to search for drugs that would bind at the IN-DNA interface as exemplified for camptothecins in the case of topoisomerase I [17].

Targeting IN helix-turn-helix folding and multimerization through an inhibition of the HHCC zinc-binding domain of the IN NTD (Fig. 2B) could represent another lever to reduce HIV replication. The HHCC domain of IN promotes multimerization and enhances catalytic activity

(for review see [9]). The use of zinc ejecting compounds to inhibit NC has provided a proof of principle for an approach that could be applied to IN inhibition [27].

Conclusion

Novel HIV inhibitors are needed to circumvent viral drug resistance or provide affordable and well-tolerated therapies. The rational for developing IN inhibitors is clear: IN is essential for viral replication and lacks a host-cell equivalent. While some aspects of the IN mechanism are not yet well defined, such as specific interactions within the PIC and the organization of concerted integration, the IN field is progressing rapidly. Diketo-acid-like inhibitors are undergoing clinical trials and several other classes of inhibitors appear promising. Significant advances in understanding intracellular interactions between IN and cellular co-factors in recent years provide opportunities for development of novel inhibitors of integration and HIV replication. Obtaining atomic (crystal or/and NMR) structures of IN-DNA complexes and IN bound to its co-factors remains an important challenge.

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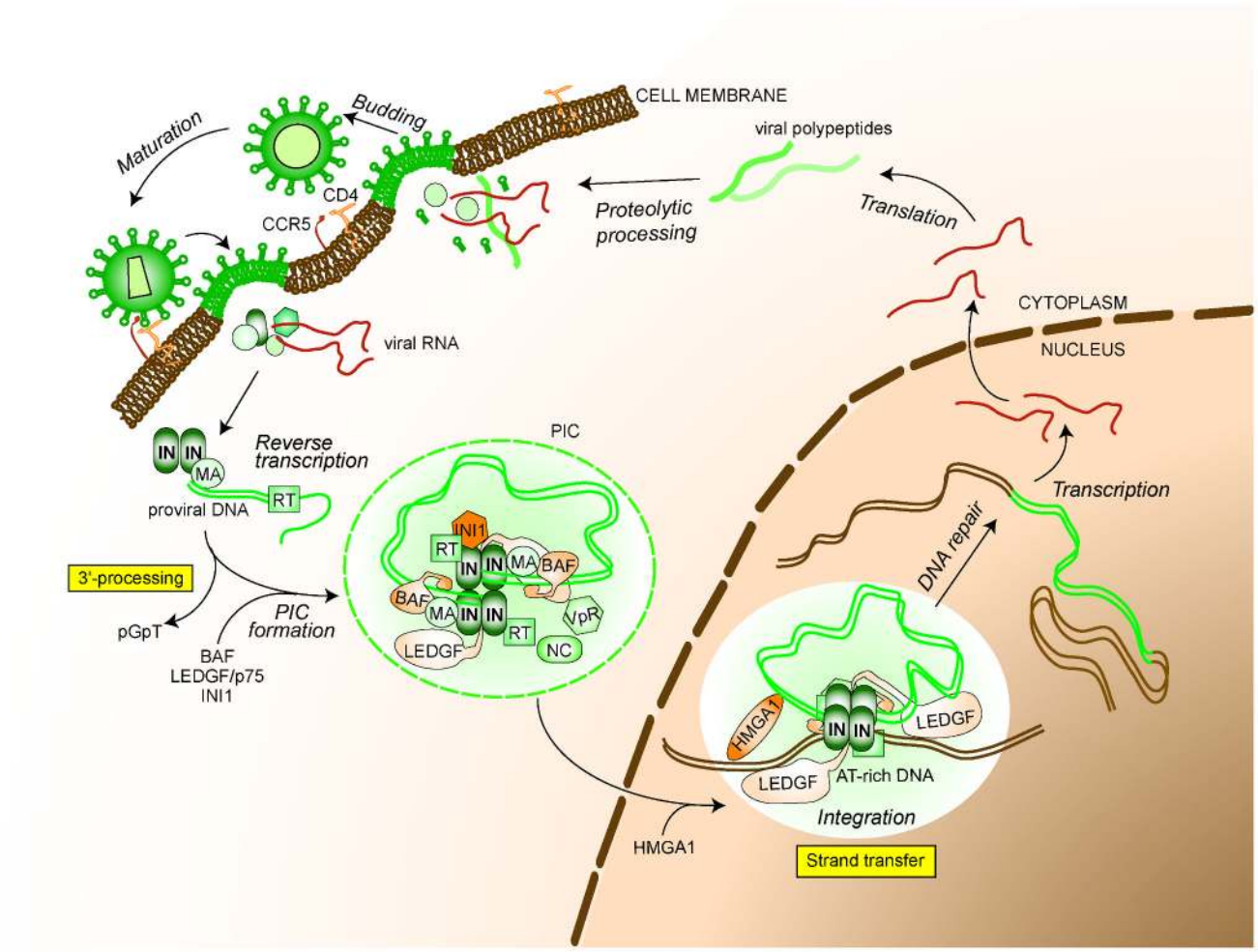

Figure 1.

Schematic diagram of the HIV-1 life cycle. After HIV-1 viral particle binding to CD-4 receptor and CCR5 co-receptor, the viral core is released into the cytoplasm. Viral RNA is processed by HIV-1 reverse transcriptase. Reverse transcription starts within 1-2 hours after viral entry and leads to the production of proviral cDNA. Integration is achieved in several steps 1) 3′-processing which consists in an endonucleolytic cleavage at the 3′ end of the proviral DNA by integrase, 2) formation of the pre-integration complex (PIC) containing viral and cellular co-factors, 3) translocation of the PIC into the nucleus, 4) insertion of processed proviral DNA ends into the host DNA during the strand transfer reaction catalyzed by integrase, 6) reparation of gaps between viral and chromosomal DNA leading to the provirus (proviral DNA integrated). The provirus is silent until triggering of DNA transcription followed by viral RNA translation, maturation, packaging and formation of new viral particles.

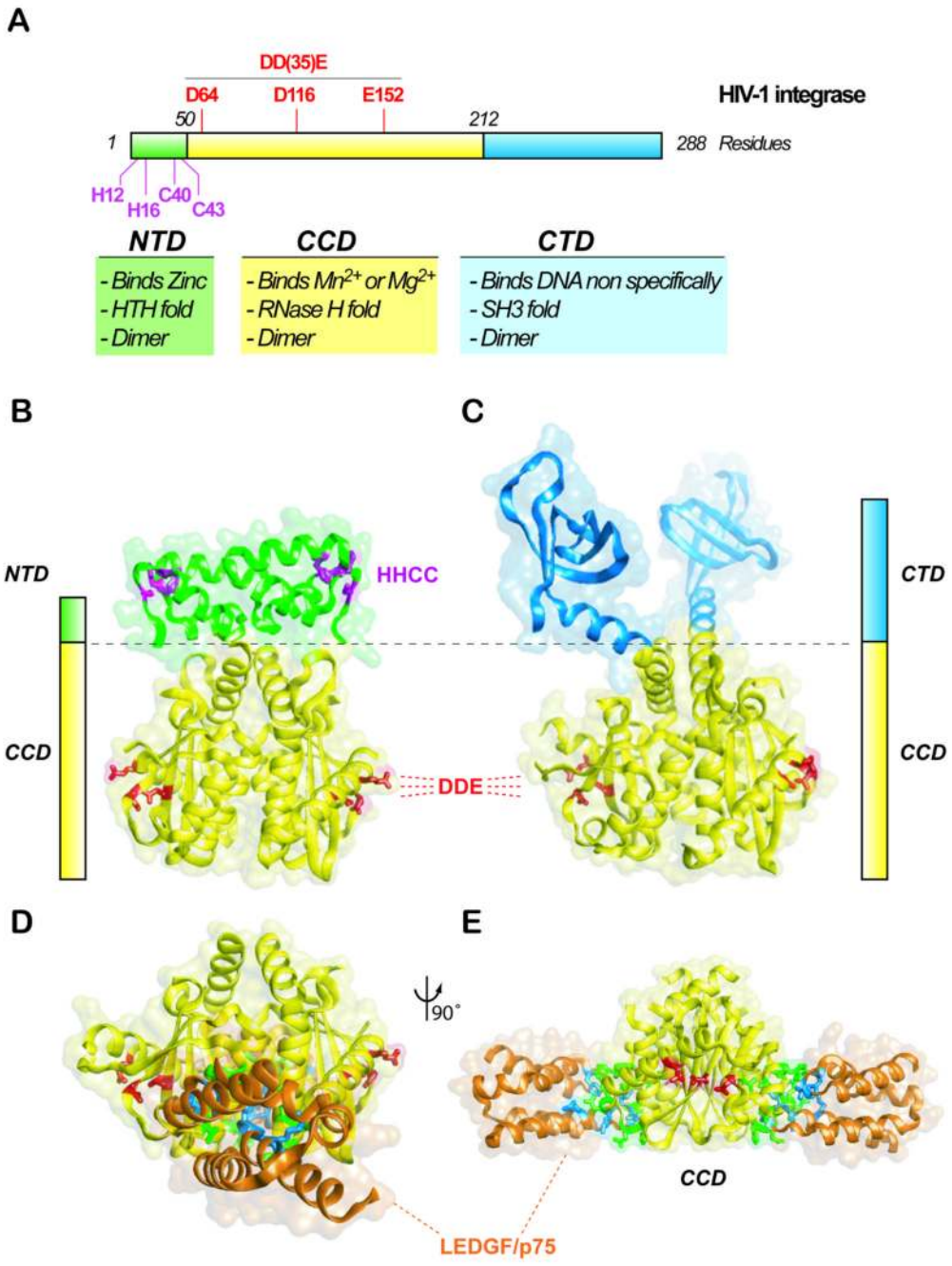

Figure 2.

HIV-1 integrase structure. **A.** Schematic diagram of the HIV-1 integrase protein domains consisting of the N-terminal domain (NTD, green), the catalytic core domain (CCD, yellow), and the C-terminal domain (CTD, blue). Zinc binding residues (purple) and catalytic residues (red) are highlighted. **B** & **C**. Integrase crystal structures of the CCD + NTD [10] (PDB code: 1K6Y) and of the CCD + CTD [11] (PDB code: 1EX4). There is no published structure of the full-length integrase. **D** & **E**. Side and top views, respectively, of the integrase CCD bound to the integrase binding domain of LEDGF/p75 [8] (PDB code: 2B4J). LEDGF/p75 residues (blue) and integrase residues (green) involved in the interaction are highlighted.

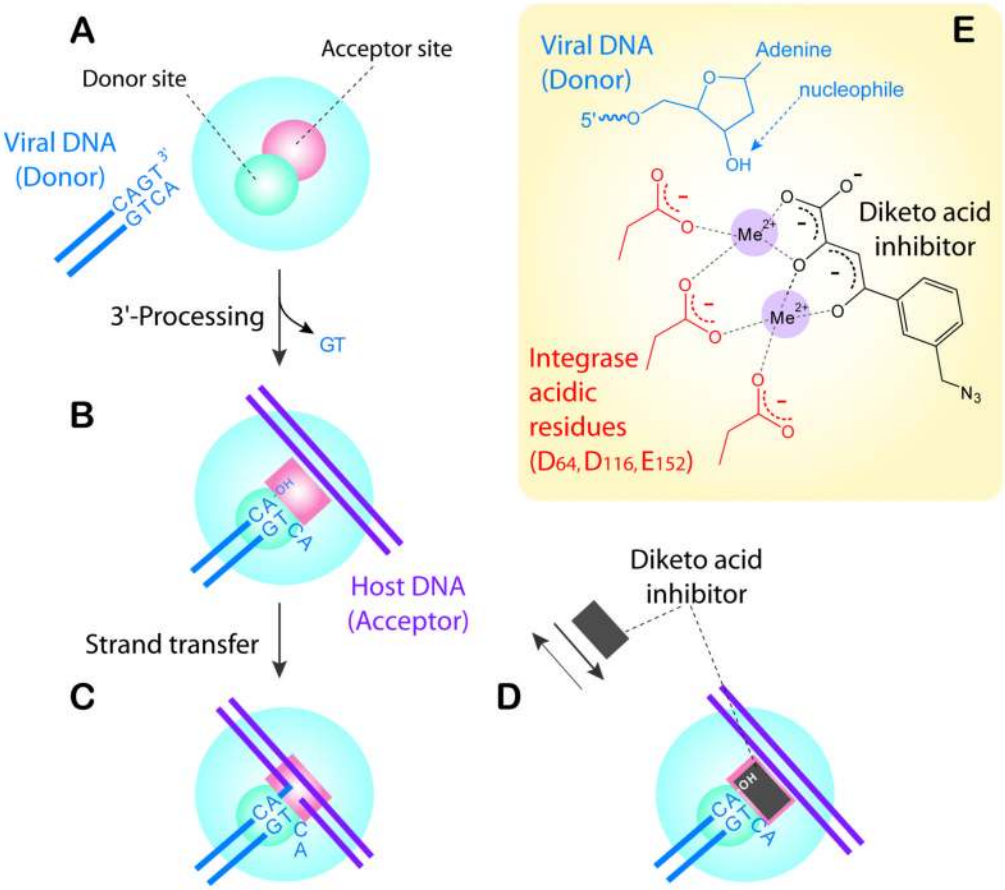

Figure 3.

Schematic diagram of integrase 3′-processing and strand transfer reactions and inhibition by diketo acid inhibitor. **A**. Integrase has two proposed binding sites: the donor site for viral DNA (blue circle) and the acceptor site for host DNA (red circle). **B** Following 3′-processing, the integrase-DNA complex is shown undergoing a structural (allosteric) change rendering the acceptor site competent (red rectangle) for binding host (chromosomal) DNA. C Under normal conditions, binding of the host (acceptor) DNA to the acceptor site leads to strand transfer. **D** The diketo acid inhibitor (gray rectangle) can only bind to the acceptor site after 3′-processing. **E** Details of the hypothetical binding of diketo acid inhibitors at the interface of the integrase-DNA-divalent metal complex. The processed viral 3′-DNA ends (in blue) are bound to integrase (in red, the 3 acidic catalytic residues [DDE]) ready to attack a host DNA phosphodiester bond. Diketo acid inhibitors have been proposed to chelate the divalent metals in the integrase catalytic site and stabilize the macromolecular integrase-DNA complex at the 3′-processing step of the reaction.

Table 1

Targets and related therapies

Target	Strategic approaches to target	Expected outcome of intervention at target	Who is working on the target	Refs
BAF[a]	DNA binding Interaction with LAP2α[b] Interaction with MA[c]	Stimulation of autointegration	Craigie Engelman	[28-30]
HMGA1[d]	DNA binding	Inhibition of chromosome tethering	Bushman Leis	[31,32]
INI1[f]	DNA Binding Interaction with IN[g]	Inhibition of chromosome tethering Inhibition of virus assembly	Kalpana	[33,34]
LEDGF/p75[h]	DNA Binding Interaction with IN	Inhibition of nuclear import and chromosome tethering	Bushman Debyser Engelman Postal	[8,35-37]
HRP2[i]	Interaction with IN	Inhibition of nuclear import and chromosome tethering	Engelman	[38]
p300 acetyltransferase	Interaction with IN IN acetylation	Impaired proviral integration	Giacca	[39]
HSP60[j]	Interaction with IN	Impaired IN folding	Litvak	[40]
Polycomb group EED[k]	Interaction with IN	Inhibition of nuclear import and chromosome tethering	Boulanger	[41]
MA	Interaction with IN Interaction with BAF	Inhibition of nuclear import	Bukrinsky Bushman Wilson	[30,42,43]
Vpr[l]	Interactions with UNG[m] Vpr-mediated cell cycle arrest Interactions with nucleoporins and importins	Inhibition of nuclear import Stimulation of viral mutation rate	Aida Benichou Burkinsky Green Zeichner	[44-48]
NC[n]	DNA & RNA binding Interaction with RT[o]	Inhibition of reverse transcription	Darlix Gorelick	[49-52]
RT[o]	RNA binding Interaction with NC Interaction with IN	Inhibition of reverse transcription Inhibition of integration	Chow Darlix Prasad Roques	[49,52-54]

[a] Barrier to Autointegration Factor,

[b] Lamina-Associated Polypeptide 2α,

[c] Matrix,

[d] High Mobility Group Protein A1,

[e] Long Terminal Repeat,

[f] Integrase Interactor 1,

[g] Integrase,

[h] Lens Epithelium-Derived Growth Factor/p75,

[i] Hepatoma-derived growth factor Related Protein 2,

[j] Heat Shock Protein 60,

[k] Polycomb Group Embryonic Ectoderm Development protein,

[l] Viral Protein R,

[n] Nucleocapsid,

[o] Reverse Transcriptase.