COMMENTARY

Integrating the HIV-1 assembly/maturation pathway

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In PNAS, Jurado et al. (1) describe an unexpected mechanism of action of a new class of HIV type 1 (HIV-1) integrase (IN) inhibitors. Several years ago it was discovered that the HIV-1 IN was targeted to sites in chromatin by the host protein lens epithelium-derived growth factor (LEDGF)/p75 (2). The site of interaction between IN and LEDGF/p75 was defined, and inhibitors to block that interaction were sought and identified. In PNAS, Jurado et al. (1) show that although these inhibitors (termed Allosteric IN inhibitors, or ALLINIs) have some potency to block steps involved in integration, their most dramatic effect is to cause the virus particle to assemble into a noninfectious structure.

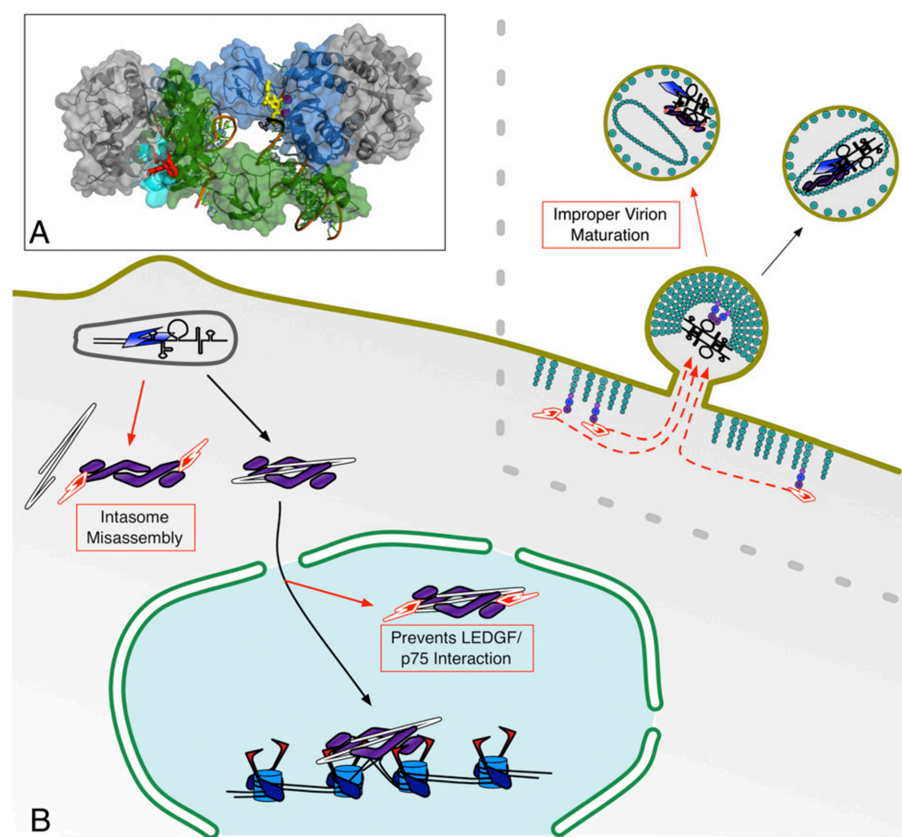

Fig. 1. Multimodal mechanisms of ALLINI action. (*A*) Model of the HIV-1 intasome bound to the INSTI Raltegravir (yellow) and ALLINI BI-D (red). The inner monomer subunits (blue/green) bind viral DNA and are catalytically active. The catalytic core domains of the outer monomers (gray) provide structural support; the N- and C-terminal domains of the outer monomers have not been resolved by X-ray crystallography. INSTIs bind the active site of the inner monomers, displacing the reactive 3′-OH group of the terminal deoxyadenosine (black). ALLINIs locate to the LEDGF/p75 binding site (cyan) found at a dimerization interface. (*B*) Schematic demonstrating the three mechanisms of ALLINI action (red arrows). ALLINIs can promote early dimerization and stabilization of integrase multimers, blocking association with viral DNA. ALLINIs also compete with LEDGF/p75 for binding to assembled intasomes, reducing the efficiency of chromatin-association and strand transfer/integration. Finally, virus particles assembled in the presence of ALLINIs result in improper virion maturation, rendering nascent particles noninfectious.

The IN protein contains three structural domains: the N-terminal domain (NTD; a series of α-helices and a zinc-binding motif), the central catalytic core domain (CCD), and the C-terminal domain (structurally similar to a SH3 β-barrel fold) (3). The CCD has a structure that makes it a member of an ancient family of polynucleotide transferases (also seen in the RNase H domain at the C terminus of HIV-1 reverse transcriptase), with a $D,DX_{35}E$ catalytic triad comprising the active site by coordinating two essential Mg^{2+} ions. IN must complete two enzymatic steps to insert viral DNA into the host cell genome. In the first reaction, IN cleaves the two terminal nucleotides at the 3′ ends of the newly synthesized linear viral DNA to create the 3′-OH nucleophiles that will be used in the second reaction. In the second reaction, the strand transfer, IN uses the new 3′ ends of viral DNA to break phosphodiester bonds in the host DNA, and simultaneously insert viral DNA.

The prototype foamy virus intasome (the IN/DNA nucleoprotein complex capable of integration) currently provides the example for retroviral integration machinery (4), and this has also allowed modeling of the HIV-1 intasome (5). Each intasome consists of a dimer of IN dimers in complex with the two viral DNA ends (Fig. 1*A*). For each individual dimer, only one CCD engages the DNA substrate; the role of the monomers that do not engage the DNA (gray in Fig. 1*A*) is likely a support function. LEDGF/p75 binds the IN dimer at an interface between the CCDs (6), although the NTD is necessary for high-affinity interactions (2). In the context of integration, this interaction must occur subsequent to assembly of the intasome and 3′ end processing of viral DNA by IN. It was this interaction that these compounds were designed to disrupt by binding to the LEDGF/p75 interface in IN.

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ALLINIs are preceded by an earlier class of IN inhibitors. Screens targeting intasomes with 3′ end-processed DNA led to the identification of a class of inhibitors that specifically blocks the strand transfer reaction (INSTIs) (7). These inhibitors include negatively charged or polar groups that allow the inhibitor to align on the Mg^{2+} ions at the active site and displace the 3′ terminal deoxyadenosine in viral DNA critical to the strand transfer reaction (4). Thus, the ALLINIs are directed at an entirely new target on the IN structure.

Dominant-negative forms of LEDGF/p75 can act as inhibitors of integration (8), validating its relevance as a target. The original small-molecule inhibitors of the IN-LEDGF/p75 interaction were discovered by pharmacophore modeling (9). ALLINIs were expected to inhibit integration by blocking the interaction with chromatin-bound LEDGF/p75, which they do (9) (Fig. 1*B*). However, the actual breadth of viral activities affected includes two additional processes. ALLINIs also promote the premature assembly and stabilization of IN dimers in the absence of viral DNA, indirectly blocking the two downstream enzymatic reactions by preventing association with the viral DNA (9–12) (Fig. 1*B*). In addition, ALLINIs surprisingly also inhibit the production of infectious virus particles (i.e., steps that occur after integration and expression of viral RNA and proteins) (Fig. 1*B*). This phenomenon was first described by Christ et al. (10), but is examined in greater detail by Jurado et al. (1).

The outline of the assembly/maturation process that results in infectious virus particles is well understood, although many important details are still unknown (13). The viral Gag precursor protein contains the structural proteins of the virion: matrix (MA), capsid (CA), nucleocapsid (NC), and p6. About 5% of the time Gag is linked to sequences encoded in an alternate reading frame to create a Gag-Pro-Pol fusion protein, where the Gag structural proteins are now linked to the enzymes needed for viral replication: protease (encoded by the *pro* gene), and reverse transcriptase and IN (encoded in the *pol* gene). Upon dimerization, protease activates and releases all of the other viral proteins in a sequence of proteolytic processing events that facilitates the assembly of an infectious virion.

ALLINIs do not inhibit the release of virus particles (1, 10) or the processing of viral proteins (1), which suggests they do not initiate premature dimerization of the Gag-Pro-Pol precursors, because premature activation of protease manifests as impaired particle production (14). Presumably the effect of ALLINIs is still at the level of IN, (as shown by resistance mutations), by inappropriately stabilizing IN dimers in the assembly process. Although not discussed directly in Jurado et al.'s report (1), it is tempting to suggest a specific defect in the assembly process. Thin-section electron microscopy of virus reveals two major processes in virion maturation: the condensation of the RNA with NC, (which, after staining, is electron dense), and the formation of the CA capsid cone, (which stains poorly and appears more wispy). In a properly assembled virion, the capsid forms around the condensed nucleoprotein core. The virions shown in Jurado et al. (1) appear to have dissociated these two processes, with apparent capsid formation squeezing the condensed nucleoprotein core to the side.

The assembly/maturation pathway is itself a potentially important target for inhibitors. Each virus must create a complex structure to exit the cell, and then successfully undergo reorganization after budding from the cell to generate a capsid structure that will allow subsequent DNA synthesis, nuclear transport, and integration to occur upon entry of a new cell. Perturbations of this structure can easily affect multiple steps. The development of the drug bevirimat, which inhibits cleavage at the C-terminal end of CA, represents a proof-of-concept for using the assembly/maturation pathway as a target (15). Similarly, a number of small molecules, such as CAP-1 (16) or CAI (17), can bind to the N- or C-terminal domain of CA, respectively, where they likely interfere with CA–CA interactions necessary for stabilizing the cone structure (18, 19). In our own work we have found that using a genetic trick to inhibit cleavage at the MA-CA site by as little at 10% results in complete loss of virion infectivity, presumably by tethering the assembling capsid to the membrane through residual MA-CA fusion proteins (20). Furthermore, a truncated version of another IN-binding protein, INI1/hSNF5, transdominantly interferes with particle production (21), possibly by facilitating the early processing of the Gag polyprotein. BI-D, the ALLINI of note from Jurado et al. (1), joins a growing list of assembly/maturation inhibitors that target this complex pathway, a pathway where complete surprises, like the ability of allosteric inhibitors of IN to create aberrant virions, still await.

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