Integrase illuminated

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The long struggle to develop effective therapies against human immunodeficiency virus (HIV) continues in earnest. New antiretroviral drugs to prevent integration of the viral genome are undergoing clinical trials. However, the drug development has relied on testing for effectiveness with only limited understanding of the structural aspects of HIV integration.

To establish successful infection, HIV must insert a DNA replica of its genome into a human chromosome—an irreversible process catalyzed by an essential viral enzyme called integrase (IN), which binds tightly to and protects viral DNA termini. On entry into the nucleus, IN inserts the viral DNA ends into cellular chromosomal DNA, making the cell a permanent carrier of the viral genome.

The initial crystal structure of the catalytic core domain of HIV IN (Dyda *et al*, 1994) established a familial link between retroviral INs and a diverse group of nucleotidyl strand transferases that notably includes Tn5 transposase and RNase H. Although the latter was instrumental to elucidate the catalytic mechanism shared by members of this extended family to great detail (Nowotny *et al*, 2005), the similarities between retroviral INs and their cousins were insufficient to build a reliable model for functional HIV IN.

As one of the three essential retroviral enzymes, IN has been targeted for inhibitor development since the early 1990s with mixed success. The first IN inhibitors capable of suppressing HIV replication were reported by Merck Research Laboratories (Hazuda *et al*, 2000), and the company produced the first clinically approved IN antagonist, Raltegravir, in 2007. Similar molecules—commonly referred to as strand transfer inhibitors—developed by Gilead and GlaxoSmithKline are currently in clinical trials. Remarkably, the structural basis behind their effectiveness remained mysterious until earlier this year, when the crystal structure of IN from little-known prototype foamy virus (PFV) in complex with its cognate viral DNA ends—a complex also known as the intasome—was reported by Hare *et al* (2010).

Why did it take so long to determine the structure of a retroviral intasome and explain how strand transfer inhibitors work? When removed from their natural environment, retroviral INs are poorly active. In addition, the relatively harsh conditions typically required to keep them in solution preclude their binding to DNA. Unfortunately, it is the DNA-bound form that is targeted by strand transfer inhibitors, enormously complicating any structural studies. The favourable biochemical properties of PFV IN allowed for its isolation in complex with viral DNA. Although crystallization of the PFV intasome required truly Herculean effort, the outcome was well worth it.

As expected from earlier work (Li *et al*, 2006), the intasome structure revealed a tetramer of IN associated with a pair of viral DNA ends. Interestingly, the topology of protein and DNA chains in the complex indicates an assembly pathway involving initial—presumably weak—binding of IN dimers to viral DNA ends, followed by synapsis of the ends through IN tetramerization. In fact, the propensity of HIV IN to tetramerize in the absence of DNA could explain its relatively poor ability to synapse viral DNA ends *in vitro*. The functional significance of DNA-independent tetramerization of retroviral INs remains, therefore, a puzzling question.

Most details of the fully ordered active site observed in the PFV intasome are identical to those in HIV, making it a good proxy for studies of HIV IN inhibitors. Indeed, soaking the clinical drugs Raltegravir and Elvitegravir into the crystals revealed how they block integration. Both drugs engage a pair of Mg^{2+} cations in the active site of the intasome, while their halobenzyl groups intercalate between viral DNA bases and the protein structure, displacing the 3′ nucleotide of the viral DNA from its natural position. Fundamentally, intasome inactivation is caused by the dislocation of the reactive 3′-end of the viral DNA from the active site of IN. In addition, the inhibitors may sterically preclude host DNA binding, which would be consistent with published observations (Espeseth *et al*, 2000).

Although Raltegravir is a highly potent antiretroviral drug, its clinical effectiveness can be compromised by viral resistance (Cooper *et al*, 2008). Comparison of the amino acids primarily responsible for HIV resistance to the structurally equivalent PFV IN residues has shown that although one of them is in direct contact with the inhibitor molecule, the remaining two residues do not interact with the drug. Rather, it appears that they subtly change the environment of the active site, probably affecting the coordination spheres of the catalytic metal cations. Co-crystal structures of wild-type or mutant PFV intasomes with second-generation IN inhibitors such as MK2048 or S/GSK1349572 may shed light on how these molecules circumvent the Raltegravir resistance pathways and help rational drug design.

Follow-up work will also need to focus on both target DNA binding and the mechanics of the active site. The results will hopefully elucidate the entire retroviral DNA integration pathway and suggest additional ways of clinically interfering with this process. Importantly, the PFV intasome structure now allows the construction of reliable structural models for its HIV counterpart. Such models will be useful for the design of HIV IN mutants that are more amenable to crystallization. With the HIV IN models in hand and ongoing work to elucidate the mechanisms behind the effectiveness of strand transfer inhibitors against IN, improved clinical applications seem likely. Nevertheless, the promise of these developments remains to be realized and there is yet much work and exciting discoveries for the years to come.

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