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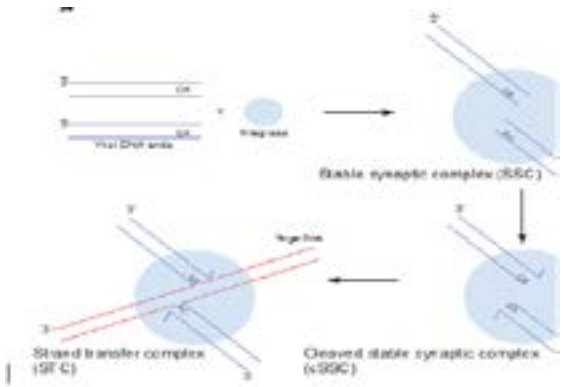
HIV-1 integrase assembles multiple discrete intasomes that are active for DNA integration *in vitro*

Robert Craigie
National Institutes of Health, USA

Statement of the Problem: Integration of retroviral DNA into host DNA is an essential step in the replication of HIV-1 and other retroviruses. Integration is mediated by a nucleoprotein complex (intasome) comprising the virally encoded integrase enzyme and a pair of viral DNA ends. The first intasome on the integration pathway is the stable synaptic complex (SSC) in which a pair of viral DNA ends is bridged by integrase. Within the SSC, integrase then cleaves two nucleotides from the 3' ends of the viral DNA to form the cleaved stable synaptic complex (cSSC). The cSSC captures a target DNA and a pair of transesterification reactions covalently joins viral to target DNA. Currently approved inhibitors of HIV-1 DNA integration target intasomes (specifically the cSSC) rather than free integrase protein, High-resolution structures of intasomes are required to understand their detailed mechanism of action and how HIV-1 can escape by acquiring resistance.

Methodology & Strategy: Although the structures of the individual domains of HIV-1 integrase were determined more than two decades ago, attempts to obtain high-resolution structures of HIV-1 intasomes were unsuccessful. The main obstacles were the propensity of both integrase and intasomes to aggregate and the low efficiency of assembly *in vitro*. We have overcome these problems by developing a hyperactive integrase mutant that assembles intasomes that are amenable to biophysical and structural studies. CryoEM studies of STCs reveal both tetrameric and higher order species that both share a common core architecture with intasomes of related retroviruses. SSCs also assemble both tetrameric and higher order intasomes and both are active for concerted DNA integration *in vitro*.

Conclusions & Significance: The results highlight how a common core intasome architecture can be assembled in different ways. Structures of cSSC intasomes in complex with inhibitors will elucidate their detailed mechanism of action and mechanisms by which HIV-1 can evolve drug resistance.



Biography

Robert Craigie is a Senior Investigator in the National Institute of Diabetes and Digestive and Kidney Diseases at the National Institutes of Health, Bethesda, MD, USA. His research has focused on the mechanism of retroviral DNA and the structure and function of proteins and nucleoprotein complexes that mediate it.

bobc@helix.nih.gov