Proc. KSAM International Symposium
June 20-22, 2001, Chonan, Korea

Macromolecular Interactions Involved In Intasome Formation By Lambda Integrase

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Bacteriophage lambda integrase is a sequence-specific recombinase that carries out site-specific recombination of lambda. Integrase contains two autonomous DNA binding domains with distinct sequence specificity, namely arm-type binding and core-type binding domains. Int binding to the high affinity arm-type site by the arm-type binding domain facilitates Int binding to the low affinity core-type site, where the cleavage and strand exchange occurs. The arm-type binding domain also modulates the core-binding and catalysis through intramolecular domain-domain interaction and/or intermolecular interactions between Int monomers. This indicates that amino-terminal domain of Int plays an important role in formation of proper higher-order nucleoprotein structure required for lambda site-specific recombination.

Introduction

High-precision DNA transactions such as those involved in initiation of DNA replication and site-specific recombination are often carried out by complexes in which two or more proteins interact with specific DNA sites to form higher-order complexes. The site-specific recombination system encoded by bacteriophage lambda provides a classic example of reactions controlled by a higher-order multiple protein-DNA complex (for a review, see Azaro and Landy 2000). The lambda recombination complex is composed of specific DNA sites in the phage and bacterial chromosomes and host- and phage-encoded proteins. Integrative recombination between specific attachment sites, *attP* on the phage DNA and *attB* on the bacterial chromosome, generates recombinant *attR* and *attL* sites flanking the prophage DNA. Both reactions are catalyzed by the phage-encoded protein integrase (Int), assisted by accessory proteins. The host-encoded integration host factor (IHF) is a protein required for both reactions. Excision requires an additional phage-encoded protein called excisionase (Xis). Excision is stimulated by the factor for inversion stimulation (FIS) supplied by the host.

Int plays a central role in recombination. Int carries out the cleavage, strand exchange and resealing of the att site DNAs. During recombination, Int recognizes to two distinct classes of DNA sequences, interacts with another Int molecule, and also interacts with Xis during excision. The two classes of Int binding sequences are called as "arm-type" binding sites and "core-type" binding sites, respectively. The core-type sites consist of imperfect inverted repeats that flank the sites of strand exchange during recombination. The arm-type sites occur five times outside the region of strand exchange on the attP. Int binding to a subset of these arm-type binding sites, in addition to accessory protein binding to their cognate sites, results in formation of a higher-order complex, called an 'intasome', that is an active substrate for the recombination reaction.

Three Functional Domains of Lambda Integrase

The Int protein can be divided into three major domains (Fig. 1a). The proteolytic fragment containing amino-terminal 64 amino acids binds to arm-type sites in vitro (Moitoso de Vargas et al., 1988). A second domain spanning amino acid residues 65 to 169 involves core-type binding (Tirumalai et al., 1998). The third domain includes amino acid residues 170 to 356 (Kwon et al., 1997). This is the catalytic domain which contains the conserved amino acids required for type I topoisomerase activity.

The Int protein of lambda is the best-characterized member of a large Tyrosine family of site-specific recombinases (Nunes-Duby et al., 1998; Esposito and Scocca, 1997). The Tyrosine recombinase family is identified by extended areas of similarity and four strongly conserved residues, including an active site tyrosine directly involved in catalysis. The catalytic domain of lambda Int contains all of the residues that define the Tyrosine recombinase family. High-resolution structure reveals that the catalytic domain of recombinases share similar overall structure despite extensive divergence in their amino acid sequence (Kwon et al., 1997; Subramanya et al., 1997; Guo et al., 1997; Chen et al., 2000).

Similarity of the amino-terminal regions of Tyrosine recombinases, however, was only detected within a handful of closely related phage integrases. Among these, similarities between lambda and HK022 Ints are striking. The sharp transition in the degree of sequence identity that is typical in lambdoid phage genomes was observed between the amino-terminal DNA binding domains (>95 % identical) and the remainder (~70 % identical) of lambda integrase and HK022 integrase (Yagil et al., 1989). Of the 64 amino acids in the arm-binding domain, the first 55 amino acids are completely identical. Because of the amino-acid homology, lambda and HK022 Ints share the same arm-type DNA binding specificity (Yagil et al., 1989; Lee et al., 1990).

The Functions of Amino-terminal Domain of Int

The lambda Int binds simultaneously to two different DNA sites (Fig. 1b). The high-affinity amino-terminal domain binds to the arm-type sequence to deliver the low-affinity core-binding domain of Int

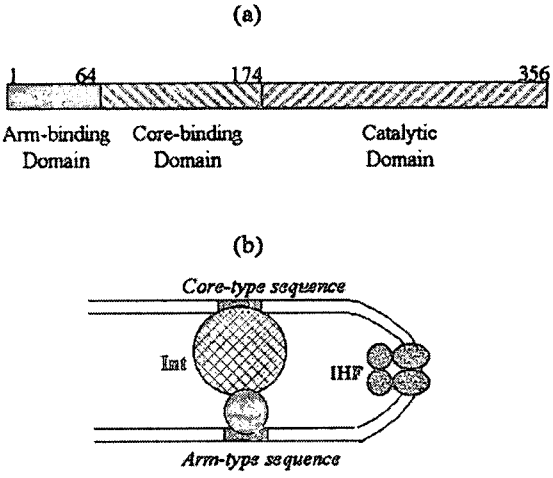

Fig. 1. (a) Domain Structure of Lambda Integrase. Numbers represent amino acid residues at the end of each domain. (b) Schematic Diagram of Intasome Formation by Lambda Integrase. The small circle represents the arm-binding domain and the catalytic domain. Only a single Int molecule which simultaneously occupies the arm-type site and core-type site is depicted in this diagram. DNA bending induced by IHF binding to its cognate binding site stabilizes the complex.

to the core-type site where actual cleavage and rejoining occur. Simultaneous binding to the two different DNA sites by a single Int molecule is further facilitated by other accessory proteins which bind and bend the sites between the arm-type and the core-type sequences (Fig. 1b).

Several recent studies, however, reveal more elaborate role of the arm-binding domain of the Int molecule in addition to the architectural role. The amino-terminal domain is implicated as the region which is involved in protein-protein interactions between Int molecules during intasome formation (Jessop et al., 2000). A few mutations in the HK022 Int which relaxe core-binding specificity without changing arm-type binding affinity locate on the arm-type binding domain, within the region that is completely conserved between the HK022 and lambda Ints (Cheng et al., 2000). A class of mutations in the core-binding domain that change arm-type binding mode is also reported (Han et al., 1994). These mutations may alter the structure of the other domain through domain-domain communication. In addition, when the amino-terminal domain is supplied separately with the rest of the protein, it stimulates core-DNA binding and cleavage (Sarkar et al., 2001).

These results suggest that the amino-terminal domain not only plays an architectural role in formation of intasome by bridging the high-affinity and the low-affinity sites, but also plays more active role in modulating the activity of core-binding and catalytic domains. The modulation of Int activity by the amino-terminal domain can be achieved through intramolecular domain-domain interaction and/or intermolecular interactions between Int monomers.

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