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# Multiple Functions of the Amino-terminal Domain of Bacteriophage Lambda Integrase: A New Member of Three-stranded β-sheet DNA-binding Proteins

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## Summary

Bacteriophage lambda integrase carries out the site-specific recombination of lambda. Integrase contains two DNA binding domains with distinct sequence specificity, namely arm-type binding and core-type binding domains. The amino-terminal arm-binding domain is structurally related to the three-stranded β -sheet family of DNA-binding domains. Integrase binding to the high affinity arm-type site by the amino-terminal domain facilitates Int binding to the low affinity core-type site, where the cleavage and strand exchange occurs. The amino-terminal domain of Int also modulates the core-binding and catalysis through intramolecular domain-domain interaction and/or intermolecular interactions between Int monomers. In addition, the amino-terminal domain interacts cooperatively with excisionase during excision. 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 2002). 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.

## The Structure of the Amino-terminal Domain of Int

The Int protein can be divided into three major domains. 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.

Recently the NMR solution structure of the amino-terminal domain containing the first 64 amino acid residues of Int was solved by Wojciak *et al.* (2002). It folds into a three-stranded, antiparallel β -sheet that packs against a C-terminal α -helix. The domain is structurally related to the N-terminal domain of the Tn*916* integrase (Wojciak et al., 1999). Modeling of the structure and a mutational analysis indicated that Arg4, Arg5, Arg27, and Glu34 of the fragment are involved in binding arm-type sites. Interestingly, the structure shows that there are three isoleucine residues (Ile45, Ile49, and Ile53) that are solvent exposed. As noted by Wojciak et al (2002) these residues could form the surface of Int that interacts with other Int monomers. Alternatively, or in addition, these residues could also be involved in interactions with Xis.

## Multiple Functions of Amino-terminal Domain of Int

Until recently, a relatively simple model was proposed for the biological function of the amino-terminal domain of the lambda Int. The high-affinity amino-terminal domain binds to the arm-type sequence to deliver the low-affinity core-binding domain of Int to the core-type site where actual cleavage and rejoining occur (Motoso de Vargas et al., 1988; Motoso de Vargase et al., 1989; Kim and Landy, 1992). Simultaneous binding to the two different DNA sites by a single Int molecule is further facilitated by accessory proteins which bind and bend the sites between the arm-type and the core-type sequences (Moitoso de Vargas et al., 1989; Kim and Landy 1992).

Several recent studies, however, revealed more elaborate roles of the amino-terminal 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). Some amino acid substitutions in one domain may alter the structure of the other domain through domain-

domain communication. A few amino-acid substitutions in the HK022 Int which relax core-binding specificity without changing arm-type binding affinity are located in 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 amino-acid substitutions in the core-binding domain that change arm-type binding mode has also been reported (Han et al., 1994). When the amino-terminal domain of Int is supplied separately with the carboxyl domain (amino acids 65 to 356) core-DNA binding and cleavage are stimulated, while in the full length protein the amino-terminal domain inhibits core binding and DNA cleavage (Sarkar et al., 2001). In addition, the amino-terminal arm-type DNA binding domain interacts cooperatively with Xis. This indicates the amino-terminal domain carries another function for regulating directionality of recombination by interacting with Xis in direct manner (Cho et al., 2002).

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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