

Structure and mechanism in site-specific recombination

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Three-dimensional structural information on the integrase family of site-specific recombinases has only recently become available, with the crystal structures of catalytic domains, full-length proteins and protein–DNA complexes of this family reported over the past two years. These results have led to a model for the overall architecture and active site stereochemistry of the recombination pathway that addresses a number of interesting mechanistic issues.

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Abbreviation

PDB Protein Data Bank

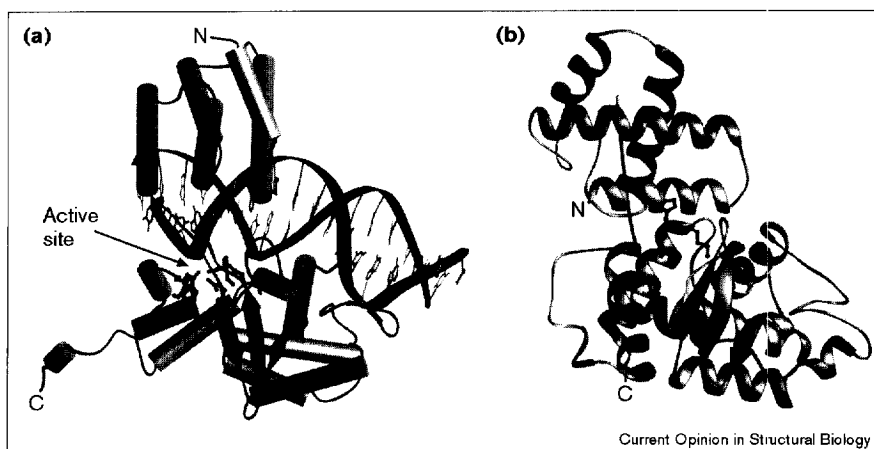
Introduction

Genetic recombination plays a number of important roles in biology, including the generation of genetic diversity, the maintenance and propagation of genomes, the control of gene expression and the movement of viral DNA into and out of host chromosomes. In site-specific recombination, recombinase proteins catalyze the cutting and rejoining of two different DNA segments at specific sequences. Bacteriophages, for example, use this cut-and-paste operation to insert their own genomes into the circular genome of a bacterial host. Two large families of site-specific recombinases have been identified in bacteria and yeast — the λ -integrase family and the resolvase/invertase family [1,2].

The λ -integrase or ‘tyrosine recombinase’ [3] family includes over 100 members identified according to sequence similarity [4]. Most biochemical studies of this family of enzymes have focused on the integrase from bacteriophage λ (Int) [5], FLP recombinase from yeast [6], Cre recombinase from bacteriophage P1 [7] and the XerC and XerD recombinases from *Escherichia coli* [8]. These proteins share only limited sequence similarity, but carry out site-specific recombination using a common mechanism that involves the formation of a Holliday junction intermediate [9]. The other major family of site-specific recombinases, the resolvases/invertases, uses a different mechanism to cut and rejoin duplex DNA molecules [2].

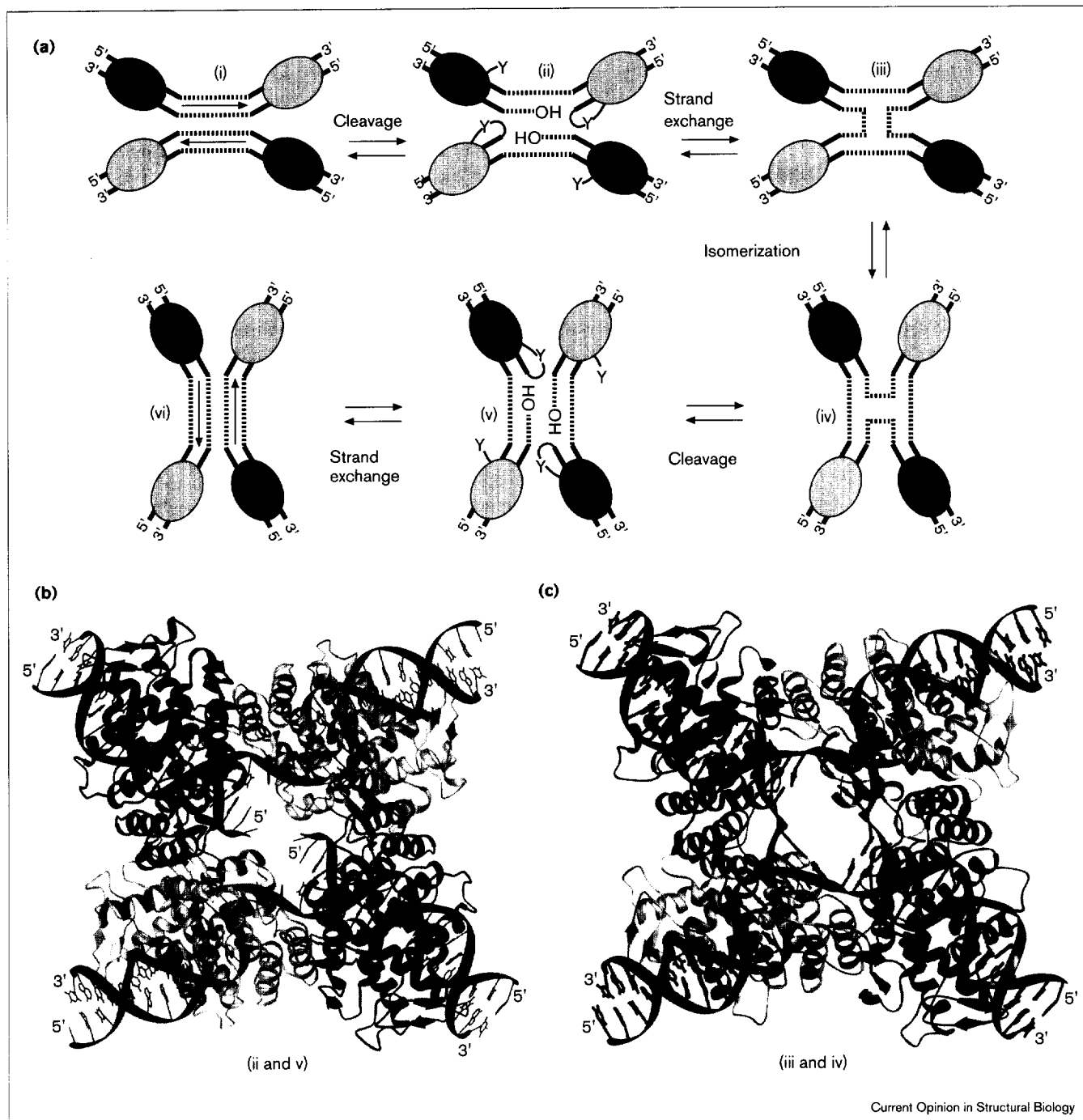
The past two years have been exciting times for the Int family, with structural models emerging for four different systems. First to be reported was the structure of the catalytic domain from λ -integrase [10**], followed by the structure of the same domain from the related bacteriophage HP1 integrase [11**]. These structures revealed a new protein fold and intriguing views of the Int family active site, where cleavage and strand exchange occur. Reported next was the structure of full-length XerD [12**], showing a second helical domain on a flexible tether, and the structure of Cre recombinase bound covalently to its DNA substrate [13**]. Together, these structural models complement the large body of biochemical work on the various Int family systems and have led to a three-dimensional understanding of how some steps in the recombination reaction are likely to proceed (the four structures are reviewed in [14*–16**]). The most recent additions to the story are structures of the Cre enzyme bound to two different Holliday junction intermediates [17**]. In this review, we begin by summarizing how structural information has influenced the current model for site-specific recombination in

Figure 1



Structures of Cre and XerD recombinases. (a) The structure of Cre recombinase bound to its recognition element, as observed in a Cre–Holliday junction complex (Protein Data Bank [PDB] entry 3CRX). Adapted with permission from [17**]. (b) The structure of full-length XerD recombinase (PDB entry 1AOP [12**]). The N and C termini are indicated and active site sidechains are drawn in black. This figure and other crystallographic drawings in this paper were prepared using the program RIBBONS [43].

Figure 2



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The Cre-loxP site-specific recombination pathway. **(a)** Current model of the Cre-loxP site-specific recombination mechanism [17**]. Two Cre monomers (black or gray ovals) bind to each recombination site and synapse the DNA substrates through protein-protein interactions (i). One protein subunit bound to each substrate cleaves the DNA using conserved tyrosine sidechains (indicated by Y) to form covalent 3' phosphotyrosine linkages (ii). The liberated 5' hydroxyl groups can either attack the adjacent phosphotyrosine linkage to return to the starting configuration or attack the partner substrates to exchange one pair of strands and form a Holliday junction intermediate (iii). Isomerization of the Holliday intermediate (iv) deactivates the first pair of Cre subunits and activates the second pair, allowing cleavage and exchange of the second pair of DNA strands to give recombinant products (v and vi). In the Cre

system, the recombination site is called loxP and is composed of two 13 base pair recognition elements (solid lines) separated by an eight base pair central strand exchange region (dashed lines). Cleavage occurs after the first base of the strand exchange region on the top and bottom strands, resulting in a total swap of six base pairs between loxP sites. Adapted with permission from [17**]. **(b)** The structure of a covalent Cre-DNA complex (PDB entry 1CRX [13**]), in which two of the four subunits have cleaved a suicide substrate to form stable 3' phosphotyrosine linkages. This structure is a model for intermediates (ii) and (v) in the reaction scheme. Adapted with permission from [13**]. **(c)** The structures of two Cre-Holliday junction complexes (PDB entries 2CRX and 3CRX [17**]) are models for intermediates (iii) and (iv) in the reaction pathway. The structure of 3CRX is shown here.

the Cre recombinase pathway, for which structures of two of the protein–DNA intermediates are now available. We then discuss from a structural point of view two specific aspects of the Int family mechanism that have been the focus of a great deal of attention — isomerization of the Holliday junction intermediate and *cis* versus *trans* cleavage of the DNA substrate.

Structures of the recombinases

The full-length Cre and XerD recombinases contain two primarily helical domains (Figure 1). A poorly conserved N-terminal domain is flexibly attached to a larger C-terminal domain, which houses the most strongly conserved sequences of the Int family. This larger catalytic domain contains the ‘RHR triad’, which, along with the conserved tyrosine nucleophile, have been the hallmark signature of the Int family [18] and form part of the active site. λ -Integrase contains an additional N-terminal domain that is responsible for binding accessory DNA sequences remote from the cleavage sites and the Flp-like recombinases contain additional C-terminal sequences [4]. Similarities in both structure and mechanism between the Int family and the eukaryotic topoisomerase I family catalytic domains have been recently discussed [3,19,20].

Cre recombinase binds to its recognition element by forming a ‘C-shaped clamp’ around the DNA duplex (Figure 1a). Orthogonal helices from the N-terminal domain lie in the major groove on one face of the DNA, while the major groove and both adjacent minor grooves on the opposite face are engaged by helices and loops from the catalytic domain. The 13 base pair recombinase-binding element in the Cre–DNA structure is bent only slightly as a result of Cre–DNA contacts, but the full recombination site (34 base pairs) is bent quite sharply in the central strand exchange region between half-sites. Although there are likely to be some differences in the details, the Cre–DNA half-site complex shown in Figure 1a is expected to be similar to the integrase, Xer (Figure 1b) and Flp systems. Although the protein–DNA interfaces will be an important part of the overall Int family story, some of the most interesting structural questions are instead concerned with how these recombinase–half-site complexes interact with one another during the recombination process.

Isomerization of the Holliday junction intermediate

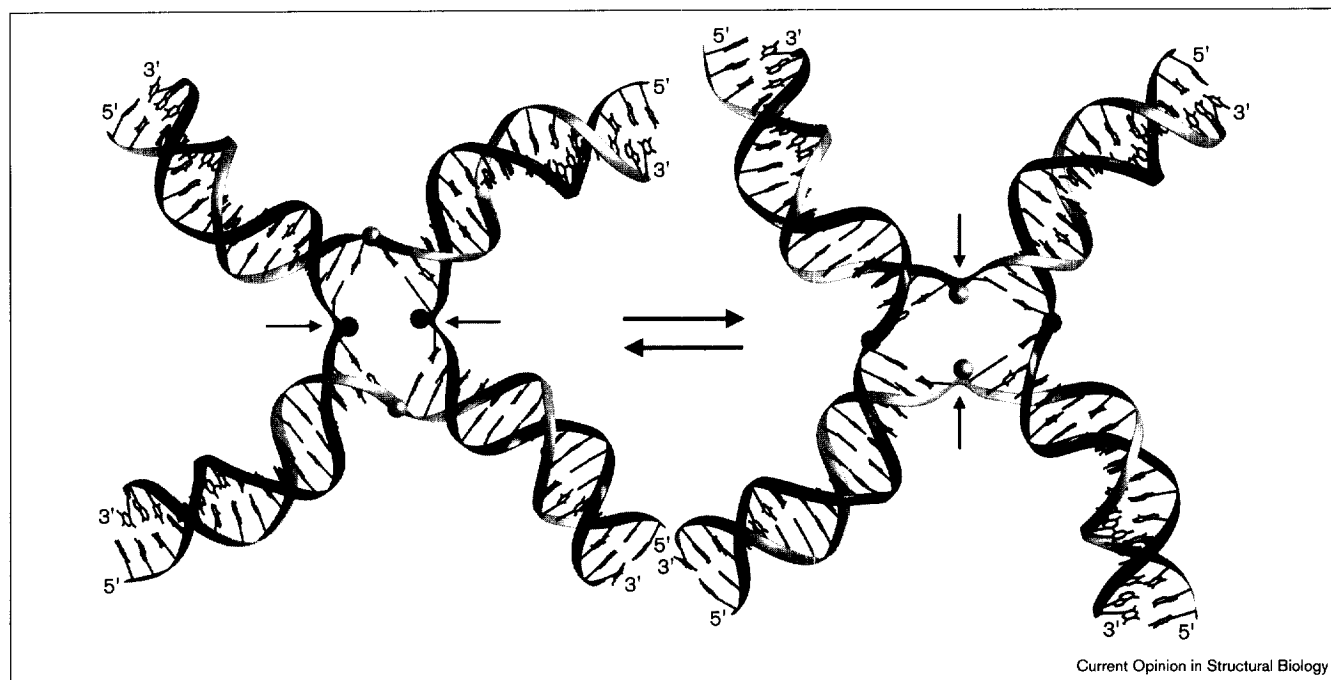
It has been known for many years that Int family proteins recombine their DNA substrates through a stepwise cleavage and strand exchange process that involves the formation of a Holliday junction intermediate [9]. A model for this pathway in the Cre–loxP system is outlined in Figure 2, along with the crystal structures of relevant Cre–DNA complexes. The most significant implication of the Cre–DNA structures is that the recombination reaction takes place within a single architectural framework. In this model, the DNA duplex substrates are synapsed in an antiparallel orientation and are bent at the

start of the reaction to provide an architecture that anticipates the formation of a nearly planar four-way junction. Both the cleavage and strand exchange steps can then take place by simply swapping the DNA strands between substrates, with only modest changes in the quaternary structure of either the protein subunits or the DNA arms throughout the entire pathway. The strand-swapping element of this mechanism was, in fact, proposed in 1995, based on biochemical experiments on the Int system [21], and was supported by related work done in several laboratories (see references in [17**]).

The isomerization step in Figure 2 (iii and iv) has been the focus of a number of recent studies [22*–24*], as it represents the crucial switching point between two symmetry-related halves of the reaction. The mechanistic question is this: once a given strand in each of the two DNA substrates has been cleaved and exchanged to form a four-way junction, how does the recombination assembly then turn off the first pair of active sites and turn on the other pair to allow cleavage and exchange of the second pair of strands? For many years, it was thought that when the Holliday junction intermediate was formed, the branch point of the junction (where the strands cross over) would be located at the first cleavage site. Migration of the branch point to the second cleavage site would then somehow trigger the second half of the pathway. This model conveniently explained the strict requirement for homology between recombining sites, since branch migration would stall at a mismatch and would not allow the second strand exchange to occur [25]. In more recent years, however, this view has been challenged and most of the current models for Int, Xer and Flp recombination involve only limited branch migration (1–3 base pairs) of the Holliday intermediate, coupled to an isomerization event that is responsible for switching the strand cleavage specificity [21,22*,24*,26].

The structure determination of a Cre–Holliday junction complex [17**] has provided an indication of the nature of this isomerization step, at least for the Cre–loxP pathway (Figure 3). The ‘arms’ of the Cre-bound Holliday junction lie approximately in a plane and adopt a pseudo-fourfold symmetric arrangement. The Cre-bound junction therefore shows some similarities to the square planar junction observed in a recently determined RuvA–Holliday junction complex crystal structure [27*]. Close inspection, however, reveals that the Cre-bound junction is actually only dyad symmetric. Some of the distortion from fourfold symmetry arises from the pairs of acute and obtuse angles between DNA arms. A more surprising feature of the junction, however, involves the asymmetric configuration of the phosphate backbones at the branch point. The central phosphates on strands that bridge the acute angles between arms adopt an inverted orientation with respect to the continuous backbone. An isomerization scheme that incorporates these structural features and accomplishes the task of switching stereochemical environments between DNA strands is shown in Figure 3. This process

Figure 3



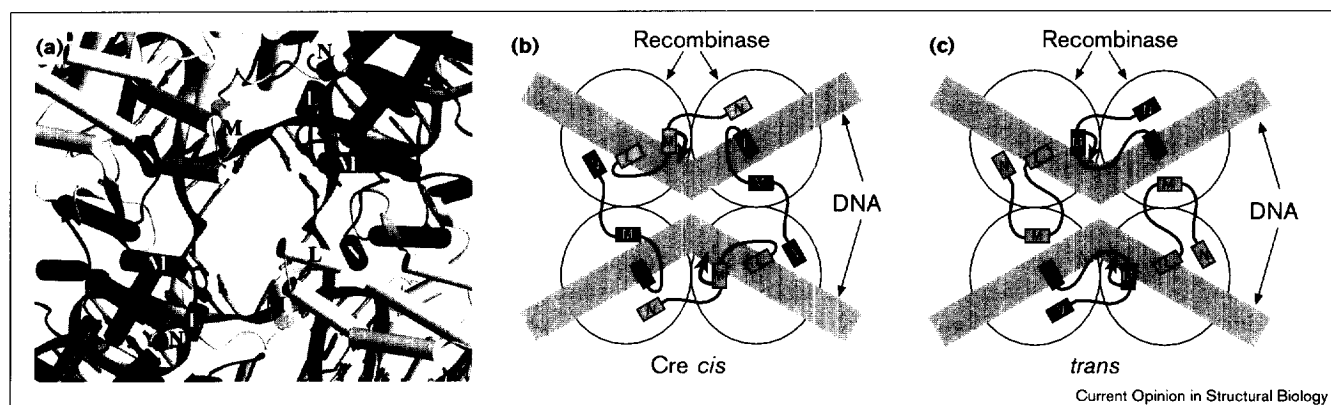
Structure and model for the isomerization of the Holliday junction intermediate in Cre-loxP site-specific recombination (PDB entry 3CRX [17**]). Central phosphates at the branch point of the junction are drawn as spheres and the bound recombinases have

been omitted for clarity. Isomerization of the Cre-junction complex involves a scissor-like motion of the DNA arms, which exchanges the roles of the DNA strands. Phosphates with an inverted backbone geometry are indicated with small arrows.

is analogous to crossover isomerization [28], with the same implications for switching the roles of DNA strands, as observed from the resolution of Holliday intermediates in homologous recombination [29]. In the context of the

Cre-DNA recombination assembly, isomerization allows the system to switch between two dyad symmetric forms, with only subtle changes in protein-protein and protein-DNA contacts. The stereochemical details of how

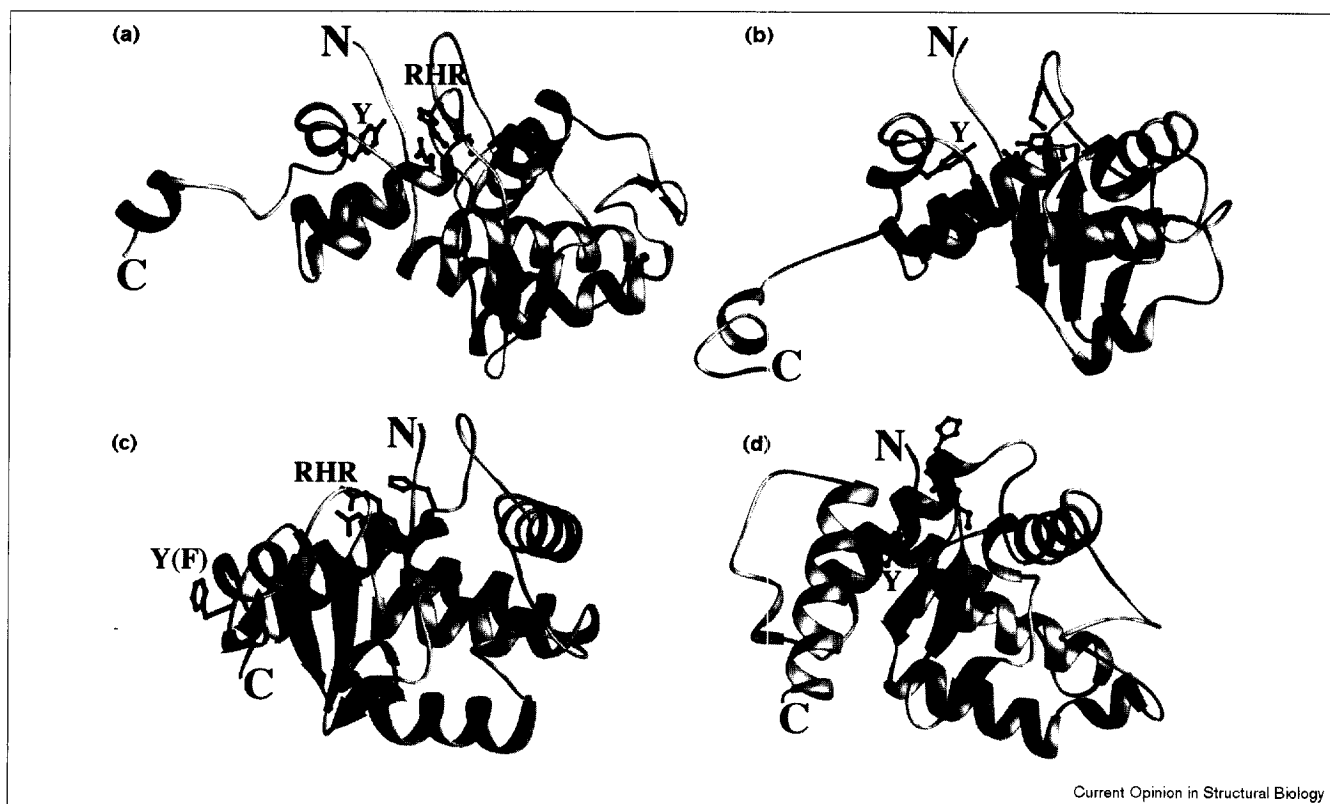
Figure 4



A *trans* cleavage model that is compatible with Cre-DNA architecture. (a) Close-up of the Cre-Holliday junction complex (PDB entry 3CRX [17**]), illustrating the cyclic organization of the three C-terminal helices (labeled L, M and N) in the DNA-bound Cre tetramer. Helix M contains the tyrosine nucleophile that cleaves the DNA. (b) Cartoon representation of the Cre-DNA complex in (a). Only helices L, M and

N are shown. Helix N is donated in *trans* to an adjacent subunit and helix M is bound in *cis*. (c) Cartoon representation of a *trans* cleavage arrangement produced by changing the connections between the L and M helices in the Cre-DNA complex. In this '*trans* cyclic' arrangement, M helices are donated in *trans* to adjacent subunits and N helices are bound in *cis*.

Figure 5



Structures of the catalytic domains of (a) Cre recombinase (PDB entry 1CRX [13**]), (b) HP1 integrase (PDB entry 1AIH [11**]), (c) λ -integrase (PDB entry 1AE9 [10*]) and (d) XerD (PDB entry 1AOP [12**]). The conserved tyrosine nucleophile (Y), the active site 'RHR triad' residues and

the N and C termini are indicated. In the Cre and HP1 structures, the tyrosine sidechain hydrogen bonds either to the DNA substrate (Cre) or to a sulfate ion (HP1) in the active site. In the Int and XerD structures, the tyrosine (phenylalanine [F] for Int) is located outside the active site.

the active sites are turned on and off in the Cre system as a result of this isomerization process are the focus of current work in our laboratory.

The isomerization scheme in Figure 3 does not involve branch migration of the Holliday junction intermediate. How then does the system sense homology between recombining sites? In the case of Cre, both strand exchange steps involve a three base pair swap between substrates. Since there are six base pairs between cleavage sites in the loxP sequence, a pair of sequential strand exchanges effectively tests the homology between recombining sites in the Cre pathway by forming (or not forming) the three base pairs required for successful exchange. In the case of Flp recombinase, for which there are eight base pairs between cleavage sites (or Int, for which there are seven), this isomerization step may be somewhat different. The simplest scenario would be that Flp just swaps four bases at a time and tests homology in the same manner as Cre. Alternatively, Flp could swap three base pairs, like Cre, and the Holliday junction could branch migrate through the central two base pairs [21,30]. An important consequence of branch migration in this isomerization step is that the DNA arms of the junction (and their bound recombinases) need

to perform coordinated rotations as base pairs melt along one helical axis and form along the other [31]. This operation would necessarily result in a remodeling of the interfaces between pairs of recombinases, but in a more drastic manner than observed in the Cre system. Unique pairings of adjacent protein subunits could then be responsible for the formation of only two cleavage-competent active sites at any given step of the reaction [30].

Which tyrosine cleaves where?

Although it seems clear that Int family members share a common catalytic machinery and recombine their DNA substrates using similar mechanisms, the issue of 'which tyrosine cleaves where' has been the focus of a great deal of debate [32–35]. In 1992, Jayaram and colleagues [36] reported that Flp recombinase cleaves its DNA substrates not as shown in Figure 2, but in a 'trans cleavage' mode. In this mode, the tyrosine nucleophile from a recombinase subunit bound to a given DNA half-site cleaves the scissile phosphate located in a different half-site. A subsequent study then concluded that λ -Int also undergoes trans cleavage [37], only to be refuted by more convincing experiments showing that Int cleaves in *cis* [38]. Similarly, a report published early in 1997 concluded

that Cre recombinase uses a *trans* cleavage mechanism [39]. The Cre–DNA structures that followed [13**,17**] instead clearly showed a *cis* cleavage geometry. In the XerC and XerD system, *cis* cleavage has been demonstrated [40]. So, what's going on?

A number of possible explanations for the conflicting biochemical data have been put forward [32,34,35,38,41], but the question we consider here is whether or not a single structural framework could accommodate both *cis* and *trans* cleavage modes. Based on the Cre–DNA structures, the answer appears to be yes (Figure 4). In each of the Cre–DNA recombination complexes, the helix (M) containing the catalytic tyrosine sits in the active site cleft in *cis* and the C-terminal helix (N) is donated in *trans* to an adjacent subunit (Figure 4a,b). By simply changing the connection between the L and M helices, an alternative domain swap and organization of active sites can be produced that results in a '*trans* cyclic' cleavage scheme (Figure 4c) [13**]. We have modeled this hypothetical structure into the Cre system and find that the *trans* cleavage geometry can just barely be accommodated by the relatively short linker between the L and M helices without distortion of the active site. The only changes in the Cre–DNA architecture that are required to accomplish this cleavage mode switch involve four residues (316–319), which must adopt an extended conformation in the *trans* cleavage arrangement. A similar scheme for the Flp mechanism was presented in a figure by Cox and co-workers [42], but with a parallel alignment of DNA sites instead.

If such a structure can be formed, the possibility then exists for competition between the two cleavage modes. The issue is even more compelling if we assume that the C-terminal regions of the catalytic domains are flexible enough to be able to adopt either configuration under the appropriate conditions. Indeed, the active site regions of Int, HP1 integrase, XerD and Cre (Figure 5) provide direct structural evidence that the catalytic tyrosine is mobile and that a tyrosine–phosphate hydrogen bond is an important component of active site assembly. In two structures, the tyrosine is located in the active site — Cre, which is bound to its DNA substrate [13**], and HP1 integrase, which binds a sulfate ion in the active site [11**]. In contrast, the Int and XerD structures demonstrate that the catalytic tyrosine (a phenylalanine mutant in the Int structure) is capable of moving out of the *cis* active site in the absence of the substrate [10**,12**]. It is interesting that in the Cre–DNA structures, a single hydrogen bond from the catalytic tyrosine to a DNA phosphate group anchors helix M in the active site. In complementation experiments designed to test *cis* versus *trans* cleavage, this residue is generally changed to phenylalanine in order to provide a protein subunit that is capable of activating, but not cleaving, a scissile phosphate. An intriguing possibility in these cases is that the removal of this

important hydrogen bond could lead to both a greater mobility of the tyrosine-containing segment and a corresponding increase in the ability to switch between cleavage modes. The suggestion that recombinases might be capable of using alternative pathways when a primary cleavage mode is disabled by mutagenesis [38] would then appear to be quite feasible on structural grounds.

Conclusions

Most of what we know about the three-dimensional structures of proteins and protein–DNA complexes of the λ -Int family of site-specific recombinases has been provided in the past two years. Although structural models for the λ -integrase and HP1 integrase catalytic domains, full-length XerD recombinase and Cre recombinase–DNA complexes have answered a number of puzzling questions about how these systems work, the story seems far from over. A general model for the stereochemistry of isomerization and the switching of strand cleavage specificity is a major goal for the future, along with a resolution of the puzzling *cis/trans* cleavage issue. The structure of Flp recombinase bound to DNA would therefore be a most welcome addition to the growing Int family of structures.

Acknowledgements

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