# Teaching a new dog old tricks?

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The recently determined crystal structures of fragments of the human and vaccinia virus type IB topoisomerases reveal unexpected similarity with the lambda family of site-specific recombinases. The conservation of structure suggests a common mechanism, indicating that topoisomerase activity may be the consequence of uncoupling DNA strand cleavage/religation from synapsis.

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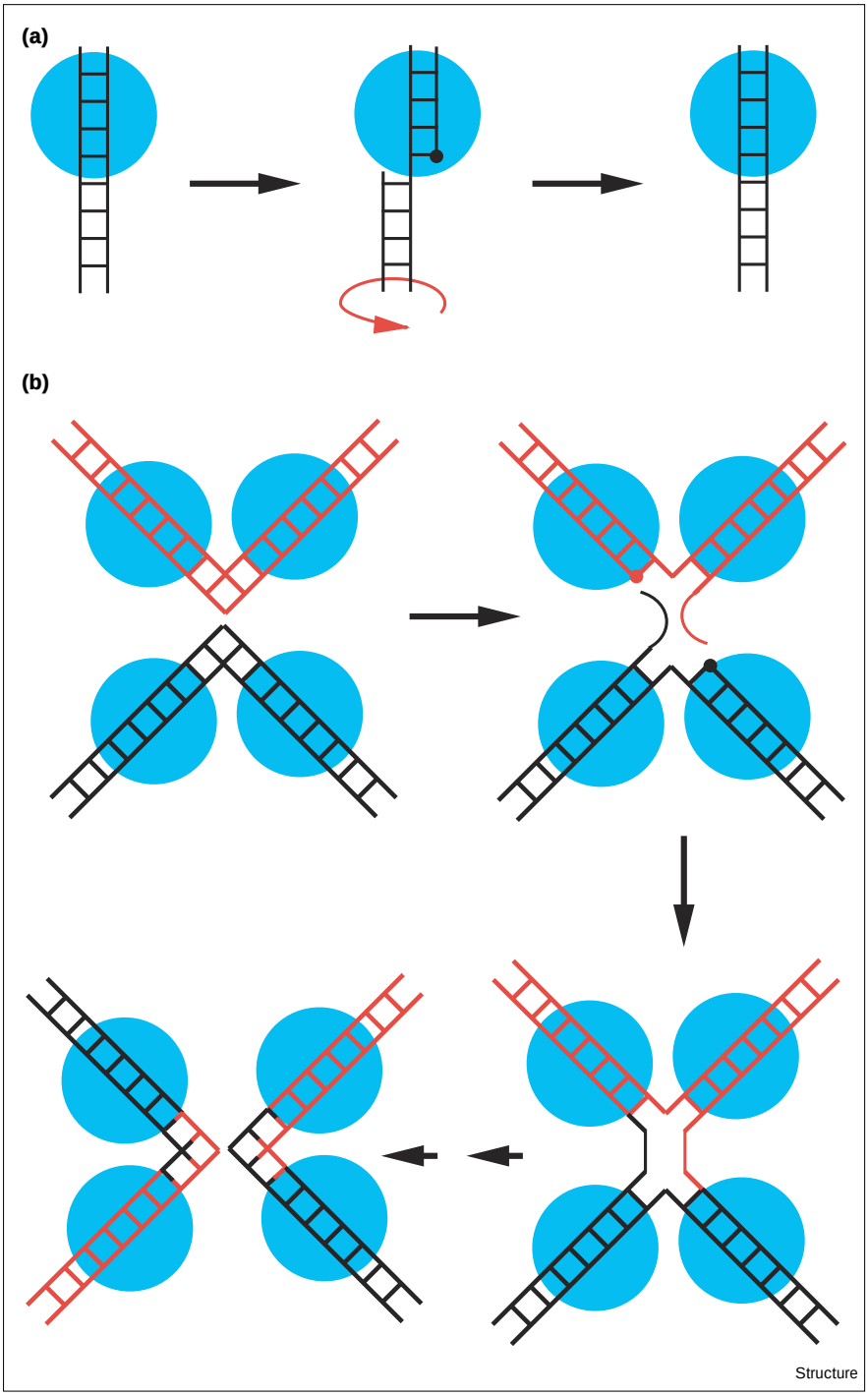
It is understandable why the maintenance of the genome should be of prime concern to an organism, and it is not surprising to discover that there are a wide variety of proteins associated with that task. Topoisomerases are one such group of enzymes which are responsible for catalysing a number of complicated interconversions of different topological states of DNA, including alteration of supercoiling and chromosome decatenation. Site-specific recombinases are equally important for a different set of complex reactions that are responsible for events as apparently diverse as chromosome segregation and genetic transposition. For a comparison of the overall reactions of type I topoisomerases and site-specific recombinases see Figure 1. Intriguingly, although both classes of enzyme catalyse a wide range of reactions, they have been shown to share a number of mechanistic similarities, most notably a magnesium ion independent reaction and the formation of a covalent 3′-phosphotyrosine enzyme–DNA intermediate.

There are two families of type I topoisomerases, designated IA and IB [1]. The crystal structure of a type IA topoisomerase from *Escherichia coli* was reported several years ago [2]. The structure revealed a large cavity within the protein that was proposed to accommodate a DNA duplex as part of the so-called ‘strand-passage’ mechanism. This mechanism involves passage of a DNA duplex through a gateway formed by cleavage of a separate single-stranded region of DNA. A strand-passage mechanism is also proposed for the type II topoisomerases (reviewed in [3]). Recent crystal structures of fragments of human topoisomerase IB [4,5] and the catalytic domain of vaccinia virus topoisomerase [6] have revealed fascinating glimpses of the catalytic mechanism of these enzymes. The structure of the human topoisomerase has been determined in two complexes with DNA: one is of the initial protein–DNA complex, whereas the other is of an intermediate on the reaction pathway in which the enzyme has cleaved the DNA and formed a 3′-phosphotyrosine covalent linkage at the nick site. The vaccinia topoisomerase structure is that of the free protein and, as expected, shows structural homology with a comparable region of the human enzyme that contains the active site and with which it shares considerable amino acid sequence homology. The overall shape of the human enzyme reveals a large central cavity reminiscent of the type IA topoisomerase structure. The folds of the IA and IB topoisomerases, however, are completely different. From a biochemical perspective there are also significant differences in the mechanism between the IA and IB enzymes, including the requirement for magnesium ions by IA topoisomerases and a difference in the nature of the phosphotyrosine intermediate in IA and IB enzymes (5′ or 3′, respectively). For these and other reasons, a different mechanism is proposed for the IB topoisomerases [5] (Figure 2). This ‘controlled rotation’ mechanism is very different from the strand-passage mechanism proposed for other topoisomerases.

A quite unexpected discovery was that the human and vaccina topoisomerases share structural homology with a family of site-specific recombinases for which four independent structures were determined last year (reviewed in [7]) (Figure 3). What is the biochemical significance of this similarity? Hints about a link between type IB topoisomerases and recombinases have been accruing in the literature for a number of years. Experimental data showing that the vaccinia topoisomerase was able to catalyse the resolution of Holliday junctions and that it was unique amongst topoisomerases in having a strict site selectivity were important clues about this linkage. The observation that by uncoupling the recombinases from recombination *per se*, it was possible to observe topoisomerase activity, albeit rather poor, was also important. However, hindsight is a wonderful thing and the extent of the underlying connection has only become apparent since the determination of the crystal structures of these enzymes. It is now evident that, despite a complete lack of detectable sequence homology, the two enzyme families promote catalysis by a similar mechanism based upon a conserved catalytic framework (Figure 4). Consequently, the biochemical outcome of the reaction appears to result from a subtle control of catalysis rather than by an altered chemical mechanism. If that is indeed the case, what might be the structural and chemical bases for this control mechanism?

**Figure 1**

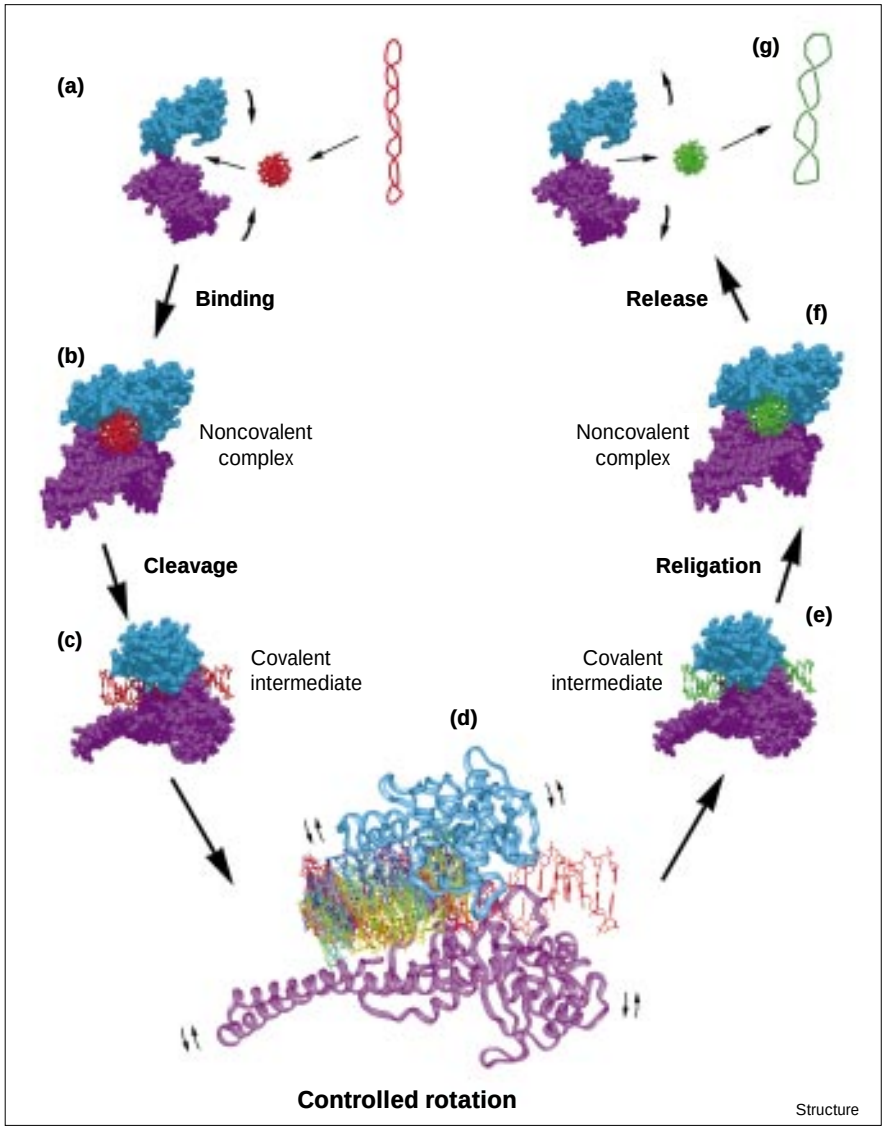



Reactions catalysed by type IB topoisomerases and site-specific recombinases. **(a)** In type IB topoisomerases the enzyme (represented by a blue circle) cleaves a single strand of the duplex and forms a covalent linkage with the DNA (denoted by a dot). The DNA is then relaxed before the DNA is rejoined. **(b)** In site-specific recombinases the reaction involves two pairs of enzyme molecules and two separate duplexes. One enzyme molecule in each pair cleaves the DNA and forms a covalent linkage (again denoted by a dot). The free 5′-end at each cleavage site is then swapped with its equivalent in the partner duplex, before rejoining of the DNA takes place. This cross-shaped DNA product is known as a Holliday junction. The final two steps of the reaction are equivalent to the first two except that it is the other enzyme molecule in each pair that performs the cleavage and religation.

Recombinases carry out a highly controlled cleavage/religation reaction that is tightly coupled to the association of two DNA duplexes prior to strand exchange (referred to as synapsis). Topoisomerase activity, on the other hand, can be regarded as the consequence of uncoupling strand exchange from cleavage and rejoining. The obvious explanation for this is that it is the association of protein monomers at synapsis that controls the nuclease and/or religation activity of the recombinases (i.e. that the activation requires cooperativity between partner protein molecules). The biochemical evidence is clear on this point, recombinases can bind to their target sequences

**Figure 2**

The proposed 'controlled rotation' mechanism for topoisomerase IB. The reaction is initiated by cleavage of a single strand of the duplex DNA as it resides within the central cavity of the enzyme (a–c). The duplex is then allowed to rotate within this central cavity (d) before rejoining of the cleaved strand (e,f) and release of the relaxed DNA (g). Each rotation of the DNA duplex at (d) results in the relaxation of one supercoil. (The figure was reproduced from [5] with permission.)




but will not cleave the DNA in the absence of their partners. By contrast, the topoisomerases appear to operate as monomers. Hints for how this 'activation' process might occur are provided by the variety of structures that

**Figure 3**

Stereoview superposition of the crystal structures of human topoisomerase IB (red and green) and HP1 recombinase (grey) in the region of the active sites. The folds of the catalytic domains of the two enzymes are remarkably similar. (The figure was reproduced from [4] with permission.)

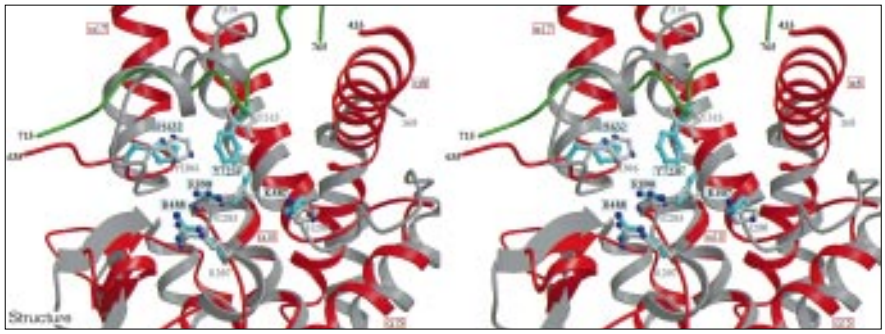
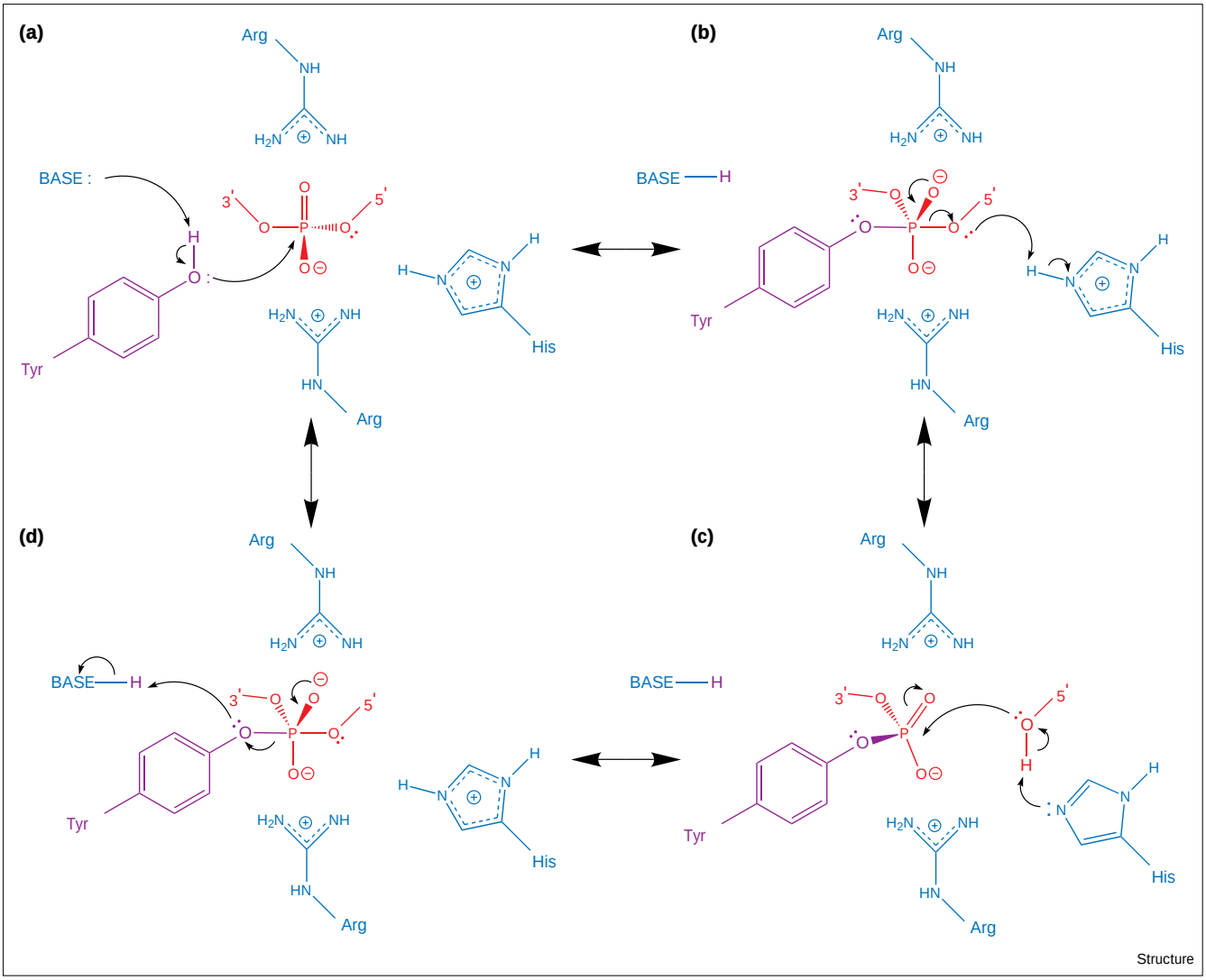


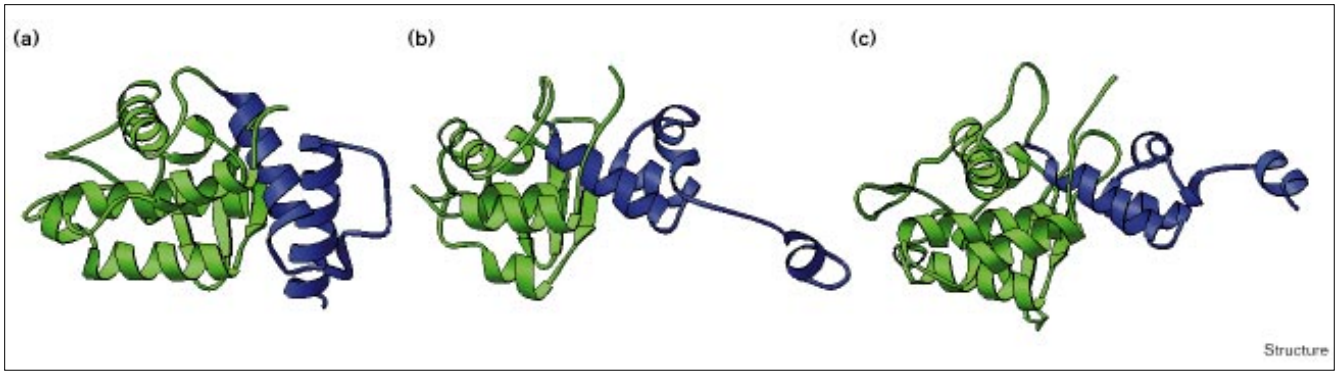
**Figure 4**




The chemical mechanism of type IB topoisomerases and lambda family site-specific recombinases. The reaction can be considered in four stages (**a–d**) with two pentavalent phosphorous transition states (b and d). The reaction begins with the initial protein–DNA complex (a) and proceeds via the transition state (b) to the stable 3′-phosphotyrosine DNA–enzyme intermediate (c). The reaction is completed by the attack of a 5′-hydroxyl from the same duplex (in the case of topoisomerases) or from a different duplex (recombinases), via a similar transition state (d) to give the products (a). DNA strand swapping (recombinases) or 'controlled rotation' (topoisomerases) occurs after formation of the phosphotyrosine adduct. The base shown in the scheme is always a histidine in recombinases, but the equivalent residue is a lysine in topoisomerases. In addition, the histidine residue shown is exceptionally a tryptophan in Cre recombinase. This means that although proton transfers are shown to be taking place at certain stages in the reaction pathway, these transfers may not be complete but might be more akin to hydrogen bonds between the enzyme and the substrates that would serve either to stabilise or activate intermediates of the catalytic pathway.

are now available. One of the recombinase structures, XerD [8], shows that the active-site tyrosine is positioned away from the other active-site residues. This observation led to a proposal for activation in which a conformational change moves the active-site tyrosine from being buried in the protein interior, to place it in position in the active site. Evidence that this conformational change is a result of protein contacts is provided by the structure of another recombinase family member, HP1 integrase [9], which crystallises as a dimer and in which the conformation of the tyrosine is in the activated position, even in the absence of DNA (Figure 5). The structure of Cre recombinase bound to a pseudo Holliday junction shows the tyrosine to be in the activated position both before and after cleavage of the DNA [10]. Intriguingly, this activation process appears to be mirrored in the topoisomerases. The structure of the vaccinia topoisomerase corresponds to the inactive conformation, while the human enzyme is

**Figure 5**




Activation in recombinases. Ribbon representations of the catalytic domains of three recombinases (a) XerD, (b) HP1 and (c) Cre. The figure illustrates the difference in conformation at the C terminus of the proteins (coloured blue in each case) that is proposed to constitute the activation of enzyme activity. A fourth recombinase structure (lambda integrase [14]) has not been included in this comparison as it is unclear whether the enzyme utilises a *cis* or *trans* cleavage mechanism – which may confuse the interpretation of the conformational differences that are observed.

in the activated conformation in both the pre- and post-cleavage DNA complexes. As all of the topoisomerase structures are monomeric, however, this suggests that activation is a consequence of binding to DNA rather than to a protein partner. Thus topoisomerase activity is uncoupled from the formation of protein–protein contacts (i.e. synapsis). The structures of the free human topoisomerase IB or of the vaccinia enzyme bound to DNA should be revealing with regard to this activation process.

Recent biochemical data have uncovered some interesting alternative catalytic activities of these recombinases and topoisomerases. It has been shown that both the vaccinia topoisomerase [11] and Flp recombinase [12] are able to act as ribonucleases on substrates in which the base next to the normal cleavage site is a ribonucleotide rather than the usual deoxyribonucleotide. The reaction proceeds by the usual chemistry until step (c) in the scheme shown in Figure 4. However, it is the 2′-hydroxyl of the ribose sugar moiety of the covalent enzyme–DNA adduct, rather than the 5′-hydroxyl of the cleaved DNA, that attacks the phosphotyrosine. This attack results in the formation of a cyclic 2′,3′-ribose phosphate on the 3′-side of the cleavage site and the normal 5′-hydroxyl on the other side. Thus the enzyme is now acting as a nuclease. As if this were not enough, it has also been shown that the vaccinia topoisomerase can take this reaction a step further by ligating the 2′,3′-cyclic phosphate to the 5′-hydroxyl of the DNA [13], by means of a mechanism that does not require the active-site tyrosine (shown by mutation to a phenylalanine). Thus the catalytic mechanism of the active site itself can be uncoupled, by using the appropriate substrates, to reveal an underlying catalytic activity which is even simpler than that usually observed with the 'natural' substrates.

These data allow us the fun of speculating about the molecular evolution of this system. One can envisage a protein capable of binding two RNA fragments and a simple active site that promoted ligation of the fragments, perhaps itself evolving from a primordial RNase. This system could have developed further to replace the attacking 2′-hydroxyl of the RNA with that from a tyrosine sidechain in the protein. This active site would then be capable of elementary breakage and reunion activity upon DNA. Later adaptation could produce a topoisomerase by taking a tight grip on the DNA duplex at least on one side of the single-strand nick. Recombination would then evolve by making a tight grip on both sides of the nick site and then coordinating the swapping of strands between partner proteins in a recombination synapse. Whatever the evolutionary pathway may have been, we are fortunate to have such marvellous insights into the mechanism of these enzymes from the mounting structural and biochemical evidence. Nevertheless, although these catalytic 'snapshots' are a fascinating preview, we are still some way from seeing the entire movie. Let us hope that further structures will help to explain the plot.

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