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Communication Between Accessory Factors and the Cre Recombinase at Hybrid *psi-loxP* sites

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By placing *loxP* adjacent to the accessory sequences from the Xer/*psi* multimer resolution system, we have imposed topological selectivity and specificity on Cre/*loxP* recombination. In this hybrid recombination system, the Xer accessory protein PepA binds to *psi* accessory sequences, interwraps them, and brings the *loxP* sites together such that the product of recombination is a four-node catenane. Here, we investigate communication between PepA and Cre by varying the distance between *loxP* and the accessory sequences, and by altering the orientation of *loxP*. The yield of four-node catenane and the efficiency of recombination in the presence of PepA varied with the helical phase of *loxP* with respect to the accessory sequences. When the orientation of *loxP* was reversed, or when half a helical turn was added between the accessory sequences and *loxP*, PepA reversed the preferred order of strand exchange by Cre at *loxP*. The results imply that PepA and the accessory sequences define precisely the geometry of the synapse formed by the *loxP* sites, and that this overcomes the innate preference of Cre to initiate recombination on the bottom strand of *loxP*. Further analysis of our results demonstrates that PepA can stimulate strand exchange by Cre in two distinct synaptic complexes, with the C-terminal domains of Cre facing either towards or away from PepA. Thus, no specific PepA-recombinase interaction is required, and correct juxtaposition of the *loxP* sites is sufficient to activate Cre in this system.

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Introduction

Site-specific recombination brings about programmed DNA rearrangements for a variety of biological purposes, including bacteriophage integration and excision, plasmid and chromosome monomerisation, and resolution of cointegrates produced by transposition.[1] Recombination is catalysed by specialised recombinase proteins that bind and cleave at short recombination core sites, and is often regulated by accessory proteins binding to accessory sequences adjacent to the core site.

Xer recombination is catalysed by the chromosomally encoded XerC and XerD proteins, two members of the tyrosine recombinase family.[2,3] Xer recombination acts at *dif* sites in the terminus regions of bacterial chromosomes to monomerise chromosomal dimers produced by homologous recombination.[4] XerC and XerD also act at plasmid resolution sites, such as ColE1 *cer* and pSC101 *psi*, to monomerise plasmid multimers generated by homologous recombination, maximising the number of independently segregating plasmid units and increasing stable plasmid inheritance.[5,6]

Plasmid recombination sites *cer* and *psi* comprise 150–180 bp of accessory sequences adjacent to a *dif*-like core site, at which XerC and XerD catalyse strand exchange. The accessory sequences contain binding sites for the accessory proteins PepA and either ArgR (in *cer*) or ArcA (in *psi*).[7–10] The accessory proteins are required for recombination, but do not participate directly in the catalysis of strand exchange. Instead, they bind to the accessory sequences to form a synaptic complex in which the two sites are wrapped around each other, bringing the core sites close together and activating strand exchange by the recombinases (Figure 1(a)).[11,12] The interwrapped synaptic complex can form only between directly repeated sites on the same supercoiled circular DNA molecule. Therefore, recombination occurs only between directly repeated sites in the same DNA molecule, and plasmid multimers

Abbreviations used: HJ, Holliday junction.

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are resolved, but not formed by Xer recombination. As a consequence of this mechanism, the two circular products of recombination are interlinked in a specific four-node catenane (Figure 1(a)).

We have reported the construction of hybrid sites that contain *cer* or *psi* accessory sequences adjacent to *loxP*, the recombination site for the well-characterised Cre recombinase. Accessory proteins were not required for Cre recombination at these hybrid sites, but addition of PepA altered the topology of recombination, so that the product was a four-node catenane.[13] We also isolated a Cre mutant (CreC1) that requires PepA and *cer* or *psi* accessory sequences for efficient recombination *in vitro*.[13] We have proposed that CreC1 has a synapsis defect that is rescued by PepA and the accessory sequences.[13] Just like the natural Xer system, recombination by CreC1 in the presence of PepA shows topological selectivity and specificity; it only recombines directly repeated sites, and the product of recombination is exclusively a four-node catenane.

The active form of Cre is a pseudo square-planar tetramer containing two *loxP* sites in an antiparallel configuration.[14,15] XerC and XerD are thought to act as a hetero-tetramer with a similar structure.[16,17] Both systems catalyse recombination by sequential strand exchange reactions, *via* a Holliday junction (HJ) intermediate. One pair of diagonally opposite recombinase subunits in the tetramer exchanges the first pair of strands to give HJ, which is resolved by the other two recombinase subunits to give recombinant product.[18] Xer and Cre recombination reactions both proceed with a defined order of strand exchanges.[19,20] The order of strand exchanges at *psi* is imposed on the recombinase proteins by the synaptic complex formed by PepA and the accessory sequences.[21] This synaptic complex brings the XerCD binding sites together with a defined geometry, such that the recombinase monomers proximal to the accessory sequences (XerC in the wild-type *psi* site) carry out the first pair of strand exchanges. The order of strand exchanges by Cre is also defined by the geometry of synapsis, but this geometry is normally dictated by sequence asymmetry in the central 8 bp of *loxP*.[22,23]

The way in which PepA and the accessory sequences activate recombination is still unknown. The synaptic complex may simply position the core sites in an appropriate configuration for recombination, or there may be specific interactions between the accessory proteins and the recombinases that activate the recombinases. Here, we investigate communication between Cre bound at *loxP* and the interwrapped synaptic complex formed by PepA and the *psi* accessory sequences by varying the spacing between *loxP* and the accessory sequences, and by changing the orientation of *loxP*. We show that adding half a helical turn of DNA between *loxP* and the accessory sequences alters the order of strand exchange by Cre without changing the product topology. We give a structural interpretation of these results and show that PepA can stimulate Cre without any requirement for specific protein–protein interactions between PepA and Cre.

Results

The effect of spacer length on recombination by wild-type Cre

In the *psi-loxP* hybrid recombination site described in our previous work, the XerC and XerD binding sites of *psi* were replaced precisely by the *loxP* recombination site.[13] In this original site, *loxP* is oriented such that the site of bottom strand cleavage on *loxP*, and hence the Cre monomer preferentially initiating strand exchange, is proximal to the *psi* accessory sequences (*psi*-acc; Figure 1(b) and (c)). Here, we refer to this original site as *psi-loxP*A0, where A denotes the orientation of *loxP* with respect to *psi*-acc, and the numeral indicates the length of the spacer between *psi*-acc and *loxP* relative to the original site, as explained in the legend to Figure 1.

To investigate whether the spacing between *psi*-acc and *loxP* is important for the efficiency and topology of recombination by Cre, we constructed a set of sites with *loxP* in the same A orientation, but with different spacer lengths between *psi*-acc and *loxP*. The *psi*-acc to *loxP* spacers in these variant sites ranged from 2 bp shorter (*psi-loxP*A-2) to 52 bp longer (*psi-loxP*A52) than in the original *psi-loxP*A0 site (Figure 1(d)). Plasmid substrates (pAA-2, pAA0, pAA2, …pAA52) containing directly repeated copies of each *psi-loxP*A variant were incubated *in vitro* with Cre in the presence or in the absence of PepA, nicked with DNase I to remove supercoiling, and run on an agarose gel (Figure 2). In the absence of PepA, all substrates yielded unlinked recombinant circles and a band migrating slightly faster than the unknotted circle as major products (Figure 2(a), lane 2 and data not shown). Restriction analysis (data not shown) demonstrated that the band migrating ahead of unknotted circles was mainly zero-node figure-of-eight HJ, an intermediate in the production of unlinked recombinant circles, with a small amount of slightly faster-migrating two-node catenane (Figure 2(b)). As reported,[13] the addition of PepA changed the topology of Cre recombination at pAA0, reducing the amount of unlinked circle and zero-node HJ products, and increasing dramatically the amount of four-node product (Figure 2(a), lane 4). This four-node product consists of four-node catenane, and some four-node HJ, an intermediate in the production of four-node catenane that co-migrates with four-node catenane[11] (Figure 2(b)). When the spacing between *psi*-acc and *loxP* was varied in small increments, the effect of PepA on the topology of recombination varied with the helical phasing between *psi*-acc and *loxP* (Figure 2(a), lanes 3–12). pAA-2 and pAA2 gave slightly less four-node