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The two functional domains of γδ resolvase act on the same recombination site: Implications for the mechanism of strand exchange

(site-specific recombination/DNA cleavage/protein subunit exchange/mutant enzymes)

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ABSTRACT **During site-specific recombination by the γδ resolvase, four DNA strands are broken, exchanged, and religated. This exchange is carried out within a DNA–protein complex, the synaptosome, in which the recombination sites, *res*, are aligned. The domain of resolvase that binds to a *res* site is distinct from the domain that breaks and rejoins the DNA. We tested whether the catalytic domain acts on the *res* site to which its binding domain is bound (in cis) or on the opposing *res* site in the synaptic complex (in trans). We constructed a hybrid synaptosome in which one *res* site is bound to wild-type resolvase and the other is bound to a mutant resolvase that binds normally but is unable to break DNA. From the pattern of strand breakage in the reaction intermediate containing resolvase covalently attached to DNA, we conclude that resolvase attacks predominantly, if not exclusively, in cis. Because cis breakage and reunion *per se* cannot lead to recombination, our results support a model in which DNA exchange is guided by an exchange of resolvase subunits between the breakage and reunion events.**

Site-specific recombination by the resolvases of the γδ/Tn*3* family of transposons (1) is remarkably fast and efficient. Within *Escherichia coli* cells (J. Bliska, H. Benjamin, and N.R.C., unpublished data) and *in vitro* (2, 3) most of the plasmid substrate is recombined within minutes. The requirements are only the 21-kilodalton resolvase and two recombination sites, called *res*, that are directly repeated (head to tail) in a negatively (−) supercoiled molecule. The reaction can be divided into two stages that are easily separated *in vitro* (4): (*i*) the formation of a synaptic complex containing two intertwined *res* sites and multiple copies of resolvase and (*ii*) the breakage and crossed reunion of DNA.

It is the second step, strand exchange, that we address here. The mechanism of strand exchange has not been established for any recombination system, but the critical questions can be framed with precision for resolvase because of the wealth of biochemical, genetic, and topological data (reviewed in refs. 1, 5, 6). A *res* site consists of three resolvase binding sites, called I, II, and III, each of which, in turn, is made up of an inverted repeat of a 12-base-pair (bp) "half site" (7–9). Although all three sites are required, exchange occurs only between the halves of site I (2). For this reason, we focus on the complex of four resolvase monomers bound to synapsed sites I, shown schematically in Fig. 1.

Strand breakage and reunion are accomplished by two successive ester exchanges in which the energy of the scissile phosphodiester bond is stored transiently as a phosphate ester to serine 10 of resolvase (10, 11). Because two double-strand breaks are engaged in exchange, all four resolvase protomers at the crossover region are involved (2). Recombination results because the attacking 3′ hydroxyls during reunion are from the paired *res* site rather than the ones bonded to 5′ phosphates in the substrates.

Clearly, substantial movement of DNA must occur during exchange. The distance traveled may be as large as 100 Å, the approximate diameter of the resolvase synaptic complex (4). The movements of DNA that accompany breakage and reunion must nonetheless be precisely choreographed because the product of one round of recombination is a (−) singly linked catenane (5) with four fewer (−) supercoils (6, 12). This unique topological change requires that during exchange the four 3′ hydroxyl terminated strands in addition to the four covalently linked 5′ termini remain bound to resolvase to prevent free swiveling of the DNA.

The catalytic and DNA binding functions of resolvase reside in different domains (13, 14). The N-terminal domain (residues 1–140) contains the transesterification active site, whereas the C-terminal domain (residues 141–183) binds specifically to each half site. In this report, we ask whether the two domains of a single monomer act on the same *res* site (i.e., in cis; Fig. 1*A*) or on opposite *res* sites (i.e., in trans; Fig. 1*C*), or whether a mixture of the two alternatives applies (Fig. 1*B*). A recombinase anchored by its C-terminal domain to one *res* site reaching out with its N-terminal domain to the other could explain the controlled long-range movement during exchange because the trans-covalent attachment might lead the broken DNA into the recombinant configuration. Trans nicking could also explain why catalytic activities of resolvase such as site I cleavage and topoisomerase activity are confined to a synaptic complex (2, 15).

To distinguish among these alternatives, we developed a method for constructing a hybrid synaptic complex in which one *res* site was bound by wild-type γδ resolvase whereas the other was complexed with a mutant blocked in transesterification. We then measured the production of an intermediate in which DNA strand breakage had occurred but no reunion occurred. Barring preferential religation of selected single-strand breaks, each of the three models shown in Fig. 1 makes distinct predictions: exclusive nicking in cis leads to a double-strand break in the *res* site bound to the wild-type protein (Fig. 1*A*), strand nicking in trans leads to a double-strand break in the *res* site bound by mutant protein (Fig. 1*C*), and mixed cis and trans nicking leads to a single-strand break in both *res* sites (Fig. 1*B*). The predominant pattern observed implies nicking only in cis. Transesterification exclusively in cis would by itself not lead to recombination. Thus, our results support a model described in the *Discussion* in which an exchange of resolvase monomers between the breakage and reunion events is required for recombination.

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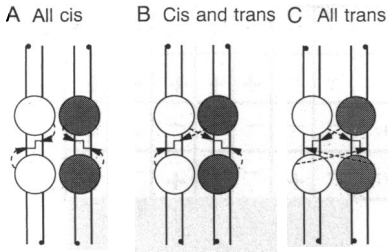

FIG. 1. Three possible DNA breakage patterns by resolvase. Only the portion of the synaptosome near the crossover sites is represented. The DNA in the crossover region (site I of *res*) is depicted as two lines with circles marking the 5′ ends. Each crossover region is bound by a resolvase dimer marked as open and shadowed circles. Resolvase can nick the DNA backbone to which it is bound (i.e., in cis, *A*) or break the opposed DNA (i.e., in trans, *C*). There can also be mixed cis and trans nicking (*B*). If the differentially shaded dimers are considered to make up a hybrid synaptosome of a wild-type resolvase dimer and a mutant dimer that cannot nick DNA, there will be a double-strand break of the DNA bound to the wild-type enzyme in *A* and of the mutant enzyme in *C*; both sites will be nicked in *B*. Other nicking patterns are also possible—e.g., each monomer may nick in cis but attack the half site that is bound by the other protomer of its dimer. Our experiments distinguish whether the two domains of resolvase act on the same *res* site but not which half site is attacked.

MATERIALS AND METHODS

Enzymes. γδ Wild-type resolvase and the mutant resolvases, R68H and S10C, were purified as described (11). The first and last letters of the mutant designation are the wild-type and substituting amino acids, respectively, and the number is the residue position. Phase λ integrase protein (Int) was the gift of H. Echols (University of California, Berkeley). The purification of *E. coli* integrative host factor has been described (3). Integration reaction mixtures contained Int and integrative host factor (16) but are referred to herein as Int reactions. Restriction enzymes were from New England Biolabs.

DNA. The plasmid substrates for recombination, pPD1 and pPD2 (Fig. 2), contained a single *res* site and either *attP* or *attB*, the phage and bacterial attachment sites for Int. pPD1 was generated from pAB7.0d (17) and pPD2 was from pAB3 (18).

Reactions. Resolvase reactions with independently complexed *res* sites were carried out in three steps (Fig. 2). In step I, 4 pmol of resolvase was preincubated separately with 0.2 pmol of pPD1 and pPD2 at room temperature for 30 min in 20 μl of 50 mM Tris·HCl, pH 7.6/20 mM KCl/50 mM NaCl/2 mM EDTA/5 mM spermidine/0.5 μg of serum albumin. In step II, the reaction mixtures were chilled to 0°C and excess integrative host factor and 4 pmol of Int were added to pPD2 and mixed with the resolvase pPD1 complex at 26°C for 20 min to permit intermolecular recombination by Int. In step III, the temperature was raised to 37°C for 5 min to promote intramolecular recombination by bound resolvase. Reactions were stopped by heating to 68°C for 10 min. After addition of 10 mM $MgCl_2$, restriction enzymes were added and incubation was continued for 30 min at 37°C. Digestion was terminated by addition of 0.5% $NaDodSO_4$ (wt/vol) followed by ethanol precipitation. DNA was dissolved in 10 mM Tris·HCl, pH 7.5/1 mM EDTA/0.5% (wt/vol) $NaDodSO_4$ and subjected to gel electrophoresis.

To increase the yield of recombination intermediates in which resolvase was attached to cleaved DNA, 47% ethylene glycol (Sigma) was added at the end of step II. A 1.2-kilobase (kb) plasmid, πres (4), which contains a single *res* site, was added (0.4 pmol) to each reaction mixture at the beginning of step II to trap unbound resolvase and to minimize protein exchange between the plasmids. The reactions were terminated by heat treatment and DNA was precipitated with ethanol. DNA was dissolved in 10 mM Tris·HCl, pH 7.5/1 mM EDTA prior to endonuclease digestions.

Gel Electrophoresis. DNA was subjected to electrophoresis through a 1.2% horizontal agarose gel in a $NaDodSO_4$-containing Tris acetate buffer (18) for 18 hr at 2 $V \cdot cm^{-1}$. Southern blots were as described (19), except that carrier DNA was omitted during hybridization. Autoradiographs were quantified using a Hoefer GS300 scanning densitometer.

RESULTS

Inside the resolvase synaptosome, the two copies of site I are each bound by a resolvase dimer and juxtaposed in preparation for cleavage (Fig. 1). We asked whether the resolvase subunits break the DNA backbones of their binding sites in cis (Fig. 1*A*) or whether one (Fig. 1*B*) or both (Fig. 1*C*) break the DNA in trans. To address this question, we used wild-type γδ resolvase and the mutant resolvase, R68H. This mutant has a specific DNA binding affinity similar to that of wild-type enzyme and forms an apparently normal complex with a single *res* site but is unable to induce cleavage and to recombine DNA (G.F.H. and N.D.F.G., unpublished results). We developed a strategy that allows independent loading of the two *res* sites that form a synaptosome. By loading one *res* site with the mutant enzyme and the other with wild-type enzyme, we can ask which of the two crossover sites is attacked by the wild-type enzyme. This question may not be addressed by simply mixing two DNAs loaded with resolvase because resolvase recombines only intramolecular sites.

We utilized plasmids pPD1 and pPD2, each containing a single *res* site (*res*1 or *res*2) and either the bacterial or the phage site (*attB* or *attP*) for Int recombination (Fig. 2). Int recombines the two plasmids to yield a (−) supercoiled dimer, pPD3, which contains two *res* sites oriented as direct repeats. In the complete experiment, resolvase is added separately to the plasmids and excess enzyme is then trapped by competitor DNA that contains a single *res* site. The plasmids are now mixed but resolvase cannot recombine the DNA until both *res* sites are on the same supercoiled molecule, which is accomplished by the Int treatment.

The results of such an experiment are presented in Fig. 3. More than 50% of both starting plasmids were recombined by Int *in vitro* within 20 min at 26°C to form the dimer, pPD3 (lanes 1–5). If the starting plasmids were preincubated with wild-type resolvase, more than 80% of the dimer, pPD3, was resolved within 5 min at 37°C (compare lanes 4 and 7).

In the next experiments, ethylene glycol was added prior to resolution to increase accumulation of recombination intermediates with exclusively double-strand breaks at the crossover sites (P.D. and N.R.C., unpublished results). The result of one such experiment is shown in Fig. 4*A*. In the control with wild-type resolvase bound to the *res* site on both plasmids, three linear DNA species with covalently attached resolvase were found (lane 4); these represent pPD3 cut at one or both *res* sites. Confirmation of the covalent attachment of both ends of the linear intermediates (2) is provided by the retardation of the electrophoretic mobility of fragments containing the ends. There was no evidence for pPD3 with resolvase bound at a nick. In another control, the mutant enzyme was preincubated with both of the *res*-containing plasmids (lane 3). None of the three bands was detected, which confirms that the mutant enzyme is completely blocked in transesterification. However, when wild-type enzyme was preincubated with either *res*1 or *res*2 and the mutant protein was bound to the other *res* site, linearized pPD3 was detected (lanes 1 and 2). This species is the product of a double-strand cleavage within one *res* site and contains resolvase covalently attached to both 5′ ends. The absence of

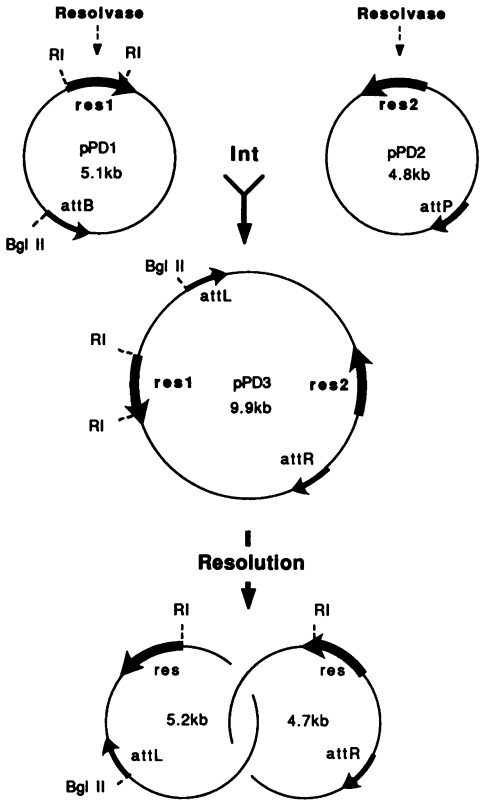

FIG. 2. Strategy of the experiment. To load two identical *res* sites with different resolvases, we constructed pPD1 and pPD2 that contain a single *res* site marked here as *res*1 and *res*2, respectively. Each plasmid also carries either a bacterial (*attB*) or a λ phage (*attP*) recombination site for the λ Int system. Int carries out intermolecular recombination to generate a negatively supercoiled dimer, pPD3, which contains the two *res* sites as direct repeats. A synaptosome may then be formed with prebound resolvase, and if both sites are bound by wild-type resolvase, pPD3 is converted into a singly interlinked catenane. After cleavage within *res*1 and restriction with *Eco*RI, fragments of 0.43 kb, 0.47 kb, and 9.0 kb are generated. Cleavage within *res*2 produces 0.9-, 4.3-, and 4.7-kb fragments. The positions of sites for *Eco*RI (RI) and *Bgl* II are indicated.

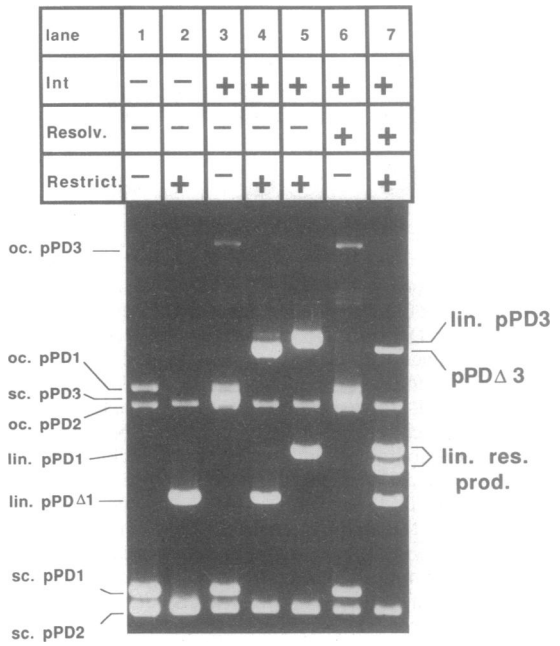

FIG. 3. Test of the experimental protocol. pPD1 and pPD2 were bound to wild-type resolvase as indicated and fused with Int. The recombination products were subjected to electrophoresis through a 1.2% agarose gel and visualized by ethidium bromide staining. The 0.9-kb *Eco*RI restriction fragment was run off the bottom. Lane 1, DNA incubated without Int and resolvase; lane 2, same as lane 1 but treated with *Eco*RI; lane 3, same as lane 1 but recombined with Int; lanes 4 and 5, same as lane 3 but digested with *Eco*RI or *Bgl* II, respectively; lanes 6 and 7, preincubation of both plasmids with wild-type resolvase and the products treated without or with *Eco*RI, respectively. oc, Open circular; lin, linear; sc, negatively supercoiled; lin. res. prod., linearized resolution products; lin. pPDΔ1 and pPDΔ3, linearized pPD1 and pPD3, respectively, lacking the 0.9-kb *Eco*RI fragment.

doubly cut DNA shows that only wild-type:mutant hybrid synaptosomes were formed.

Our results indicate that a double-strand cut is made at just one of the crossover sites when wild-type and mutant proteins oppose each other in a synaptosome, thereby eliminating the possibility of mixed cis and trans cleavage as depicted in Fig. 1*B*. To determine which crossover site is cut, we analyzed the DNA by *Eco*RI restriction. If the cut occurs within *res*1, three restriction fragments of 0.43, 0.47, and 9.0 kb are expected. If the cleavage occurs within *res*2, the predicted lengths of the fragments are 0.9, 4.3, and 4.7 kb (Fig. 2).

When wild-type resolvase is prebound to both *res* sites, a doublet of 0.43 and 0.47 kb and a 4.3-kb band are detected, confirming that strand cleavage occurs at both crossover sites (Fig. 4*B*, lane 4). We used the relative intensities of these bands to normalize the cutting within hybrid synaptosomes. Preincubation of wild-type protein with *res*2 and mutant with *res*1 results in the production of the 4.3-kb and 4.7-kb bands. This indicates a double-strand break at the site bound by wild-type enzyme. The 0.43/0.47-kb band was also detected, although at a reduced level (lane 1). When the preincubation protocol is reversed so that wild-type enzyme is on *res*1 and mutant is on *res*2, the 4.3-kb band is detected only after long exposures, whereas the appearance of the 0.43/0.47-kb band is enhanced (lane 2). This again shows preferential cutting at the site bound by wild-type enzyme. None of these bands was visible when mutant enzyme alone was prebound to both sites (lane 3). Quantitation of the signals and comparison with the control showed a 3-fold preference for cleavage at *res*2 over *res*1 in lane 1, whereas the preference in lane 2 is reversed to at least 5-fold in favor of cleavage at *res*1. Also observed in Fig. 4*B*, lane 4, are the products of resolution resulting from digestion of catenanes with *Eco*RI (see Fig. 2). Recombination was highly active when wild-type enzyme was present at both *res* sites (lane 4) but was completely suppressed in both wild-type/mutant cases (compare lanes 1 and 2 with lane 3). The two species in lane 3 migrating close to the position of recombination products correspond to residual linear pPD1 and pPD2, which are inert to cutting by *Eco*RI. These species are also visible in lanes 1 and 2. By isolating linearized pPD3 out of a gel and subsequently restricting it with *Eco*RI, we confirmed that the observed restriction fragments are indeed the result of resolvase cleavage at one of the two *res* sites in the hybrid synaptosome (data not shown).

We performed experiments such as shown in Fig. 4*B* a total of seven times, using various ratios of wild-type to mutant protein and, in some instances, replacing R68H with S10C in which the active site serine is mutated (11). We observed a 2- to 7-fold preferential cleavage at the site to which wild-type enzyme was prebound, with a mean of 3.5-fold preference. The similar results with S10C eliminate the possibility that the R68H enzyme was activated by association with wild-type enzyme in the hybrid synaptosome. In all of the experiments, no significant single-strand breakage of resolution substrate pPD3 was observed. Our results therefore imply that a first step in strand exchange is the attachment of both subunits of

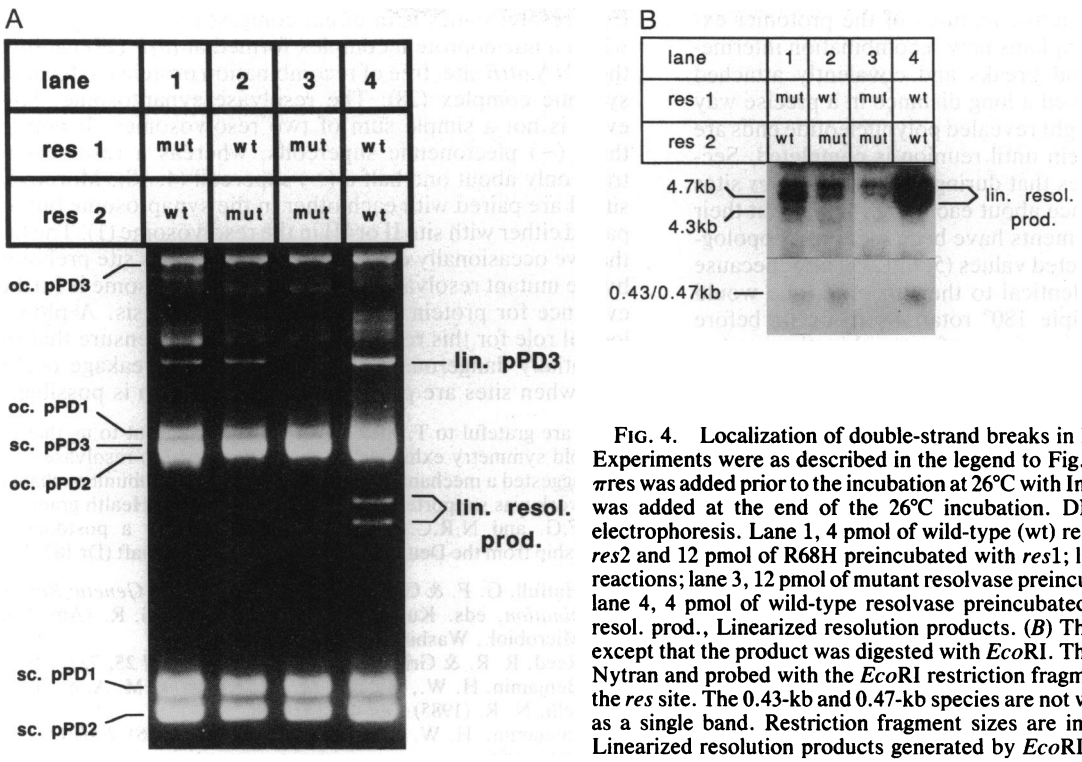

FIG. 4. Localization of double-strand breaks in hybrid synaptosomes. (*A*) Experiments were as described in the legend to Fig. 3 except that 0.4 pmol of πres was added prior to the incubation at 26°C with Int, and 47% ethylene glycol was added at the end of the 26°C incubation. DNA was analyzed by gel electrophoresis. Lane 1, 4 pmol of wild-type (wt) resolvase preincubated with *res*2 and 12 pmol of R68H preincubated with *res*1; lane 2, reciprocal of lane 1 reactions; lane 3, 12 pmol of mutant resolvase preincubated with both plasmids; lane 4, 4 pmol of wild-type resolvase preincubated with both plasmids. lin. resol. prod., Linearized resolution products. (*B*) The experiment was as in *A* except that the product was digested with *Eco*RI. The DNA was transferred to Nytran and probed with the *Eco*RI restriction fragment of pPD1 that contains the *res* site. The 0.43-kb and 0.47-kb species are not well resolved and are taken as a single band. Restriction fragment sizes are indicated. lin. resol. prod., Linearized resolution products generated by *Eco*RI.

a resolvase dimer to the backbones of their DNA binding sites in cis (Fig. 1*A*).

DISCUSSION

DNA replication, transcription, and recombination often require multiple protein-bound DNA sites in cis. A difficulty in the study of these interactions, particularly if the same DNA site is repeated, is that the requirement for the sites on the same DNA molecule precludes separate addition and manipulation of the components. This problem prevented us from targeting different resolvases to distinct *res* sites with the conventional recombination substrates.

Our solution was to bind separately the wild-type and mutant resolvases to distinct plasmids and then to join the plasmids together by Int recombination to create the intramolecular configuration needed for resolution (Fig. 2). Competitor DNA was added after the initial binding step to trap unbound resolvase. When wild-type resolvase was prebound to the two unlinked *res* sites, the efficiency of resolution following Int-mediated linkage was generally about 70%. We conclude that there was no fast exchange between bound and unbound resolvase from the lack of recombination or double cleavage of the substrate with wild-type enzyme bound to one *res* site and mutant bound to the other (Figs. 3 and 4). The success of this procedure implies that the decay of the resolvase complex at each *res* site, the resolvosome (1), is slow compared to Int-mediated plasmid fusion, resolvase synaptic complex formation, and resolution.

Our major result is that the two double-strand breaks necessary for recombination are made by nicking in cis by the four resolvase monomers at the crossover region of the synaptosome (Fig. 1*A*). In the experiments with the hybrid mutant:wild-type synaptosome, this was indicated by the double-strand break at the *res* site bound by wild-type resolvase. The double-strand break was at the *res* site bound by the mutant enzyme 3.5-fold less frequently, on average. This most likely results from exchange of the wild-type and mutant dimer within the synaptosome, but more complex mechanisms involving, for example, occasional double-strand breaks in trans have not been eliminated. The third alternative, nicking at each *res* site, was not observed under the conditions used. This would have been the result if equal cis and trans nicking had occurred (Fig. 1*B*). We conclude that bound resolvase can act locally so that both functional domains of resolvase operate on the same recombination site.

If the synaptosome has a fixed quaternary structure, nicking and religation in cis would not result in recombination but would merely restore the parental nucleotide sequence; recombination requires that breakage exclusively in cis is followed by an all trans reunion. Thus, to achieve recombination via all cis breakage and reunion, there must be a reorganization of the synaptosome between breakage and reunion. This reorganization is most readily accomplished by a 180° rotation of one-half of the synaptic complex about a dyad axis between the paired *res* sites (Fig. 5), as has been suggested on topological grounds (6, 12, 18, 20). This makes reunion in effect a trans operation even though it occurs biochemically in cis.

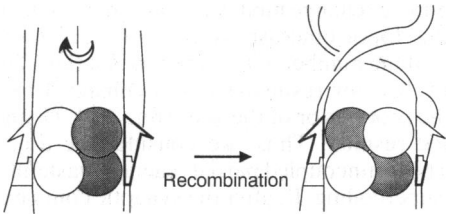

FIG. 5. Model for protein subunit exchange during DNA exchange. Schematized here is the portion of the resolvase synaptosome that contains site I (arrows) and the bound resolvase dimers (circles). The jog in the middle of the arrows is the crossover site, and the surrounding DNA is shown as double lines. One site and its bound dimer are differentially marked. Resolvase subunits attack the DNA generating two double-strand breaks in cis. The upper half of the synaptosome now rotates 180° about the dyad axis (left) and the two double-strand breaks are resealed (right). Note the concomitant twisting and writhing of the DNA. The consequence of this model is DNA strand exchange and resolvase subunit exchange. The alternative method of rotation of just the top half of the DNA during exchange is precluded by the covalent cis attachment of each DNA to a resolvase monomer.

There are several attractive features of the protomer exchange model. First, it explains how recombination intermediates with double-strand breaks and covalently attached resolvase (2) can be moved a long distance in a precise way to affect exchange. All eight revealed polynucleotide ends are firmly anchored to protein until reunion is completed. Second, the model postulates that during exchange the *res* sites are positively twisted once about each other and about their axis (Fig. 5). Both movements have been measured topologically and have the predicted values (5, 6, 12). Third, because the product is locally identical to the substrate, one would expect occasional multiple 180° rotations to occur before religation. This processive form of recombination makes unique predictions about the topology of secondary products of recombination, which have been verified out to four rounds of recombination (5).

The subunit exchange model also explains recombination by site-specific invertases such as Gin. It gives the correct values for the two components of site movement during exchange (21, 22). A different model, however, has been proposed for enzymes of the Int family, which have a Holliday structure intermediate and different topological changes (6, 16).

What is responsible for the movement of the resolvase protomers and the coordination with the catalytic events, and what holds the complex together during strand rotation? Protomer exchange requires breakage of the original contacts between the two monomers bound to the half sites of a site I. Because the contacts can be remade after a 180° rotation, the change in protein quaternary structure could be isoenergetic, but there could be kinetic problems. The contacts between adjacent monomers might be broken in two steps. Resolvase exists as a dimer in solution (15). Nonetheless, although the K_a for binding to each half-site is about 10^6, it is only about 10^8 for binding to a complete site (13). Thus, much of the energy of binding the resolvase dimer to DNA may have gone into inducing DNA distortions, such as bending (23), and weakening dimeric contacts. Conversion of some of the energy of substrate binding into catalysis is a generally important feature of enzymes (24). The new protein–protein contacts established by formation of the synaptosome may trigger a conformational transition that brings the active site serines to the scissile bond and weaken further the interaction between the original partners of the resolvase dimer.

Because the synaptic complex does not dissociate after DNA cleavage, it seems likely that some bonding between resolvase monomers persists during rotation of the subunits. The direction of rotation is very likely governed by the energetics of the changes in DNA structure (6, 18, 25).

The subunit exchange model may also mechanically accommodate the topoisomerase activity of recombinases: the change in linking number without recombination. This activity is generally low for resolvase but is enhanced by alteration either of the proteins or of the sites (ref. 26; P.D. and N.R.C., unpublished results). Thus, we consider the topoisomerase activity to be an uncoupled partial reaction wasteful of the free energy of supercoiling. If, after the synaptic complex is formed as in Fig. 5, one site I detaches from the protein tetramer and a double-strand break is made in the remaining bound site I, rotation can now occur. After a 360° turn about the dyad axis of the tetramer, religation results in a change of Lk by +1. An alternative plausible mechanism for relaxation requires a more massive disruption of the synaptosome: after a nick or double-strand break in site I, the DNA along with its bound resolvase separates from the synaptosome and swiveling about the helix axis changes Lk in steps of 1 (15, 26).

Our results also imply that resolvosomes are stable complexes. The resolvosomes neither break down during the 20-min Int treatment nor exchange with exogenously added DNA containing a *res* site. Formation of the synaptosome from resolvosomes is in direct contrast to the Int system in which a nucleoprotein complex formed at *attP* (27) captures the DNA *attB* site, free of recombination proteins to form the synaptic complex (28). The resolvase synaptosome, however, is not a simple sum of two resolvosomes. It entraps three (−) plectonemic supercoils, whereas a resolvosome traps only about one-half a (−) supercoil (4, 18). Moreover, sites I are paired with each other in the synaptosome but are paired either with site II or III in the resolvosome (1). The fact that we occasionally observe cutting of the *res* site prebound by the mutant resolvase in the hybrid synaptosome is further evidence for protein reorganization at synapsis. A physiological role for this reorganization may be to ensure that the potentially dangerous double-strand DNA breakage occurs only when sites are paired and recombination is possible.

We are grateful to T. Steitz, who first pointed out to us that the two-fold symmetry exhibited by the packing within resolvase crystals suggested a mechanism for recombination via subunit exchange. This work was supported by National Institutes of Health grants (to N.D.F.G. and N.R.C.). P.D. was supported by a postdoctoral fellowship from the Deutsche Forschungsgemeinschaft (Dr 187/1-1).

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