Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 9642–9646, October 1992
Biochemistry

Symmetry in the mechanism of bacteriophage λ integrative recombination

(site-specific recombination/DNA strand exchange/DNA topoisomerase)

ALEX B. BURGIN, JR.*, AND HOWARD A. NASH

Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, MD 20892

Contributed by Howard A. Nash, May 4, 1992

ABSTRACT **During the strand-exchange events of bacteriophage λ integration, pairs of phosphodiester bonds are broken and then rejoined to form novel DNA linkages. The reaction proceeds *in vitro* in the absence of an external energy source; the bond energy needed to rejoin broken strands of DNA must therefore be conserved during cleavage. Although some of this conservation involves a covalent intermediate between DNA and the recombinase Int, it is possible that such an intermediate is formed with only one of the two phosphodiesters. In such an asymmetric mechanism, the second phosphodiester would be attacked by a nucleophile that is exposed by cleavage of the first DNA strand. In contrast, a symmetric mechanism hypothesizes nucleophilic attack by Int on both phosphodiesters. We have distinguished these two mechanisms by removing potential nucleophiles from the integrative recombination reaction. Our data are inconsistent with an asymmetric mechanism. We conclude that during strand exchange both phosphodiesters proceed through a covalent protein–DNA intermediate.**

Site-specific recombination is the process by which the DNA of bacteriophage λ integrates into and excises out of the *Escherichia coli* chromosome. The elements of these reactions are well characterized genetically and biochemically (for reviews see refs. 1 and 2). Integrative recombination takes place between two attachment sites, *att*B and *att*P, located on the bacterial and phage chromosomes, respectively. Both sites share an essential region of sequence identity, termed the overlap region, that is bounded by the sites of strand breakage and rejoining. Flanking this crossover region are elements essential for attachment site function. Each of these is unique; thus each *att* site can be written as a tripartite structure consisting of the overlap region, O, and two flanking segments of DNA: *att*P = POP′ and *att*B = BOB′. The phage attachment site (≈240 base pairs) is complex, with the flanking arms containing multiple binding sites for the two proteins required for integrative recombination, Int and IHF. However, the bacterial attachment site is relatively simple (≈24 base pairs) and contains only two Int binding sites.

To generate a recombinant molecule, four strands of DNA must be broken and then joined to new partners. Several lines of evidence indicate that the reaction proceeds according to the following scheme. One strand of *att*P and one strand of *att*B is cleaved at one border of the overlap region. Ligation of the two partner strands results in a binary strand transfer event (i.e., two out of four strands are exchanged) with the consequent generation of a Holliday intermediate (3). This intermediate is then resolved into a completed recombinant by a second strand-exchange event 7 base pairs away at the other end of the overlap region. Recombination proceeds with a defined order of strand exchanges: "top" strands are exchanged first, and exchange of the "bottom" strands resolves the Holliday intermediate. (By convention, the top strand has 5′ to 3′ polarity when *att*B is written in the orientation BOB′; see Fig. 3.)

What is the mechanism of strand exchange? Strand exchange proceeds *in vitro* in the absence of an external energy source such as ATP. Thus the breakage and reunion steps in recombination must be coupled in a way that preserves the energy of the phosphodiester backbone. Such coupling is inherent to topoisomerase mechanisms (4) in which cleavage of DNA is not achieved via hydrolysis but by a transesterification reaction involving a nucleophilic residue of the enzyme. The covalent bond between enzyme and DNA that is formed in this step can be subsequently attacked by the broken DNA so as to reform a continuous backbone. Int protein has topoisomerase activity; it can cleave DNA at the edges of the overlap region by forming a covalent intermediate between an enzyme residue (Tyr-342) and a 3′-phosphate of DNA (5, 6). To show that this topoisomerase activity is used as part of the strand-exchange mechanism, Mizuuchi and Adzuma (7) followed the stereochemistry of a chiral center in the top strand of *att*B through a single round of recombination. The chirality of the phosphodiester was retained, implying that breakage and reunion indeed proceeds with the intervention of a covalent intermediate. While this work established the topoisomerase activity of Int as the catalyst for breakage and reunion at the top strand of *att*B, it did not address whether the same mechanism is used at the positions of strand exchange in the other partner, *att*P. As described below, a plausible alternative mechanism can be considered for cleavage of *att*P that is consistent with the overall conservation of energy of the phosphodiester backbone. In the present study, we have distinguished between these alternatives and present evidence that for *att*P, just as for *att*B, a nucleophile from Int attacks the phosphodiester backbone during both top- and bottom-strand exchange events.

MATERIALS AND METHODS

*att*B DNA substrates were constructed from the following synthetic oligonucleotides (written 5′ to 3′): oHN66, TCCGTTGAAGCCTGCTTT; oHN67, TTTATACTAACTTGAGC; oHN68, TCGCTCAAGTTAGTATAAAAAAGCAGGCTTCAACG; oHN69, TCCGTTGAAGCCTGCTTTTTTATACTAACTTGAGC; oHN70, TTTATACTAACTTGAGCGAA; oHN72, GTTTCGCTCAAGTTAGT; oHN82, TCCGTTGAAGCCTGCTTTTT; oHN83, GTATAAAAAAGCAGGCTTCAACG. Oligonucleotides were graciously synthesized by M. Brownstein (National Institute for Mental Health) and purified by reverse-phase

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*To whom reprint requests should be addressed at: National Institute for Mental Health, Building 36, Room 1B-08, 9000 Rockville Pike, Bethesda, MD 20892.

HPLC [Rainin Dynamax, C_{18}, 4.6 × 250 mm, 300-Å column with a 50 mM triethylammonium acetate/0–50% (vol/vol) acetonitrile gradient]. The oligonucleotides were 5′ end-labeled by using [γ-^{32}P]ATP and T4 polynucleotide kinase. Typically, a 50-μl reaction mixture containing 200 μCi (1 Ci = 37 GBq) of [γ-^{32}P]ATP (800 Ci/mmol; NEN), 10 units of T4 polynucleotide kinase (New England Biolabs), 0.5 μmol of oligonucleotide, 50 mM Tris (pH 7.6), 10 mM $MgCl_2$, 5 mM dithiothreitol, 0.1 mM spermidine, and 0.05% Nonidet P-40 was incubated at 37°C for 30 min, heated to 80°C for 5 min, and passed over a Bio-Gel P-30 spin column (Bio-Rad). The specific activity of the oligonucleotide was determined following polyethylenimine thin-layer chromatography analysis and scintillation counting. The oligonucleotides were 3′ end-labeled by using [α-^{32}P]dATP and terminal deoxynucleotide transferase. Typically, a 50-μl reaction mixture containing 200 μCi of [α-^{32}P]dATP (800 Ci/mmol; NEN), 20 units of terminal deoxynucleotide transferase (Pharmacia LKB), 0.5 μmol of oligonucleotide, 60 mM sodium cacodylate (pH 7.0), 5 mM $MgCl_2$, and 1 mM dithiothreitol was incubated at 37°C for 20 min and heated to 80°C for 5 min. Reaction products resulting from a single nucleotide addition were purified on a 20% acrylamide/8 M urea gel, passively eluted into 10 mM Tris (pH 8.0), 0.1 M NaCl, and 1 mM EDTA, and then passed over a Bio-Gel P-30 spin column. *att*B substrates were prepared by mixing the appropriate oligonucleotides (5 molar excess of unlabeled oligonucleotide) in 0.1 M KCl and 10 mM Tris (pH 8.0) at 37°C for 10 min and then were added to recombination reactions as a 10-fold dilution. The concentration of the *att*B DNA was based on the specific activity of the limiting oligonucleotide. Wild-type *att*B DNA was formed from oHN68 and oHN69. Nicked *att*B DNA was formed from oHN66, oHN67, and oHN68. B half-sites were formed from oHN82 and oHN83. B′ half-sites were formed from oHN70 and oHN72.

The supercoiled *att*P DNA was purified on a cesium chloride equilibrium gradient. The wild-type *att*P DNA used (plasmid HN894) is described in ref. 8, and the *saf*G variant (plasmid HN716) is described in ref. 9. Int and Int(Y342F) were purified as described (10). The Int(Y342F) clone was a generous gift of J. Gardner and is a derivative of pSX1-2 described in ref. 11. IHF was purified as described (12). Recombination reactions were performed as described (13), except where noted. Reactions were quenched by adding SDS to 0.05% and heating to 65°C, which also releases any noncovalent topological constraints on the reaction products.

RESULTS AND DISCUSSION

Experimental Rationale. Two mechanisms (outlined in Fig. 1) can explain the available set of facts about strand exchange in integrative recombination. The mechanisms are identical in the proposed way in which cleavage of *att*B is initiated, but they differ in the proposed role for enzyme catalysis during cleavage and strand transfer of *att*P. In both mechanisms, strand exchange is initiated with a nucleophilic attack on a phosphodiester linkage of *att*B by the phenolic oxygen of the Tyr-342 residue of Int. This attack generates a covalent 3′-phosphotyrosine intermediate and free 5′-hydroxyl (5′-OH). In mechanism I, the same sequence occurs at *att*P. Thus, strand exchange is initiated by two active-site tyrosine residues; both *att*P and *att*B proceed through a covalent intermediate. Both 5′-OH groups generated by the cleavage events can then displace the active-site tyrosine residues on the partner strands, thereby producing a binary strand transfer product. In mechanism II, the 5′-OH generated by Int cleavage of *att*B directly attacks the *att*P phosphodiester, resulting in a unitary strand transfer product and a 5′-OH in *att*P. The latter residue can then attack the phosphodiester at *att*B, displacing the covalently linked tyrosine and completing strand exchange. According to this mechanism, the attacking nucleophile at the *att*B phosphodiester is Tyr-342 and the attacking nucleophile at the *att*P phosphodiester is the 5′-OH from *att*B.

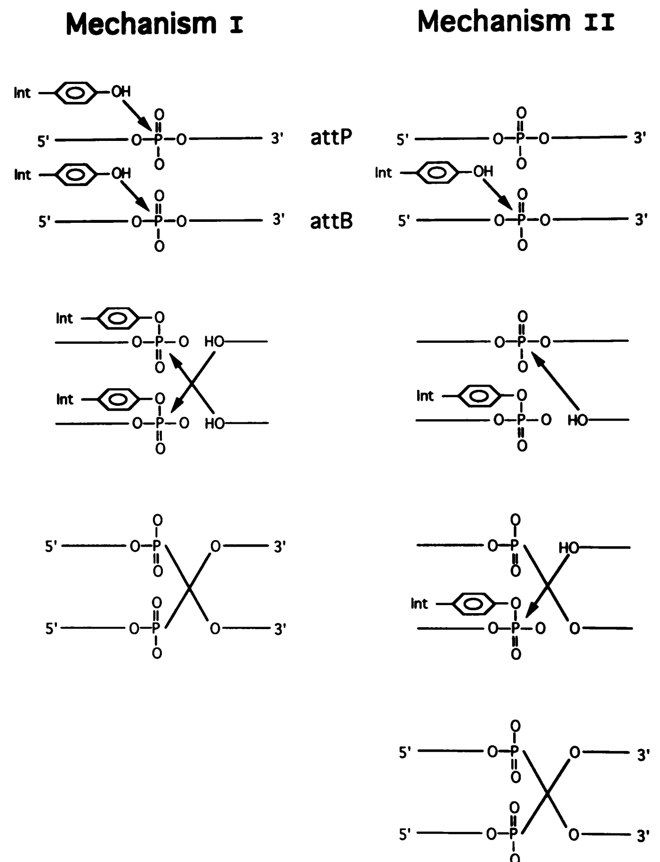

FIG. 1. Alternative mechanisms for strand exchange. The phosphodiester linkage at the site of Int-catalyzed cleavage and transfer is diagrammed. The tyrosine phenoxy of Int is also depicted. The top line represents one strand of *att*P DNA, and the bottom line represents the corresponding strand of *att*B DNA. Arrows signify nucleophilic attack. The final step depicts one strand-exchange event—i.e., a binary-strand transfer.

It has been widely assumed that reactions at *att*B and *att*P are equivalent, as outlined in mechanism I. However, several arguments make mechanism II worthy of consideration. First, cleavage–ligation reactions involving attack by a nucleophile from a nucleic acid on a phosphodiester bond are precedented in self-splicing reactions of RNA (14) and in the strand-transfer activity of Mu transposase (7). Second, there is no need to assume symmetry in the way the two attachment sites are chemically manipulated during recombination, especially since the cleavage sites of *att*P and *att*B are flanked by very different arrays of bound recombination proteins (2). Finally, mechanism II would ensure that phage DNA does not undergo cleavage until it is synapsed to a partner site. This feature would not only tend to protect the integrity of the viral genome but also explain the observation that, in the absence of a partner, the yield of *att*P duplexes cleaved by Int is low (5).

To distinguish these two mechanisms, we have designed experiments to identify the attacking nucleophile at *att*P. In mechanism I, the nucleophile is from the enzyme (Tyr-342). In mechanism II, the nucleophile is a 5′-OH from *att*B. By removal of one or the other of these two nucleophiles, we have tested predictions of both mechanisms.

Recombination in the Absence of Tyr-342: Top-Strand Exchange. Mechanism II predicts that once the top strand of *att*B has been cleaved to generate a free 5′-OH, a unitary

strand transfer product should no longer depend upon the presence of an active-site tyrosine. In contrast, mechanism I predicts that strand transfer always requires an active-site tyrosine, irrespective of the state of *att*B. The components needed to fashion an experimental test of these predictions have been described. *att*B substrates with preexisting nicks at one or the other sites of strand exchange are known to be functional substrates for integrative recombination with wild-type Int protein (15). In addition, a mutant form of Int in which the active-site tyrosine is replaced with phenylalanine has been characterized. This mutant protein, Int(Y342F), binds to DNA with the same specificity and affinity as wild-type Int but fails to sponsor recombination, resolution of Holliday junctions, or cleavage of suicide substrates (6).

To test mechanism II, recombination reactions with Int or Int(Y342F) and nicked or intact *att*B DNAs were incubated under standard reaction conditions. Unitary or binary strand transfer of labeled *att*B to *att*P creates a product that migrates at the position of relaxed circular *att*P DNA (Fig. 2). If three or four strands are exchanged, label is transferred to the position of linear *att*P DNA. As expected, linear recombinant products were obtained when wild-type Int was incubated with nicked or intact *att*B DNA (lanes 1 and 3), and no recombinant products (lane 2) were observed when Int(Y342F) was incubated with an intact *att*B. Importantly, no strand-exchange products were observed with Int(Y342F) even when it was used with an *att*B engineered to have a 5′-OH (lane 4) at the site of strand exchange.

This result fails to support mechanism II, but it does not eliminate it. Even if Int(Y342F) were competent for all the activities required for recombination except nucleophilic attack, the strand-transfer product predicted by mechanism II still might not be observed in this assay due to the reversible nature of top-strand exchange. This point is illustrated in reactions 5–8 (Fig. 2, lanes 5–8) in which *att*P DNA

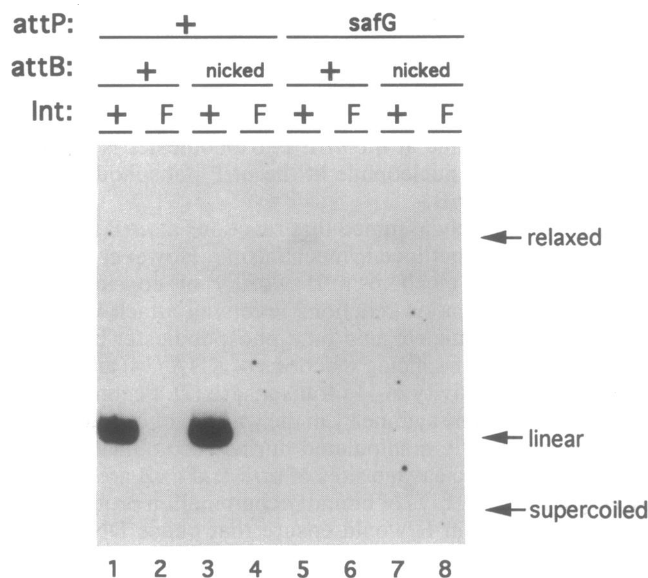

FIG. 2. Recombination in the absence of Tyr-342. Wild-type (+) or Y(342F) mutant (F) Int protein was incubated with wild-type (+) or *safG att*P DNA and either intact *att*B DNA (+) or *att*B DNA containing a nick at the site of top-strand transfer (nicked), under standard reaction conditions ([*att*B] = 500 nM, 2-hr incubation). The 3′ end of the top strand of the *att*B DNA was ^{32}P-labeled. The reaction products were heated to 65°C and resolved on a 1% agarose gel. The gel was dried and the autoradiogram is shown. The positions at which supercoiled, linear, and relaxed circular *att*P DNA migrate are indicated. The *safG* mutation (16) changes the sequence of the overlap region, thereby disrupting the perfect sequence identity between *att*B and *att*P in this region and interferes with the progression from top to bottom strand exchange (9, 15).

with a different overlap sequence replaced wild-type *att*P DNA (see figure legend for description). As expected, binary strand transfer products were observed when wild-type Int was present (lanes 5 and 7), but at a much lower yield than the yield of linear recombinant using wild-type *att*P (lanes 1 and 3). Presumably, top-strand exchange occurred efficiently, but because bottom-strand exchange was prevented, the reaction was reversed to regenerate substrate DNA (9). Similarly, strand exchange may have occurred in the experimental reactions (lanes 4 and 8), but if the products were subject to a very efficient reverse reaction, they would not accumulate. Although the negative result of Fig. 2 must therefore be interpreted with caution, the complete absence of Int(Y342F)-promoted products despite a very sensitive assay leads us to disfavor mechanism II.

Recombination in the Absence of the *att*B 5′-OH: Top-Strand Exchange. One important prediction of mechanism I is that the 5′-OH from *att*B is dispensable for the generation of strand-exchange products. According to this hypothesis, even if the *att*B 5′-OH were unavailable, an active-site tyrosine could still attack the *att*P phosphodiester, generating a 3′-phosphotyrosine intermediate and a 5′-OH in *att*P. This *att*P 5′-OH could then attack an attB 3′-phosphotyrosine intermediate, yielding a unitary strand transfer product. According to mechanism II, however, if the *att*B 5′-OH were removed from the reaction, no recombinant products would be observed since the 5′-OH is required to attack the *att*P phosphodiester before the reaction can proceed.

It is possible to remove the *att*B 5′-OH from the reaction by using "half-site" substrates (diagrammed in Fig. 3). When Int attacks the *att*B phosphodiester of these substrates, the fragment produced is so short (lowercase letters in Fig. 3) that it is predicted to diffuse away, thereby removing the reactive 5′-OH.

To inspect top-strand exchange, B half-sites were used. The reaction pathways according to mechanisms I and II are diagrammed in Fig. 4*A*. In the diagram, the 5′ end of the bottom strand is shown capped by phosphorylation. This removes one potential nucleophile from the reaction and prevents bottom-strand exchange, allowing analysis of top-strand exchange independently of a second-strand exchange event. The results in Fig. 4*B* show that recombinant products

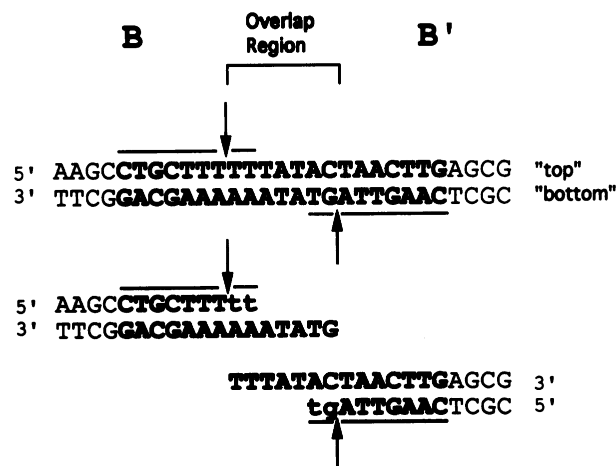

FIG. 3. *att*B DNA substrates. A wild-type *att*B substrate is shown at the top. Vertical arrows indicate the positions of Int-catalyzed strand cleavage and transfer. The Int binding sites are marked with a horizontal line. The B half-site (*Middle*) contains the Int B-binding site of *att*B but is missing the B′-binding site and five nucleotides in the top strand of the overlap region. Similarly, a B′ half-site (*Bottom*) contains the Int B′-binding site of *att*B, but is missing the B-binding site and five nucleotides in the bottom strand of the overlap region.

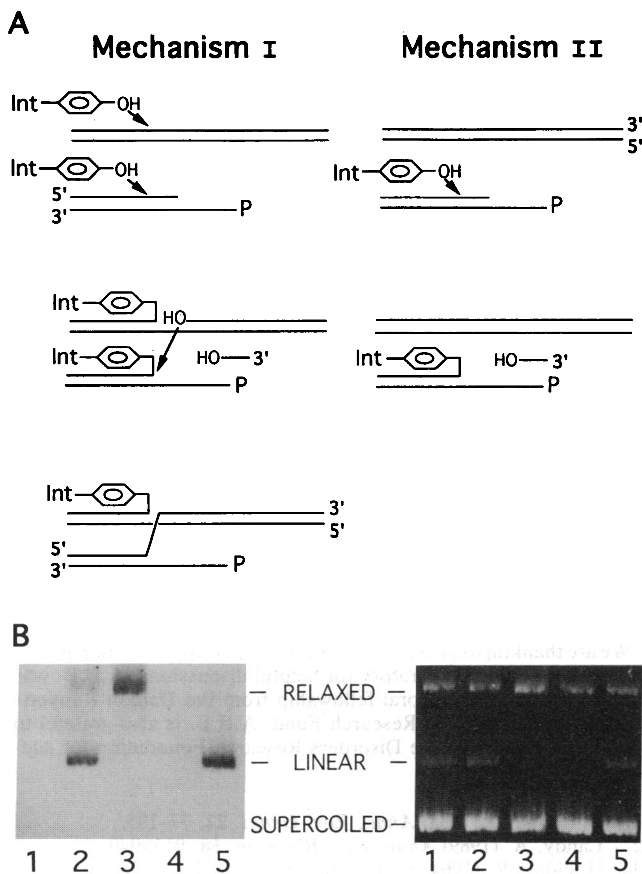

FIG. 4. Recombination in the absence of the *att*B 5'-OH: top-strand exchange. (*A*) The alternative reaction pathways of integrative recombination using a B half-site, in which the 5' end of the bottom strand is capped by phosphorylation, are diagrammed. Arrows indicate nucleophilic attack of Tyr-342 or a 5'-OH on the DNA phosphodiester. (*B*) Various ^{32}P-end-labeled B half-sites were incubated with wild-type Int under standard reaction conditions ([*att*B] = 50 nM, 30-min incubation), heated to 65°C, and then resolved on a 1% agarose gel. Lane 1, the top strand was 3'-end-labeled; lane 2, the top strand was both 3'- and 5'-end-labeled (same specific activity); lane 3, identical to lane 2 except the 5' end of the bottom strand was capped. Lane 4, the bottom strand was 3'-end-labeled and 5'-capped; lane 5, the bottom strand was 3'-end-labeled. The gel was stained with ethidium bromide and photographed (*Right*) and then dried and autoradiographed (*Left*). The positions at which supercoiled, linear, and relaxed circular *att*P DNA migrate are indicated; because the labeled *att*B DNAs and Int are small relative to *att*P DNA, all recombinant products are expected to comigrate with these species. The unreacted *att*B substrates have migrated off the gel.

were obtained; they migrated at the position of relaxed *att*P DNA if bottom-strand exchange was prevented (lane 3) or at the position of linear *att*P DNA if the bottom strand was not capped so that the second strand-exchange event was allowed (lane 5). No strand-exchange products were obtained if Int(Y342F) was used (data not shown). Comparison of lanes 4 and 5 shows that bottom-strand exchange was efficiently blocked by 5'-phosphorylation of the bottom strand. More importantly, comparison of lanes 1 and 2 shows that the 3' end of the top strand, which is connected to the critical 5'-OH, is barely detectable among the recombinants. We have considered the possibility that this small oligonucleotide does become transiently joined to *att*P but is subsequently removed; however, even at early time points no such intermediates are observed (data not shown). We therefore conclude that the critical 5'-OH is virtually eliminated from the reactions with *att*B half-sites.

The data in Fig. 4 show that removal of a 5'-OH from the top strand of *att*B does not prevent cleavage of the top strand of *att*P. This result eliminates the version of mechanism II diagrammed in Fig. 4*A*. We have considered other versions of this mechanism that invoke alternate sources for nucleophilic attack. However, no other DNA-attached nucleophiles are available since *att*P is provided as a closed circle and the only obvious nucleophile in *att*B is blocked by phosphorylation of the bottom stand. In addition, an enzyme-bound nucleophile cannot be invoked since this is a defining feature of mechanism I. The only alternate nucleophiles that are available come from solvent (e.g., the top strand of *att*P could be hydrolyzed). Although this would generate a 5'-OH on *att*P that could attack the Int attached to *att*B, this mechanism could not be part of the normal pathway of integrative recombination. As explained in the Introduction, all strand joining takes place without an external energy source; hydrolysis of the phosphodiester backbone would demand that the joining of at least one strand be driven by an energy-dependent DNA ligase.

Recombination in the Absence of the *att*B 5'-OH: Bottom-Strand Exchange. Just as for top-strand exchange, two possible mechanisms can be considered for exchange of the bottom strands (Fig. 1). Again, half-site substrates provide a critical test to distinguish between these mechanisms. When B' half-sites (Fig. 3) recombine with *att*P, the initial event is the joining of the 5'-OH of the top strand of the half-site to the corresponding strand of *att*P. Formation of such a unitary exchange of top strands can be accommodated by either mechanism I or II. However, according to mechanism II, bottom-strand exchange cannot occur because Int cleavage of the bottom strand releases a small oligonucleotide fragment, removing the 5'-OH that is needed in this mechanism to attack the *att*P phosphodiester. Mechanism II therefore predicts that only relaxed circular recombinant products, generated by unitary strand exchange, will be obtained with B' half-sites. On the other hand, mechanism I predicts that Tyr-342 of an Int protomer can attack the *att*P phosphodiester, generating a 5'-OH, which, in turn, can displace Tyr-342 from another Int protomer at *att*B and create a strand transfer product, irrespective of a 5'-OH from *att*B. Mechanism I therefore predicts that linear recombinant products, generated by a three-strand exchange, will be obtained with B' half-sites.

Fig. 5 shows that linear recombinants were obtained by using B' half-sites as substrates for the recombination reaction (lane 3). Comparison of lanes 2 and 3 indicates that, as for B half-sites, the critical 5'-OH was essentially removed

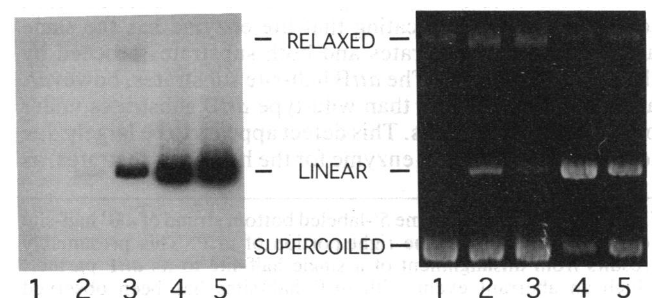

FIG. 5. Recombination in the absence of the *att*B 5'-OH: bottom-strand exchange. Various *att*B sites, ^{32}P-end-labeled on their bottom strands, were recombined with *att*P under standard reaction conditions ([*att*B] = 100 nM, 30-min incubation) and then treated as in Fig. 4. Reactions contained the following *att*B sites: lane 1, no *att*B present; lane 2, 3'-end-labeled B' half-site; lane 3, 5'-end-labeled B' half-site; lane 4, 3' end-labeled full site; lane 5, 5'-end-labeled full site. The positions at which supercoiled, linear, and relaxed circular *att*P DNA migrate are indicated. The unreacted *att*B substrates have migrated off the gel.

from the reaction. As a control, ^{32}P-labeled linear recombinants were obtained with a full *att*B substrate (lanes 4 and 5). No recombinants were observed if Int(Y342F) was used (data not shown).

Thus, loss of the 5′-OH from the bottom strand of *att*B does not prevent attack on *att*P. Just as in the experiment described earlier, which focuses on cleavage of the top strand of *att*P (Fig. 4), we must consider the possibility that attack of the bottom strand of *att*P involves a nucleophile other than Tyr-342 of Int. This analysis is complicated because bottom strands are the second set to be exchanged and 5′-OH groups that are involved in the first-strand exchange event cannot be blocked or removed. The most obvious candidate for an alternative nucleophile is a 5′-OH from a second copy of the half-site. If this were used in place of Tyr-342, for every half-site joined to the left portion of the *att*P, there would have to be a second half-site joined to the right side of *att*P. We have tested this possibility with 3′ end-labeled top strand and eliminated it.† Another possible nucleophile comes from the 5′-OH created at the top strand of *att*P by the first-strand exchange. Although it is hard to imagine how this would be used in a normal recombination pathway, the expected product, a hairpin formed at the right side of *att*P, is detected (data not shown). This observation does not provide strong support for mechanism II because such hairpins are also expected as a terminal event in mechanism I.

Implications for Recombination Mechanism. The work of Mizuuchi and Adzuma (7) strongly implies that one of the initial events in integrative recombination is the attack on the top strand of *att*B by a nucleophile carried by Int protein. In this work, we have used altered recombination components in which nucleophiles were exposed or removed to ask about the source of the nucleophile that attacks the top strand of the other partner in recombination, *att*P. These artificial systems all indicate that the nucleophile that attacks *att*P does not come from *att*B. Our results eliminate one class of *a priori* possible models for recombination and strongly implicate a symmetric model in which each attachment site is attacked by a nucleophile from a separate protomer of Int. In each case, the nucleophile is most probably Tyr-342. First, no strand-exchange products were observed when Int(Y342F) was used. Second, covalent complexes between Tyr-342 of Int and DNA have been isolated (6). Third, Tyr-342 is the only potential nucleophile that is conserved among the integrase family of recombinases (17).

It is important to ask if the artificial substrates used in these studies reflect the behavior of normal attachment sites. For nicked and intact *att*B substrates, the rate of recombination is identical over a wide range of substrate concentrations (data not shown), indicating that the enzyme has the same affinity for both substrates and both substrates proceed by the same mechanism. The *att*B half-site substrates, however, are much less efficient than wild-type *att*B substrates under our standard conditions. This defect appears to be largely due to decreased affinity of enzyme for the half-site substrates, as might be expected since one Int binding site is missing. At high substrate concentrations, the rate of recombination with half-sites ranges from 30% to 50% of that seen with full sites, indicating that the same catalytic mechanism applies to both substrates (data not shown).

We have also used half-sites to study bottom-strand cleavage of *att*P, and others have used half-sites during excisive recombination of bacteriophage λ (18) and site-specific recombination of the yeast 2-μm circle DNA (19–21). All these cases tend to support the view that the attacking nucleophile for strand cleavage does not come from a previously cleaved partner DNA, but from the recombinase itself. It must be pointed out, however, that in none of these cases has it been established that a particular partner is always cleaved by the recombinase, as has been shown for the top strand of *att*B (7). This weakens the interpretation of results with partial sites because either mechanism I or II can be invoked if one is not sure which partner is initially cleaved by an enzymic nucleophile. Nevertheless, in the case of λ integration, since our data clearly implicate a nucleophile from Int in top-strand cleavage, parsimony strongly suggests that our experiments on bottom-strand cleavage should be interpreted the same way.

†In these experiments, some 5′-labeled bottom strand of a B′ half-site did become joined to the right portion of *att*P. This presumably results from misalignment of a single half-site to its *att*P partner. Such an aberrant event with *att*B half-sites has been observed before (S. E. Nunes-Düby and A. Landy, personal communication), but since it does not involve the strand with a free 5′-OH, it does not influence our conclusions.

We are thankful to M. Jayaram for critical review of the manuscript and members of our laboratory for helpful discussions. A.B.B. was supported by a postdoctoral fellowship from the Damon Runyon–Walter Winchell Cancer Research Fund. A.B.B. is also grateful to the Mental and Addictive Disorders Research Foundation for support.

1. Craig, N. L. (1988) *Annu. Rev. Genet.* **22,** 77–105.
2. Landy, A. (1989) *Annu. Rev. Biochem.* **58,** 913–949.
3. Holliday, R. (1964) *Genet. Res.* **5,** 282–304.
4. Gellert, M. (1981) *Annu. Rev. Biochem.* **50,** 879–910.
5. Craig, N. L. & Nash, H. A. (1983) *Cell* **35,** 795–803.
6. Pargellis, C. A., Nunes-Düby, S. E., Moitoso de Vargas, L. & Landy, A. (1988) *J. Biol. Chem.* **263,** 7678–7685.
7. Mizuuchi, K. & Adzuma, K. (1991) *Cell* **66,** 129–140.
8. Nash, H. A. & Robertson, C. A. (1989) *EMBO J.* **8,** 3523–3533.
9. Kitts, P. A. & Nash, H. A. (1987) *Nature (London)* **329,** 346–348.
10. Nash, H. A., Bauer, C. E. & Gardner, J. F. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4049–4053.
11. Numrych, T. E., Gumport, R. I. & Gardner, J. F. (1991) *J. Bacteriol.* **173,** 5954–5963.
12. Nash, H. A., Robertson, C. A., Flamm, E., Weisberg, R. A. & Miller, H. I. (1987) *J. Bacteriol.* **169,** 4124–4127.
13. Nash, H. A. & Robertson, C. A. (1981) *J. Biol. Chem.* **256,** 9246–9253.
14. McSwiggen, J. A. & Cech, T. A. (1989) *Science* **244,** 679–683.
15. Nunes-Düby, S. E., Matsumoto, L. & Landy, A. (1987) *Cell* **50,** 779–788.
16. Weisberg, R. A., Enquist, L. W., Foeller, C. & Landy, A. (1983) *J. Mol. Biol.* **170,** 319–342.
17. Argos, P., Landy, A., Abremski, K., Egan, B., Haggard-Ljungquist, E., Hoess, R. H., Kahn, M. L., Kalionis, B., Narayana, S. V. L., Pierson, L. S., Sternberg, N. & Leong, J. M. (1986) *EMBO J.* **5,** 433–440.
18. Nunes-Düby, S. E., Matsumoto, L. & Landy, A. (1989) *Cell* **59,** 197–206.
19. Amin, A., Roca, H., Luetke, K. & Sadowski, P. D. (1991) *Mol. Cell. Biol.* **11,** 4497–4508.
20. Qian, X., Inman, R. B. & Cox, M. (1992) *J. Biol. Chem.* **267,** 7794–7805.
21. Serre, M., Evans, B. R., Araki, H., Oshima, Y. & Jayaram, M. (1992) *J. Mol. Biol.* **225,** 621–642.