The Role of Single-stranded DNA in Flp-mediated Strand Exchange*

(Received for publication, July 29, 1997, and in revised form, November 3, 1997)

Xu-Dong Zhu‡ and Paul D. Sadowski§

From the Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada

The Flp recognition target site contains two inverted 13-base pair (bp) Flp binding sequences that surround an 8-bp core region. Flp recombinase has been shown to carry out strand ligation independently of its ability to execute strand cleavage. Using a synthetic activated DNA substrate bearing a 3'-phosphotyrosine group, we have developed an assay to measure strand exchange by Flp proteins. We have shown that wild-type Flp protein was able to catalyze strand exchange using DNA substrates containing 8-bp duplex core sequences. Mutant Flp proteins that are defective in either DNA bending or DNA cleavage were also impaired in their abilities to carry out strand exchange. The inability of these mutant proteins to execute strand exchange could be overcome by providing a DNA substrate containing a single-stranded core sequence. This single-stranded core sequence could be as small as 3 nucleotides. Full activity of mutant Flp proteins in strand exchange was observed when both partner DNAs contained an 8-nucleotide single-stranded core region. Using suicide substrates, we showed that single-stranded DNA is also important for strand exchange reactions where Flp-mediated strand cleavage is required. These results suggest that the ability of Flp to induce DNA bending and strand cleavage may be crucial for strand exchange. We propose that both DNA bending and strand cleavage may be required to separate the strands of the core region and that single-stranded DNA in the core region might be an inter- mediate in Flp-mediated DNA recombination.

The Flp gene of the 2-μm circle plasmid of Saccharomyces cerevisiae encodes a conservative site-specific recombinase (Flp) that is involved in amplification of the plasmid (1–3). The Flp protein is a member of the integrase family of recombinases whose members share a tetrad of conserved residues (arginine 191, histidine 305, arginine 308, tyrosine 343; numbers represent amino acid numbers of Flp; see Refs. 4 and 5). The mechanism of Flp action is similar in many respects to that of the bacteriophage λ integrase and the Cre recombinase of bacteriophage P1 (6–8).

Flp mediates a recombination event between two 599-bp inverted repeats. Embedded in each repeat are two Flp recognition target (FRT) sites that are the targets of action of Flp (Fig. 1). The FRT site contains two inverted 13-bp symmetry elements (a and b) which surround an 8-bp core region (open box). A third 13-bp symmetry element (c) is in direct orientation with element b but is dispensable for recombination both in vivo and in vitro (9–11). Flp binds to each of the symmetry elements in an ordered fashion; binding to the b element occurs first, then binding to a, and finally binding to c (12, 13).

Binding of an Flp molecule to a single symmetry element results in a bend of approximately 60º, whereas binding of Flp molecules to two symmetry elements (a and b) flanking the core region results in a severe bend of greater than 144º (referred to as a type II bend; see Refs. 14 and 15). Intermolecular protein-protein interactions between Flp molecules bound to FRT sites bring two partner FRT sites together to form a synaptic complex (16). Nucleophilic attack on the DNA phosphodiester bond at the margins of the core region by tyrosine 343 of Flp results in strand cleavage, generating a free 5'-OH and a phosphotyrosine bond between the 3'-phosphoryl end of the nicked DNA and the protein (17, 18). Strand exchange and ligation take place when the free 5'-OH of the nick from one of the partner DNAs acts as a nucleophile to attack the phosphotyrosine bond of the other partner and generate a new phosphodiester bond. As a result of this event, a Holliday junction is formed (19–22). This junction is resolved to yield recombinant products by a second set of Flp-mediated strand cleavages and ligations (23, 24). The active site of Flp is constituted from two molecules of Flp (25). Cleavage by Flp takes place in trans- horizontal fashion: an Flp molecule activates the scissile phosphodiester bond for a nucleophilic attack by tyrosine 343 of the Flp molecule that is bound across the core from the bond to be cleaved (26, 27). However, ligation occurs in cis, i.e. the monomer bound adjacent to the site of cleavage executes ligation at that site (28). Flp can carry out strand ligation independently of its ability to cleave and attach covalently to the DNA (29).

Although we have learned a considerable amount about the mechanisms of Flp-mediated DNA binding, bending, strand cleavage, ligation, and resolution (6, 7, 30), little is known about the mechanism of DNA strand exchange, the process by which two strands are exchanged and ligated to the partner DNAs in the synaptic complex. One possible mechanism that may contribute to this process could be the separation of DNA strands of the 8-bp core regions of the FRT sites and reestablishment of base pairings in the newly made hybrid core regions. It has been suggested that Flp-mediated DNA bending and cleavage may facilitate the process of separation of DNA strands in the core region (31).

In this study we report the development of an assay to measure Flp-mediated strand exchange that occurs independently of DNA cleavage. We have shown that a single-stranded core region in the FRT site can overcome the defect in strand exchange of certain mutant Flp proteins that are deficient in either bending or strand cleavage. These results suggest that both DNA bending and strand cleavage may be required to separate the strands in the core region and that single-
FIG. 1. Diagram of the Flp recognition target site (FRT site).

The FRT site is characterized by three 15-bp symmetry elements (a, b, and c, horizontal arrows). Elements a and b are in inverted orientation, flanking an 8-bp core region (open box). Element c is in direct orientation with element b and is dispensable for DNA recombination both in vivo and in vitro. Flp-mediated cleavage sites are indicated by two vertical arrows.

TABLE I

Synthetic oligonucleotides used in this study

The underlined sequence represents the FRT site. The sequence of the symmetry elements is shown in italics, and the core sequence is in bold. Where appropriate, oligonucleotides 2, 5, 11 were phosphorylated at their 5′-ends using T4 polynucleotide kinase (New England Biolabs) and cold ATP to give oligonucleotides 2′, 5′, and 11′.

| Name | Sequences (5′ to 3′) |
|------|---------------------|
| 1    | TGGACTTCGATATCTCTAGAAGATGAGAAGTCCGACTCCTAGCTT (40-mer) |
| 2    | TGTAGCTTAAGATAGTCTCTAGAAGATGAGAAGTCCGACTCCTAGCTT (40-mer) |
| 3    | AGGTGCGGATGGCTCCATATCGAAGATGAGAAGTCCGACTCCTAGCTT (40-mer) |
| 4    | AGGTGCGGATGGCTCCTATACAGAAGATGAGAAGTCCGACTCCTAGCTT (40-mer) |
| 5    | GTTCTAGGCTCCATATCTCTAGAAGATGAGAAGTCCGACTCCTAGCTT (40-mer) |
| 6    | GTTCTAGGCTCCATATCTCTAGAAGATGAGAAGTCCGACTCCTAGCTT (40-mer) |
| 7    | GTTCTAGGCTCCATATCTCTAGAAGATGAGAAGTCCGACTCCTAGCTT (40-mer) |
| 8    | GTTCTAGGCTCCATATCTCTAGAAGATGAGAAGTCCGACTCCTAGCTT (40-mer) |

stranded DNA in the core region may play a role in the process of strand exchange by Flp.

MATERIALS AND METHODS

Flp Preparations—Flp proteins were purified as described previously by Pan et al. (32). The purity of wild-type Flp was >90%, and mutant Flp proteins were greater than 60% pure. Mutant proteins with purity of greater than 60% exhibit the same activity as those purified to >95% when assayed for various steps of recombination such as DNA binding, strand cleavage, and covalent attachment, strand exchange, and ligation. The concentration of Flp was estimated by densitometric comparison with highly purified Flp standards on a Coomasie Blue-stained SDS-polyacrylamide gel. The Bradford (33) assay was used to determine the concentration of the homogeneous Flp standards. Flp plasmids encoding Flp proteins Y343F and Y343S were obtained from M. Jayaram (Ref. 41). Y343F means that the tyrosine normally present at position 343 has been replaced by a phenylalanine. The Flp gene bearing a 4-amino acid insertion at position 115 (Ins115) was isolated in cold ATP to give oligonucleotides 2′, 5′, and 11′.

RESULTS

Mutant Flp Proteins Are Defective in Strand Exchange—To examine whether the abilities of Flp proteins to induce DNA type II bending and to execute DNA cleavage were correlated with their abilities to catalyze strand exchange, we carried out strand exchange assays with wild-type Flp and mutant Flp proteins that are defective in DNA type II bending and strand cleavage. As diagrammed in Fig. 2a, assays were carried out between two full-FRT sites, one being the DNA acceptor containing a 3′-phosphotyrosine and the other being the DNA donor. The two substrates were designed such that they both contained a nick at the Flp cleavage site adjacent to symmetry element a. The nick of the DNA acceptor contained the 3′-phosphotyrosyl group at the nick (28). As shown in Fig. 2a, assays were carried out with wild-type Flp as well as three mutant Flp proteins, Flp Ins115, Y343F, and Y343S (Table III). Flp Ins115 is competent for strand cleavage but is deficient in DNA type II bending (34), whereas Flp Y343F is deficient in strand cleavage but competent for DNA type II bending (28). Flp Y343S is deficient in both strand cleavage and DNA type II bending (14, 15). All proteins were competent for intramolecular strand ligation as assayed by hairpin substrates and linear nicked substrates containing a 5′-OH and a 3′-phosphotyrosyl group at the nick (28). As shown in Fig. 2b, wild-type Flp was able to promote strand exchange, converting about 19.0% of substrate to strand ex-
A Single-stranded Core Region in the DNA Acceptor Substrates Mitigates the Defects of Mutant Flp Proteins in Strand Exchange—If DNA type II bending and strand cleavage play a role in separation of DNA strands of the core region and facilitate strand exchange, then the defects in strand exchange resulting from the inabilities of mutant Flp proteins either to induce DNA bending (Ins115) or to execute strand cleavage and covalent attachment (Y343F) or both (Y343S) might be suppressed by providing substrates containing single-stranded core regions. To test this hypothesis, we carried out strand exchange assays between the DNA acceptor (A1) containing a single-stranded core region and the DNA donor (D) containing a duplex core region as illustrated in Fig. 3a. DNA donor D was the same as that diagrammed in Fig. 2. However, DNA acceptor A1 differed from the DNA acceptor A shown in Fig. 2 in that A1 had an 8-nucleotide single-stranded core region, whereas acceptor A had an 8-bp duplex core region.

As shown in Fig. 3b, when the DNA acceptor in the reaction contained an 8-nucleotide single-stranded core region, the level of strand exchange products made by wild-type Flp was increased by about 2-fold compared with that when the DNA acceptor containing a duplex core region was used (Fig. 3b, lane 3 versus Fig. 2b, lane 4); approximately 37.1% of the substrate was converted to products (Table IV). More importantly, the single-stranded core region of acceptor A1 stimulated strand exchange reactions by Flp Ins115 and Flp Y343F by more than 10-fold (Fig. 3b, lanes 4 and 5 versus Fig. 2b, lanes 6 and 8). As summarized in Table IV, approximately 23.5% of the substrate was converted to products by Flp Ins115, whereas about 15.0% of the substrate was converted to products by Flp Y343F. Furthermore, when the core region of the acceptor was single-stranded, Flp Y343S was also able to convert about 4.9% of the substrate into strand exchange products (Fig. 3b, lane 6 and Table IV). These results show that defects of mutant Flp proteins in strand exchange accompanied by their inabilities either to form DNA type II bend or to execute strand cleavage, or both, can be overcome by the presence of the single-stranded core region of the DNA acceptor. This suggests that a single-stranded core region of the DNA acceptor might be involved in Flp-mediated DNA recombination.

A Single-stranded Core Region in the DNA Donor Also Overcomes the Defects of Mutant Flp Proteins in Strand Exchange—Because a single-stranded core region of the acceptor facilitated strand exchange by Flp proteins, it was of interest to investigate whether a single-stranded core region of the DNA donor had a similar effect on strand exchange. To answer this question, we carried out strand exchange assays between an acceptor DNA containing a full-FRT site and a donor DNA containing a half-FRT site (Fig. 4a). The latter substrate enabled us to make single-stranded DNA in the 5’-OH terminus of the core region independent of cleavage of the donor DNA. As shown in Fig. 4a, DNA donors D1 and D5 differed in that D1 contained an 8-nucleotide protrusion of the core region with a 5’-OH, whereas donor D5 contained an 8-bp duplex core region with a 5’-OH. The DNA acceptor was the same as that described in Fig. 2, bearing a nick at the cleavage site adjacent to element a. The nick contained a 3’-phosphotyrosine and a 5’-PO\(_4\). As shown in Fig. 4b, when the half-FRT site donor DNA with an 8-nucleotide protrusion was used in the reaction, wild-type Flp and mutant Flp proteins produced similar levels of strand exchange products, converting about 26.0% (wild-type Flp), 22.1% (Ins115), 18.2% (Y343F), and 18.1% (Y343S) of the substrates to products (Fig. 4b, lanes 3, 6, 9 and 12, respectively, and Table IV). However, when the core region of donor DNA was rendered double-stranded, the level of strand exchange products decreased by approximately 3.6-fold for wild-

**TABLE III**

Properties of Flp proteins

| Flp proteins | DNA binding | DNA type II binding | Cleavage | Ligation | Recombination |
|-------------|-------------|---------------------|----------|----------|---------------|
| Wild-type Flp | +           | +                   | +        | +        | +             |
| Ins115\(^a\) | +           | +                   | +        | -        | -             |
| Y343F\(^b\) | +           | +                   | +        | -        | -             |
| Y343S\(^c\) | +           | -                   | -        | +        | +             |

- \(^a\) + means that the activity is equivalent to that displayed by wild-type Flp. – means defective.
- \(^b\) Ins115 is a four-amino acid insertion at position 115 (34).
- \(^c\) Y343F means that tyrosine at position 343 is replaced by phenylalanine (28, 41).
- \(^d\) Y343S means that tyrosine at position 343 is replaced by serine (14, 41).

exchange products (lane 4 and Table IV), whereas both Flp Ins115 and Flp Y343F exhibited reduced strand exchange activity, converting less than 3% of substrate to products (lanes 6 and 8 and Table II). No strand exchange product was detected with Flp Y343S (lane 10 and Table II). These results suggest that the abilities of Flp proteins to induce DNA type II bending and to carry out strand cleavage are crucial for strand exchange. Alternatively, it is possible that these mutant proteins are defective in strand exchange independent of their defects in bending and cleavage.
versus lanes 3

Table IV
Summary of strand exchange by Flp proteins

| Substrates | Wild-type Flp | Ins115b | Y343Fb | Y343Sb |
|------------|--------------|---------|--------|--------|
| A+D        | 19.0 (18.0, 20.0) | 2.3 (2.1, 2.4) | 1.3 (1.2, 1.3) | 4.9 (4.8, 5.0) |
| A1+D       | 37.1 (36.7, 37.5) | 23.5 (22.3, 24.6) | 15.0 (14.7, 15.3) | 6.8 (6.6, 7.0) |
| A+D1       | 26.0 (26.0, 26.0) | 22.1 (22.0, 22.2) | 18.2 (17.5, 18.9) | 18.1 (17.3, 18.8) |
| A+D5       | 7.2 (7.1, 7.3) | 4.3 (4.1, 4.4) | 2.2 (2.0, 2.3) | 0.5 (0.5, 0.5) |
| A1+D1      | 75.5 (74.0, 77.0) | 71.6 (69.3, 73.8) | 72.4 (72.0, 72.8) | 64.6 (62.1, 67.0) |

a The percentage of substrate converted to strand exchange product was determined by PhosphorImager analysis as described under "Materials and Methods." Numbers represent the means of two determinations; raw numbers are in parentheses.

b The properties of mutant Flp proteins are given in Table III.

type Flp, 5.2-fold for Ins115, 8.5-fold for Y343F, and 36-fold for Y343S (Fig. 4b, lanes 4, 7, 10, and 13, respectively, and Table IV). These results demonstrate that a single-stranded core region of donor DNA had an effect on strand exchange similar to that of a single-stranded core of acceptor DNA, overcoming the defects of mutant Flp proteins in strand exchange.

Defects of Mutant Flp Proteins in Strand Exchange Can Be Overcome by the Core Region Containing a 3-Nucleotide Protrusion—To investigate the minimum degree of single-stranded DNA in the core region required for strand exchange, assays were first carried out between a full-FRT site acceptor DNA and a half-FRT site donor DNA containing various degrees of single-stranded core region as diagrammed in Fig. 5a. Although a complete 8-nucleotide single-stranded donor core permitted strand exchange by mutant Flp proteins, it was not essential. As shown in Fig. 5b, a donor core containing only a 3-nucleotide protrusion was also able to stimulate strand exchange by mutant Flp proteins to a level similar to that achieved with the 8-nucleotide donor core (lanes 6, 11, and 17 versus lanes 3, 8, and 14). Similar results were also obtained by wild-type Flp (data not shown).

Unlike mutant proteins Ins115 and Y343F, mutant protein Flp Y343S seemed to use the donor DNA (D4) containing a 3-nucleotide single-stranded protrusion more efficiently than the donor DNA (D3) containing a 4-nucleotide single-stranded protrusion (Fig. 5b, lane 17 versus lane 16). It is not clear whether Flp Y343S behaves differently from mutant proteins Ins115 and Y343F.

Similar experiments were also carried out to examine the requirement of single-stranded DNA in the core region of the acceptor to allow strand exchange by mutant Flp proteins. As shown in Fig. 6, the acceptor core containing 3-nucleotide single-stranded DNA also overcame defects of mutant Flp proteins in strand exchange. These results suggest that DNA strands of the core region might be at least partially separated during strand exchange.

Strand Exchange Is Stimulated Greatly When Both Donor and Acceptor Contain a Single-stranded Core Region—Because a single-stranded core region in either the donor or the acceptor DNA was able to stimulate strand exchange by Flp proteins, it
was of interest to know whether the presence of single-stranded core regions in both the donor and the acceptor DNA would maximize strand exchange. We found that strand exchange reactions by Flp proteins were stimulated greatly when both donor and acceptor DNA contained a single-stranded core (Fig. 7). Wild-type Flp and mutant Flp proteins were able to convert approximately 70% of substrates to products (Table IV). This exceeded the sum of levels of products when either the donor or the acceptor core was single-stranded. These results strongly suggest that the single-stranded core region plays an important role in strand exchange. Furthermore, the fact that mutant Flp proteins that are defective in either cleavage or type II bending or both can carry out efficient strand exchange in the presence of the single-stranded core region in both the donor and the acceptor DNA indicates that Flp-mediated type II bending and cleavage activities may play an important role in separating the core sequence of DNA before strand exchange.

These results are not simply attributable to intermolecular strand ligation facilitated by base pairing between the single-stranded core regions of donor and acceptor DNA because the levels of strand exchange exceed those obtained between acceptor A1 and a single-stranded donor DNA that lacks Flp binding symmetry element b (data not shown and Ref. 35). These results imply that Flp bound to duplex substrate D1 brings it into a synaptic complex and therefore enhances the reaction.

A Single-stranded Core Region Also Plays a Crucial Role in Cleavage-dependent Strand Exchange by Mutant Protein Flp Ins115—The experiments described so far have used activated substrates containing a 3'-phosphotyrosine leaving group. These substrates do not require the covalent attachment of Flp to the 3'-phosphoryl group to carry out strand exchange and ligation. It was of interest to know whether single-stranded DNA might also play a role in a strand exchange reaction where DNA substrates need to be cleaved (cleavage-dependent
strand exchange). To answer this question, we carried out strand exchange assays by Flp Ins115 between the nicked DNA donor D and a “suicide substrate” (37). Flp Ins115, like wild-type Flp, is able to cleave and attach covalently to the linear FRT site but fails to carry out strand exchange between two linear FRT sites as well as between a linear FRT site and the nicked DNA donor D shown in Fig. 2 (34). We wished to know whether single-stranded DNA might overcome the defect of this mutant protein in a cleavage-dependent strand exchange reaction. As shown in Fig. 8a, suicide substrates enabled us to couple single-stranded DNA with Flp-mediated strand cleavage and covalent attachment. Suicide substrates Su-1, Su-2, and Su-3 contain a nick on the bottom strand of the core region 3, 4, and 5 nucleotides away from the a cleavage site, respectively. Upon Flp-mediated cleavage and covalent attachment, a single-stranded region containing 3, 4, and 5 nucleotides was generated in the core region of Su-1, Su-2, and Su-3, respectively (Fig. 8a). As shown in Fig. 8b, Flp Ins115 was able to execute strand exchange between suicide substrates and donor D (lanes 3, 6, and 9). These results suggest that single-stranded DNA also plays an important role in the strand exchange reaction where strand cleavage is required. Flp Ins115 was able to use suicide substrates Su-3 and Su-2 for strand exchange more efficiently than substrate Su-1 (Fig. 8b, lanes 6 and 9 versus lane 3), indicating that the amount of single-stranded DNA in the core region of the FRT site may also be crucial for strand exchange. Consistent with the data obtained in Figs. 5 and 6, a 3-nucleotide single-stranded DNA in the core was also able to suppress the defect of Flp Ins115 in strand exchange where strand cleavage is required. Wild-type Flp was able to carry out strand exchange and ligation readily regardless of whether the suicide substrates contained a nick 3, 4, or 5 nucleotides away from the a cleavage site (Fig. 8b, lanes 2, 5, and 8). These results are also consistent with the data obtained when 3′-phosphotyrosine containing substrates that bore 3-, 4-, or 5-nucleotide single-stranded DNA in the core region was used in strand exchange reactions by wild-type Flp (data not shown).

DISCUSSION

Although single-stranded DNA is known to play an important role in homologous recombination, little is known about the role of single-stranded DNA in site-specific recombination (38, 39). The studies presented in this paper have addressed the importance of single-stranded DNA in strand exchange by Flp, a conservative site-specific recombinase. We show that single-stranded DNA in the core region of the FRT site plays an

---

3 A. C. Shaikh and X.-D. Zhu, unpublished results.
important role in strand exchange either dependent on or independent of Flp-mediated strand cleavage and covalent attachment. Defects in strand exchange exhibited by certain mutant Flp proteins can be overcome by providing DNA substrates containing single-stranded core regions.

It has been shown previously that Flp is able to carry out strand ligation independently of its ability to execute strand cleavage (29). Using activated FRT substrates bearing a 3'-phosphotyrosine group, we have developed an assay to monitor specifically the strand exchange activity of Flp proteins. Whereas wild-type Flp could catalyze strand exchange, mutant Flp proteins Ins115, Y343F, and Y343S were impaired in their ability to carry out strand exchange when FRT substrates contained duplex core sequences (Fig. 2).

Cleavage is required for strand exchange to generate a free 5'-OH end that can attack a phosphotyrosyl bond in a partner DNA that is cleaved similarly. We propose that Flp-mediated cleavage and covalent attachment may induce a conformational change in the protein-DNA complex which leads to the separation of the strands in the core region to promote strand exchange. Although Flp Y343F is inactive in strand cleavage because it lacks the catalytic tyrosine residue, it is able to carry out strand separation of the strands in the core region to promote strand exchange (29). Using substrates containing a single-stranded 3'-end that can attack a phosphotyrosyl bond in a partner DNA, we have developed an assay to monitor specifically the strand exchange activity of Flp proteins.

Because Ins115, which has been shown to be defective in inducing the type II bend in DNA (34), would not be able to separate the strands in the core region, we suggest that the inability to induce a bend may be the reason that Flp Ins115 is incapable of carrying out strand exchange.

Because Flp Ins115 is able to promote strand cleavage and covalent attachment but not DNA binding and Flp Y343F is able to promote DNA binding but not DNA cleavage and covalent attachment, it is likely that either DNA binding or cleavage and covalent attachment alone may not be sufficient to separate the strands of the duplex core region in the FRT site that is required for strand exchange by Flp. For the Y343S protein, the defects in strand exchange may be a composite of its inability to induce a bend in the DNA and its inability to cleave and attack covalently to the DNA.

Using substrates containing a single-stranded core, we first demonstrated that defects in strand exchange exhibited by mutant Flp proteins can be partially overcome by the presence of a single-stranded core region of one partner DNA. The presence of single-stranded core regions in both partner DNAs allowed the mutant Flp proteins to achieve the levels of strand exchange observed for wild-type Flp. This suggests that denaturation of the duplex core region might occur during Flp-mediated recombination. Previous attempts to detect single-stranded DNA in the core region by chemical probing were unsuccessful, possibly because of limitations in the techniques used (40). However, recent studies have revealed that Flp exhibits an intrinsic single-strand-specific DNA binding activity.4 Therefore, it is possible that this specific single-stranded DNA binding activity of Flp may play a role in facilitating strand exchange.

A 3-nucleotide single-stranded region in the core immediately adjacent to the cleavage site was sufficient to permit the mutant Flp proteins to engage in strand exchange. This suggests that the core region of the FRT site may be only partially separated during strand exchange. A partially single-stranded core is consistent with the finding that only a limited amount of branch migration is required for the resolution of a Holliday junction as well as the 3-nucleotide swapping model (24, 42–44). Further evidence supporting the idea that the core region may be partially separated during strand exchange was presented by Guo et al. (45) who solved the crystal structure of a synaptic complex of the Cre recombinase covalently attached to its target DNA sequence. They showed that upon cleavage of the DNA target by Cre, 3-nucleotide single-stranded segments toward the core side of the cleavage site were released. Because like Flp, Cre is also a member of the integrase family of site-specific recombinases, it is likely that the single-stranded DNA in the core region plays an important role in strand exchange catalyzed by these enzymes.

Acknowledgments—We thank Linda Beatty, Rick Collins, Helena Friesen, Marc Perry, Arkady Shaikh, and John Walker for critical and insightful comments.

REFERENCES
1. Fouter, A. G. B. (1986) J. Theor. Biol. 119, 197–204
2. Volkert, F. C., and Broach, J. R. (1986) Cell 46, 515–550
3. Reynolds, A. E., Murray, A. W., and Szostak, J. W. (1987) Mol. Cell. Biol. 7, 3566–3573
4. Zhu, X.-D., and Sadowski, P. D. (1998) Nucleic Acids Res., in press.

4 Zhu, X.-D., and Sadowski, P. D. (1998) Nucleic Acids Res., in press. 

4 Zhu, X.-D., and Sadowski, P. D. (1998) Nucleic Acids Res., in press.
