Genetic Recombination
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Chapter 13

FLP Site-Specific Recombination System of *Saccharomyces cerevisiae*

Michael M. Cox

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I. 2μm PLASMID

The 2μm plasmid is an autonomously replicating, circular DNA present in most strains of the yeast *Saccharomyces cerevisiae* at 50 to 100 copies per diploid cell (Broach, 1982). It is located in the nucleus. The plasmid contains 6,318 base pairs (bp), and the complete sequence is known (Hartley and Donelson, 1980). General properties of the plasmid were recently reviewed (Volkert et al., 1987). The plasmid is a prototype of a widespread class of plasmids in a variety of yeast species (Toh-e et al., 1987; Araki et al., 1985). The presence of the plasmid confers no important identifiable selective advantage to its host.

Important features of the 2μm plasmid with respect to this chapter are diagrammed in Fig. 1. Replication of the plasmid has been shown to be under stringent cell cycle control (Livingston and Kupfer, 1977; Zakian et al., 1979). The plasmid nevertheless exhibits high mitotic and meiotic stability. Replication is carried out by the host replication apparatus. The plasmid encodes two systems

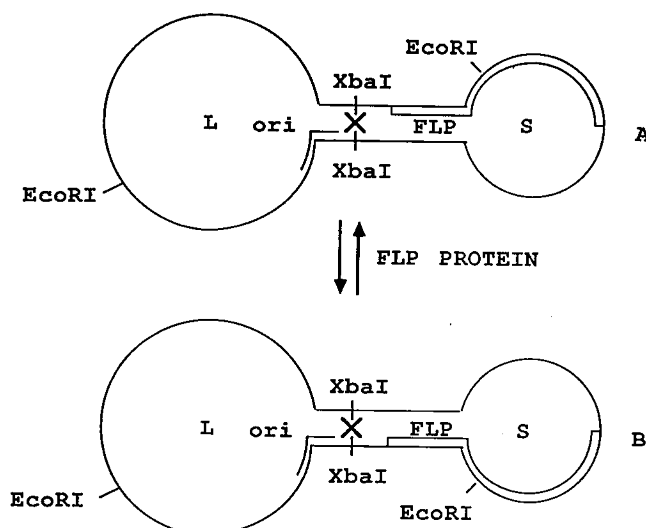

FIGURE 1. Yeast 2μm plasmid. The open reading frame encoding the FLP protein is shown. The FRT site includes base pairs spanning the *Xba*I restriction site. The interconversion between A and B forms involves site-specific recombination at the FRT site. L and S denote large and small unique sequence regions, respectively. The *Eco*RI sites serve as points of reference for the DNA inversion event. See text for details.

that enhance its stability. One of these is responsible for efficient partitioning of the plasmid during meiosis and mitosis. This system includes the products of two open reading frames designated REP1 and REP2 (not shown) and two *cis*-acting elements, i.e., the replication origin and a second site several hundred base pairs away from the origin, designated REP3 or STB (Jayaram et al., 1983; Kikuchi, 1983; Volkert et al., 1987). The REP3 locus appears to be the site of action of the REP1 and REP2 proteins (Jayaram et al., 1985; Murray and Cesareni, 1986). The second system is a plasmid-encoded capability to increase the plasmid copy number when it is low. This system consists, at least in part, of the site-specific recombination system described in this chapter.

A notable structural feature of the 2μm plasmid and related plasmids is the presence of a pair of long inverted repeats (599 bp in the case of the 2μm plasmid). The plasmid is generally found as a 1:1 mixture of two forms of equal size, designated A and B (Fig. 1). The A and B forms are related by a site-specific recombination event which occurs at a point localized to a short sequence within the 599-bp repeats (Broach et al., 1982). The recombination site has recently been designated FRT (FLP recombination target) (McLeod et al., 1986). Recombination is mediated by the product of an additional open reading frame on the plasmid designated FLP (Broach et al., 1982). No other yeast proteins are required for this reaction (Cox, 1983).

II. FLP SYSTEM

A. Historical Perspective

Efficient recombination between the 599-bp repeats in vivo, which produces an inversion of the unique regions separating the repeats, was first demonstrated by Beggs (1978). The demonstration that the 2μm plasmid encodes a protein required for this recombination event and that the protein acted in *trans* followed soon after (Gerbaud et al., 1979; Broach and Hicks, 1980). Broach et al. (1982) located the recombination site within a 65-bp region within each 599-bp repeat. Biochemical characterization was facilitated by the cloning and expression of the FLP gene in *Escherichia coli* (Cox, 1983; Vetter et al., 1983). This work established that no other yeast proteins are required for the reaction. The subsequent development of in vitro systems for the recombination event (Vetter et al., 1983; Meyer-Leon et al., 1984; Sadowski et al., 1984), by using extracts of *E. coli* strains expressing the FLP protein, has led to the extensive biochemical characterization described below. Complementary in vivo studies have also continued in *S. cerevisiae*.

B. Biological Function

Until recently, the function of the FLP site-specific recombination system was obscure. A number of similar plasmids have been found in several *Zygosaccharomyces* species, and each of them possesses a similar site-specific recombination system (Toh-e et al., 1987; Araki et al., 1985). These plasmids share little or no homology. Each possesses, however, a pair of long inverted repeats and encodes a site-specific recombination system which promotes inversion. The only detected homology occurs in the FLP genes at the amino acid level (Toh-e et al., 1987). The lack of homology at the nucleotide level, in spite of similarities in general structure, suggests that most components of these plasmids evolved independently. This further suggests that the general architecture of the plasmids, including the inversion system, has adaptive significance. Disruption of the FLP gene in the 2μm plasmid, however, has only a modest effect on plasmid stability (Broach and Hicks, 1980). Recently, Futcher (1986) proposed that the inversion mediated by the FLP system is required for plasmid copy-number amplification. His model explains how site-specific recombination can circumvent the cell cycle restriction on multiple replication initiation events. In this model (Fig. 2) the theta-form replicative intermediate of the plasmid is inverted by the FLP recombination system. The result is a double rolling circle in which the replication forks never converge, permitting the generation of many tandem copies of the plasmid from a single initiation event. This multimeric species can also be resolved into monomers by the FLP system. Experimental evidence for the central features of this model was quickly provided by Volkert and Broach (1986), who demonstrated that the FLP system is required for plasmid amplification in yeast cells grown under nonselective conditions.

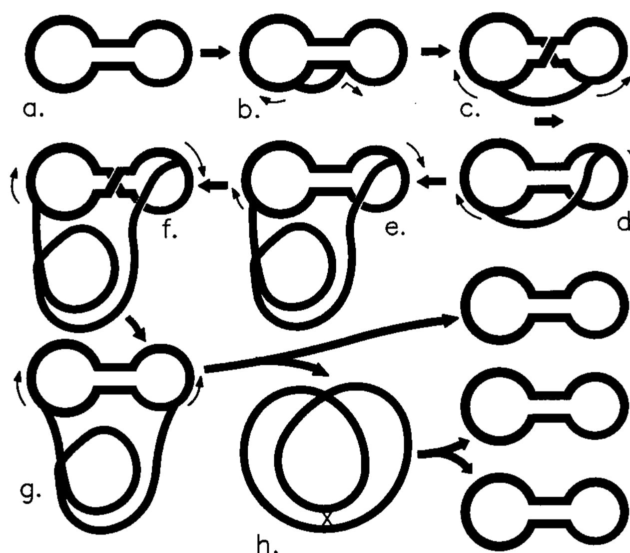

FIGURE 2. Double rolling circle model for amplification of 2μm plasmid copy number (from Futcher, 1986). Inversion (c) following replication of one 599-bp repeat (b → c) results in a double rolling circle (d). This leads to production of multiple copies of 2μm plasmid from a single replication initiation event. Resolution of this species into monomeric circles (h → k) can be achieved by FLP protein-promoted recombination between FRT sites in the same orientation on the multimer. Three copies of the plasmid are shown as products, although the number may be much larger. See text for details. (Adapted from Volkert and Broach, 1986.)

C. Fundamental Properties In Vivo and In Vitro

The properties of the FLP system place it in a class with the most straightforward of the known site-specific recombination systems. In many respects, it shares general properties with the *cre-lox* system of bacteriophage P1 (Hoess and Abremski, 1985). As described above, there is no evidence that any protein besides the FLP protein plays a specific role in this reaction, in contrast to a number of systems discussed elsewhere in this volume. As described below, the recombination site is also relatively simple. There is no evident restriction on the type of recombination reaction permitted. The FLP protein will promote inversions, deletions, or intermolecular recombination either in vitro (Meyer-Leon et al., 1984; Vetter et al., 1983) or in vivo (Cox, 1983; Royer and Hollenberg, 1977; Falco et al., 1982), if appropriate substrates are provided. The in vitro reaction requires only FLP protein, a substrate, and a simple set of buffer and ionic conditions. No high-energy cofactor or divalent cation is required. To a first approximation, there also appears to be no requirement for a supercoiled DNA substrate, with efficient recombination observed for relaxed substrates (this observation must now be amended as described below). The in vitro systems using FLP protein synthesized in *E. coli* have been successful in duplicating the key properties of the system observed in vivo in *S. cerevisiae*, especially with respect to the structure and properties of the recombination site.

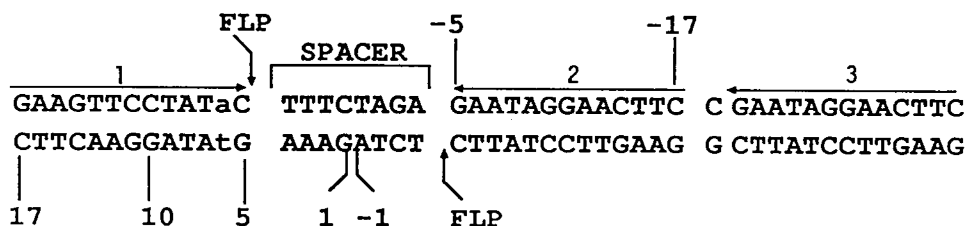

FIGURE 3. FRT site. Position numbers are as presented by Bruckner and Cox (1986). See text for details.

III. RECOMBINATION SITE

A. General Structure

Within the 65-bp region identified by Broach et al. (1982) as the region within which recombination occurs are several prominent structural features (Fig. 3). The most important are a set of three 13-bp repeats. The second and third repeats are separated by 1 bp and are in the same orientation. The first repeat is inverted with respect to the other two and is separated from the second repeat by an 8-bp spacer. The first repeat also has a 1-bp mismatch relative to the first two.

Deletion analysis demonstrated that the third repeat is unnecessary for recombination in vitro (Andrews et al., 1985; Senecoff et al., 1985). The function of this repeat is still unclear, but recent work suggests that it may have a slight effect on the reaction in vivo (Jayaram, 1985). Additional deletions revealed that most, but not all, of the first and second repeats (those flanking the spacer) are required. Deletion of 3 bp from the distal ends of one or both of these repeats has no detectable effect on the reaction. Further deletion leads to a gradual reduction in site function, with complete loss of site function occurring (in vitro) with deletions of 8 bp or more from either end (Andrews et al., 1985; Senecoff et al., 1985). The minimal site required for full function in vitro is therefore relatively small: 28 bp, including the spacer and the proximal 10 bp of each flanking repeat.

B. Spacer

The 8 bp of the spacer include the recognition sequence for the restriction endonuclease *Xba*I. Broach et al. (1982) observed that destruction of this restriction site prevented recombination in *S. cerevisiae*. Similar observations have been made in *E. coli* (Cox, 1983) and in vitro (Meyer-Leon et al., 1984), providing an experimental link between the latter systems and the recombination events in *S. cerevisiae*. The methods employed to destroy the restriction site either increased or decreased the size of the spacer, suggesting that spacer size is important for recombination site function. It has subsequently been demonstrated that an alteration of spacer size by ±1 bp is tolerated in the FLP system, although a reduction in site function is observed in reactions between these sites and sites with normal spacers. The site is inactivated by the addition of 2 bp in the spacer (Senecoff et al., 1985).

The reduction in site function observed when spacer size is altered by ±1 bp is restored in part when sites with identical alterations react with each other (Senecoff et al., 1985). This result suggested that homology between spacers of reacting sites is an important requirement for recombination. This interpretation has been confirmed with a variety of spacer mutations that do not alter spacer size (Senecoff and Cox, 1986; Andrews et al., 1987). The results of these experiments demonstrate that FLP protein does not recognize the central 6 bp of the spacer. Base substitutions can be made at any of these positions without affecting reaction efficiency as long as the reacting sites have homologous spacers.

The requirement for spacer homology implies that DNA-DNA pairing occurs in this region at some point during the recombination reaction. Similar homology requirements are observed in other site-specific recombination systems (Kikuchi and Nash, 1979; Bauer et al., 1985; Johnson and Simon, 1985). This pairing could occur before the cleavage event and reflect the formation of a four-stranded DNA intermediate such as that suggested by Kikuchi and Nash (1979). Alternatively, it could reflect events which occur subsequent to strand cleavage. In the case of the FLP system, the question has not yet been subjected to a clear experimental test.

The requirement for spacer homology serves an additional function in this system, that of proper alignment of two reaction sites which gives the reaction a directionality. In a normal intramolecular recombination reaction, a deletion will occur if the recombination sites are in the same orientation in the DNA molecule, while an inversion will occur if they are inverted with respect to each other. In some manner, the asymmetry of the sites is detected and utilized to align two sites in the same orientation during recombination. In the FRT site, the only asymmetric elements (once the third repeat is removed) are (i) a single-base-pair difference in the two remaining inverted repeats and (ii) the spacer sequence. The single mismatch in the repeats does not play a role in site alignment (Senecoff et al., 1985). The spacer, then, is the sole determinant of the directionality of the reaction. This property of the spacer is illustrated by results obtained with a recombination site in which 5 bp of the central 6 bp of the spacer were changed to produce a symmetrical spacer. This recombination site is fully functional, but the resulting reactions no longer exhibit directionality; i.e., inversion and deletion occur with equal frequency (Senecoff and Cox, 1986).

These results led to a hypothesis that the sequence of the central 6 bp of the spacer was irrelevant as long as homology was maintained in this region between reacting sites (Senecoff and Cox, 1986). Further mutational analysis, however, has revealed a number of spacer sequences which produce a site with reduced reaction efficiency. These studies (S. Umlauf and M. Cox, unpublished data) determined that no single-base-pair changes inactivated the recombination site; therefore, there is still no indication of protein-DNA interaction in this region. Instead, the effect of any given mutation depended on sequence context. Two patterns were evident. Mutations were generally deleterious if (i) they increased the G+C content of the spacer or (ii) they disrupted a polypurine tract which begins in the spacer and proceeds into the adjoining repeats. In most cases, two or more base substitutions with these properties were necessary before a significant deleterious effect was observed. In several cases in which the central

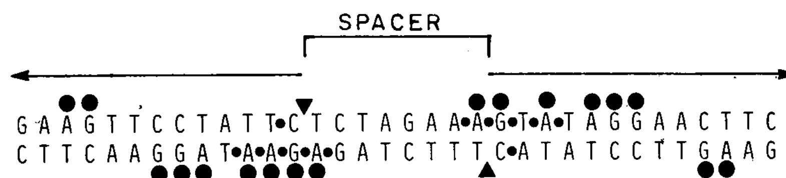

FIGURE 4. Identified contacts between FLP protein and purines and phosphate groups within the FRT site. Triangles, Points of cleavage; large circles, purine contacts; small circles, phosphate contacts.

6 bp of the spacer were all G+C, the site was inactivated in reactions employing nonsupercoiled DNA substrates. Interestingly, these mutations did not affect FLP protein binding. In fact, the binding affinity appeared to improve as the recombination efficiency decreased with these spacer mutants. In addition, the reactivity of each mutant site was improved when supercoiled rather than relaxed DNA substrates were used. These results suggest several things. The general sequence structure of the site is important for site function. This structure facilitates a recombination step subsequent to FLP protein binding. This step may involve unwinding of the DNA within the spacer region, as suggested by the effects of G+C content and supercoiling. The results permit a clear distinction to be drawn between the role of this sequence in site function as opposed to site recognition and binding by FLP protein.

C. Protein-DNA Interactions

The binding sites for FLP protein are the repeats that flank the spacer. Footprinting studies (Andrews et al., 1985) demonstrated that FLP protein protects the spacer and the two flanking repeats from DNase digestion. The binding sites were outlined in more detail by use of methylation protection and interference protocols (Bruckner and Cox, 1986). These studies mapped apparent purine and phosphate contacts within two 12-bp regions including each external base pair of the spacer and the first 11 bp of the adjacent repeats on each side of the recombination site. These contacts are summarized in Fig. 4. The phosphate contacts are clustered near the site at which FLP protein cleaves the DNA. The identified purine contacts are localized on one 180° face of a B-DNA helix. Both this work and the footprinting studies of Andrews et al. (1985) demonstrated that FLP protein binds to the third 13-bp repeat as well as the other two, even though this repeat is not required for recombination. Whether this binding has functional significance remains to be determined.

These results are consistent with and complementary to the results of the deletion analysis described above and the observed effects of mutations in the FLP binding sites. Mutations in this region have similar effects on recombination in vivo and in vitro. A few mutations in the FLP binding site have large effects on the recombination efficiency of the site (Prasad et al., 1986; Andrews et al., 1986; McLeod et al., 1986; Senecoff et al., 1988). Most of the mutations characterized in these studies, however, produce negligible effects on recombination. The

FLP PROTEIN BINDING SITE

INVERTED REPEAT

	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
		*	*	*	*		*	*	*			*	*		
WILD TYPE DNA SEQUENCE	A	A	G	A	A	T	A	G	G	A	A	C	T	T	C
MUTATIONS			A	G	G	C	G	A	A	G	G	T	C	C	
		T	T	C	C	G	C	C	T	C	C	G	A	G	
			C	T	T	A	T	T	C	T	T	A	G	A	
BASE NUMBER	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
MUTANT X MUTANT RECOMBINATION			1	4	5-10	5-10	10	1	>100	5	10	5	5	2	
		3-5	5	5	2-5	5	5-10	2-5	20	2	2	2	2	2	
			>100	40	>100	10	10	5	50	5	5	5	5	2	
RECOMBINATION WITH A WILD TYPE SITE			1	4	5	1	5	1	5	2-5	5	2	2	1	
			2-5	2.5	1-2	2	5	2	2	2	2	2	2	2	
			10	10	10	5	5	2	5	2	2.5	2	2	2	

FIGURE 5. Effects of mutations in the FLP protein-DNA binding site. One FLP binding site is shown. Asterisks denote the purine contacts illustrated in Fig. 4. Numbers denote the factor by which the FLP protein concentration must be raised to produce a minimal reaction relative to a normal recombination site; i.e., higher numbers correspond to less reactive sites. See text for details.

results of a comprehensive mutational analysis carried out in vitro with pure FLP protein (Senecoff et al., 1988) are summarized in Fig. 5. Most of the base pair substitutions (31 of 37) within the FLP binding site have modest or negligible effects in vitro, although a change of at least 1 bp at each position produces a significant (fivefold or greater) effect. Several interesting patterns are evident. The results suggest at least three types of protein-DNA interaction. These are reflected by three different patterns of effects observed at different positions: (i) all three possible base pair changes produce small effects, (ii) all three changes produce large effects (position 11 only), and (iii) different changes produce different effects (positions 5 to 7). The patterns at positions 5 to 7 are particularly interesting. In each case one change, a G→C or A→T transversion, produces a large effect, while the effects of the other two changes are small. Finally, it is noteworthy that transition mutations generally have very small effects on recombination (with the exception of position 11). This reinforces the notion that the polypurine (polypyrimidine) tracts extending through the FRT site (positions −1 to 13, and −2 to −13) are important to site function.

As shown in Fig. 4, FRT sites with mutations in one of the two FLP binding sites react better with a normal site than with an identical mutant site. In some cases the improvement is greater than 10-fold. A wild-type site can therefore "rescue" a mutant site, suggesting protein-protein contacts in the complex within

which recombination occurs (Prasad et al., 1986; Senecoff et al., 1988). Additional interactions between FLP monomers bound to the same FRT site are suggested by the much greater than additive effect observed when some mutations are present in both FLP binding sites rather than only one (Prasad et al., 1986; Senecoff et al., 1988).

D. Site of DNA Cleavage by FLP Protein

The FLP protein cleaves the two DNA strands at sites that are staggered by 8 bp. The cleavage points are at the spacer repeat junctions shown in Fig. 3. The protein becomes covalently attached to the DNA via a 3′ phosphate (Andrews et al., 1985; Senecoff et al., 1985) linked to a tyrosine (Gronastajski and Sadowski, 1985b). The eight-nucleotide single-stranded overhangs terminate in free 5′ hydroxyl groups.

E. Recombination Site Summary

The results described above thoroughly characterize the FLP recombination site. These studies reveal that the required site is relatively small, and a surprising degree of sequence flexibility is evident. In spite of the limitations described above, a wide variety of spacer sequences result in sites which are fully functional but do not cross-react. Sites with symmetrical spacers abolish reaction directionality. These properties of the site are useful experimentally (Bruckner and Cox, 1986; Senecoff et al., 1988; C. Gates and M. Cox, unpublished data).

IV. FLP PROTEIN

A. Expression in *E. coli* and Purification

Whereas expression of FLP protein in *E. coli* has been easy to detect, the levels of the protein obtained have been disappointing. Initial expression efforts employed either the *lac* or $\lambda\ p_R$ promoters (Vetter et al., 1983; Cox, 1983). Upon induction, FLP protein is generally less than 0.1% of the total soluble protein. A wide variety of classical approaches to protein expression in *E. coli* have been utilized without success in attempts to improve the level of expression (Meyer-Leon et al., 1987; E. Wood and M. Cox, unpublished data). The reason for this limited expression remains obscure.

The levels of protein obtained to date have, however, been sufficient to permit purification of significant amounts of FLP protein. Purification to near homogeneity was accomplished with the aid of site-specific DNA affinity chromatography. The strategy employed the information outlined above with respect to the FRT site. Attempts to use the intact FRT site as an affinity ligand failed because of a tendency for FLP protein to form covalent adducts with the DNA. The observation that FLP protein bound specifically to the third 13-bp repeat, however, suggested an alternative strategy. FLP protein does not cleave DNA

between the second and third 13-bp repeats. A polymer of 13-bp repeats in the same orientation, each linked to its neighbors as the second and third repeats in the FRT site are (Fig. 3), proved to be a successful affinity ligand (Meyer-Leon et al., 1987; C. A. Gates, L. Meyer-Leon, J. M. Attwood, E. A. Wood, and M. M. Cox, *in* R. Burgess, ed., *Protein Purification: Micro to Macro*, in press). Current protocols generate 1 mg of nearly homogeneous FLP protein from 100 g of *E. coli* host cells in 1 week.

B. Properties

The predicted molecular weight of the protein based on the DNA sequence of the gene is about 48,000. Migration of the protein in denaturing polyacrylamide gels and the amino-terminal sequence of the protein are consistent with the gene sequence. The purified protein is free from detectable nuclease activity and is stable for many months at −70°C. At 0°C the protein is stable for several weeks when stored in the presence of high (1 M) concentrations of salt. Under conditions previously determined to be optimal for activity (200 mM NaCl, 30°C, pH 7.5), the protein exhibits considerable instability, with complete loss of activity observed in the absence of DNA substrates in 20 to 30 min. Stability is improved significantly with the addition of bovine serum albumin and glycerol (Gates and Cox, unpublished data).

The pure protein promotes efficient recombination at levels which appear to be approximately stoichiometric with the number of recombination sites (one or two FLP monomers per site) in the reaction mixture under most conditions. When substrates with two recombination sites are employed, reactions are almost exclusively intramolecular regardless of the FLP protein concentration employed. Intermolecular recombination is enhanced in the presence of low levels of polyethylene glycol and in the presence of a number of basic DNA-binding proteins (see below).

Efforts have begun in several laboratories to generate mutations in the *flp* gene that affect the activity of the FLP protein. A strategy for isolating random mutations that inactivate FLP protein has been published (Govind and Jayaram, 1987). Mutations which change Tyr-343 to a serine or phenylalanine have also been described (Prasad et al., 1987). The inactivity of these mutants suggests that this tyrosine residue is the site of covalent attachment of the protein to the DNA.

V. RECOMBINATION REACTION

Site-specific recombination reactions provide an opportunity to examine many aspects of protein-DNA interactions. The enzyme must locate a specific binding site, juxtapose two such sites, cleave the DNA within each of the two sites, and then ligate the resulting ends with new partners in a precise way to yield recombinants. Each of the known enzymes promoting site-specific recombination is, in effect, a restriction enzyme and ligase in one package. As one of the simplest site-specific recombination systems, the FLP system recommends itself as an

experimental vehicle to probe chemical details of this process. Until recently, however, progress was slowed by the lack of pure protein, although many properties of the system have been outlined with partially purified preparations. Unless noted, the information presented below was obtained with FLP protein fractions which were approximately 5% pure (Meyer-Leon et al., 1984; Andrews et al., 1987).

Nothing is yet known about the mechanism by which FLP protein locates its binding sites. A DNA-binding assay based on gel retardation has been used to resolve three binding complexes, which apparently correspond to the binding of one, two, or three FLP monomers to available 13-bp repeats (Andrews et al., 1987; Prasad et al., 1986; Senecoff et al., 1988). If a recombination site with two 13-bp repeats is employed, and a strong deleterious mutation is present in one repeat, FLP protein binds and cleaves the DNA adjacent to the wild-type repeat only (Senecoff et al., 1988). The efficiency of intramolecular recombination appears to decrease as the distance between the sites increases, suggesting that tracking of the enzyme along the DNA may be involved in site juxtaposition (Gronastajski and Sadowski, 1985a). This relationship is abolished, however, if KCl is omitted from the reaction, suggesting that under at least some conditions site juxtaposition may involve random collisions (Sadowski et al., 1987). The cleavage of the DNA and formation of a covalent adduct was described above. As with other site-specific recombination systems and topoisomerases, this covalent adduct preserves the high-energy bond and obviates the need for high-energy cofactors in the reaction.

A common observation with site-specific recombination systems is that levels of recombinase which are at least stoichiometric with the concentration of available recombination sites are generally required to produce an optimum reaction in vitro. This suggests that these proteins do not turn over and therefore are not true enzymes. Similar observations were made in the early in vitro experiments with FLP protein. Recent results, however, indicate that FLP protein turns over slowly when relaxed DNA substrates are employed (Gates and Cox, unpublished data). The observation of turnover depends in part on the use of conditions which enhance the stability of the protein. The turnover rate is increased 1.5- to 3-fold if supercoiled substrates are employed. The apparent turnover number is low, $\sim 0.1 \text{ min}^{-1}$ under one set of conditions, and the slow step in the process has not yet been identified.

A number of recombination sites that harbor spacer alterations affected the rate of recombination but not the rate of FLP protein binding (Umlauf and Cox, unpublished data). These mutations therefore do not affect recognition of the site by FLP protein, and their existence suggests that DNA structure plays a significant role in a kinetically significant step of this reaction. The reactivity of these mutant sites is increased if the DNA is supercoiled, indicating that the affected step involves DNA unwinding in the spacer region. These results suggest an intricate relationship between DNA structure or topology and the catalytic mechanism employed by the recombinase.

VI. EFFECTS OF OTHER PROTEINS ON FLP PROTEIN-PROMOTED RECOMBINATION

Purification of FLP protein was hampered to some degree by variations in the behavior of the protein from one fraction to the next. The variation involved the relative efficiencies of inter- and intramolecular recombination as well as increases and decreases in overall activity (Meyer-Leon et al., 1984, Gronastajski and Sadowski, 1985a). High levels of FLP protein led to a two- to threefold enhancement of intermolecular recombination, while even higher concentrations inhibited the reaction almost completely. These effects have been traced to *E. coli* proteins present as contaminants of the FLP protein preparations (Meyer-Leon et al., 1987). At least four *E. coli* proteins are capable of producing these effects, and each of the four has recently been purified to homogeneity (R. C. Bruckner and M. M. Cox, submitted for publication). One of these proteins (molecular weight, 27,000) has been identified as the protein designated the H protein (Hübscher et al., 1980), which is a histonelike protein that cross-reacts immunologically with histone H2A. Another is a prominent contaminant in many H protein preparations (molecular weight, 26,000). The other two proteins are smaller (molecular weights, 24,000 and 16,000) and presumably are DNA-binding proteins. Each of these proteins increases the rate of recombination two- to fivefold when present at low concentrations. Increasing the levels of these proteins leads first to an enhancement of intermolecular recombination relative to intramolecular recombination and ultimately to inhibition of both reactions. All four proteins are prominent contaminants at early stages of the FLP protein purification. N-terminal sequence analysis of each of these proteins has produced the surprising result that all four are ribosomal proteins. The *E. coli* H protein, in fact, is ribosomal protein S3. The others are L2, S4, and S5 in order of decreasing size (R. C. Bruckner and M. M. Cox, submitted for publication). The functional significance of this observation is not yet clear. Interestingly, histones produce similar effects on FLP protein-promoted recombination. These effects almost certainly do not reflect a specific interaction of these proteins with the FLP protein or FRT site. It is noteworthy, however, that the 2μm plasmid is packaged as chromatin in *S. cerevisiae*, and it is conceivable that histones play a role in regulating this recombination event in vivo.

VII. PROSPECTS

The FLP system has been thoroughly characterized in vivo and in vitro and can now be used to address more general questions related to the detailed mechanism of site-specific recombination. As a vehicle for the investigation of chemical steps in this process, it has the advantage of simplicity. No additional proteins or factors which could complicate experimental design or interpretation are required, and no restrictions on the type of reaction (inversion, deletion) are superimposed on the basic chemistry of site-specific recombination. The availability of pure protein, protein mutants, and a well-defined recombination site

should ensure rapid progress. The properties of the system also recommend it as a potential reagent. The recombination site required is large enough that it is unlikely to occur at random in most DNA molecules but is small enough for convenient laboratory manipulation. The site exhibits considerable sequence flexibility, and suitable sites may exist in many chromosome-sized DNAs. Thus, it may be possible to use the FLP system to insert exogenous DNA into specific chromosomal sites.

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