Resolution of Multimeric Forms of Circular Plasmids and Chromosomes

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ABSTRACT One of the disadvantages of circular plasmids and chromosomes is their high sensitivity to rearrangements caused by homologous recombination. Odd numbers of crossing-over occurring during or after replication of a circular replicon result in the formation of a dimeric molecule in which the two copies of the replicon are fused. If they are not converted back to monomers, the dimers of replicons may fail to correctly segregate at the time of cell division. Resolution of multimeric forms of circular plasmids and chromosomes is mediated by site-specific recombination, and the enzymes that catalyze this type of reaction fall into two families of proteins: the serine and tyrosine recombinase families. Here we give an overview of the variety of site-specific resolution systems found on circular plasmids and chromosomes.

One of the serious disadvantages of circular plasmids and chromosomes is their high sensitivity to rearrangements caused by homologous recombination. Odd numbers of recombinational exchanges occurring during or after replication of a circular replicon result in the formation of a dimeric molecule in which the two copies of the replicon are fused in a head-to-tail configuration (Fig. 1). If they are not converted back to monomers, the dimers of replicons may fail to correctly segregate at the time of cell division.

Resolution of multimeric forms of circular plasmids and chromosomes is mediated by site-specific recombination, an efficient and tightly controlled DNA breakage and joining reaction that occurs at specific DNA sequences (Fig. 1A). Site-specific recombinases, the enzymes that catalyze this type of reaction, fall into two families of proteins: the serine and tyrosine recombinase families (1).

This chapter, which is an update of a previous version (2), provides an overview of the variety of site-specific resolution systems found on circular plasmids and chromosomes. After an introduction about the formation and the incidence of replicon multimers, we present the two families of resolution systems, based on serine or tyrosine recombinases. The most recent advances in understanding of the molecular mechanisms that control the recombination reactions catalyzed by these systems will be discussed. These illustrate how different molecular actors have evolved to achieve the same function but also how some are devoted to plasmid dispersion, whereas some are devoted to chromosome or to mobile genetic element dispersion.

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FORMATION AND INCIDENCE OF PLASMID MULTIMERS

Multimerization of circular DNA molecules is largely due to homologous recombination. Studies of high-copy-number plasmids, such as pBR322, revealed that multimerization primarily occurs by RecF-dependent recombination (3). RecBCD activity on double-strand ends would lead to rapid degradation and inefficient recombination of small DNA molecules. To our knowledge, similar studies have not been performed on large low-copy-number plasmids, although these plasmids harbor multimer resolution systems whose inactivation causes a defect in plasmid stability.

The net result of homologous recombination between sister plasmids is the production of multimers (Fig. 1A). However, the formation of multimers may have different effects depending on the mode of replication and partition of the replicon. Unresolved chromosome dimers are certainly lethal in most cases and are broken in a desperate effort of resolution due to the so-called guillotine effect. Plasmid multimers affect plasmid stability by lowering the number of independently segregating units.

FIGURE 1 Formation of plasmid dimers and their stability in the cell. (A) Dimers of plasmids or chromosomes are formed by homologous recombination (HR) during replication and are later resolved by site-specific recombination (SSR). Origins are shown by a circle, and site-specific recombination sites by a black and white square. (B) The accumulation of plasmid dimers in the cell leads to an increase in plasmid loss compared to monomeric plasmids. doi:10.1128/microbiolspec.PLAS-0025-2014.f1
at the time of cell division. They may also interfere with the control of replication and with the activity of the plasmid segregation system. High-copy-number plasmids segregate randomly so that the probability of generating plasmid-free cells is inversely proportional to their copy number at division time (4, 5) (Fig. 1B). Replication of these plasmids is controlled by origin counting, which means that a dimer counts as two plasmids for replication but only as a single unit for segregation. Thus, the formation of multimers lowers the number of freely segregating DNA molecules per origin, thereby raising the frequency of plasmid loss (4, 6, 7) (Fig. 1B). In addition, replication of high-copy-number plasmids follows a random copy choice mode so that a dimer replicates twice as frequently as a monomer. This replicative advantage of multimers causes their rapid accumulation in the progeny of the cells in which they appeared. This phenomenon, called the dimer catastrophe, is responsible for the largest part of the segregation defect due to plasmid multimerization since it leads to the formation of a subpopulation of cells that mostly contain multimers (4, 6, 7).

Although they are likely to have adverse effects on partition systems, multimers of actively partitioned replicons such as F or P1 have complex and poorly understood effects since the stability of these plasmids does not solely depend on the number of independently segregating units at cell division (for review see reference 8).

DNA SITE-SPECIFIC RECOMBINATION: THE GOOD RESOLUTION OF PLASMIDS AND CHROMOSOMES

Conservative DNA site-specific recombination is a carefully orchestrated reaction during which four DNA strands are broken, exchanged, and resealed to equivalent positions of separate sequences (see below). This is mediated by relatively simple molecular machines in which specialized enzymes, termed site-specific recombinases, catalyze the essential DNA breakage and joining reactions. The recombination reaction can lead to the integration, excision, or inversion of a DNA fragment, depending on the relative positioning of the recombination sites. However, recombination between directly repeated sites on a circular DNA molecule leads to deletion of the intervening DNA segment.

Besides its role in the stable inheritance of circular plasmids and chromosomes, site-specific recombination is exploited in a range of programmed DNA rearrangements in bacteria including integration and excision of temperate bacteriophages into and out of the genome of their host, the movement of different classes of mobile genetic elements (e.g., transposons, integrons, insertion sequences, and integrating conjugative elements [ICE]), the variable expression of virulence genes in pathogens (by means of simple or combinatorial DNA inversion switches), and the control of developmentally regulated genes (9–13).

Recombinases that mediate these different rearrangements, including dimer resolution, are often termed resolvases, integrases, transposases, or DNA invertases to designate the type of reaction they catalyze. These enzymes fall into two major families of unrelated proteins using different mechanisms to cleave and rejoin DNA molecules. These two groups of enzymes are now commonly referred to as the serine recombinase family and the tyrosine recombinase family according to the conserved residue that provides the primary nucleophile in the DNA cleavage reaction (1). Recombinases of both families mediate recombination at the level of short (∼30 base pairs) DNA segments termed the core or crossover site onto which two recombinase molecules bind, usually by recognizing specific sequences with dyad symmetry. The recombinase recognition motifs are separated by a central region at the borders of which the DNA strands are cut and exchanged by the protein (see below and Figs. 2 and 3). With a few exceptions that will be outlined below, this minimal core site is usually insufficient to mediate recombination. The recombination sites of most characterized systems have a more complex organization, with additional binding sites for accessory proteins. These accessory sequences and proteins are used to control the recombination reaction, allowing recombination systems to achieve their biological function without generating undesirable and potentially deleterious DNA rearrangements. For plasmid and chromosome resolution systems this control is important to convert multimers to monomers and not vice versa.

Plasmid Resolution Systems of the Serine Recombinase Family

Recombinases belonging to the serine recombinase family are present on a number of plasmids from both Gram-negative and Gram-positive bacteria, but only a few of them have been shown to contribute to plasmid maintenance by converting multimers into monomers. Here, we report several characterized plasmid resolution systems of the serine recombinase family, most of them being clearly derived, during recent evolution, from the γδ and Tn3 resolvases from replicative transposons (for review see references 14 and 15).
Genetic organization of serine recombinase-catalyzed resolution systems

RK2/RP4 resolution systems: Tn3 and γδ recombinases

An example of an integrated plasmid resolution system is provided by the ParA/res system of the IncP-1α plasmid RK2 (identical to RP4) (15, 17). Plasmids of this family have a relatively low copy number (between five and eight copies per chromosome) and can be stably propagated in a wide range of Gram-negative bacteria (17). Important determinants of this stability are encoded in the 3.2-kb par locus of the plasmid. This locus is comprised of two divergently transcribed operons (parCBA and parDE), separated by a short intergenic region of about 180 bp. The parDE operon encodes a post-segregational killing system (toxin-antitoxin system) homologous to those found in other plasmids and in the chromosome of various bacterial species (19, 20).

In addition to the serine recombinase ParA, the parCBA operon encodes two other proteins that have no apparent role in site-specific recombination. This region also contains the recombination site at which the ParA recombinase acts to resolve plasmid multimers (15, 20, 21). This site, designated RK2 res, has an organization similar to that of many Tn3-family transposon res sites, with three inverted repeats being bound by the recombinase (17) (Fig. 2A).

The ParA/res recombination system significantly contributes to the segregational stability of the RK2 plasmid, but recombination was insufficient to account for this contribution. Resolution of dimers may thus not be the only function of the ParA/res system (21). In addition, its replacement by another multimer resolution system (i.e., the Escherichia coli XerC/mer system or the bacteriophage P1 Cre/loxP system; see below) only partially complements the defects in plasmid stability (22).
Together, these observations suggest that the plasmid resolution function of the Par/Res system forms a part of a more complex stabilization process involving additional host- and/or plasmid-encoded factors.

**pSM19035 and pAMβ1 resolution system:**

Large theta-replicating plasmids of Gram-positive bacteria encode different subfamilies of serine recombinases, which together form the β recombinase family. This family is a group of relatively well-conserved proteins that are present on broad-host-range plasmids. The best-studied members of this family are the β protein of the Streptococcus pyogenes plasmid pSM19035 and the pAMβ1 Resβ recombinase from Enterococcus faecalis (for review see reference 23). These proteins are 40% identical to Sin, the prototype of a second group of highly homologous recombinases found on large staphylococcal plasmids (2, 24, 25). Sin and the β recombinases have been characterized at a genetic and biochemical level. The recombination target site of these recombinases exhibits a similar two-subsite structure, with the protein recognizing inverted repeats at the crossover site I and direct repeats at the accessory site II (26, 27) (Fig. 2A). In vitro studies have shown that recombination by Sin and the β recombinases also requires the nucleoid-associated protein Hbsu or any equivalent nonspecific DNA-binding protein, such as HU from E. coli or the HMG proteins from eukaryotes (27, 28). This protein plays an architectural role in the formation of the synaptic complex by inducing substantial DNA bends in the recombining partners (see below) (27).

**Mechanism of serine recombinase-catalyzed resolution:**

Serine recombinases are characterized by the presence of a relatively well-conserved catalytic domain of about 120 amino acid residues. This domain contains the catalytic serine and several other conserved residues clustered into specific motifs (Fig. 2B). Some of these residues are part of the active site pocket, whereas others are involved in protein-protein interactions between separate recombinase molecules in the recombination complex (29–32).

Although functional and structural diversity among serine recombinases is likely to reflect important variations in the molecular organization of the recombination complex, all the family members are thought to mediate recombination by using the same strand exchange mechanism (27, 30) (Fig. 2B). Recombination by serine recombinase is a concerted process in which all four DNA strands of the two recombination sites are cut, exchanged, and then rejoined in the recombinant configuration (for review see reference 1). The reaction starts when the active serine of each of the four recombinase molecules in the complex attacks the phosphodiester bond adjacent to its binding site. Cleavage of each duplex is staggered by two base pairs, generating protruding 3′-OH overhangs and recessed 5′ ends to which the recombinase is attached through a covalent phosphoseryl bond. Catalysis of these reactions is highly coordinated, and the complex in which the four cleaved half-sites are solely held together by protein-DNA and protein-protein interactions is an obligate intermediate in the recombination pathway (33–35). Topological changes occurring during recombination mediated by serine recombinase are consistent with the DNA strands being exchanged by a simple 180° right-handed rotation of one pair of cleaved ends relative to the other. In this mechanism, the extended two bases that are exchanged between the two duplexes must be complementary to allow base pairing between rejoining ends. Thus, the two base-pair overlap regions define the polarity of the recombination site (Fig. 2B).

Crystallographic studies on the resolvase of γδ, together with the biochemical characterization of specific recombinase mutants, have provided important insights into the molecular organization of serine recombinases and the protein-protein and protein-DNA interactions within the recombination complex supporting a detailed molecular model for the 180° rotation (36–39). The arrangement of the active site residues in the resolvase dimer is consistent with the previous finding that the recombinase cleaves the DNA in cis, by acting at the nearest position from its binding site (33). In this model, the strand exchange is coupled to a rotational rearrangement of the DNA-linked recombinase subunits within the tetramer (39). This mechanism requires that the dimer interface that holds the cleaved half-sites in the complex is transiently disrupted during the dissociation/reassociation process. To make this possible, a flat and hydrophobic interface is created within the tetramer, which permits the rotation of the synapsed subunits relative to each other (29, 39). This class of mechanism is supported by data suggesting that the DNA strands are at the outside of the synaptic complex and are thus too far apart to be exchanged without involving substantial motions of the proteins (30, 37). However,
**FIGURE 3** Site-specific recombination by tyrosine recombinases. (A) Structure of principal recombination sites from tyrosine recombinase family. Symbols are the same as in Fig. 2. (B) The primary structure of a typical tyrosine recombinase (top), showing the catalytic tyrosine in a circle; other amino-acids implicated in catalysis are also indicated. Two distinct protein domains are shown: the DNA binding domain (DBD) and another DNA binding domain containing the catalytic domain (DBD + CD) (bottom). The scheme depicts the principal steps of recombination by tyrosine recombinase: four recombinases bind on their sites and form the synapse; cleavage occurs first on only two DNA strands, catalyzed by one active pair of recombinases in the teramer (XerC or XerD in the case of dif, cer, mwr, and psi). A transient Holliday junction is formed after a first strand exchange and is isomerized to allow the cleavage of the two other strands by the other pair of recombinases. The second strand exchange takes place, and the complex dissociates.

based on homology between serine recombinases and topoisomerases, an alternative model, without subunit rotation, has been recently proposed and is still under debate (40).

**Plasmid Resolution Systems of the Tyrosine Recombinase Family**

**Genetic organization of tyrosine recombinase-catalyzed resolution systems**

Site-specific resolution systems that function with a tyrosine recombinase exhibit variable levels of complexity. Large conjugative plasmids and replicative prophages generally encode their own recombinase adjacent to the recombination site, allowing them to be transferred with a fully functional resolution system among different bacteria. In contrast, small plasmids, such as those of the CoLE1 family, utilize the chromosome dimer resolution system of their host. The recombination site of these different systems may be limited to a simple crossover sequence, as in the bacteriophage P1 Cre/loxP system, or may be more complex, containing additional DNA binding sites for regulatory proteins (Fig. 3A).

**Xer: a universal and multipurpose recombination system for the resolution of replicon dimers**

Xer recombination provides a good example illustrating how relatively simply DNA site-specific recombination mechanisms can be adapted to accomplish different biological functions. The Xer recombinase is unusual in that it functions as a heterotetramer comprising two proteins of the tyrosine recombinase family, XerC and XerD (41, 42). Homologues of these two proteins are found in the genome of virtually all bacteria and archaea harboring circular chromosomes, consistent with Xer recombination having an important and conserved function in chromosome segregation (43–46). The xerC and xerD genes are generally found in separate regions of the genome where they are sometimes associated with other genes involved in DNA repair and recombination (47). They were first demonstrated to increase the stability of naturally occurring multicycopy plasmids, such as CoLE1, by converting multimers into monomers (5, 48). This function was shown to require the presence of a specific site termed cer in CoLE1. Related recombination sites were subsequently identified in a number of E. coli plasmids (49), as well as in pSC101 (psi) from Salmonella typhimurium (50) and pJHCW1 (muv) from Klebsiella pneumoniae (51, 52). It was later found that the primary function of the Xer system is to resolve dimers of the chromosome by acting at the dif site, located in the replication terminus region of the E. coli chromosome (see below) (53–55).

The different target sites for Xer recombination share a conserved ~30-bp core sequence containing 11-bp XerC and XerD recognition motifs separated by a central region of 6 to 8 bp (53, 56). In addition to XerC and XerD, recombination at plasmid resolution sites depends on additional host-encoded proteins and on the presence of ~160 to 180 bp of accessory sequences adjacent to the core (Fig. 3A). Interactions between the accessory sequences and proteins are important to control the outcome of recombination, ensuring that it will convert plasmid multimers to monomers and not the converse (see below and Fig. 4) for a recent review see reference 57). A special section is devoted to the XerCD-dif chromosome dimer resolution (see below and Fig. 5).

**Individual resolution systems:** Cre/loxP of P1 and ResD/ifs of F

The Cre/loxP recombination system of E. coli bacteriophage P1 was discovered for its role in the circularization of the infecting phage genome by performing a recombination reaction between the terminally redundant P1 DNA ends (58–60). The recombination system was subsequently shown to contribute to the proper segregation of the prophage (61). In P1 derivatives lacking either the loxP site or a functional cre gene, products were lost 20 to 40 times more frequently than in the wild-type lysogenic form of the phage at each generation. This segregational defect disappeared when plasmids were propagated in a RecA+ strain, indicating that it resulted from the formation of dimers and higher multimeric forms by homologous recombination. This finding provided the first demonstration that site-specific recombination can be used to counteract damaging effects caused by homologous recombination on circular replicons (61).

The Cre recombinase is distantly related to the integrase Int of bacteriophage λ, the archetype of the tyrosine recombinase family. This is consistent with biochemical data showing that both proteins use the same mechanism to cut and rejoin DNA molecules (for reviews see references 1, 62). The loxP site, which is located 434 bp upstream of the cre gene, has the minimal structure of a recombination core site, with two inversely oriented 13-bp recombinase-binding motifs flanking an asymmetrical sequence of 8 bp (Fig. 3A). Both in vivo and in vitro studies have demonstrated that this 34-bp loxP sequence is a sufficient substrate for Cre (63, 64). In addition, in contrast to most recombinases,
Mechanism of tyrosine recombinase-catalyzed resolution

Tyrosine recombinases differ widely in amino acid sequence. However, the ternary structure of the catalytic domain is remarkably conserved among different members of the family (70–75). The catalytic signature of the family is composed of six residues, RKHRH/WY, which are clustered in specific regions of the C-terminal catalytic domain of the proteins (Fig. 3B) (1, 76).

The crystal structures of protein-DNA complexes reported for Cre and Flp, together with biochemical studies of different recombination systems, have given conclusive information on how the recombination complex is organized and how it works (Fig. 3B) (1). The assembly of the synapse is crucial for the regulation of the reaction (see below and references 65, 66) within this complex; the two recombination sites are aligned in an antiparallel conformation, and the DNA is bent in a roughly square planar configuration. It has been proposed that the DNA is bent in such a way that only one pair of recombinases is ready to cut DNA. Indeed, DNA bending “hides” two of the four scissile phosphate groups, one in each DNA duplex, allowing nucleophilic attack by catalytic tyrosine residues on the two other scissile phosphates (Fig. 3B; red circles). This creates a 3′-phosphotyrosyl covalent link between the DNA and the protein and liberates a 5′-OH end at the cleaved strand. DNA strands are then exchanged by melting three or four nucleotides from the parental duplexes before re-annealing them to the complementary bases in the partner (Fig. 3B). Watson-Crick base-pairing between the cleaved and uncleaved DNA strands tests the homology between the two recombination sites and helps to orient the invading 5′-OH ends for ligation (77). This ligation is the result of the nucleophilic attack on the 3′ phosphotyrosyl group by the partner 5′-OH end after strand exchange and forms a first Holliday junction intermediate (HJ1). HJ1 isomerizes into a second Holliday junction intermediate, HJ2, within which the second pair of recombinases is activated (66, 78–80). The resolution of HJ2 into the recombination product is performed via a second cycle of cleavage, strand exchange, and ligation reactions (Fig. 3B) (for a more detailed description see reference 1).

The choice of the first active pair of recombinases is crucial for the control of the reaction. It is thought that this choice is made during the assembly of the synapse (66, 81). Within the Cre-loxP synapse, the four recombinase molecules forming the tetramer are held together by a cyclic network of protein-protein interactions involving both the C and N-terminal domains of the protein. This cyclical donor-acceptor interaction between Cre monomers connects the four recombinase active sites in the tetramer, providing a means of communication between them (71, 72). Deviation from the perfect 4-fold symmetry in the Cre-DNA complexes provides a simple model for the isomerization mechanism that leads to sequential activation (and reciprocal inactivation) of pairs of recombinase subunits during a complete recombination reaction. In this mechanism, which is also true for other tyrosine recombinases such as λint, small readjustments of the angles formed by the arms of the
Holliday junction, coupled with little conformational changes in the C-terminal donor-acceptor interaction, suffice to explain the reciprocal activation/inactivation between pairs of recombinases (65, 66). Accordingly, in the case of Cre, this isomerization step recently appeared to be very rapid (79, 80, 82).

For XerCD-dif recombination, the synapse contains two units of XerC and two units of XerD, and the two pairs, XerC or XerD, are active sequentially (81, 83–85). In this case the model for the isomerization mechanism is supported by experiments showing that alteration of the recombinase C-terminus affects catalysis by the partner recombinase (84, 86).

A remarkably similar organization of the recombination complex is observed for Flp, although the N-terminal domains have totally different structures (87). A fundamental difference between Cre and Flp lies in the nature of the allosteric interactions that regulate the recombinase activity. In the Flp-DNA structures, the α-helix carrying the tyrosine nucleophile of one monomer is donated to the active site of an adjacent monomer of the complex. This active site sharing mechanism of Flp and the allosteric donor-acceptor interaction proposed for Cre and other cis-acting recombinases represent alternative, but functionally equivalent, strategies for coordinating catalysis in the recombination complex.

**SYNAPTOSOME AND TOPOLOGY**

**Convergent Mechanisms To Impose Resolution Selectivity on the Recombination Reaction**

As mentioned above, for most site-specific recombination systems, the minimal core site at which the cognate recombinase acts to catalyze the strand exchange reaction is usually insufficient to carry out normal recombination at a normal frequency. The target sites of these systems contain additional accessory sequences to which further recombinase molecules or other auxiliary proteins bind, thereby forming part of the functional recombination complex. Formation of this complex may be needed to facilitate the pairing of the core sites and/or to activate the recombinase catalytic activity within the tetramer. The specific architecture of the recombination complex provides a means of controlling the reaction, ensuring that recombination will only occur at the correct time or between appropriately positioned recombination sites. It is crucial for the biological function of site-specific resolution systems to selectively carry out recombination between directly repeated copies of the recombination site in order to prevent the formation of multimers and to avoid other undesirable DNA rearrangements. In these systems, discrimination between different arrangements of the recombination sites is achieved by imposing a specific local DNA geometry within the synaptic complex. Requirements for this complex act as a topological filter because it cannot readily assemble if the two recombination sites are in an wrong configuration or on separate DNA molecules. The recombination products of these systems are characterized by a single topology that is dictated both by the topology of the initial synapse and by the strand exchange mechanism used by the recombinases (for a comprehensive discussion on the mechanisms of topological selectivity, see reference 57).

**A Common Synapse Architecture for Resolvases of the Serine Recombinase Family**

The mechanism of topological selectivity was first established for the resolution systems of replicative transposons belonging to the Tn3 family (88). The resolvase is the only protein required to resolve cointegrates in vitro. Recombination only takes place if two complete copies of the transposon resolution res sites are present in an appropriate head-to-tail orientation on the same supercoiled DNA molecule. The major products of the reaction are two molecules interlinked twice (four-node catenane). This observation, combined with a number of biochemical and topological data from different laboratories, led to the conclusion that resolvase binding to the three subsites of res results in the formation of a specific synaptic complex, termed the synaptosome, in which the two recombination sites are interwrapped in a structure trapping three negative supercoils from the initial DNA substrate (Fig. 4). Assembly of this complex is a stepwise process that initiates with the antiparallel pairing of the accessory sites II and III of the two res sites. DNA wrapping around the resolvase accessory subunits acts as a checkpoint (i.e., topological filter) that dictates whether the reaction can proceed further by correctly positioning the subsite I-bound resolvase subunits for the strand exchange reaction (36, 88).

In the most recent model for the Tn3 synaptosome, the recombination sites are wrapped around a pair of interlocked protein filaments comprised of six resolvase dimers, each dimer being bound to a different res subsite (36) (Fig. 4). The site III-bound dimer forms the central unit of each filament, making equivalent contacts with its two neighbors. An important implication of this model is that the crossover sites lie at the outside of the protein core formed by the recombinase catalytic
domains, which is consistent with the view that there must be substantial structural rearrangements within the complex to carry out strand exchange.

The topology of the recombination reaction catalyzed by other resolvases of the serine recombinase family, such as the ParA protein of RP4/RK2 (17), the Sin recombinase of S. aureus (31, 89) and the β recombinase of pSM19035 (90) (Fig. 4), was found to be same as that reported for the cointegrate resolution system of Tn3 and γδ, generating four-node catenane products. Consistently, a similar overall architecture of the synaptic complex has been proposed for the plasmid resolvases Sin and β (31, 35, 37, 90). This arrangement thus represents a common structural unit in the synaptic complex of different resolution systems. Hbsu plays an important role in the formation of the complex by stabilizing the DNA bends between the two regions that are bound by the recombinase (Fig. 4). Note that in the case of the β recombinase, alternative configurations of the synapse were proposed to account for its ability to mediate DNA inversion under certain circumstances (90).

**Topological Selectivity in Xer Recombination of the Tyrosine Recombinase Family**

To satisfy its dual role in chromosome and plasmid segregation, the Xer system has evolved separate mechanisms to control recombination at the chromosomal site dif, or at plasmid resolution sites such as cer and psi (for review see reference 13). In the case of cer and psi, binding of the accessory proteins (i.e., PepA and ArgR, and PepA and ArcA, respectively) to the recombination site accessory sequences promotes the assembly of a topologically defined synaptic complex in which, as in the resolvase synaptosome, three negative DNA supercoils are trapped (Fig. 4). A fourth node is introduced.
to align the recombination core sites in an antiparallel configuration. Consequently, recombination between directly repeated psi sites produces a four-noded catenane (Fig. 4). Recombination at cer follows the same pathway but stops after the first strand exchange, generating a catenated Holliday junction-containing molecule that is resolved to products by a Xer-independent mechanism in vivo (for review see reference 57).

Based on genetic, structural, and topological data, a model of the cer synaptic complex was proposed (Fig. 4) (91–93). In this model, one ArgR hexamer is sandwiched between two PepA hexamers, and the recombination sites wrap around the accessory proteins as a right-handed superhelix. The ArgR hexamer bridges the two recombination sites by binding to the ArgR box from either partner, whereas PepA specifies the topology of the complex by directing the DNA across three large grooves running from the lower face to the upper face of the hexamer. Recombination at psi is thought to occur within a similar synaptic structure, in which an oligomer of ArcA would take the place of ArgR (57). Formation of the synaptic complex at psi was found to position the recombinases in a specific configuration so as to activate the XerC protomers for the first strand exchange reaction (94).

A similar topological filter mechanism was more recently found to control the recombination reaction catalyzed by the tyrosine recombinase TnpI from the Tn3-family transposon Tn4430, providing another example of convergent strategies to mediate selectivity in plasmid multimers and transposition cointegrate resolution systems (95, 96). However, in the case of the Tn4430 resolution system, additional subunits of the TnpI protein itself provide the architectural regulatory elements of the recombination complex without requiring any other host- or transposon-encoded proteins. In addition, recombination by TnpI produces two-noded catenane products instead of four-noded catenanes as observed for Xer, indicating that the topological organization of the recombination complexes differ between the two systems (95, 96).

RESOLUTION OF CHROMOSOME DIMERS

E. coli Model

The best-studied case of multimer formation is dimerization of circular chromosomes, and most of the work has been done in E. coli. Ten to fifteen percent of the chromosomes require Xer recombination for correct segregation (97, 98). This requirement is suppressed in a RecA-deficient strain, consistent with the view that the vast majority of chromosome dimers form by homologous recombination (97). It is generally admitted that most recombination events are a consequence of recombinational repair of stalled, broken, or collapsed replication forks. Collapse or processing of stalled forks creates DNA ends that need recombination to be resealed with the circular molecule, allowing replication to restart (99–101). In the case of an odd number of these events, this leads to the formation of a dimer of chromosomes. It is believed that unresolved dimers are trapped by the cell septum at division, leading to chromosome breakage by an unknown mechanism (100).
To avoid random cutting of its chromosome, *E. coli* uses the tyrosine recombinases XerC and XerD, but with a different control than for plasmid dimer resolution. The recombination site, called *dif*, possesses the same characteristics as a plasmid core recombination site (Figs. 3A and 5), with oppositely oriented XerC and XerD recognition elements separated by a 6-bp central region (Fig. 3A). However, the mechanism that controls recombination at the chromosome site *dif* and the plasmid resolution sites differs significantly. There is no need for accessory sequences around *dif*. Instead, the reaction is under the control of the DNA-translocase FtsK. This ATP-dependent translocase is anchored at the septum through its N-terminal transmembrane domain, which interacts with the early proteins of the divisome. This restricts FtsK action to a specific cellular location, the septum, and a short period of time following replication (97, 98, 102). Septal localization of FtsK is also important to recruit late divisome proteins that are required for septum completion and closure (103). Analogues of FtsK are found in almost all bacteria sequenced so far (104). The main ones studied are SpoIIE and SftA from *Bacillus* (105, 106), but their role in site-specific recombination is still unclear (107). Recombination activation involves direct and specific interactions between the very C-terminal domain of FtsK, called the γ-domain, and XerD bound to *dif* (Fig. 5) (108–110). When activated, XerD cuts and exchanges the first pair of strands, and the resulting Holliday junction intermediate is then resolved by XerC.

Finding two *dif* sites within a dimer of chromosomes might not be an easy task for FtsK. In addition to interacting with XerD, the γ-domain of FtsK also recognizes 8-bp oriented sequences on DNA, called KOPS (for FtsK oriented polar sequences), which point toward the *dif* site (105, 111). FtsK recognizes these sequences in an oriented manner, and therefore translocates toward the direction of *dif* (112, 113). During its high-speed translocation (up to 17 kb/s), FtsK removes any protein bound to DNA (114, 115). It only stops when it reaches a *dif*-XerCD complex, but it is still unclear whether FtsK contributes to synapase assembly by recruiting another XerCD-*dif* complex or whether the synapase is already formed at this point (81, 85, 114).

It has been long questioned whether the translocase activity of FtsK could play a more general role in chromosome segregation, since the presence of the C-terminal motor does not appear to be essential for proper cell division (103). Recent results have shown that FtsK does indeed participate in chromosome segregation, by ordering segregation of the terminal domain (116), independently of the monomeric or dimeric state of the replicated chromosomes. Therefore, FtsK ensures that the last genetic locus to remain at the septum is *dif*, since the KOPS converge at this site.

Another remaining question concerns the ability of the FtsK/XerCD system to resolve dimers by generating only free products (not catenated). Experiments done on plasmids containing two *dif* sites show that the γ-domain of FtsK (free or linked to XerC or XerD) is sufficient to activate recombination. However, such reactions lead to products with complex topologies (catenated or knotted). These topologically complex products never arise when the reaction is carried out with a full-length FtsK motor (110). FtsK thus either prevents the formation of interlinked products before activating recombination and/or resolves them by further rounds of recombination. This latter hypothesis has been demonstrated *in vitro* and *in vivo* (117, 118).

**Multichromosomal Bacteria**

The only experimental work on multichromosomal bacteria has been done with *Vibrio cholerae*, which has two chromosomes (119) but only one XerC, XerD, and FtsK. This work revealed that chromosome dimer resolution in *V. cholerae* followed the same mechanism as in *E. coli*. The key point is the difference in the sequences of the two *dif* sites carried by the two chromosomes. The XerD binding motifs of both sites are conserved from other bacterial species, as are most of the XerC ones. The main difference lies in the central region: the central region of *dif1* is highly conserved with respect to the *dif* site of other bacteria, whereas the central region of *dif2* is much more divergent. This is also the case for most of the multichromosomal bacteria. A plausible explanation for this observation is selective pressure to avoid recombinational fusion between two, nonhomologous, chromosomes (43, 44). Despite this, chromosome fusions appear to happen sporadically within populations of *V. cholerae* (120), suggesting that this mechanism is not totally error-proof.

**Alternative, Single-Recombinase Xer Systems in Bacteria**

Despite the broad conservation of the XerCD system in bacteria, some bacteria have acquired another system that functions with a single recombinase. Examples include the firmicutes *Lactococcus* and *Streptococcus* and some of the ε-proteobacteria such as *Helicobacter* spp. and *Campylobacter* spp. The Xer5/dif5 and XerH/difH systems acting in the two groups of bacteria (121, 122) function on the same principle as XerCD/*dif*. However,
protomers of the same recombinase bind the two arms of the dif sites, and the control imposed by FtsK seems to be looser than that described for canonical systems involving two different recombinases (122–124).

Resolution of Dimers in Archaea

Despite their eukaryotic-type machineries for replication and DNA repair, archaea also possess a Xer/dif recombination system to maintain their circular chromosomes. A survey covering about 20 archaeal genomes suggested that archaea possess only one Xer protein, later named XerA (45, 125). The XerA proteins from Pyrococcus abyssi and Sulfolobus solfataricus have been characterized biochemically (45, 46). Both display recombination activity on their respective dif sites without the help of any accessory protein, which is unusual for Xer-type proteins but is consistent with the lack of a FtsK homologue in archaea. It is therefore unclear yet how archaea couple the end of replication with cell division.

DIMER RESOLUTION AND HORIZONTAL GENE TRANSFER

Beyond their demonstrated role in the maintenance of genetic information, site-specific resolution systems have been found to be involved in horizontal gene transfer. As mentioned earlier, Tn3-family transposable elements use a replicative transposition mechanism, which generates a cointegrate structure made of the fusion of the donor and target DNA molecules. Resolution of this cointegrate by intramolecular-site-specific recombination between the two copies of the element regenerates the initial donor molecule and releases a copy of the target into which the transposon is inserted. For most Tn3-family transposons the recombination reaction is catalyzed by a serine recombinase, while in the case of Tn430 and a few other elements, resolution of the cointegrates is mediated by a tyrosine recombinase (2, 96, 126).

More recently, integrative mobile elements exploiting Xer recombination systems (13) have been described to hijack the chromosomal XerCD-dif dimer resolution system. First discovered for their involvement in toxic conversion in V. cholerae (127), it appears that integration/excision of these mobile genetic elements depends on XerC and/or XerD (128–130).

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REFERENCES
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complex.


