REVIEW

A broadening view of recombinational DNA repair in bacteria

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Recombinational DNA repair is both the most complex and least understood of DNA repair pathways. In bacterial cells grown under normal laboratory conditions (without a DNA damaging treatment other than an aerobic environment), a substantial number (10–50%) of the replication forks originating at oriC encounter a DNA lesion or strand break. When this occurs, repair is mediated by an elaborate set of recombinational DNA repair pathways which encompass most of the enzymes involved in DNA metabolism. Four steps are discussed: (i) The replication fork stalls and/or collapses. (ii) Recombination enzymes are recruited to the location of the lesion, and function with nearly perfect efficiency and fidelity. (iii) Additional enzymatic systems, including the ϕX174-type primosome (or repair primosome), then function in the origin-independent reassembly of the replication fork. (iv) Frequent recombination associated with recombinational DNA repair leads to the formation of dimeric chromosomes, which are monomerized by the XerCD site-specific recombination system.

Introduction

Recombinational DNA repair represents a cross-roads where virtually every aspect of DNA metabolism comes together. When a bacterial cell is subjected to UV irradiation or other DNA damaging treatment, DNA replication rapidly comes to a halt. After 30–40 min, replication is restored to its original level. Replication restart (Khidhir *et al*. 1985; Echols & Goodman 1990; Echols & Goodman 1991) requires both recombination and replication functions. In the meantime, a wide array of DNA repair processes are induced as part of the SOS system, including many that facilitate the recombinational DNA repair and replication restart. Information about what occurs during the 30–40 min required for replication to recover is still limited, but available experimental data can provide some insight.

The transient abatement of DNA synthesis is almost certainly not limited to environmental stress producing unusual levels of DNA damage. Instead it is a manifestation (albeit dramatic and readily observed) of a process that makes an important and regular contribution to bacterial DNA replication under *normal* growth conditions. A summary of the likely fate of a replication fork in the *Escherichia coli* chromosome can serve as an overview to organize this discussion (Fig. 1). Once initiated at *ori*C, some replication forks complete their task, while others encounter either an unrepaired DNA lesion or a DNA strand break at a lesion undergoing repair. At these encounters, the replication complex halts and/or collapses. The resulting gap or double-strand break is processed by recombination enzymes. The branched DNA replication fork is re-established after a lag of some minutes. A replication complex which may be distinctly different from that assembled at *oriC*, comes together in an origin-independent manner, and replication again proceeds unimpeded. Any DNA lesions left behind are now within double-stranded DNA and can be processed by excision repair pathways. The improper resolution of recombination intermediates (Holliday structures) producing a dimeric bacterial chromosome is countered by a distinct and specialized site-specific recombination system.

The pathways presented in Fig. 1 are not intended to be comprehensive or unique, and the precise

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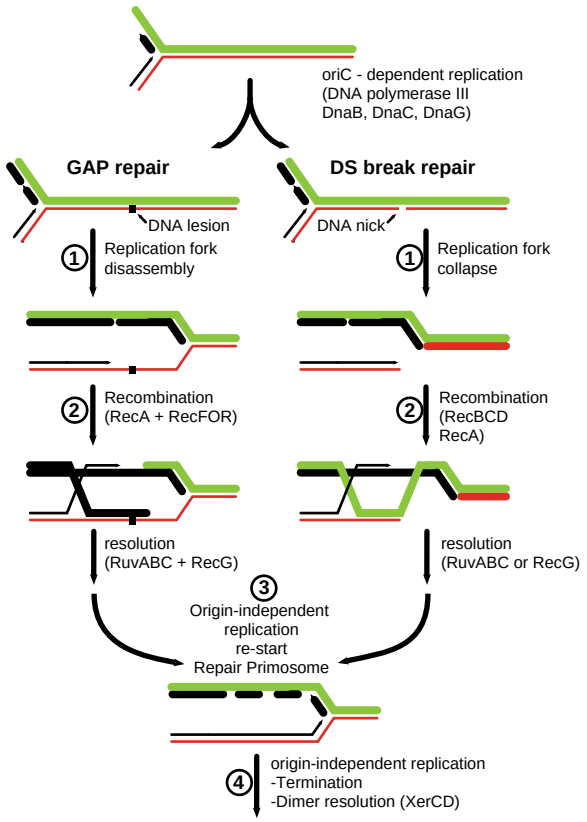


Figure 1 Pathways of recombinational DNA repair. The steps described in the text are outlined. The listing of proteins involved in each step is not meant to be exhaustive. Circled numbers correspond to the four steps highlighted in the text.

mechanisms by which these processes occur are left intentionally vague. Many of the ideas in Fig. 1 can be traced to recombinational repair models presented by West and Howard-Flanders (West *et al.* 1981) and Szostak *et al.* (Szostak *et al.* 1983). Many of the same ideas have also been developed in a number of recent articles, books and reviews (Zavitz & Marians 1991; Cox 1993; Livneh *et al.* 1993; Asai *et al.* 1994a; Friedberg *et al.* 1995; Sherratt *et al.* 1995; Kuzminov 1996a; Kogoma 1997; Roca & Cox 1997). Drawing from different perspectives, each contributes to the synthesis attempted here and should be consulted for more detailed discussions of individual points and for some alternative views.

Bacteria have made an extraordinary evolutionary investment in recombinational DNA repair. At some point in the scheme of Fig. 1, almost every bacterial protein known to play a role in DNA metabolism leaves its mark. Many of these proteins, particularly those with the designations Rec, Pri and Xer, may have evolved primarily to address the requirements of recombinational DNA repair. The importance of this process is also reflected in an extraordinary concentration of facilitating sequences (*chi* sites) seen throughout the *E. coli* genome.

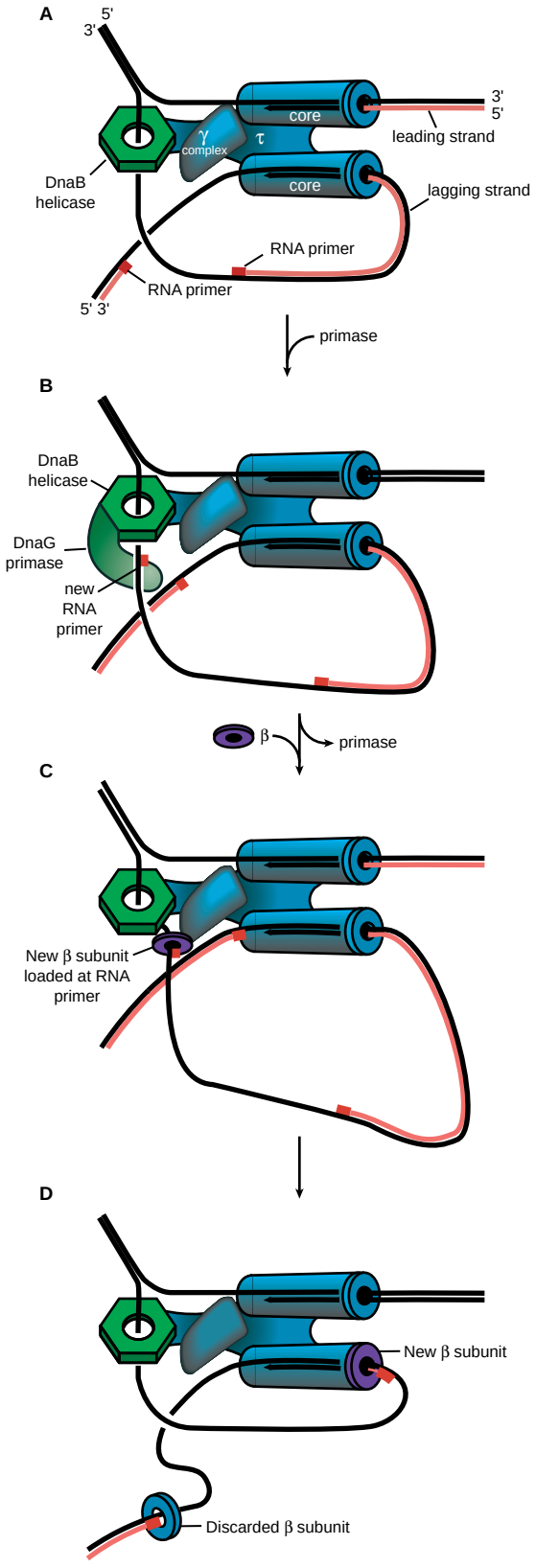
A concise integration requires a focus on several themes. First, a premature termination of replication fork movement which requires recombinational DNA repair is a very common occurrence *even in the absence of treatments designed to elevate DNA damage*. Second, the re-initiation of replication after recombinational repair requires a specialized enzymatic system and mechanisms distinct from those applied at the genomic origin. Third, the organization of the replication fork may be altered after recombinational DNA repair. Fourth, homologous genetic recombination is a required step in this repair pathway, bringing with it an array of genomic and cellular consequences.

The paradigm outlined in Fig. 1 can enhance our understanding of the genetics and biochemistry of the proteins playing a direct or indirect role in recombinational DNA repair. It also has the potential to illuminate a surprising number of biological systems, enzymatic activities and cellular phenomena that have sometimes been difficult to place in an appropriate functional context.

The structure of replication forks assembled at oriC

Following an oriC-dependent initiation process that relies on the activities of the DnaA and DnaC proteins, replication forks proceed bidirectionally from the *E. coli* origin (Kornberg & Baker 1992; Marians 1992). The contiguous protein complex at each fork consists of the asymmetric DNA polymerase III holoenzyme and the DnaB helicase (Fig. 2). The DnaG primase plays an intermittent role in the priming of lagging strand DNA synthesis (Tougu & Marians 1996). Auxiliary proteins (DNA topoisomerases, single-strand DNA binding protein (SSB)) play important roles that do not necessarily require direct physical contact with the replication fork complex. The result is an integrated complex that carries out DNA synthesis on both DNA template strands (Fig. 2).

Continuous DNA synthesis on the leading strand is complemented by the coordinated synthesis of Okazaki fragments on the lagging strand. The DnaG primase interacts transiently with the DnaB helicase (Tougu & Marians 1996) to effect routine priming of lagging strand DNA synthesis. For replication which is initiated at oriC, the DnaB and DnaG proteins constitute a



minimal (oriC-type) lagging strand primosome, both *in vivo* and *in vitro* (Kornberg & Baker 1992; Marians 1992). Both of these proteins are essential in *E. coli*, and ts mutants exhibit a rapid-stop phenotype with respect to DNA synthesis (Wechsler & Gross 1971). The DnaB protein is the only one of the multiple *E. coli* helicases that is absolutely required for chromosomal replication (Baker *et al.* 1986).

Conspicuously absent from the complex at the replication fork are five of the proteins defined as components of a larger *E. coli* primosome, often referred to as the ϕX174-type primosome. These include the PriA, PriB, PriC, DnaC and DnaT proteins, originally discovered during *in vitro* studies of the replication of the bacteriophage ϕX174 genome (Arai & Kornberg 1981; Zavitz & Marians 1991). This more elaborate primosome is required for initiation of replication and lagging strand synthesis in bacteriophage ϕX174 and also for plasmids with ColE1 origins (Zavitz & Marians 1991). However, a ϕX174-type primosome is not required for replication originating at oriC, either *in vivo* or *in vitro*. This opens the intriguing question of the cellular function of a ϕX174-type primosome, which presumably did not evolve to serve the needs of bacteriophages or plasmids.

The PriA protein plays a central role in the assembly of the ϕX174-type primosome, and also exhibits DNA-dependent ATPase and DNA helicase activities *in vitro* (Wickner & Hurwitz 1975; Shlomai & Kornberg 1980). The issue of primosome function has been addressed in the isolation of mutant cells deficient in PriA activity (Lee & Kornberg 1991; Nurse *et al.* 1991). Cells lacking PriA exhibit growth defects, filamentation, and an induction of the SOS response. They have a reduced viability, but the mutation is not lethal (Nurse *et al.* 1991). Combining the *priA* defect with an *sulA* mutation that relieves the filamentation phenotype, resulted in cells with improved viability and growth rates that were 60% of wild-type (Nurse *et al.* 1991). A priA mutation that eliminated the ATPase function but retained the primosome assembly activity restored cell

Figure 2 A replication fork assembled at oriC. (A) An asymmetric DNA polymerase III complexed with the DnaB helicase moves along the DNA. (B) At intervals, the DnaG primase binds to the DnaB helicase and synthesizes an RNA primer on the lagging strand. (C) The γ complex them loads a new β subunit complex at the new primer. (D) Once the synthesis of one Okazaki fragment is complete, the old β subunit complex is released along with the replicated DNA, and a new Okazaki fragment is initiated at the new RNA primer utilizing the new β subunit complex.

growth and viability to normal levels (Zavitz & Marians 1992). Overall, the present work indicates that oriC-dependent replication does not depend on a functional PriA protein *in vivo*. The reconstitution of oriC-dependent DNA replication has demonstrated no need for PriA protein *in vitro* as well (Baker *et al.* 1986; Funnell *et al.* 1987).

It has been recognized for some time that replication forks might be halted at DNA damage (Kuzminov 1995a). Complementing many other experimental paths, indicating a link between recombination and DNA repair (Cox 1993; Clark & Sandler 1994; Kuzminov 1996a; Roca & Cox 1997), the analysis of *priA* mutants led to the suggestion that replication forks originating at oriC might be halted at DNA damage and disassemble surprisingly often (Zavitz & Marians 1992). The ϕX174-type primosome would then function to re-establish a viable replication fork to complete the DNA synthesis. In effect, the replication fork that completed DNA synthesis would often be distinct from the replication fork generated at oriC. Although this is not the only insight leading to a broader synthesis of recombinational DNA repair pathways, it serves as an entry point for a more detailed examination of the steps outlined in Fig. 1.

Step 1: the replication fork is halted at DNA damage

The first suggestion that a replication fork might collapse at the site of a DNA strand break came in 1974 (Skalka 1974). The general idea that replication fork progress is halted by many types of DNA damage is now supported by an array of experimental observations (Kuzminov 1995a). The response to a UV challenge provides ample evidence that damage halts the progression of replication forks. As already noted, UV triggers a transient pause in DNA synthesis (Livneh *et al.* 1993). DNA fragments produced after UV irradiation have sizes that correspond to the average inter-dimer distance in template strands, as though the replication forks halted, then started up again so as to leave discontinuities (Sedgwick 1975; Youngs & Smith 1976). The resulting single-strand gaps almost certainly represent the signal for induction of the SOS response (Livneh *et al.* 1993; Friedberg *et al.* 1995; Kuzminov 1996a), and studies of the requirements for SOS induction can therefore reinforce the link between the appearance of the gaps and replication. A variety of reports have demonstrated that replication is required for the induction of SOS (Sassanfar & Roberts 1990; Friedberg *et al.* 1995). DNA damage alone is insufficient. For example, the introduction of a damaged but unreplicated viral or plasmid genome into a cell does not induce SOS (D'Ari & Huisman 1982; Sommer *et al.* 1991). The general conclusion of many studies is that replication forks stall at the sites of DNA damage and that single-strand gaps open up in the DNA as a result. The gaps provide binding sites for RecA protein, which in turn leads to an induction of the SOS response. Alternatively, and at least as important, the replication forks might encounter DNA strand breaks and thereby generate double-strand breaks. As outlined below, double strand breaks may form at stalled replication forks. The processing of the DNA ends for recombinational repair could also lead to RecA protein binding and SOS induction, as well as to DNA repair (Anderson & Kowalczykowski 1997a,b). The same RecA protein that binds to single-stranded DNA and induces the SOS response also plays a direct role in repair. It is important to note that it is the recombinational activities of RecA that are required for recombinational DNA repair, as opposed to the RecA-mediated induction of repair functions associated with the SOS system (Smith & Wang 1989; Roca & Cox 1990; Asai *et al.* 1993).

Quantifying step 1 under normal growth conditions

What happens if the cells are not subjected to a DNA damaging treatment? Under normal aerobic growth conditions, an *E. coli* cell suffers 3000–5000 DNA lesions of oxidative origin per cell per generation (Park *et al.* 1992). Good estimates are not available for other types of damage, although oxidative damage is almost certainly the major source of lesions in the absence of other environmental challenges. The effects of oxidative damage are evident in the phenotypes of many bacterial strains lacking key activities in DNA metabolism. For example, *recB polA* or *recA xth* double mutants are nonviable when grown aerobically, but survive under anaerobic conditions (Morimyo 1982; Imlay & Linn 1986).

Although most lesions are repaired quickly, a substantial number of replication forks have their progress arrested at DNA damage. Without DNA damaging treatments, and under conditions which are generally used for the growth of bacteria in the laboratory, it can be estimated that 10–50% of the replication initiations at oriC are not completed without such interruption. This estimate is derived from a variety of indirect observations. Some of the best clues can be derived from studies of cells lacking the *recA* or *recBC* functions.

If a replication fork encounters a lesion at a stage in repair in which a DNA strand break exists, the

encounter will result in the formation of a double strand break that cannot readily be repaired in a *recA* or *recBC* mutant. A careful look at linearized *E. coli* chromosomal DNA (Michel *et al.* 1997) indicated that double strand breaks appeared in about 15% (after correction for background) of the chromosomes of a *recB* or *recC* mutant, and over 20% of the chromosomes from a *recA recD* double mutant. In this study, cells were grown aerobically in a minimal media. The frequency of double strand breaks can be interpreted as one measure of the encounters between replication forks and genomic strand breaks, or other replication-associated events that generated double strand breaks.

Additional studies of RecA-deficient cells yield similar clues. A defect in DNA replication and cell division in *recA* cells was first reported by Inouye (Inouye 1971). Over 50% of the cells in a culture of a *recA* null mutant are nonviable under at least some conditions (Capaldo *et al.* 1974). The frequency of productive replication initiation at oriC is significantly reduced (Skarstad & Boye 1988). More than 10% of *recA* cells contain no DNA (Zyskind *et al.* 1992; Horiuchi & Fujimura 1995), and many more have abnormal numbers of chromosomes (Skarstad & Boye 1988; Skarstad & Boye 1993). The observation of anucleate cells has been explained as reflecting a defect in chromosomal partitioning (Zyskind *et al.* 1992), but chromosome loss due to the degradation of chromosomes with breaks provides a quantitative explanation for the appearance of anucleate cells as well as those with odd numbers of chromosomes (Skarstad & Boye 1993). These studies were carried out with bacterial cells grown aerobically in a variety of standard media.

A stalled replication fork can lead to a double strand break (Kuzminov 1995b). Artificially halting replication forks (by including a ts mutation in a replication helicase like DnaB) increased the amount of linearized DNA observed in *recBC* mutant cells (Michel *et al.* 1997). The increase was not observed without replication. Placement of a replication termination site (*ter*) at the lac operon so as to halt replication prematurely leads to a substantial increase in the generation of anucleate cells in *recA* strains (Horiuchi & Fujimura 1995). This is consistent with the generation of double-strand breaks at the stalled replication forks followed by a degradation of the broken chromosomes. High levels of homologous recombination have been noted in the region near the terminus of replication (Louarn *et al.* 1994), an observation that can be explained by the introduction of breaks at the site of stalled replication forks (Kuzminov 1995b).

The estimate of 10–50% for the premature arrest of replication forks is obviously approximate, but serves to underline that these events are common and highly significant to bacterial DNA metabolism. To the extent that alternative pathways for the repair of stalled replication forks exist that are independent of RecA and/or RecBCD (Cao & Kogoma 1995; Bi & Liu 1996; Saveson & Lovett 1997), the frequencies estimated from the DNA structures seen in *recA* and *recBC* cells could underestimate the severity of the problem. In addition, the frequencies are likely to vary greatly as a function of growth conditions. The presence or absence of oxygen is a major factor. Even the choice of media, to the extent that it affects oxidative metabolism (Galitski 1996; Roca & Cox 1997), may alter the fate of replication forks. Finally, the most straightforward message of the cell survival curves published in numerous studies of *rec* mutants should be noted: even a modest challenge with a DNA damaging agent kills the vast majority of rec^- cells (Livneh *et al.* 1993; Clark & Sandler 1994; Kowalczykowski *et al.* 1994; Friedberg *et al.* 1995; Kuzminov 1996a; Roca & Cox 1997). More systematic studies of the status of the bacterial chromosome as a function of growth conditions and the presence or absence of *rec* mutants could be highly informative. A fascinating corroboration of these estimates for the frequency of replication fork collapse and recombinational DNA repair is evident in recent work on the XerCD site-specific recombination system (step 4, below).

Step 2: replication gives way to recombination

The pathways of Fig. 1 are an oversimplification in more than one respect. There are doubtless more than two pathways for recombinational DNA repair, along with overlapping pathways and pathway variants. There are also many more steps and proteins involved in individual pathways. The recombination functions of recombinational DNA repair can be viewed as an adaptable and changing assemblage that can address a wide range of DNA structural realities. The hierarchy of pathways and enzymatic activities defined to date for conjugational and transductional recombination (Smith 1991; Clark & Sandler 1994; Kowalczykowski *et al.* 1994) reflect the DNA substrates presented to the cell under those specialized conditions, and need not be exactly replicated in recombinational DNA repair. For example, the DNA damage sensitivity conferred by mutations in RecF pathway mutant genes (Roca & Cox 1997) suggests that this pathway may be more important to recombinational DNA repair than it is to conjugational recombination in wild-type cells. New

assays examining recombination between chromosomal direct repeats also suggest that sexual recombination assays under-estimate the importance of the RecF pathway (Galitsky & Roth 1997).

Once a replication fork halts at a DNA lesion or encounters a DNA strand break, it is believed to disassemble (Kuzminov 1996a). Direct evidence for this outcome is limited. DNA polymerases are halted by a variety of DNA lesions *in vitro* (Livneh 1986; Banerjee *et al.* 1988; Lawrence *et al.* 1990; Bonner *et al.* 1992). The interruption is followed by polymerase dissociation (Livneh 1986). However, these are simple model systems that do not reproduce a complete replication fork. At a minimum, the disassembly of a replication fork upon encountering a nick or lesion is logical. The available evidence is insufficient to preclude the possibility that stalled replication forks remain partially or entirely intact under some circumstances.

Repair of the double-strand break resulting from an encounter with a nick is dependent on the RecBCD enzyme and follows a pathway outlined in Fig. 1. Large parts of this proposed pathway have been reconstituted *in vitro* (Kowalczykowski 1994; Anderson & Kowalczykowski 1997b; Eggleston *et al.* 1997). In short, the RecBCD enzyme binds to a double-stranded DNA end, unwinds the DNA and degrades the two strands asymmetrically, with the 5′-ending strand remaining relatively intact. Upon encountering the 8-nucleotide sequence called *chi*, the enzyme's 3′ to 5′ nuclease activity is abated, a 5′ to 3′ exonuclease is up-regulated, and RecBCD facilitates the loading of RecA protein on to the prepared single strand (Anderson & Kowalczykowski 1997a,b). A RecA-mediated strand invasion and strand exchange then follows, with the resulting crossover resolved by some combination of the RuvABC, RecG, and perhaps other enzymes (West 1994).

The *chi* sites recognized by the RecBCD enzyme function in only one orientation relative to a RecBCD enzyme unwinding and degrading a linear DNA from one end (Bianco & Kowalczykowski 1997). In the *E. coli* genome, the *chi* sites are highly over-represented (Burland *et al.* 1993; Blattner *et al.* 1997; Tracy *et al.* 1997). Furthermore, most of the chi sites are orientated so that they would alter the activity of RecBCD enzymes moving only in the direction toward oriC (Burland *et al.* 1993; Blattner *et al.* 1997; Tracy *et al.* 1997). The *chi* sites are therefore positioned to function in recombinational DNA repair (Kuzminov 1995a). This is true regardless of which template strand is broken. They can modulate the activity of RecBCD enzymes entering a linear DNA molecule at the site of a replication-generated double-strand break, and are spaced to prevent extensive degradation of the chromosome by RecBCD. The chi sites also appear to be located within islands of sequences shown to be preferred DNA binding sites for the RecA protein (Tracy *et al.* 1997). The evolution and conservation of this highly facilitative positioning of *chi* sites in the *E. coli* genome can be viewed as indirect evidence that most double-strand breaks, subject to recombinational DNA repair in bacteria, are generated in the course of replication.

The repair of DNA gaps which are generated when a replication fork encounters a DNA lesion follows a pathway dependent on the RecF, RecO and RecR proteins (Fig. 1). At least one major function of these proteins is to modulate the assembly of RecA protein filaments in the single-strand gap. RecA filaments assemble and disassemble 5′ to 3′ in an end-dependent fashion, with a protein being added at one end and deleted at the other (Roca & Cox 1997). Certain mutants of RecA protein suppress the defects of recFOR mutants (Thoms & Wackernagel 1988; Madiraju *et al.* 1992; Wang *et al.* 1993). *In vitro* work to date indicates that the RecR protein forms alternative complexes with the other two proteins, with different functions (Fig. 3). The RecOR complex facilitates the binding of RecA protein to SSB-coated DNA (Umezu & Kolodner 1994), and prevents the end-dependent disassembly of the RecA filament (Shan *et al.* 1997). The RecFR complex binds primarily to double-stranded DNA and can prevent excessive extension of the filament into the adjoining duplex DNA (Webb *et al.* 1997). If this activity of RecFR faithfully mimics a function of the proteins *in vivo*, some mechanism would be necessary to position the RecFR complexes near the gaps where they are needed. Neither RecF protein or RecFR complexes bind specifically to the ends of DNA gaps *in vitro* (Webb *et al.* 1997; B. Webb & M.M. Cox, unpublished data). A stalled replication complex would be positioned in part in the exact location where the RecFR complex would be required to modulate RecA filament assembly, and an interaction of RecFR with replication proteins is an intriguing possibility. The importance of the RecFOR proteins in a RecA filament assembly can be seen in the RecA-mediated induction of the SOS response, which is delayed in *recFOR* mutant cells (Hegde *et al.* 1995; Whitby & Lloyd 1995).

Following RecA-mediated DNA strand exchange, the crossover would be resolved as for double-strand break repair. The RuvABC proteins and the RecG helicase provide alternative pathways for the resolution of Holliday intermediates (Kuzminov 1996b). The RuvC protein is a Holliday junction resolvase (West

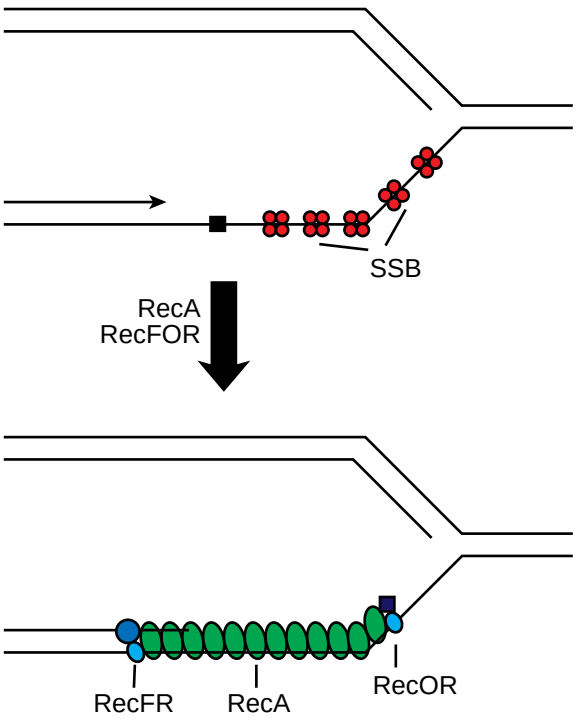


Figure 3 Postulated role of RecFOR proteins in RecA filament assembly at a DNA gap. RecA protein does not readily nucleate the filament assembly on single-stranded DNA that is prebound with SSB. A complex of the RecOR proteins facilitates this nucleation and also prevents the end-dependent disassembly of the filament. Filament extension at the other end is halted by a complex of the recFR proteins, bound to duplex DNA near the gap.

1994), and is one of several enzymes with this activity in *E. coli* (Sharples *et al.* 1994). A deficiency in RecG protein greatly increases the sensitivity to DNA damage and the recombination defects conferred by *ruvABC* mutations (Lloyd 1991; Kuzminov 1996b). The RecG protein has helicase activity which promotes the migration of a DNA branch or crossover in the direction opposite to that promoted by the RecA protein (Lloyd & Sharples 1993; Whitby *et al.* 1993; Kuzminov 1996b). The action of RecG following RecA-mediated DNA strand exchange and DNA repair could move the crossover backwards and ultimately reconstruct the framework of a replication fork without the action of RuvC or a similar Holliday junction resolvase (Kuzminov 1996b). The RuvA and RuvB proteins also displace RecA protein from DNA under some *in vitro* conditions, suggesting an additional function for these proteins *in vivo* (Adams *et al.* 1994). However, the RecFOR proteins also appear to modulate RecA filament assembly and disassembly, and the fate of RecA filaments may turn out to be a more complex affair involving interactions with the RuvAB, RecFOR, and perhaps other proteins.

Two additional points are worth noting. First, the pathways outlined in Fig. 1 are not necessarily as distinct as shown. For example, some evidence exists that the RecF pathway functions may participate in RecBCD-mediated recombination pathways under at least some conditions (Miesel & Roth 1996). Second, the resolution of the recombination crossover in either pathway can occur in two ways. One of the possibilities leads to the formation of a chromosome dimer (Fig. 4). If recombinational DNA repair is required as often as already postulated, then the formation of dimeric chromosomes should represent a barrier to the segregation of chromosomes at cell division in a large fraction of cells, even under normal growth conditions. This is actually observed, and the problem is addressed by the XerCD site-specific recombination system (step 4 below).

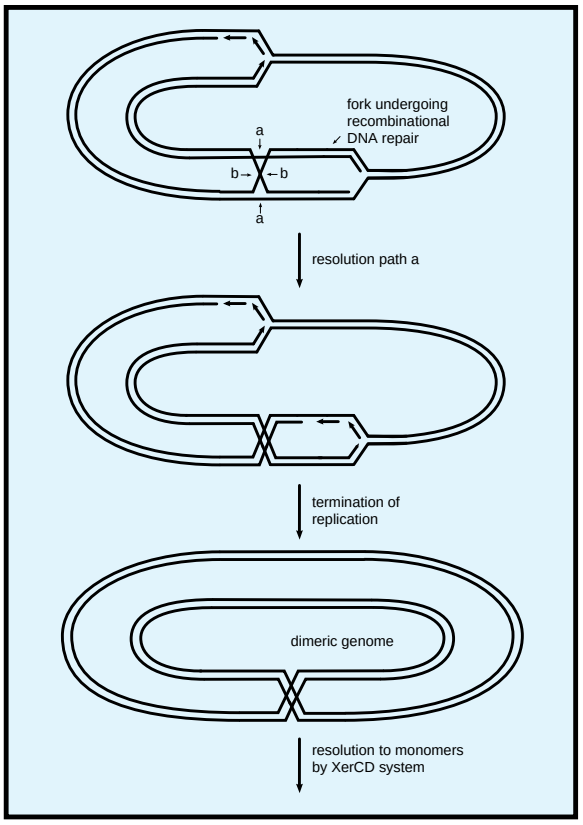


Figure 4 Resolution of a recombination crossover during recombinational DNA repair of a circular chromosome can lead to dimerization of the chromosome.

Step 3: replication restart following recombination

Just as replication resumes some 30–40 min after the cell is exposed to heavy UV irradiation, replication must resume after recombination under normal growth conditions. The resumption of DNA replication does not rely on new initiations at oriC, since replication recovers even in a ts *dnaA* mutant at the restrictive temperature (Jonczyk & Ciesla 1979; Khidhir *et al.* 1985). Much genetic evidence links the ϕX174-type primosome to this function. As already noted, primosome components such as the PriA protein are not required in oriC-dependent replication. Instead, *priA* mutations confer deficiencies in recombination and recombinational DNA repair (Kogoma *et al.* 1996; Sandler *et al.* 1996). Following recombination, a new replication fork is assembled in an origin-independent manner, mediated in this case by the ϕX174-type primosome. The genetics suggests a complex interplay between recombination and replication functions in replication restart, although the information available on these interactions is limited. The new replication forks are distinct from those originating at oriC, at least to the extent that they possess a different primosome for lagging strand DNA synthesis. In effect, the ϕX174-type primosome plays a central role in bacterial DNA metabolism, and might be referred to more descriptively, economically and accurately as the repair primosome.

Formation of the repair primosome (as defined in studies of ϕX174 replication) requires the PriA, PriB, PriC, DnaC and DnaT proteins, along with DnaB and DnaG. The primosome normally assembles in a stepwise manner at the primosome assembly site (PAS) in ϕX174 and ColE1 origin DNA (Allen & Kornberg 1993; Ng & Marians 1996a,b). PAS is recognized by PriA protein, and the PriA-PAS complex is stabilized by PriB protein. DnaT protein then joins the complex. DnaB protein is transferred from a DnaB-DnaC complex in an ATP-dependent reaction to form a preprimosome containing PriA (2 monomers), PriB (two dimers), DnaB (one hexamer) and DnaT (one monomer). PriC (one monomer) is also present, although the stage at which it associates is not known. The preprimosome can translocate along the DNA in a reaction requiring ATP hydrolysis. The transient interaction of DnaG primase with the preprimosome produces a completed primosome and leads to the synthesis of RNA primers (Allen & Kornberg 1993; Ng & Marians 1996a,b).

Analysis of the now-completed sequence of the *E. coli* genome (Blattner *et al.* 1997) has revealed an apparent absence of PAS sites related to those found in the ϕX174 and ColE1 plasmid origins. Instead, the PriA protein binds to branched recombination intermediates such as D-loops in order to nucleate the assembly of a primosome for replication restart following recombination (McGlynn *et al.* 1997). The PAS sites found in the ϕX174 and ColE1 genomes can take up structures mimicking branched DNA in some respects, and it has been postulated that they represent an evolutionary device to expropriate the repair primosome to effect the initiation of viral or plasmid replication (McGlynn *et al.* 1997).

Genetic studies have suggested numerous connections between recombination functions and replication restart, although the underlying biochemical mechanisms in most cases remains obscure. The RecG and PriA proteins bind to the same branched DNA structures, and may compete for binding sites *in vivo* (McGlynn *et al.* 1997). A deficiency in RecG protein can be suppressed by mutations that reduce the activity of PriA protein, indicating that the balance between these proteins is important *in vivo* (Al Deib *et al.* 1996). The activity of the RecF protein, but not the RecOR proteins, is essential in *priA* null mutant cells (Sandler 1996), suggesting that the RecF and PriA functions overlap. Mutations that eliminate PriA activity are suppressed by a number of *dnaC* mutations, which presumably permit the loading of DnaB helicase and some kind of primosome complex in the absence of PriA. Many recombination functions, including the RecA, RecF and RecR proteins, are needed for replication restart (Skarstad & Boye 1988; Smith & Wang 1989; Livneh *et al.* 1993; Courcelle *et al.* 1997; Kogoma 1997). It is the recombination activities of RecA that are required for recombinational DNA repair, as opposed to the function of RecA protein in the induction of SOS (Smith & Wang 1989; Roca & Cox 1990; Asai *et al.* 1993). In general, it is not clear whether the requirement reflects a direct interaction between the recombination proteins and the replication apparatus, or the fact that recombination must simply precede replication restart. In the case of the RecF and RecR proteins, it is tempting to speculate about the possibility of a direct interaction with the replication proteins. The recF and recR genes are both found in operons that include the genes for some subunits of DNA polymerase III and other replication functions (Ream *et al.* 1980; Flower & McHenry 1991; Perez-Roger *et al.* 1991)—perhaps a coincidence of evolution and perhaps not.

Step 4: Resolution of dimeric chromosomes by the XerCD site-specific recombination system

Recombinational DNA repair involving nearly half of the replication forks originating at oriC should result in the formation of numerous dimeric chromosomes via a resolution of the recombination crossover (Fig. 4). The conversion of chromosome dimers to monomers is mediated by a specialized site-specific recombination system in *E. coli*, the XerCD system (Sherratt *et al.* 1995). Mutations that inactivate the XerCD site-specific recombinase, or its chromosomal binding site called *dif*, lead to a lengthening of the average cell, severe filamentation of a substantial fraction of the cells, and abnormal nucleoids (Blakely *et al.* 1991; Kuempel *et al.* 1991). These and other observations have led several groups to propose that the function of the XerCD system is to convert chromosomal dimers, resulting from recombination, in to monomers (Sherratt *et al.* 1995; Kuzminov 1996a). The requirement for Xer-mediated recombination is reduced greatly in *recA*$^{-}$ cells, reinforcing a connection between the XerCD system and recombinational DNA repair (Blakely *et al.* 1991; Kuempel *et al.* 1991). Using a variety of growth conditions, there appears to be a direct relationship between the levels of recombinational DNA repair and the requirements for Xer-mediated site-specific recombination (D. Sherratt, personal communication).

Within the *E. coli* chromosome, the dif site functions only when positioned within a relatively short region near the terminus of replication (Leslie & Sherratt 1995; Tecklenburg *et al.* 1995; Cornet *et al.* 1996; Kuempel *et al.* 1996). In this location, it can be replaced by some other site-specific recombination systems (Leslie & Sherratt 1995). When replication is complete, site-specific recombination at dif sites might proceed repeatedly, with final chromosome segregation occurring at a point where the chromosomes are monomeric. Such a model works if the dif sites are positioned within the last part of the chromosome to be separated at segregation (Baker 1991). The detailed *in vivo* function of the XerCD system may also rely upon the properties of at least some sequences surrounding dif (Cornet *et al.* 1996).

One of the most striking effects of mutations that inactivate the XerCD system is the sheer number of cells affected. The large fraction of cells with defects in chromosomal segregation is consistent with the idea that a large fraction of replication forks originating at oriC undergo recombinational DNA repair. Certain bacterial plasmids also contain sites at which the XerCD enzymes can function and correct the dimerization of plasmids brought about by homologous genetic recombination (Cornet *et al.* 1994). Excluding the special circumstances of conjugation and transduction, little homologous recombination occurs within bacterial cells in the absence of DNA damage, even with multicopy small genomes (Feng *et al.* 1991; Hays & Hays 1991; Cornet *et al.* 1994; Feng & Hays 1995; Touati *et al.* 1995). The evolution of plasmid sequences to take advantage of a host system to resolve dimers provides yet another indication of the central place of recombinational DNA repair in DNA metabolism.

A unified view of DNA metabolism in bacteria

The replication, recombination and repair of DNA in bacteria have often been presented as distinct topics. A useful integration of these processes is evident in a consideration of recombinational DNA repair, as reflected in the related work of many laboratories (Zavitz & Marians 1991; Cox 1993; Livneh *et al.* 1993; Friedberg *et al.* 1995; Sherratt *et al.* 1995; Kuzminov 1996a; Kogoma 1997; Roca & Cox 1997). In many respects, the study of bacterial DNA metabolism is taking on the integration long evident in the study of the DNA metabolism of bacteriophage T4 (Karam 1994). The outlines of recombinational DNA repair pathways (Fig. 1) offer a way to integrate a variety of phenomena, in addition to those already discussed.

A system mediating the efficient origin-independent replication restart as a part of frequent recombinational DNA repair should be manifested in elevated levels of detectable origin-independent DNA replication under conditions in which DNA is damaged and/or recombination is stimulated. Phenomena of this kind have been reported, with a particularly detailed characterization contributed by Kogoma and colleagues (Kogoma 1997).

The initiation of replication at oriC is dependent on the DnaA protein and requires new RNA and protein synthesis (von Meyenburg *et al.* 1979). DNA replication occurring in the absence of protein synthesis is called stable DNA replication (SDR)(Kogoma & Lark 1970, 1975). Stable DNA replication is normally repressed, but can be induced by conditions that invoke the SOS response, giving rise to induced stable DNA replication (iSDR) (Kogoma *et al.* 1979). Other forms of stable DNA replication observed in some mutant backgrounds will not be considered here (see Kogoma 1997).

Although iSDR is presented as an alternative or back-up mechanism for the initiation of DNA replication, the links between iSDR and recombinational DNA repair are very evident and acknowledged (Asai *et al.* 1994a; Kogoma 1997). The requirements for iSDR are almost identical to those for recombinational DNA repair and its associated phenomena, such as replication restart following UV irradiation. The chromosomal origin oriC is not needed. The recombination proteins RecA, RecBC, RecF and RecN are required for iSDR, as are the repair primosome components PriA, DnaB, DnaC, DnaG and DnaT (Kogoma 1997). A potential requirement for the PriB and PriC proteins has not been explored. The same proteins are intimately associated with recombinational DNA repair. One distinction that has been noted between iSDR and the repair-associated phenomenon of replication restart is the lack of a requirement for RecBC protein in the latter (Kogoma 1997). However, given the multiple pathways for repair prior to replication restart, and the possibility that oriC-mediated replication could contribute to replication restart, the requirements for recBC could well be a function of the conditions used to halt replication and measure its recovery.

The relationships extend to mechanisms of initiation. The mechanism of iSDR initiation is proposed to involve the processing of a double-strand break by RecBCD, followed by a RecA-mediated invasion such as that which is envisioned for double-strand break repair in Fig. 1 (Kogoma 1997). The iSDR is considered a special form of recombination-dependent replication. In spite of a reported lack of a requirement for oriC (Kogoma 1997), iSDR cannot be considered a complete back-up replication system. A partially replicated chromosome (which must ultimately come from oriC-mediated initiation) is essential to provide the invading DNA needed to initiate iSDR via recombination. *De novo* replication of a monomeric genome by iSDR is not envisioned.

The one property of iSDR that is difficult to rationalize within the context of recombinational DNA repair is the observed initiation of iSDR at defined origins (Magee *et al.* 1992; Asai *et al.* 1993; Asai *et al.* 1994b). These origins have been localized very close to oriC and the ter (termination) regions of the chromosome. The stimulation of recombination and accompanying replication in the ter region might simply reflect stalled replication forks as already described. The origins near oriC (called oriM (Kogoma 1997)) are more difficult to rationalize unless some specialized mechanism exists to introduce double-strand breaks in DNA in the oriM region. The requirement for defined origins is not absolute. The real requirement for iSDR is the introduction of a double-strand break in replicated DNA and the presence of a nearby *chi* site (Asai *et al.* 1994a). Livneh (Livneh *et al.* 1993) has suggested a unified view in which iSDR and replication restart following UV irradiation represent related and overlapping responses to DNA damage. Under this response, DNA lesions are overcome either by the activation of DnaA-independent origins (oriM) and recombinational repair of stalled replication forks as outlined above. The activation of DnaA-independent origins is simply a matter of providing a double-strand break that can be exploited by the vigorous resident recombinational repair system.

The impact of recombinational DNA repair also extends to models for homologous genetic recombination during conjugation. As already noted, the mechanisms of conjugational recombination are likely to reflect the structure of the DNA substrates presented to the cell. The observation of extensive replication associated with conjugational recombination (Smith 1991) can be viewed as a manifestation of the very active system for origin-independent replication restart within recombinational repair. The connections can also be seen in the requirements for functions such as PriA protein in conjugational recombination (Kogoma *et al.* 1996).

Finale

The effects of a wide range of mutations in DNA metabolism functions lead directly or indirectly to the same conclusion. Recombinational DNA repair is a frequent occurrence in cells, even under standard growth conditions. As a central process in DNA metabolism, recombinational DNA repair can be viewed as the primary function guiding the evolution of the recombination enzymes, the repair primosome, and the XerCD site-specific recombination system. DNA damage is the sometimes unseen cause of phenomena affecting every aspect of DNA metabolism.

Acknowledgements

I am grateful to Ken Marians, Andrei Kuzminov and David Sherratt for helpful email discussions during the preparation of this manuscript. Described work from the author's laboratory is supported by grants GM32335 and GM53575 from the National Institutes of Health.

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