Critical Reviews in Biochemistry and Molecular Biology, 43:347–370, 2008

ISSN: 1040-9238 print / 1549-7798 online
DOI: 10.1080/10409230802485358

informa
healthcare

Mechanisms of Recombination: Lessons from *E. coli*

Nicole S. Persky and Susan T. Lovett
Department of Biology and Rosenstiel Basic Medical Sciences Research Center MS029, Brandeis University, Waltham, MA, USA

The genetics and biochemistry of genetic recombination in *E. coli* has been studied for over four decades and provides a useful model system to understand recombination in other organisms. Here we provide an overview of the mechanisms of recombination and how such processes contribute to DNA repair. We describe the *E. coli* functions that are known to contribute to these mechanisms, step by step, and summarize their biochemical properties in relation to the role these proteins play *in vivo*. We feature areas of investigation that are newly emerging, as well as work that provides a historical perspective to the field. Finally, we highlight some of the questions that remain unanswered.

Keywords genetic recombination, DNA repair, genetic rearrangements, replication fork repair

INTRODUCTION

Genetic recombination between homologous DNA sequences permits rescue of broken or incompletely replicated chromosomes in all organisms. The core proteins in homologous recombination are related in the three domains of life, RecA in bacteria, RadA in archaea and Rad51 in eukaryotes, suggesting that homologous recombination is an ancient process and is likely to be similar in all cells. Nowhere is the process of genetic recombination better understood than in the bacterium *Escherichia coli*. This review is designed to present an introduction to the mechanisms of recombination and a portal to more detailed information concerning the biochemical and genetic properties of *E. coli*'s recombination proteins. In particular, we will feature the biological consequences of these functions. Finally, we will address some of the persistent or emerging questions that remain to be answered.

RECOMBINATION MECHANISMS AND THEIR ASSOCIATION WITH DNA REPAIR

The Idea of Recombination as a Form of DNA Repair

The connection between homologous recombination functions and DNA repair was evident from the first recombination mutants isolated in *E. coli* in what would become known as the *recA*, *recB*, and *recC* genes (Clark and Margulies, 1965; Howard-Flanders and Theriot, 1966; Emmerson and Howard-Flanders, 1967). Mutants which failed to yield genetic recombinants after Hfr crosses or P1 transduction were found to be uniformly sensitive to ultraviolet light, which produces replication-blocking lesions, and to X-irradiation, which produces DNA strand breaks. Recombination mutants also poorly survive treatment with reactive oxygen species (including hydrogen peroxide), alkylating agents (including MMS), cross-linking agents (including Mitomycin C and cisplatin) and replication inhibitors (hydroxyurea and azidothymidine) (Sargentini and Smith, 1986; Linn and Imlay, 1987; Beam *et al*., 2002; Nowosielska *et al*., 2004; 2006; Foti *et al*., 2005). This property, sensitivity to DNA damage, is seen for many different recombination mutants in all genetically tractable organisms. It can be argued that the emergence of homologous recombination derives from its primary importance for repair of damaged chromosomes, especially damage that arises inevitably during replication.

We now appreciate that three types of recombination reactions can mediate the repair of damaged chromosomes: broken fork repair, double-strand break repair and recombinational gap-filling repair. The reader is directed to a number of recent reviews on this topic for more details (Kuzminov, 1999; Cox, 2001; Cromie *et al*., 2001; Wyman *et al*., 2004; Kreuzer, 2005; Michel *et al*., 2007). It is useful to consider these mechanisms prior to discussion of specific recombination enzymes to understand how these functions may be specialized for one or more of these repair reactions. Recent evidence suggests that recombinational repair is particularly important during DNA replication; in higher organisms the lack of recombination causes accumulation of catastrophic DNA breaks during replication resulting in cell death (Lim and Hasty, 1996; Tsuzuki *et al*., 1996). In *E. coli* the inactivation of recombination proteins leads to

Address correspondence to: Susan T. Lovett, Department of Biology and Rosenstiel Basic Medical Sciences Research Center MS029, Brandeis University, Waltham, MA 02454-9110, USA. E-mail: lovett@brandeis.edu

reduced cell viability (Capaldo and Barbour, 1975) and, if the burden of DNA lesions is elevated, lethality (Kouzminova *et al*., 2004).

Common to all these recombinational repair mechanisms is the pairing of homologous DNA strands from two different molecules. This involves DNA strand transfer to form "heteroduplex" DNA, which, through base-pairing interactions, provides precision to the joining processes that will lead to repair. The RecA protein plays a central role in this synapsis reaction. In addition, another common feature of these recombinational mechanisms is the formation of branched DNA structures that must ultimately be resolved into duplex DNA molecules for the completion of recombination.

Broken Fork Repair

The first reaction we will discuss allows a broken replication fork (also known as a "collapsed" fork) to be repaired, restoring an intact fork upon which replication can be reinitiated. Such broken forks can arise by replication on a nicked template, as has been demonstrated *in vivo* (Kouzminova and Kuzminov, 2004). Alternatively, stalled replication forks may be severed by endonucleases (Michel *et al*., 2007). Breakage of the fork appears to be stimulated by difficulties in replication (such as those afforded by mutations to the replisome complex.) In the absence of recombination to repair a broken fork, the broken chromosome would be degraded.

In this model (Figure 1), one strand of the broken arm is digested ("resected") to reveal a 3′ single-stranded tail (Figure 1B). This end invades the homologous duplex, causing displacement of one of the two strands and formation of a branched intermediate known as a D (displacement)-loop (Figure 1C). Extension of the heteroduplex region (see below) between the invading strand and its partner, with concomitant displacement of the other strand, moves the junction in a process known as "branch migration" (Figure 1C'). [Note that branch migration in the opposite direction will decrease the region of heteroduplex and ultimately dissolve the joint; see Figures 5B and 6B.] Branch migration that extends the heteroduplex into the duplex region of the invading DNA molecule produces a 4-strand branched structure known as a Holliday junction. Cleavage or "resolution" of the appropriate strands of these branched molecules, either the D-loop (Figure 1D) or Holliday junction (Figure 1D') and ligation of strands restores a fork structure.

Broken fork repair has been well studied in *E. coli*, where the process has been called "recombination-dependent replication" (RDR) (Kogoma, 1996), or double-strand end repair (DSE repair) (Cromie *et al*., 2001). After the completion of replication fork repair, the replisome must be reassembled to reinitiate replication. In *E. coli*, special replication restart factors are required to reload the replisome's DNA helicase after such repair reactions (Marians, 2004). In eukaryotes, this type of mechanism constitutes a process known as "break-induced replication" (Symington, 2002; McEachern and Haber, 2006).

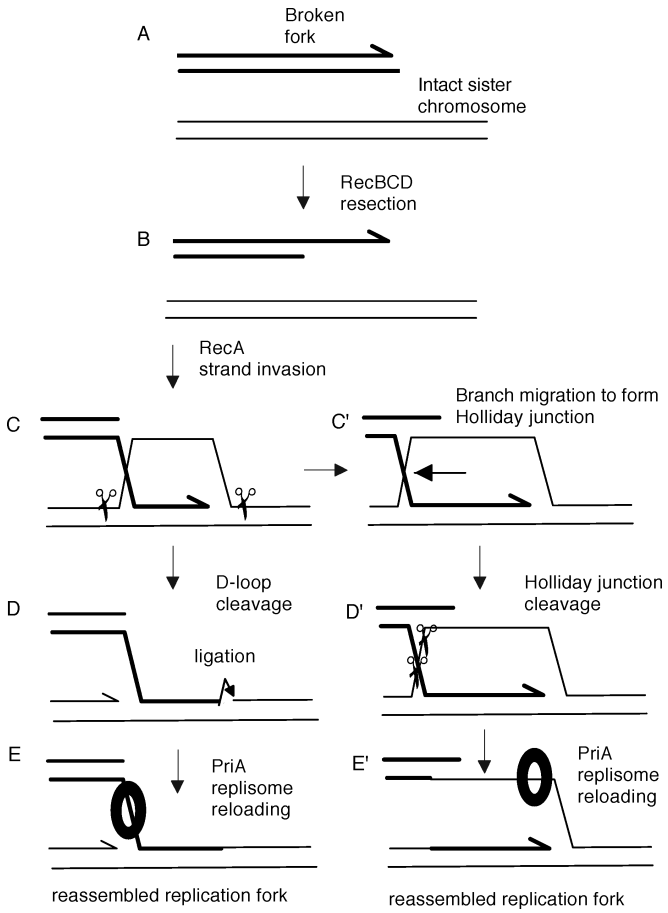

FIG. 1. Broken fork repair–a one-ended repair event. (A) The broken chromosome is resected to reveal 3' single-strand DNA by RecBCD. (B) RecA is loaded onto the ssDNa and catalyzes strand invasion at the region of homology in an intact sister chromosome. (C) This produces the structure known as the "D-loop". (C') Branch migration of the D-loop can extend the heteroduplex region and generate a 4-crossed strand intermediate, the Holliday junction. The branched structures are resolved by D-loop cleavage (D) or Holliday junction cleavage (D'). (E) and (E') Fork structures formed by resolution. PriA re-establishes a replication fork by loading of DnaB helicase on the lagging strand template.

Double-Strand Break Repair

In another mechanism, frank double-strand breaks can be repaired by interactions with a homologous chromosome. This differs from the first in that two ends, rather than one, are recruited into the repair reaction, but is similar in other respects. Because *E. coli* lacks a pathway to join non-homologous ends, homologous recombination is the only means to salvage broken chromosomes.

The double-strand break repair model (Szostak *et al*., 1983), proposed to explain yeast meiotic recombination, is a useful starting point (Figure 2). A broken end invades to

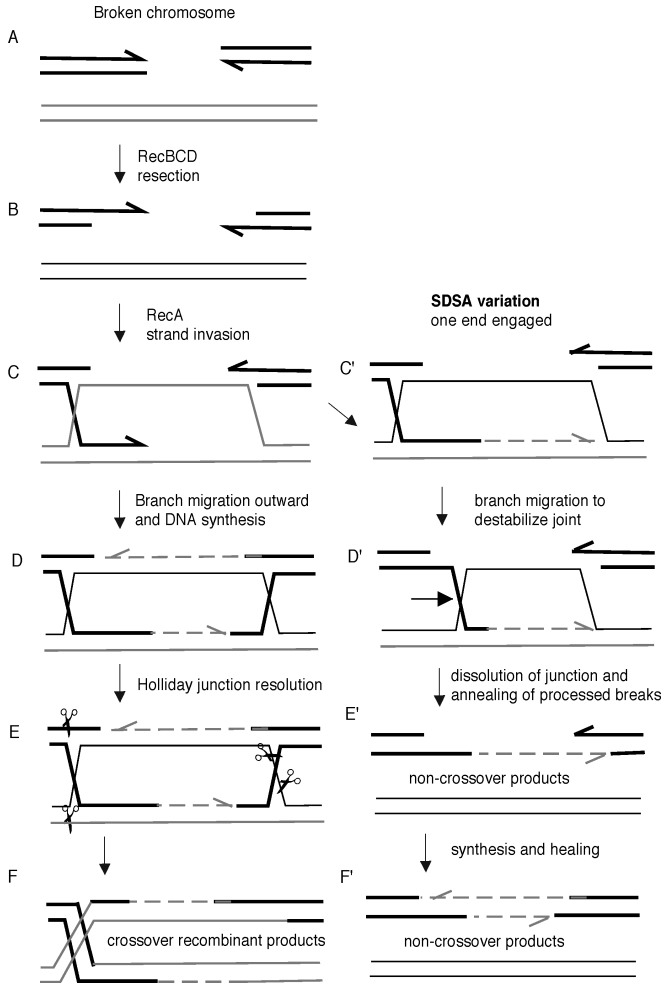

FIG. 2. Double-strand break repair – a two-ended repair event. (A) A broken chromosome. (B) Resection by RecBCD to reveal 3′ single-strand ends. (C) RecA catalyzes strand invasion, potentially of both ends of the break. (D) DNA synthesis from the invading strands regenerates any missing information from the break, and produces a two-Holliday junction intermediate. (E) Holliday junctions are resolved by cleavage. Shown is a cleavage pattern, in a different sense at each junction, that will give rise to crossover products. Non-crossovers can be produced by cleavage in the same sense at each junction, not shown. (F) Mature crossover products, with exchange of DNA flanking the site of repair. (C') In the SDSA ("synthesis dependent strand annealing) variation of the mechanism, one broken end is engaged, and primes DNA synthesis. (D') Branch migration in the direction to destabilize the joint. (E') Dissolution of the joint permits the two broken strands to anneal. (F') The second end primes synthesis from the annealed intermediate to seal to the two strands. Note that genetic information can be transfered to the broken chromosome from the synthesis in part C', although non-crossover products are exclusively formed.

form a D-loop or Holliday junction; the displaced strand in this intermediate can recruit and pair with the second resected broken end. Lost information from either broken end can be restored by DNA synthesis in this paired intermediate. Two Holliday junctions are formed by this process (a feature clearly demonstrated for yeast meiotic recombination intermediates; see Schwacha and Kleckner, 1995). Their resolution yields two intact chromosomes, which may or may not have exchanged the flanking information, ("crossover" or "non-crossover" products). Because *E. coli* possesses a circular chromosome, crossovers between sister chromosomes will form a dimeric circular chromosome. A site-specific recombination system, reliant on protein XerCD at the *dif* site near the terminus, resolves such dimeric chromosomes into monomers for proper segregation to daughter cells (Blakely *et al*., 1993).

In a variant version of double-strand break repair, known as "synthesis-dependent strand annealing" (SDSA), capture of the two broken ends occurs sequentially. The synapsis of the second end is dependent on successful synapsis of the first end. When the first end invades, DNA synthesis from this invading strand extends it beyond the point of the original break. Dissolution of this strand exchange intermediate frees the invading strand so that it may now anneal to the other processed broken end. This accomplishes repair of the break, without the requirement for cleavage of any branched structure, and so does not yield crossover products. The SDSA variation of double-strand break repair is believed to underlie mating-type switching in yeast (Haber, 1998), repair of transposon-induced breaks in *Drosophila* (Nassif *et al*., 1994), and extreme resistance to radiation-induced breaks in the bacterium, *Deinococcus radiodurans* (Zahradka *et al*., 2006).

In *E. coli*, DSB repair has been studied by introduction of double-strand breaks from sequence-specific endonucleases and excision of transposable elements. Ionizing radiation also produces DSBs, a particularly lethal lesion to bacteria, but it is important to remember that other lesions such as base and sugar oxidation and single-strand breaks are also produced by such radiation. In most of these repair events, the partner for repair is the intact sister chromosome, although recombination with other chromosomes sharing homologous sequences can be detected.

In practice, two-ended double-strand break repair can be difficult to distinguish from one-ended replication fork break repair. Double-strand break repair is not necessarily associated with the replication fork, although many agents that produce DSBs will produce broken forks. (For example, nicks or gaps are converted to DSBs at the fork by replication). Transposon excision is also targeted to newly replicated DNA and coordinated with the passage of the replication fork (Roberts *et al*., 1985; Yin *et al*., 1988), presumably so that the breaks may be more efficiently repaired. DSB repair also differs from replication fork break repair in that repair of two ends could be coordinated, so may require specific functions not necessary for single-end break repair. Alternatively, repair of the two broken ends could involve independent and uncoordinated one-ended repair, with each event

establishing a replication fork. The "classical" recombination measured after Hfr conjugation or P1 transduction involves ds-DNA ends and is thought to occur in the latter way, with each end of the transferred DNA fragment independently repaired to establish a new replication fork (Smith, 1991; Kogoma *et al*., 1996).

Gap-Filling Recombinational Repair

In the third type of DNA repair associated with homologous recombination, single-strand DNA gaps can be filled by strand transfer reactions between sister or homologous chromosomes (Figure 3). A daughter-strand gap may be produced by incomplete replication, in which synthesis was blocked by template or nascent strand damage, DNA secondary structure or tightly bound proteins. Although daughter strand gap repair (DSGR) was one of the first proposed mechanisms for recombinational repair (Rupp *et al*., 1971), it is the most poorly understood (Smith, 2004). The recombinational gap repair process has been best studied in *E. coli* after UV irradiation, where it has been called "post-replication repair." Gap-filling was historically assayed by conversion of low molecular weight newly-replicated DNA into larger species by thymidine labeling and denaturing sucrose gradient centrifugation (Rupp and Howard-Flanders, 1968; Rupp *et al*., 1971). Such studies demonstrated that newly replicated DNA became associated with parental DNA concomitant with repair, suggesting a recombinational mechanism for gap-filling. Newer studies involve the transformation of synthetically produced gapped plasmid molecules in which a replication-blocking lesion has been introduced into the gap (Berdichevsky *et al*., 2002). Although recombination clearly contributes to gap-filling and in *E. coli* is arguably the predominant mechanism of gap-filling (Berdichevsky *et al*., 2002), note that other lesion tolerance mechanisms can also fill gaps, such as translesion DNA synthesis (Goodman and Tippin, 2000). This latter mechanism is sometimes referred to as "error-prone post-replication repair" because of the involvement of low-fidelity polymerases and its tendency to produce mutations.

A model for recombinational gap-filling is shown in Figure 3. The single-strand region of the gap pairs with its complement on an intact DNA molecule. This strand invasion may be aided by displacement by one or both of the strands that flank the gap. Because this synapsis does not involve a free end, this

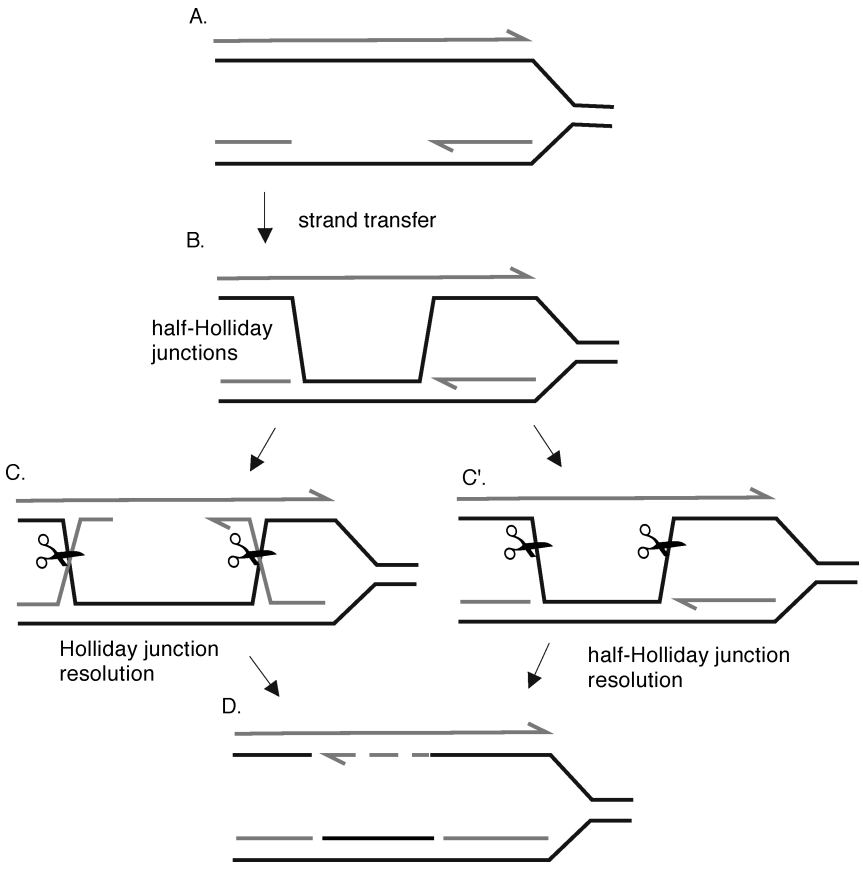

FIG. 3. Recombinational gap-filling repair. No dsDNA ends involved. (A) Gapped substrate molecule. (B) Strand exchange by RecA produces a joint molecule. Note that this pairing must be accompanied by topological changes to interwind the DNA strands. (C) Recruitment of ends flanking the joint produces Holliday junctions that can be resolved by cleavage. (C') Alternatively, the crossed strand of the half-Holliday junctions can be cleaved. (D) Recombinant product, with gap filled.

pairing presents topological problems that may be relieved by topoisomerases. DNA synthesis primed from the ends replaces the transferred strand, setting up a branched molecule, consisting of one or more crossed strands. Cleavage of these restores two duplex molecules and the gap is filled. Like the double-strand break model, cleavage of these junctions can also yield crossover or non-crossover products.

Recombination at gaps differs from the above double-strand break recombination mechanisms in the nature of the initiating substrate. It is therefore not surprising to find that it depends on a different set of proteins from those that mediate DSB repair. Gap-filling may not necessarily require resection, although some small gaps may have to be widened to allow cooperative binding of recombination proteins (see below). In addition, single-strand gaps left by incomplete replication are likely to be bound stably by single-strand DNA binding protein, SSB, which must be removed to allow access to recombination proteins.

Early genetic studies underestimated the contributions of the gap-filling mechanism of recombination. This is, in part, because the classical methods of measuring recombination in *E. coli* after conjugation and transduction, involving dsDNA ends, report DSB-mediated recombination and not that associated with gap-filling mechanisms. In repair of DNA damage, recombinational gap repair can be difficult to distinguish in practice from repair of double-strand breaks. Many agents that produce gaps, such as UV irradiation, will also produce double-strand breaks. In the repair of DNA damage, we likely underestimate the contribution of gap repair to survival: gaps that fail to be repaired are likely converted to double-strand breaks and repaired by DSB-specific repair mechanisms.

Conclusion

These idealized mechanisms of recombinational repair provide us with a framework to understand the proteins involved in different recombination reactions and their influence on different types of repair reactions and events involving genetic exchange.

RECOMBINATION PROTEINS AND THEIR FUNCTIONS

Introduction

In thinking about recombination mechanisms, it is useful to break down the process into three distinct steps in which the DNA substrates are processed prior to strand invasion, paired to form heteroduplex DNA intermediates and are matured to recombinant products. The first step, "pre-synapsis" involves the production of recombinogenic single-strand DNA and the cooperative binding of the strand exchange protein, RecA. The second-step, "synapsis" requires RecA-mediated homologous pairing, strand invasion and formation of heteroduplex intermediates. These heteroduplexes are subject to processing which may stabilize or destabilize these recombination intermediates. The third step "post-synapsis" or "resolution" involves the dissolution of intermediates, including any branched molecules involved in the process, removal of recombination proteins and formation of intact duplex DNA recombinant products. Reassembly of the replication fork may also be required post-synaptically. In all the aforementioned recombination mechanisms, RecA protein plays a central role. A number of these steps are fluid and can be catalyzed in alternative ways. In addition, some proteins appear to be specialized for particular recombination mechanisms: for instance, there are proteins involved in gap repair that are not required for double-strand break repair and vice versa.

In this context, we will introduce the functions in *E. coli* that have been implicated in genetic recombination and briefly summarize their biochemical properties. For more details of the biochemical mechanisms, please consult recent reviews (Cox, 2007b; Kowalczykowski, 2000). Additional discussion of the genetic properties of *E. coli* recombination mutants can be found dispersed in several reviews (Clark, 1973; 1991; Smith, 1987; Smith and Wang, 1989; Clark and Sandler, 1994; West, 1997; Kuzminov and Stahl, 2005).

A. Presynapsis

To prepare for recombination, DNA substrates are processed by nucleases and helicases to reveal single-strand DNA regions, which are bound by the central strand exchange protein RecA. *In vivo*, RecA is actively loaded by at least two distinct mechanisms involving RecBCD or RecFOR. This filament both protects the DNA and potentiates subsequent synapsis and strand exchange. A number of other proteins modulate the stability of the RecA helical presynaptic filament. Special structural proteins may also coordinate or tether DNA ends.

Single-Strand DNA: the Signal for Recombination

Persistent single-strand DNA in the cell is recombinogenic. Although ssDNA is produced transiently during replication, its persistence signals the presence of gaps produced by incomplete replication. Binding of RecA to this ssDNA (see below) will initiate the recombination process. Persistent ssDNA may be bound with single-strand DNA binding protein, SSB, which must be removed and replaced with RecA.

Double-strand breaks in DNA, other lesions that require recombination to be repaired, must be converted to partial single strands to initiate recombination. In this case, single-strand DNA is produced actively by exonuclease digestion of one of the two strands, a process known as resection. Once the ends are single-stranded, they will bind RecA protein to initiate the homology search process (see below.) Because a 3′ end can prime new DNA synthesis, thereby aiding the recombination reaction and stabilizing the heteroduplex joint, (see Figure 5F below) resection of DSBs appears to occur universally in the 5′ to 3′ direction, to reveal a 3′ ssDNA tail at the break site.

RecBCD and Resection of DSBs

Double strand breaks are processed into recombinogenic single strands by the RecBCD nuclease. The RecBCD nuclease

is a complex enzyme that couples ATP-dependent DNA unwinding to DNA digestion (see reviews by Smith, 2001; Spies, 2005). RecBCD is a particularly potent exonuclease, degrading hundreds of bases per second from a double-strand end. The voracity of its nuclease is thought to act as a defense against viral infection and replication; many bacteriophages with linear genomes survive by encoding a specific inhibitor of RecBCD, the most well-known of which is the Gam protein of bacteriophage lambda (Enquist and Skalka, 1973). RecBCD's nuclease activity is tempered when the enzyme encounters a specific octamer DNA sequence (known as "Chi"). These sequences are over-represented in the *E. coli* genome in one orientation relative to the progression of the replication fork (Blattner *et al*., 1997). The interaction of RecBCD with Chi promotes recombination in its vicinity (Lam *et al*., 1974).

What happens at Chi? The structure of RecBCD (Singleton *et al*., 2004), bulk reaction and single-molecule studies of the complex have elucidated the following scenario. Unwinding of DNA is accomplished by two helicase motors in the RecB and RecD subunits, contacting the 5′ and 3′ ended strands, respectively (Taylor and Smith, 2003; Dillingham *et al*., 2005). Upon loading at a double-strand end, the RecD motor is faster than the RecB motor, causing a loop to extrude from the complex as the enzyme translocates on DNA. When the RecC subunit of the enzyme encounters Chi, forward movement arrests, the RecD motor disengages from the DNA and the complex moves more slowly, powered solely by the RecB subunit (Spies *et al*., 2005; 2007). This encounter also switches the dispensation of the strands relative to the nuclease active site in RecB (Dixon and Kowalczykowski, 1991; Dixon and Kowalczykowski, 1993). Whereas before Chi, the 3′ ended strand was extensively cleaved, after Chi recognition, digestion is targeted to the 5′ ending strand. This allows 3′ single-stranded tails to accumulate, which will become the strand invasion substrate when loaded with RecA (see section below).

This central role of RecBCD in converting double-strand ends to 3′ single-strand recombinogenic tails, we believe, causes all recombination in *E. coli* arising normally from double-strand breaks to be highly dependent on a functional RecBCD complex. This correlation is sufficiently strong that the dependence of a recombination or repair event on RecBCD has been used to infer the presence of a DSB end in the process.

The initiation of RecBCD nuclease activity on a DNA end requires it to be flush; RecBCD nuclease is inhibited by 3′ or 5′ single-strand tails of as little as 25 nucleotides (Prell and Wackernagel, 1980; Taylor and Smith, 1985). Therefore, in the processing of naturally-arising chromosome breaks by RecBCD, there may be a requirement for "end-blunting" through the action of single-strand DNA exonucleases. Effects on RecBCD nuclease activity on linear DNA phage suggest that single-strand exonucleases Exo I, RecJ and SbcCD (see below) play a role in potentiating DNA substrates for RecBCD (Thoms and Wackernagel, 1998).

Resection of DSBs by Other Nucleases in the Absence of RecBCD

In the absence of RecBCD, double-strand break repair and recombination involving double-strand ends appears to be inefficient. This efficiency can be improved by inactivation of 3′ exonucleases Exo I (also known as SbcB) and SbcCD, so-called "suppressors of *recBC*" (Kushner *et al*., 1971; Lloyd and Buckman, 1985; Gibson *et al*., 1992). This suggests that 3′ single-strand ends can be produced by enzymes other than RecBCD, but are unstable and subsequently digested by Exo I or SbcCD nuclease. Recombination at double-strand ends in *recBC sbc* mutant strains is catalyzed by the RecFOR pathway (see below), which provides an alternative mechanism for RecA loading. Resection of double-strand ends in this case is apparently accomplished by the combined action of RecQ helicase (Umezu *et al*., 1990), to unwind DNA at the ends, and RecJ exonuclease, a 5′ to 3′ single-strand DNA exonuclease (Lovett and Kolodner, 1989). Inactivation of RecJ and RecQ reduces the efficiency of recombination as measured by conjugation or transduction, both of which involve double-strand ends, in *recBC sbcB sbcCD* strains but not in *recBCD*$^+$ strains (Lovett and Clark, 1984; Nakayama *et al*., 1984; Lovett and Sutera, 1995).

Resection of Gaps?

Replication gaps are already single-stranded and so do not necessarily require exonuclease action to initiate recombination. However, some gaps may be too small to nucleate a RecA filament and may require some widening (Viswanathan and Lovett, 1998). The single-strand exonucleases Exonuclease I (Lehman and Nussbaum, 1964) and RecJ (Lovett and Kolodner, 1989) may be required for this step, in concert with a DNA helicase such as RecQ. After UV irradiation, RecJ in concert with RecQ promotes degradation of the nascent strand (Courcelle and Hanawalt, 1999) and defects in these genes lead to defects in recovery after irradiation (Courcelle *et al*., 2006). In a temperature-sensitive mutant of the fork helicase, DnaB, both Exo I and RecJ contribute to extensive degradation of the nascent strand that is induced upon shift to the non-permissive temperature (Belle *et al*., 2007). The relationship of this degradation to recombination, however, has not been demonstrated. In strains that accumulate recombination intermediates and fail to process them, both RecQ and RecJ promote toxic levels of recombination (Magner *et al*., 2007), suggesting that efficient initiation of recombination at spontaneous lesions (replication gaps?) requires both functions.

RecA: the Presynaptic Filament

As can be seen from the brief discussion of recombinational repair mechanisms, strand invasion and heteroduplex formation are central to these processes. These key reactions are catalyzed by the RecA protein. *E. coli* mutants in *recA* are strongly

deficient for many types of recombination processes, including a greater than 10,000-fold decrease in recombination after Hfr crosses, and are exquisitely sensitive to many forms of DNA damaging agents, including UV and ionizing-radiation (Clark and Margulies, 1965; Howard-Flanders and Theriot, 1966). RecA (reviewed in Bianco *et al*., 1998; McGrew and Knight, 2003; Bell, 2005; Cox, 2007a) is an ATPase that forms a right-handed helical filament on DNA, with a preference for single-strand DNA, which it binds at 3 nucleotides per RecA monomer. *In vitro*, RecA-ssDNA filaments can catalyze synapsis and strand exchange between homologous DNA molecules. The RecA filament also promotes the self-cleavage of the LexA repressor, an activity called "co-protease", and thereby controls the onset of the SOS regulatory response to DNA damage (Walker, 1987).

A recent crystallographic study reveals its structures bound to single or double-strand DNA (Chen *et al*., 2008). One of the properties of this RecA filament, conserved in its archaeal and eukaryotic orthologs, is the stretching of the DNA to a helical pitch about 50% greater than that found in B-form double-strand DNA (Yu *et al*., 2004), exposing the bases in a way that aids in the homology search process. Although initially, from RecA's ability to catalyze multiple important recombinational reactions *in vitro*, much focus of the field was on the properties of RecA itself, we now appreciate that the formation, dissolution and activity of the RecA filament is extensively regulated by other proteins in the cell. Much of this information is newly emerging (reviewed recently by Cox (2007b)).

Conflict and Cooperation Between RecA and Single-Strand DNA Binding Protein, SSB

Single-stranded DNA binding protein (SSB) is an abundant protein that plays a ubiquitous role in many DNA processing activities, including replication and repair (see reviews by Lohman and Ferrari, 1994; Meyer and Laine, 1990). SSB binds to single stranded DNA with high affinity, removing its secondary structure (Meyer and Laine, 1990). SSB exists as a tetramer in *E. coli* and wraps the DNA around itself in a fashion reminiscent of histones and dsDNA (Chrysogelos and Griffith, 1982; Meyer and Laine, 1990; Raghunathan *et al*., 1997). Its C-terminal domain is disordered in the crystal structure (Savvides *et al*., 2004) and is the site of interaction with various replication and recombination proteins.

SSB has both stimulatory and inhibitory actions on RecA strand exchange *in vitro*. If single-strand DNA is pre-bound by SSB, it inhibits RecA filament formation (Kowalczykowski *et al*., 1987). This inhibition appears to be at the initial nucleation step (Thresher *et al*., 1988; Joo *et al*., 2006). However, if RecA is allowed to bind first, SSB is stimulatory to strand exchange, presumably by its ability to melt secondary structures that would be inhibitory to RecA binding (Kowalczykowski and Krupp, 1987; Muniyappa *et al*., 1984). Once RecA has successfully nucleated on ssDNA, the presence of SSB does not inhibit RecA filament extension (Joo *et al*., 2006).

In vivo, the presence of SSB on recombinogenic ssDNA may direct its future processing. Mutants in *ssb*, including those in the C-terminal interaction domain, are defective in genetic recombination and highly sensitive to DNA damaging agents such as UV light (Glassberg *et al*., 1979; Wang and Smith, 1982; Quinones and Piechocki, 1987; Carlini and Porter, 1997). SSB interacts with a number of proteins, potentiating their activities (Molineux *et al*., 1974; Chase and Williams, 1986; Srivenugopal and Morris, 1986; Meyer and Laine, 1990; Umezu *et al*., 1993; Sandigursky and Franklin, 1994; Harmon and Kowalczykowski, 2001; Han *et al*., 2006; Shereda *et al*., 2007), including some that participate in recombination. A new concept is that, rather than acting merely as passive bystander, SSB may form a scaffold to assemble DNA processing enzymes. Although SSB has been assumed to protect DNA from nuclease attack, it dramatically stimulates the digestion of ssDNA by exonucleases Exo I (Molineux and Gefter, 1975) and RecJ (Han *et al*., 2006). SSB also recruits and stimulates the RecQ helicase (Umezu and Nakayama, 1993; Shereda *et al*., 2007). A genome-wide study shows a whole system of DNA modification enzymes, including toposimerases, helicases and nucleases, that interact with SSB (although potentially indirectly) (Butland *et al*., 2005) and there is much to be learned about how these interactions are managed.

Loading the RecA Filament by RecBCD

Although RecBCD's role in generating single-strand DNA for initiation has been long appreciated, its involvement in establishing the RecA presynaptic filament is a fairly recent discovery. The coordination of DNA digestion with RecA loading is a regulated feature of the RecBCD complex. As discussed in the previous section, the RecBCD nuclease degrades DNA until the octamer Chi site in DNA is encountered. Upon recognition of Chi RecBCD's nuclease activity is attenuated, its helicase activity is reduced two-fold, and preferential degradation of the 5′ strand of the duplex produces a 3′ single stranded DNA tail (Spies, 2005). Recognition of Chi also triggers the RecBCD complex to direct loading of RecA onto the emergent 3′ single stranded tail (Anderson and Kowalczykowski, 1997). This loading occurs through the RecB subunit C-terminal domain, which by itself makes stable complexes with RecA (Spies and Kowalczykowski, 2006). Through the active loading of RecA immediately after the creation of ssDNA end, RecBCD is able to exclude SSB from binding, explaining why the RecBCD pathway is not inhibited by SSB overproduction (Moreau, 1988). Supporting a role in RecA filament formation, RecBCD is required for full induction of the SOS response following DSB-inducing treatments such as nalidixic acid (Chaudhury and Smith, 1985).

Loading the RecA Filament by RecFOR

An alternative mechanism for RecA loading is provided by the RecFOR proteins and they provide a solution for RecA to gain access to SSB-coated single-stranded DNA. The *recF* gene

was discovered as a component of a recombination pathway, initially called the "RecF pathway," that functions independent of the RecBCD nuclease (Horii and Clark, 1973). Subsequent analysis identified two other genes, *recO* and *recR* (Kolodner *et al*., 1985; Mahdi and Lloyd, 1989), with somewhat similar effects, now defining the "RecFOR pathway". Genetic analysis suggested that RecF, RecO and RecR act in some common fashion to promote RecA function. These mutants have similar phenotypes, and their recombination and repair deficiency could be suppressed by a lambda protein, known as "*orf*" (Sawitzke and Stahl, 1992), or by mutations in RecA (known as "*srf*" for suppressor of recF) (Volkert and Hartke, 1984; Wang and Smith, 1986; Madiraju *et al*., 1988). One of these *srf* alleles, RecA803, altered strand exchange properties of RecA such that it became less susceptible to inhibition by SSB, leading the authors to propose that the RecF was involved in overcoming the inhibitory effects of SSB on RecA (Madiraju *et al*., 1988). Supporting this idea, overproduction of SSB leads to phenotypes similar to RecF deficiency (Moreau, 1988). A number of genetic studies suggested that the RecFOR pathway is specialized for single-strand gap repair (Wang and Smith, 1984; Galitski and Roth, 1997), in contrast to the RecBCD-promoted DSB repair, and one might imagine unrepaired replication gaps would be pre-bound with SSB. Mutants in RecFOR also are defective in induction of the SOS response by UV irradiation (Sandler and Clark, 1994), consistent with a defect in RecA filament formation on replication gaps caused by DNA polymerase arrest at UV lesions in the template.

In vitro characterization of these proteins confirms genetic expectations that RecFOR proteins act to control formation of the RecA filament and direct it to single-strand DNA gaps. Unlike RecBCD, these proteins appear not to work as a stoichiometric machine. Instead, RecF, RecO, and RecR work either independently or in pairs. Fulfilling the expectations from their genetic properties, the RecO and RecR proteins act as "mediators" to allow RecA to replace SSB on ssDNA. The RecO protein can bind directly to SSB-coated single stranded DNA as well as naked single stranded DNA (Luisi-DeLuca and Kolodner, 1994; Umezuv, 1993; Umezu and Kolodner, 1994). The RecOR pair promotes the dissociation of SSB and its replacement with RecA in the single-stranded DNA regions of the gap (Morimatsu and Kowalczykowski, 2003; Umezu *et al*., 1993; Umezu and Kolodner, 1994). SSB lacking its C-terminal interaction domain blocks the RecA-mediator function of RecOR, suggesting that specific interactions between RecO and SSB are required (Hobbs *et al*., 2007). The role of RecF appears to direct and confine RecA filament formation to the gapped region. The RecF protein binds to ssDNA/dsDNA junctions preferentially and in conjunction with RecR prevents RecA loading onto the dsDNA regions (Webb *et al*., 1997; Morimatsu and Kowalczykowski, 2003). RecF mutant phenotypes can be partially suppressed by RecOR overproduction (Sandler and Clark, 1994), supporting the idea that RecOR provide the RecA-mediator function, which RecF directs to the right location.

Dynamics of the RecA/DNA Filament and its Control

Like other self-assembling structures such as actin and tubulin, the RecA filament is a dynamic structure. RecA filaments on ssDNA are formed in two steps: first, the rate-limiting nucleation of 4–5 monomers (Galletto *et al*., 2006), then followed by filament extension. Net filament growth is 5′ to 3′ on ssDNA (Register and Griffith, 1985), although single-molecule studies have shown that the filament can be extended in either direction (Galletto *et al*., 2006). ATP binding is required for RecA filament nucleation (Menetski and Kowalczykowski, 1985; Galletto *et al*., 2006) and ATP hydrolysis promotes dissociation of RecA from the filament, preferentially from the 5′ end (Lindsley and Cox, 1990).

A number of proteins have been identified that modify the stability of the RecA:DNA filament (see review by Cox (2007)). These mechanisms may prevent RecA from initiating recombination during the exposure of ssDNA during replication or during certain excision repair processes. The RecA filament may also be dismantled after an unsuccessful search for homology, to allow other gap-filling mechanisms to operate or for a broken chromosome to be degraded. After the completion of recombination, destabilization of the RecA filament may also aid its removal from recombination products.

One of the most important modifiers of RecA function, as revealed by both *in vitro* and *in vivo* analysis, is the UvrD helicase, a superfamily-1 3′ to 5′ DNA helicase. UvrD can dismantle the RecA nucleoprotein filament (Veaute *et al*., 2005) and acts as an anti-recombinase when added to *in vitro* RecA DNA strand transfer reactions (Morel *et al*., 1993). Accordingly, mutants in *uvrD* are hyper-recombinational in a number of assays (Zieg *et al*., 1978; Arthur and Lloyd, 1980; Feinstein and Low, 1986; Bierne *et al*., 1997). These results are consistent with the idea that UvrD may act as a "proofreader" of recombination so that it cannot occur between short homologous sequences; in its absence, chromosomal rearrangements are stimulated (Feschenko *et al*., 2003; Kang and Blaser, 2006). When late stages of recombination are blocked (such as RuvABC mutants, see below), loss of UvrD is lethal (Magner *et al*., 2007)–this results from increased accumulation of lethal intermediates in recombination, since blocks in early functions (such as RecA, RecF, RecQ and RecJ) relieve this toxicity.

The RecOR proteins, in addition to their role as RecA mediator proteins, act to stabilize the RecA:ssDNA filament, especially at its 5′ end, (Shan *et al*., 1997; Bork *et al*., 2001), either by inhibiting dissociation or encouraging reassociation of RecA. *In vivo*, the importance of this filament stabilizing activity of RecOR versus their mediator activity in displacing SSB from ssDNA is unclear.

Recently, two other proteins have been identified that modulate RecA filament formation: RecX and DinI. RecX was found as an open reading frame, immediately downstream of the RecA gene in many organisms (Sano, 1993); DinI is a DNA damage inducible gene whose overexpression produced inhibitory effects on the SOS response (Yasuda *et al*., 1998). Visualization of RecA

cytologically suggest the two proteins have opposite effects on RecA:DNA structures: mutants in *dinI* had fewer spontaneous RecA-GFP foci whereas *recX* mutants exhibited more foci than wild-type strains (Renzette *et al*., 2007). Both RecX and DinI have been shown to directly bind to RecA (Voloshin *et al*., 2001; Stohl *et al*., 2003; Lusetti *et al*., 2004b) and have opposing effects on the stability of the RecA:DNA filament. RecX is able to inhibit RecA strand exchange and RecA filament extension (Stohl *et al*., 2003; Drees *et al*., 2004). Interestingly, RecF and SSB can suppress this inhibition of RecA strand extension by RecX (Baitin *et al*., 2008; Lusetti *et al*., 2006). DinI, at stoichiometric concentrations, can stabilize already formed RecA filaments and prevent them from dissociating (Lusetti *et al*., 2004a). At these concentrations DinI does not inhibit RecA's ability to catalyze strand exchange nor its co-protease activity.

RecN and Organization of DNA Ends

The RecN protein is a predicted coiled coil protein of the SMC ("structural maintenance of chromosomes") family of proteins found in bacteria, archaea and eukaryotes, whose members play various roles in chromosome architecture, DNA repair and recombination (Hirano, 2005; Strunnikov, 2006). SMC proteins exhibit dumbbell structures, with two head domains connected via long coils. The *recN* gene was identified as a gene required for optimal recombination via the RecFOR pathway, whose expression is strongly induced by the SOS response to DNA damage (Picksley *et al*., 1984; Finch *et al*., 1985). In *E. coli*, RecN, a normally unstable protein and degraded by protease ClpXP, forms GFP-labeled foci after DNA damage. After completion of repair, aggregates of RecN must be removed by ClpXP to restore viability (Nagashima *et al*., 2006).

Further investigation has established RecN as particularly important for repair of double-strand breaks. Mutants in *recN* are sensitive to ionizing radiation, bleomycin, EcoK restriction following 2-amino-purine incorporation and induction of the I-SceI nucleases (Picksley *et al*., 1984; Sargentini and Smith, 1986; Cromie and Leach, 2001; Kosa *et al*., 2004; Meddows *et al*., 2005), all agents that produce double-strand breaks. During conjugational recombination in *E. coli*, RecN may protect single-strand DNA or in some way promote single-strand annealing, since sectored colonies that presumably arise by annealing of genetically distinct strands and formation of heteroduplex DNA are highly RecN-dependent (Lloyd and Buckman, 1995).

In analogy to the role of other SMC proteins, RecN may play a role in coordinating DNA ends during double-strand break repair and the subsequent recruitment of other repair factors. Although little direct evidence for this exists in *E. coli*, cytological studies of RecN protein in *Bacillus subtilis* suggests that RecN is one of initiating factors in establishment of "repair centers", visible assemblies of recombination proteins at sites of DNA damage (Kidane *et al*., 2004). After treatment with agents that induce double-strand breaks, RecN-YFP can be seen to form distinct foci; the later assembly of RecO and RecF fluorescent foci requires RecN. During the process of natural competence and DNA transformation, *Bacillus subtilis* RecN is seen to oscillate dynamically throughout the cell; upon entry of ssDNA RecN foci become fixed at the cell pole at the the DNA uptake machinery is found (Kidane and Graumann, 2005). This stage is later followed by the appearance of RecA threads that emanate from this pole.

Although the biochemical properties of the *E. coli* RecN have been difficult to determine because of the insolubility of the protein, a number of studies of the *Bacillus subtilis* protein have elucidated RecN as a DNA binding protein. *In vitro*, *Bsu* RecN is an ATP-dependent ssDNA binding protein (Kidane and Graumann, 2005; Sanchezv, 2008). Among the SMC proteins, this preference for ssDNA, rather than dsDNA, appears to be unique. As ascertained by atomic force microscopy, RecN binding to ssDNA is insensitive to *Bacillus* SSB, SsbA, but reversed by RecA (Sanchezv, 2008). RecN forms aggregates (Kidane *et al*., 2004), and promotes rosette structures of protein and DNA (Sanchez *et al*., 2008). This activity could easily explain how RecN may protect single-stranded DNA and assist strand annealing during double-strand break repair. One might imagine that this activity would be quite useful in the annealing step of SDSA-mode of DSB repair, but this has yet to be confirmed by *in vivo* experiments. Specific interactions of other recombination proteins with RecN have not been reported but appear likely, given its function in the establishment of cytologically defined "repair centers" in *Bacillus*.

SbcCD, an SMC Nuclease

SbcCD is also an SMC-like protein and is arguably the structural and functional equivalent of the eukaryotic Mre11-Rad50-Xrs2/Nbs1 complex (reviewed in Stracker *et al*., 2004). The SbcC subunit is a coiled coil protein with an ATPase domain; SbcD composes the nuclease activity of the protein. (Unlike the complex in eukaryotes, there is no third subunit.) *In vitro*, SbcCD acts as an ATP-independent single-strand DNA endonuclease and an ATP-dependent 3′ to 5′ exonuclease (Connelly and Leach, 1996; Connellyv, 1998; 1999). SbcCD also shows preference for hairpin secondary structures in DNA, which it cleaves close to the unpaired tip.

In vivo, SbcCD can initiate recombination between sister chromosomes by the production of double-strand breaks, the result of its cleavage at secondary structures formed by inverted repeats, or palindromic DNA sequences (Cromie *et al*., 2000; Bzymek and Lovett, 2001; Eykelenboom *et al*., 2008). The exonuclease activity of SbcCD may also process ends of broken chromosomes. This activity could be recombinogenic, potentially helpful in the removal of aberrant structures or tightly bound proteins at DNA ends. Indeed, *in vitro*, SbcCD can remove a streptavidin/biotin moiety at a 5′ end by its endonuclease activity (Connelly *et al*., 2003). Conversely, SbcCD may also act to inhibit recombination by digestion of 3′ single-strand tailed recombination substrates, an activity that may account for its

discovery as a function inhibitory to the RecFOR-mediated recombination of double-strand ends (Gibson *et al*., 1992; Lloyd and Buckman, 1985). In a clever assay designed to assay the repair of double-strand chromosome breaks with its sister chromosome (Eykelenboom *et al*., 2008), SbcCD was found to be necessary for repair, leading to the suggestion that the SMC character of the molecule may assist the coordination of two DNA ends to allow healing specifically by DSB repair recombination. This coordination may channel the substrates into the two-ended DSB repair mechanism involving limited DNA synthesis (Figure 2) rather than one-ended recombination (broken fork repair) that would be accompanied by initiation of new replication forks (Figure 1).

B. Synapsis and Strand Exchange

Once the RecA filament has been formed on ssDNA, it catalyzes three important processes to accomplish strand pairing and synapsis: (1) a search for homology by transient interactions; (2) once homologous sequences have been located, strand invasion and synapsis; and (3) extension of heteroduplex regions by branch migration. The recent crystal structure of RecA bound to single and double-strand DNA (Chen *et al*., 2008), and a single-molecule analysis of the strand transfer reaction (van der Heijden *et al*., 2008) offer some new insights.

Homology Assessment and Strand Exchange

Evidence suggests that RecA filament has two sites for DNA binding: a high affinity, primary site that is occupied when the filament forms on ssDNA and a lower affinity, secondary site in which the donor dsDNA will be sampled for pairing-potential (Mazin and Kowalczykowski, 1998). Once homology is sensed, pairing of the donor duplex is destabilized and a strand from the duplex is transferred to the recipient single-strand for pairing (see diagram in Figure 4). In the crystal structure (Chen *et al*., 2008), both dsDNA and ssDNA when bound by RecA in the primary site are stretched overall and underwound; however local areas resemble B-form DNA, explaining how Watson-Crick base-pairing can be assessed in the synaptic complex. This is in agreement with the idea that the stretching of the donor dsDNA breaks base-pairing and stacking interactions; the structure reveals that these bases can be sampled for pairing, via the local B-form conformations, with the recipient strand for pairing in the primary site. (See the schematic illustration of this process in Figure 4.)

Immediately after strand exchange, the newly formed heteroduplex remains in the primary DNA binding site of the RecA filament, while a single strand is left behind in the secondary site (Chow *et al*., 1986). Single molecule studies of RecA in the act of strand transfer have revealed several critical features (van der Heijden *et al*., 2008). Surprisingly, in the presence of ATP, the region of synapsis, with three strands being protein-bound, occurs only over about 80 nucleotides at any given time, even when large regions of homology are present. ATP hydrolysis promotes

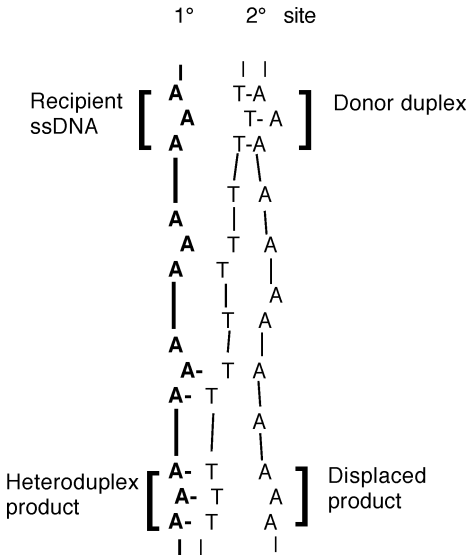

FIG. 4. Schematic of the RecA-catalyzed strand exchange reaction. RecA binds ssDNA in high-affinity site 1 (A strand in bold) and recruits partner duplex in low-affinity site two, top of figure. Stretching of the DNA destabilizes the pairing of the donor duplex and allows the recipient strand to sample pairing by local Watson-Crick base-pairing, middle of figure. Stable pairing, bottom of figure, causes strand-transfer, with the heteroduplex product in site 1 and a displaced single-strand in site 2.

release of RecA from the DNA products — as a wave of strand transfer proceeds, the complex releases its products and leaves behind an unpaired displaced strand that is wrapped around the duplex. In the presence of poorly hydrolyzable ATPgammaS, strand exchange occurs at the same rate, but the DNA is not released from the filament and a long ternary complex is formed between RecA and the two DNA molecules.

Topological Issues in Strand Exchange

Strand exchange initiated internally on a gapped molecule (as in Figure 3) presents potential topological problems, since the ends are not free to rotate to establish interwound joint molecules, so-called plectonemic joints, via the RecA filament. RecA can pair DNA strands that cannot intertwine, and such "paranemic" joints are stable but disrupted by deproteinization (DasGupta *et al*., 1980). These paranemic joints can be converted to plectonemic joints with the addition of topoisomerase I (Cunningham *et al*., 1981), suggesting that paranemic joints are precursors of plectonemic joints. Experiments with oligonucleotides with non-homologies at the ends shows that strand exchange can indeed occur when axial rotation is obstructed (Adzuma, 1992). Type I topoisomerases (in *E. coli*, topoisomerase I and III) could in theory function to allow interwinding during strand exchange at a gap. This topological problem is not unique to single-strand gaps: even recombination involving dsDNA linear molecules could initiate pairing somewhat

removed from the dsDNA end. However, how topological problems are solved during recombination has not been well investigated. In a plasmid recombination assay, presumably involving gaps because of its dependence on RecF, a requirement for TopoI has been reported (Fishel and Kolodner, 1984).

The RecO protein can also anneal single-strand DNA, even that pre-bound by SSB, and can catalyze strand exchange to form D-loops (Luisi-DeLuca and Kolodner, 1994; Luisi-DeLuca, 1995; Kantake *et al*., 2002). This suggests that RecO could assist RecA in synapsis at gaps that may have topological constraints and pair less strongly; alternatively, RecO could help recruit the strands flanking the gap into pairing to form Holliday junctions (Figure 3C'). In either case, we await genetic data to support this role for RecO.

Making Strand Exchange Irreversible

After strand displacement, the resulting single strand can be bound by SSB. *In vitro*, this sequestration drives the exchange to completion and prevents the product from re-initiating the reverse reaction (Lavery and Kowalczykowski, 1992; Mazin and Kowalczykowski, 1998). In addition, the displaced strand can be subject to exonuclease digestion, which stimulates branch migration by removing the competitor strand for pairing and removes the possibility of reaction reversal (See Figure 5D). *In vitro*, both RecJ and Exo I single-strand exonucleases stimulate RecA strand exchange between linear duplex and circular single-strand molecules and so may have a synaptic-stabilization role *in vivo* (Bedale *et al*., 1993; Corrette-Bennett and Lovett, 1995; Konforti and Davis, 1992). In assays for conjugational recombination that proceeds by the RecBCD-dependent DSB mechanism, inactivation of both RecJ and Exo I (but neither singly) causes a reduction of recombination by about 10-fold (Viswanathan and Lovett, 1998). This is best explained by a post-synaptic role in which digestion by either RecJ or Exo I helps promote strand exchange, even when recombination is initiated by RecBCD. *In vivo*, RecJ and Exo I appear to act post-synaptically on heteroduplex joints, as determined by physical analysis of the recombinant products formed after linearization of plasmid DNA with terminal repeats (Friedman-Ohana and Cohen, 1998; Friedman-Ohana *et al*., 1999). RecJ also stimulates branch migration past regions of non-homologies *in vitro* (Corrette-Bennett and Lovett, 1995).

Branch Migration by RecA

Once strand exchange has been initiated by joint molecule formation, RecA can extend the heteroduplex region by a reaction known as branch migration. Branch migration can also be driven between two duplex molecules, with formation of the crossed 4-strand structure, the Holliday junction. RecA can catalyze this phase of the reaction but is relatively inefficient when compared to other branch migration helicases (Adams and West, 1996; see below), such as RuvAB and RecG. RecA also is poor at traversing regions of non-homology between the reacting molecules (Morel *et al*., 1994) and a 22 nt non-homology can be sufficient to block further exchange by RecA.

C. Postsynaptic DNA Processing

An Introduction to D-loop Processing and Holliday Junctions

The product of strand exchange between a resected DSB and an intact duplex is a D-loop ("Displacement loop"), where a heteroduplex region is formed between the single strand tail of the linear molecule and its complement on the recipient dsDNA molecule (Figure 5A). Although all models of recombination involving double-strand ends show initial strand exchange through a D-loop intermediate, the ultimate processing of these intermediates is not well understood and could be quite complicated. The D-loop heteroduplex joint is intrinsically unstable — branch migration (Figure 5B) can diminish the heteroduplex region and ultimately disrupt the joint structure. In contrast, branch migration to extend the region of heteroduplex will stabilize the joint (Figure 5C), and will eventually form a 4-stranded branched

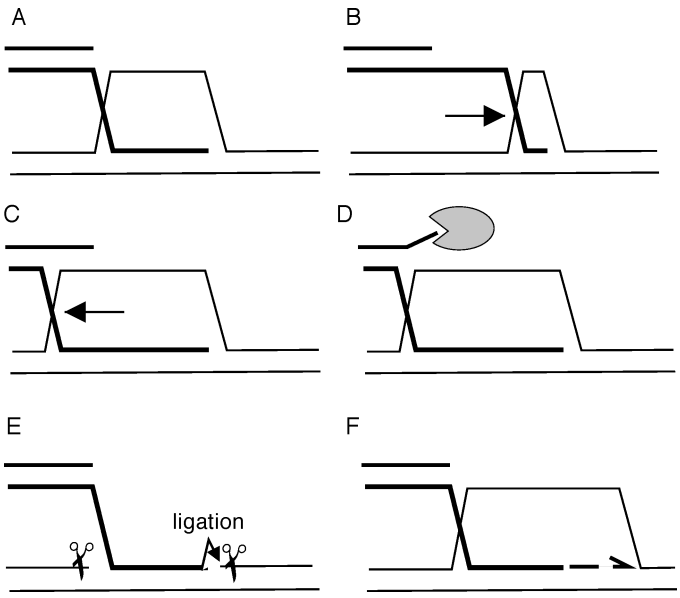

FIG. 5. Stabilization and destabilization of D-loop recombination intermediate. (A) The D-loop produced by strand exchange between a resected dsDNA end and an intact homologous duplex molecule. (B) Branch migration rightward will destabilize the D-loop by reducing the length of heteroduplex and will ultimately dissove the joint. (C) Conversely, branch migration leftward stablizes the joint by extending the heteroduplex, concomitant with formation of a Holliday junction (4-strand joint molecule). Cleavage of this junction, not shown) will produce a fork structure. (D) Exonuclease digestion associated with branch migration will also stabilize the joint, without Holliday junction formation. (E) Cleavage of the D-loop stabilizes the joint and produces a fork structure. (F) Priming of DNA synthesis from the invading 3′ strand will also stabilize the joint.

molecule (the Holliday junction). Not all recombination intermediates will include true Holliday junctions. If exonuclease activity accompanies the branch migration phase, no such Holliday junction is formed concomitant with heteroduplex extension (Figure 5D) and the D-loop just grows larger. Cleavage of the displaced strand of the D-loop can also stabilize the intermediate and can allow the transferred strand to be ligated to its recipient, producing a covalent recombinant strand and a fork structure (Figure 5E). Finally, the 3′ end of the invading strand can be extended by DNA polymerase, extending and stabilizing the heteroduplex joint (Figure 5F).

Intermediate Structures Posed by DSB Repair vs. Gap Repair

Repair of double-strand breaks, the DSB repair model, involves D-loop formation by the invading dsDNA end, followed by recruitment of the second end into the D-loop, by pairing with the displaced strand. This mechanism can produce two Holliday junctions (Figure 2C), whose resolution can yield crossovers (Figure 2D–F) or non-crossover products. During yeast meiotic recombination, double Holliday junctions have been detected (Schwacha and Kleckner, 1995). In the SDSA variant of DSB repair, only one end need invade to form a D-loop and initiate DNA synthesis from the 3′ end (Figure 2C'–D'). The synaptic intermediate is then dissolved and the 3′ ends of each dsDNA end can anneal to heal the break, without the production of crossover products between the donor and recipient (Figure 2E'–F'). The DNA synthesis in the annealed intermediate (dashed lines in Figure 2E'–F') can, however, produce some genetic exchange from the intact donor DNA molecule to the recipient broken strand even without crossover at the flanks.

In gap repair, initial pairing forms synaptic structures involving junctions with a single crossed strand (half-Holliday junctions) connecting donor and recipient DNA molecules (Figure 3B). Engagement of one or both of the broken strands that flank the gap and branch migration can convert one or both of these to four-strand Holliday junctions (Figure 3C). Although either half-Holliday or Holliday junctions in gap repair, in theory, can be resolved to create recombinant products (Figure 3D), it not clear what forms exist *in vivo*.

Reverse versus Forward Branch Migration/RuvAB versus RecG

RuvABC is a coordinated protein machine that acts in the late stages of replication to migrate and resolve Holliday junctions. RuvA is a specificity factor that targets RuvB to Holliday junctions, RuvB is the Holliday junction branch migration helicase, and RuvC is an endonuclease that specifically cleaves four-way junctions (West, 1997). RuvA exists as one tetramer or two tetramers that sandwich the junction, holding the four strands of DNA in an open square configuration. RuvA is able to protect the junction from being modified by other DNA processing enzymes while recruiting RuvB (Kaplan and O'Donnell, 2006). RuvB itself exists as a hexamer, and two of these hexamers bind to opposite arms of the Holliday junction, pumping the DNA to power migration by its ATPase activity. RuvC is finally recruited to the complex to cleave the Holliday junction DNA. RuvC has a preferred site for cleavage, and it is thought that one function of branch migration is to identify and present these sequences to RuvC for cleavage (West, 1997). RuvC exists as a dimer, and the mechanism for loading of RuvC remains unclear. The nicks created by RuvC can be re-ligated into two DNA duplexes by DNA ligase, and the Holliday junction becomes completely resolved (West, 1997). In the absence of RuvAB or RuvC *in vivo*, Holliday junction recombination intermediates have been shown to accumulate in the cell (Donaldson *et al*., 2006). Mutations in RuvAB or RuvC can be suppressed by expression of RusA, a cryptic bacteriophage-encoded Holliday junction endonuclease, that is normally not expressed in *E. coli* (Mandal *et al*., 1993; Sharples *et al*., 1994; Mahdi *et al*., 1996).

RecG is a helicase implicated in recombination that can bind to Holliday junctions, D-loops and other branched structure, from which it promotes ATP-dependent branch migration. RecG can not only migrate Holliday junctions but can also form Holliday junctions from by fork regression *in vitro*, a mechanism thought to aid in restarting replication at blocked replication forks (McGlynn and Lloyd, 2002; Briggs *et al*., 2004; Baharoglu *et al*., 2008), but not discussed further here. RecG binds well to D-loop structures and its helicase activity is directional on such substrates (Whitby *et al*., 1993). In contrast to RuvAB, RecG's helicase activity acts to disrupt D-loop structures (Figures 5B and 6B) and can reverse RecA-mediated strand exchange in vitro (McGlynn and Lloyd, 2002; West, 1997).

Genetic Effects of RuvABC and RecG: Genetic Independence and Synergy

Both RuvABC and RecG contribute to recombination *in vivo*. Inactivation of either complex has little effect on recombination, as measured after P1 transduction and conjugation, which occurs by a RecBCD-dependent DSB-repair pathway. Inactivation of both has some synergistic effects, with reduction of recombination approximately 100-fold (Lloyd, 1991). This suggests that RecBCD mediated recombination requires the function of either RuvABC or RecG for maximal efficiency. Survival of cells to X-irradiation and to chromosomal cleavage by the meganuclease I-SceI likewise requires either RecG or RuvABC, suggesting that the two functions define separate and redundant mechanisms of resolution after double-strand break repair (Meddows *et al*., 2004).

Although the branch migration and cleavage activity of RuvABC can act to stabilize and resolve heteroduplex joints *in vitro* and *in vivo*, it is much less clear how RecG's helicase activity contributes to recombination, since its preferred directionality dissolves heteroduplex joints (Figure 6AB). One idea is that RecG promotes the dissolution step of the SDSA variant of double-strand break repair (Meddows *et al*., 2004)

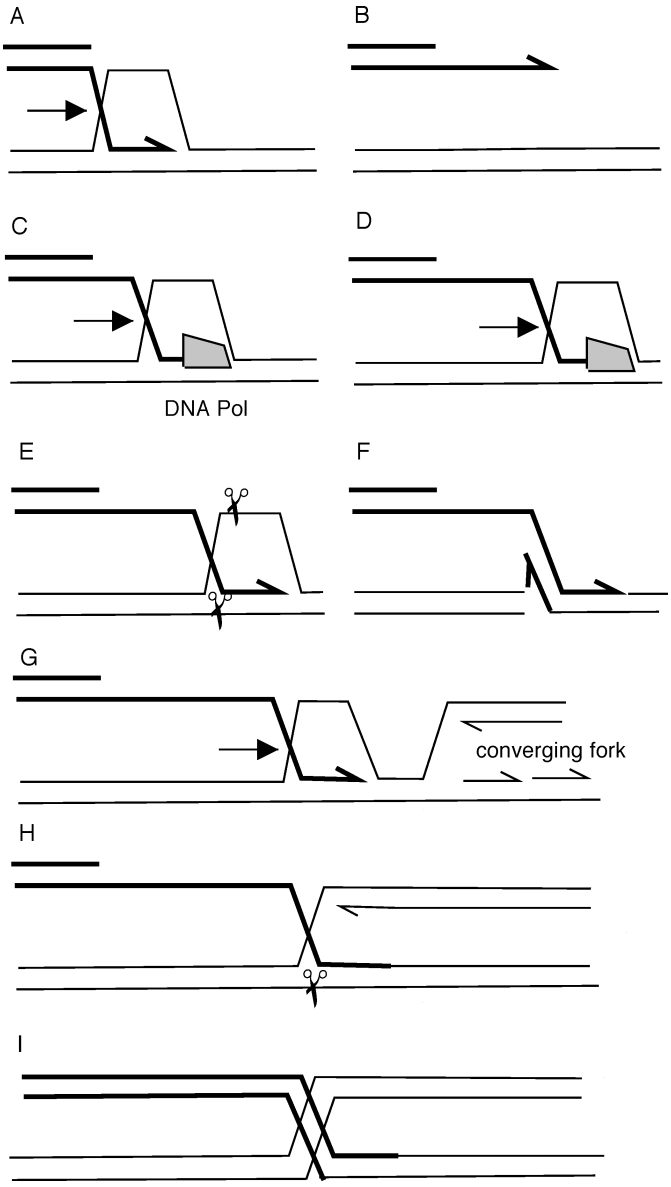

FIG. 6. How reverse branch migration by RecG can yield recombinants. (A) RecG catalyzes reverse branch migration on D-loops. (B) Joint dissoved by RecG. (C) Extension of 3′ end by a DNA polymerase. (D) Bubble migration, D-loop translocated by reverse migration coupled to DNA synthesis. (E) Appropriate cleavage of D-loop to generate recombinants. (F) Recombination product, with donor DNA covalently joined to recipient. (G) Bubble migration converging on replication fork. (H) Resolution of half Holliday junction formed by the collision. (I) Recombinant product, after healing.

(Figure 2D'–E'), where after DNA synthesis in the recombination intermediate, synapsis is reversed to permit annealing of the broken chromosomes. This mechanism could clearly contribute to recombinational DNA repair of breaks but, because it does not promote genetic crossovers, it is difficult to understand how this mechanism would contribute to inheritance of markers after conjugation or transduction. Another notion to explain RecG's role is that RecG's helicase activity is coupled to DNA synthesis (Meddows *et al*., 2004), such that the D-loop is not dissolved but migrates with the polymerase in a manner termed "bubble migration" (Figure 6CD). Appropriate resolution of these structure or convergence of the D-loop bubble onto another replication fork can yield crossover products (Figure 6E–I). Recombination and repair defects in *recG* mutants can be corrected by inactivation of the helicase activity of the PriA replication restart protein (see below), and it has been suggested that both proteins compete for access to the 3′ strand of the D-loop (Al-Deib *et al*., 1996).

Migration and cleavage of Holliday junctions appear to be more critical for the gap repair recombinational mechanism than for double-strand break repair. The RuvABC functions have more pronounced effects on recombination promoted by the RecFOR pathway than on the RecBCD pathway and were identified as a classic "RecF pathway" genes for conjugational recombination (Lloyd *et al*., 1984). This may be because RecBCD-mediated strand exchange at ends through D-loops are more stabilized, for example by larger heteroduplex joints (Figure 5D–F) and do not obligately form Holliday junctions. In addition, RecG appears to exhibit more anti-recombinational action on gap-mediated recombination. In another assay for recombination induced by DNA damage, the RecFOR pathway is stimulated four-fold by inactivation of RecG (Bichara *et al*., 2006), supporting the idea that RecG exerts anti-recombinational effects by reverse junction migration.

Although RuvABC and RecG can be somewhat redundant for effects on recombination in some assays, both functions are required for efficient recombination in other assays, especially those that appear to involve breaks induced after passage of the replication fork. In an assay of repair of chromosomal breaks induced by cleavage at palindrome sequences, efficient survival required both RuvABC and RecG (Eykelenboom *et al*., 2008). In another assay for recombination between sister chromosomes at short tandem repeats, elevated recombination caused by defects in DnaB (the replication fork helicase), requires both RuvAB and RecG (Lovett, 2006) in a mechanism involving RecBCD (Saveson and Lovett, 1999).

A Role for a RecA Paralog in Late Stages of Recombination

All organisms appear to encode one or more proteins that are paralogous to their strand exchange proteins (Lin *et al*., 2006). A bacterial RecA paralog is encoded by a gene known as Sms (for sensitivity to MMS) or RadA (named for its sensitivity to radiation), found in virtually every bacterial genome, as well as in plants. [This nomenclature is unfortunate, since bacterial RadA protein is not orthologous to archaeal RadA strand exchange protein and is more similar, in fact, to the archaeal paralog protein, RadB.] Genetic studies of *radA*/*sms* of *E. coli* show that the strain is mildly sensitive to certain form of DNA damage (Beam *et al*., 2002; Felzenszwalb *et al*., 1984; Neuwald *et al*., 1992;

Sargentini and Smith, 1986; Song and Sargentini, 1996). However, it has strong synergistic effects on DNA damage survival when combined with mutants in other recombination genes, particularly *recG* (Beam *et al*., 2002).

In measurements of conjugational recombination, RadA/Sms appears to have a role somewhat redundant to RuvAB and RecG: single mutants are not substantially reduced in recombination, whereas double mutants are more strongly deficient. The triple *radA recG ruvABC* mutant is severely recombination defective, comparable to mutants in *recA* (Beam *et al*., 2002). Because of the known role of RuvABC and RecG in late stages of recombination, this implicates RadA/Sms likewise in post-synaptic events. RadA/Sms is required, as are RecG and RuvAB, for recombination at tandem repeats associated with replication fork helicase defects (Lovett, 2006) and *radA* mutants are particularly sensitive to the replication chain-terminator, azidothymidine (Cooper and Lovett, unpublished results). *E. coli* RadA, by itself, has no effect on RecFOR-mediated recombination (Beam and Lovett, unpublished data) and affects only RecBCD-mediated events (Beam *et al*., 2002; Lovett, 2006). The effects of RadA are most pronounced in assays that report recombination associated with replication, leading to the speculation that it, in some unknown way, stabilizes D-loops associated with replication fork repair (Lovett, 2006).

Bacterial RadA possesses, in addition to a domain homologous to RecA, an N-terminal putative Zn finger and a C-terminal domain related to the Lon protease. As the catalytic triad of Lon is not present in bacterial RadA sequences, RadA is unlikely to function as a protease, leaving the function of this domain mysterious. The putative Zn finger is clearly important for function, as the founding mutation, *radA100*, affects a conserved cysteine residue in this site (Song and Sargentini, 1996). There is no published biochemical characterization of the protein: our unpublished results (Cooper and Lovett, unpublished results) confirm a weak ATPase activity and binding to single-strand DNA.

An Alternative for Resolution Involving RecQ and Topoisomerase III

In yeast, double-Holliday junctions appear to be resolved, without crossing over, by a mechanism involving Sgs1 (a RecQ-family helicase) and the type 1 topoisomerase, topoisomerase III (Topo III), which specifically interact with each other (Gangloff *et al*., 1994). RecQ helicase and Topo III have been indicated as partners in the resolution of replication intermediates. The mammalian counterparts resolve double Holliday junction structures *in vitro* (Wu and Hickson, 2003; Lopez *et al*., 2005). In *E. coli*, RecQ and Topo III have not been shown to interact directly. However, both proteins interact directly with SSB, as with other SSB interacting proteins, through the C-terminal tail of SSB (Shereda *et al*., 2007; Suski and Marians, 2008). RecQ directly stimulates Topo III activity (Harmon *et al*., 1999), and Topo III stimulates RecQ activity (Suski and Marians, 2008). RecQ, Topo III, and SSB together in solution can both catenate and decatenate plasmids *in vitro* (Harmon *et al*., 1999). These three proteins together also can resolve a plasmid with a structure that resembles converging replication forks *in vitro* (Suski and Marians, 2008). Topo III mutants show genetic synergy with those of RuvABC for DNA damage survival; furthermore, Topo III mutants are synthetically lethal with defects in topoisomerase IV (a type II decatenating topoisomerase) and such lethality can be rescued by mutations in RecQ or RecA (Lopez *et al*., 2005). These findings support a role for Topo III and RecQ in a recombination pathway alternative to RuvABC.

Polymerization of 3′ Invading Ends and the Necessity for Replication Restart

Strand exchange provides 3′ DNA ends that can be elongated by DNA synthesis. This synthesis can stabilize joint molecules (Figure 5F) and restore lost information from breaks (Figure 2D and D'). DNA synthesis following recombination may involve single DNA polymerase molecules or be associated with re-establishment of new bona fide replication forks, with coordinated leading and lagging strand synthesis (Figure 1E and E').

The coordination of late stages of recombination with replication is likely to be subject to regulation, depending on the nature of the recombination intermediate (see Cromie *et al*. (2001), Lovett (2003) and Briggs *et al*. (2004) for discussion of these issues.) For example, it makes sense for one-ended recombination events, such as replication fork break repair (Figure 1), to be obligately associated with establishment of new forks to allow efficient resumption of replication after fork breakage. However, double-strand break repair events (Figure 2) and gap repair recombination (Figure 3) may need only limited amounts of DNA synthesis in the recombination intermediates to restore missing information — it might be unnecessary to establish replication forks from these. The factors and mechanisms that might allow this discrimination are still incompletely understood.

E. coli has five DNA polymerases: during chromosomal replication, DNA III synthesizes the bulk of DNA, with DNA polymerase I (and its associated 5′ flap endonuclease activity) involved in the maturation of Okazaki fragments (Kornberg and Baker, 1991). DNA polymerases II, IV and V are induced by the SOS response to DNA damage and function in translesion synthesis reactions (Pham *et al*., 2001; Tippin *et al*., 2004). The involvement of these latter repair polymerases in recombination events is still not clear, potentially because of redundant roles. The properties of DNA polymerase II make it particularly well-suited to synthesis from DNA gaps (Tomer *et al*., 1996) as might be presented by recombination intermediates: it has high fidelity due to associated 3′ exonuclease activity and Pol II is specifically stimulated by SSB-coated ssDNA (Sigal *et al*., 1972). There is evidence that DNA polymerase IV (DinB) is required for certain recombination events triggered

by replication stalling (Lovett, 2006) and that DinB-dependent polymerization accompanies dsDNA break repair (Ponder *et al*., 2005).

Reestablishment of replication forks requires certain specific factors to load the replication fork helicase, DnaB onto repaired structures (reviewed in Marians, 2004). Once DnaB has been loaded, the DNA polymerase III replisome and DNA primase are believed to reassemble automatically, via interactions with DnaB. *E. coli* uses two systems for replication restart, which depend on the nature of the substrates presented (Heller and Marians, 2005). For structures in which a 3′ end, equivalent to the newly synthesized leading strand, abuts the fork, a PriA system is used to recruit DnaB and its escort proteins DnaC and DnaT. PriA seems to prevent access of DNA polymerase to the 3′ end until DnaB is loaded (Xu and Marians, 2003), providing a mechanism by which polymerase extension of the invading end is disfavored and establishment of symmetric forks is promoted. For a structure in which a gap is present between the 3′ end and the fork, a PriC-dependent mechanism reloads DnaB. *In vivo*, loss of both PriA and PriC is lethal (Sandler and Marians, 2000).

PriA and Recombination

In *E. coli*, the PriA system appears to be important for certain recombination reactions, particularly those involving double-strand ends. Mutants in *priA* are strongly reduced for recombination after transduction and more modestly reduced for recombination after Hfr conjugation (Kogoma *et al*., 1996; Sandler *et al*., 1999). This finding and other considerations have led to the idea that recombination as measured by these processes occurs by two independent one-end events (broken fork repair), requiring re-establishment of replication forks (Figure 7) (Smith, 1991; Kogoma *et al*., 1996). Although one might imagine that DSB break repair (Figure 2) would not necessarily require establishment of new replication forks, *in vivo* double-strand break repair appears to be PriA-dependent. Recombinational repair of I-SceI induced breaks in the *E. coli* chromosome require PriA, regardless of whether they are processed by RuvABC or RecG (Meddows *et al*., 2004). Likewise, breaks induced by SbcCD-induced cleavage at palindromic sequences requires PriA (Eykelenboom *et al*., 2008), contradictory to an early report from the same group (Cromie *et al*., 2000). The requirement for PriA for these events may be because RecBCD exonuclease processing causes the ends to be quite far apart by the time they are made competent for recombination by loading of RecA – the ends may have lost sight of each other and each end recombines independently to re-establish a new fork. Based on its proposed mechanism (Figure 3) we would not expect that daughter-strand gap repair would require PriA-dependent replication fork reassembly. Mutants in PriA require RecFOR for viability, suggesting that gap-repair is indeed intact in such strains and required to repair replication forks (Grompone *et al*., 2004).

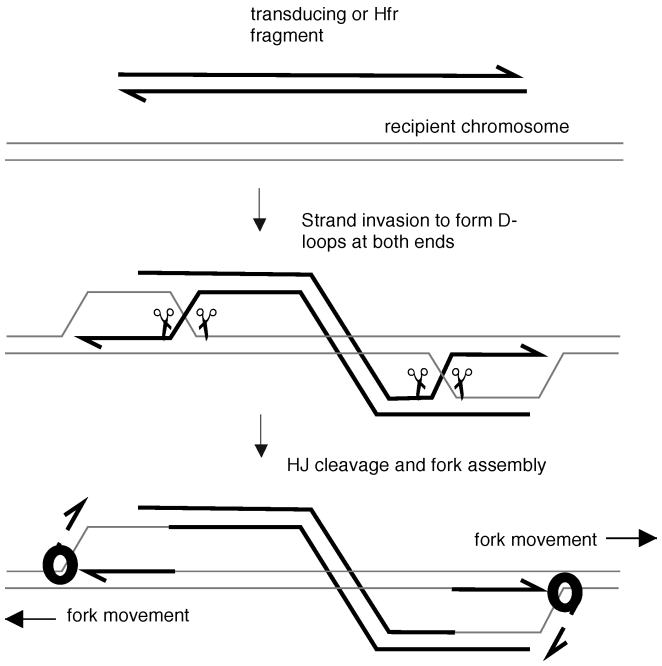

FIG. 7. Replication fork model for conjugational and transductional recombination. Two separate one-ended break repair events give rise to D-loops each end. Holliday junction intermediate is resolved by cleavage. (Other resolutions possible, for instance by D-loop cleavage, as shown in Figure 1C.) New replication forks are established at each joint by PriA mediated loading of DnaB (ovals), yielding recombination between in the incoming fragment and the recipient chromosome.

ISSUES REMAINING IN *E. COLI* RECOMBINATION

Although a great deal has been deduced about recombination using *E. coli*, several issues still remain unsettled. In particular, the diversity and regulation of recombination reactions *in vivo* raises new questions.

How is Recombination Regulated, in Time and Place?

Recent studies in *E. coli* suggest that multiple mechanisms exist to regulate recombination, via effects on the RecA filament. For many of these, the biological wherewithal is not yet clear. By these mechanisms, recombination, particularly at DNA replication gaps, may be restrained until other repair mechanisms have failed to remove the problem. Single-strand DNA is exposed naturally and transiently during the course of DNA replication where it is inappropriate to initiate recombination reactions. The RecOR mechanism to remove SSB from single-strand DNA and replace it with RecA may be a deliberately slow reaction *in vitro* (Hobbs *et al*., 2007), reflecting the fact that the cell exercises caution in replacing SSB with RecA *in vivo*.

In addition, recombination reactions must be regulated to insure that they effect repair, without producing undesirable chromosomal rearrangements. Recombination must be restricted to extensively homologous DNA sequences so that it does not occur between spurious homologies or short repetitive elements such as insertion sequences. Therefore, the recombination system must sense the extent of DNA homology, both its length and quality, and recombination between homeologous (imperfectly homologous) sequences or between short sequence repeats must be aborted. A minimal effective processing segment ("MEPS") has been defined in *E. coli* to be 30–90 bp in double-strand break associated recombination; below this length recombination efficiency falls dramatically (Shen and Huang, 1986). For RecA-dependent recombination occurring by a RecFOR pathway that resembles gap repair (Lovett *et al*., 2002), the minimal homology required appears to be somewhat larger, at approximately 200 bp; RecA-dependent recombination between tandem repeats on plasmids, presumably mediated by RecFOR (Kolodner *et al*., 1985; Lovett *et al*., 1993), is ineffective when homologies are shorter than 300 bp (Bi and Liu, 1994). This may reflect: (1) the extent of DNA pairing in synaptic intermediates; (2) instability of RecA filaments at short lengths; and (3) unwinding and destabilization of synaptic intermediates such as D-loops. Mismatches between sequences ("homeology") also deter recombination between them, as a result of the action of the MutLS UvrD-dependent mismatch repair pathway (Petit *et al*., 1991; Rayssiguier *et al*., 1989). Some of this inhibition of recombination may be direct on RecA-dependent strand exchange (Worth *et al*., 1994); some appears to be independent of RecA, with mismatch repair acting to dissolve recombination intermediates (Lovett and Feschenko, 1996).

How Many "Pathways" are There for Recombination?

Early genetic analysis suggested that RecA-dependent recombination could be catalyzed by two genetically distinct pathways (Clark, 1971), one that is dependent on RecBCD (the "RecBC pathway", before discovery of the RecD subunit of the complex) and the other dependent on RecFOR, (the "RecF pathway", before discovery of RecO and RecR). [A third pathway, the RecE pathway, was discovered to be dependent on prophage-encoded recombination genes (Gillen *et al*., 1981) and will not be discussed further.] The distinction between the RecBCD and RecFOR pathways has been made clearer by the biochemical finding that both function to load RecA on ssDNA (see above). Therefore, these pathways merely reflect alternative modes of formation of RecA pre-synaptic filaments. Initially, the RecFOR pathway was considered to be a minor pathway for recombination, which could only be "activated" by mutations in nucleases SbcB and SbCD. However, this was based on assays for DSB-mediated conjugational recombination, for which the RecFOR pathway is mechanistically ill-suited. Other analysis suggested that the RecFOR pathway operates efficiently in wild-type cells (without "activation") and mediates recombination at single-strand gaps (reviewed in Clark, 1973; Wang and Smith, 1984). Recombination between homologies on plasmids, either intra- or inter-molecular, is RecFOR-dependent and RecBCD-independent (Cohen and Laban, 1983; Kolodner *et al*., 1985; Lovett *et al*., 2002), probably because they are small and cannot escape RecBCD destruction if they are broken; these plasmids naturally lack Chi sequences to attenuate RecBCD nuclease activity and to promote RecA loading by RecBCD. Measurements of recombination between homologous sequences resident on the *Salmonella* chromosome during normal growth suggests that the RecFOR pathway and RecBCD contribute roughly equally (Galitski and Roth, 1997); delivery of double-strand breaks stimulates the RecBCD-dependent events but not those dependent on RecFOR. Likewise, crossing over between *E. coli* sister chromosomes is promoted equally by both RecFOR and RecBCD pathways (Steiner and Kuempel, 1998). As eukaryotic cells and many bacteria seem to lack a RecBCD-like nuclease, a RecFOR-like pathway may be the more universal and primordial mechanism.

Can these two mechanisms of loading RecA account for all recombination? Certain analyses suggest that there could be missing ways to load RecA, especially on DNA gaps. In measurements of gap-filling, "post-replication" recombinational repair (Wang and Smith, 1984), the RecF(OR) pathway was found to be the major contributor but there was some component independent of both RecBCD and RecF. This pathway appeared to require the 5′ exonuclease activity of DNA polymerase I (Sharma and Smith, 1987). Likewise, our laboratory observed that RecA-dependent crossing-over between homologies carried on distinct plasmids was primarily dependent on RecFOR, but a substantial fraction was RecBCD- and RecFOR-independent (Lovett *et al*., 2002).

Probably most mysterious are the RecA-independent recombination mechanisms, which can be observed in various assays and whose genetic basis is not fully understood. A RecA-independent template-switch reaction leading to crossover recombination products has been documented (Lovett *et al*., 1993; Goldfless *et al*., 2006) but appears to be restricted to sister chromosomes. Obligately intermolecular recombination not involving sisters that is independent of RecA can also be detected and is especially prominent in reactions involving limiting homologies, less than 100 nucleotides (Lovett *et al*., 2002; Dutra *et al*., 2007). Although it has often been claimed that all homologous recombination is dependent on the RecA strand transfer protein, efficient recombination via a RecA-independent mechanism occurs as long as exonucleases degrading single-strand DNA are inactivated (Dutra *et al*., 2007). This suggests that RecA-independent recombination is merely limited by availability of ssDNA substrates and that RecA plays in important role, not only in the catalysis of strand-transfer but also in the protection of ssDNA substrates for recombination. The RecA-independent pathway may involve assimilation of single-strand DNA into replication forks, as inheritance of genetic markers from oligonucleotides introduced into the cell by electroporation is more efficient

when they are single-stranded and when complementary to the corresponding lagging strand DNA template (Dutra *et al*., 2007).

Are There Additional Ways to Resolve Branched Recombination Intermediates?

As discussed above, RuvABC branch migration and cleavage can resolve Holliday junction recombination intermediates and RecG can resolve structures such as D-loops by reverse branch migration. Surprisingly, an assay designed to detect crossover products between two plasmids did not detect any decrement of recombination in *ruvC recG* mutants (Lovett *et al*., 2002), suggesting that there could be alternative mechanisms for junction resolution. (We rule out the RecQ Topo III alternative resolution mechanism because it does not yield crossover products.)

In addition, although cleavage of D-loops can stabilize replication fork break repair intermediates and models for DSB recombination (Figure 1) invoke such processing, the existence of such an activity in *E. coli*, or any other organism, remains unknown. A D-loop cleavage activity was identified and purified (Chiu *et al*., 1997) but its genetic basis has not been established.

How are Late Events in Recombination, Including DNA Synthesis, Coordinated?

Early steps in recombination involve "hand-off" mechanisms, such as the recruitment of RecA to single-strand gaps via RecO bound to SSB. Our knowledge of the coordination and regulation of the later steps of recombination is less clear. Does RecA eventually fall off post-synaptic structures or is it stabilized or removed by specific factors? Are resolution proteins recruited to recombination intermediates through the early recombination proteins? What DNA polymerases are required for gap repair? How is the need for re-establishment of replication forks after recombination assessed and fulfilled?

SUMMARY AND CONCLUSIONS

A little over four decades of genetic and biochemical analysis has provided key insights into the mechanisms of recombination in *E. coli* that have relevance for other organisms. A number of paradigms established for the function of *E. coli* recombination proteins have held true in eukaryotic systems. The connection between genetic recombination and DNA repair mechanisms was discovered in *E. coli* by early genetic analysis, and has been codified into three mechanisms, "broken fork repair", "DSB repair" and "recombinational gap-filling repair". Genetics and biochemistry have established functions that operate in three stages of recombination: prior to synapsis of two DNA molecules, during their synapsis and in the processing that occurs afterwards. Work in *E. coli* established the RecA protein as a key protein mediating homologous pairing and strand exchange during recombination. Two mechanisms or "pathways" of RecA loading have been deduced by the combined power of genetics and biochemistry, one specific to double-strand ends, RecBCD, and the other specific to replication gaps, involving RecA mediator proteins, RecFOR. The latter proteins appear to be recruited to substrates through interaction with single-strand DNA binding protein, SSB. The pathway choice *in vivo* is dictated by the nature of the initiating DNA substrate: double-strand breaks for RecBCD and single-strand gaps for RecFOR. Exonucleolytic processing of recombination substrates involves either RecBCD at double-strand breaks or RecJ exonuclease and RecQ helicase at replication gaps. At least two mechanisms for resolution of recombination intermediates have been deduced in late stages of recombination: one involving migration and cleavage of Holliday junctions via RuvABC and the other involving RecG helicase; still others may exist. Recombination intermediates such as D-loops can be matured into replication forks via the action of the PriA replication restart protein. It is clear from *E. coli* genetics that recombinational repair of broken or damaged replication forks plays a vital function for the cell.

As the proteins involved in recombination and replication in *E. coli* are better understood mechanistically and likely to be less complex than their eukaryotic counterparts, it is our hope that continued biochemical and genetic analysis in this system will provide further lessons in the difficult questions that remain concerning coordination and regulation of genetic recombination, applicable to all cells.

ACKNOWLEDGMENTS

We thank Tom Silhavy for suggesting that a basic review of *E. coli* recombination genetics and biochemistry would be useful. STL is grateful for early training in the joys and complexities of*E. coli* recombination genetics by A. J. Clark and for many interactions over the years with members of the recombination field. Unpublished work cited in this article was supported by NIH award GM51753.

REFERENCES

Adams, D.E., and West, S.C. 1996. Bypass of DNA heterologies during RuvAB-mediated three- and four-strand branch migration. *J Mol Biol* 263:582–596.

Adzuma, K. 1992. Stable synapsis of homologous DNA molecules mediated by the *Escherichia coli* RecA protein involves local exchange of DNA strands. *Genes Dev* 6:1679–1694.

Al-Deib, A.A., Mahdi, A.A., and Lloyd, R.G. 1996. Modulation of recombination and DNA repair by the RecG and PriA helicases of *Escherichia coli* K-12. *J Bacteriol* 178:6782–6789.

Anderson, D.G., and Kowalczykowski, S.C. 1997. The translocating RecBCD enzyme stimulates recombination by directing RecA protein onto ssDNA in a chi-regulated manner. *Cell* 90:77–86.

Arthur, H.M., and Lloyd, R.G. 1980. Hyper-recombination in *uvrD* mutants of *Escherichia coli* K-12. *Mol Gen Genet* 180:185–191.

Baharoglu, Z., Bradley, A.S., Le Masson, M., Tsaneva, I., and Michel, B. 2008. *ruvA* Mutants that resolve Holliday junctions but do not reverse replication forks. *PLoS Genet* 4:e1000012.

Baitin, D.M., Gruenig, M.C., and Cox, M.M. 2008. SSB antagonizes RecX-RecA interaction. *J Biol Chem* 283:14198–14204.

Beam, C.E., Saveson, C.J., and Lovett, S.T. 2002. Role for *radA*/*sms* in recombination intermediate processing in *Escherichia coli*. *J Bacteriol* 184:6836–6844.

Bedale, W.A., Inman, R.B., and Cox, M.M. 1993. A reverse DNA strand exchange mediated by *recA* protein and exonuclease I. The generation of apparent DNA strand breaks by *recA* protein is explained. *J Biol Chem* 268:15004–15016.

Bell, C.E. 2005. Structure and mechanism of *Escherichia coli* RecA ATPase. *Mol Microbiol* 58:358–366.

Belle, J.J., Casey, A., Courcelle, C.T., and Courcelle, J. 2007. Inactivation of the DnaB helicase leads to the collapse and degradation of the replication fork: a comparison to UV-induced arrest. *J Bacteriol* 189:5452–5462.

Berdichevsky, A., Izhar, L., and Livneh, Z. 2002. Error-free recombinational repair predominates over mutagenic translesion replication in *E. coli*. *Mol Cell* 10:917–924.

Bi, X., and Liu, L.F. 1994. *recA*-independent and *recA*-dependent intramolecular plasmid recombination. Differential homology requirement and distance effect. *J Mol Biol* 235:414–423.

Bianco, P.R., Tracy, R.B., and Kowalczykowski, S.C. 1998. DNA strand exchange proteins: a biochemical and physical comparison. *Front Biosci* 3:D570–603.

Bichara, M., Pinet, I., Origas, M., and Fuchs, R.P. 2006. Inactivation of *recG* stimulates the RecF pathway during lesion-induced recombination in *E. coli*. *DNA Repair (Amst)* 5:129–137.

Bierne, H., Seigneur, M., Ehrlich, S.D., and Michel, B. 1997. *uvrD* mutations enhance tandem repeat deletion in the *Escherichia coli* chromosome via SOS induction of the RecF recombination pathway. *Mol Microbiol* 26:557–567.

Blakely, G., May, G., McCulloch, R., Arciszewska, L.K., Burke, M., Lovett, S.T., and Sherratt, D.J. 1993. Two related recombinases are required for site-specific recombination at *dif* and *cer* in *E. coli* K12. *Cell* 75:351–361.

Blattner, F.R., Plunkett, G., 3rd, Bloch, C.A., Perna, N.T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J.D., Rode, C.K., Mayhew, G.F., Gregor, J., Davis, N.W., Kirkpatrick, H.A., Goeden, M.A., Rose, D.J., Mau, B., and Shao, Y. 1997. The complete genome sequence of *Escherichia coli* K-12. *Science* 277:1453–1474.

Bork, J.M., Cox, M.M., and Inman, R.B. 2001. The RecOR proteins modulate RecA protein function at 5′ ends of single-stranded DNA. *EMBO J* 20:7313–7322.

Briggs, G.S., Mahdi, A.A., Weller, G.R., Wen, Q., and Lloyd, R.G. 2004. Interplay between DNA replication, recombination and repair based on the structure of RecG helicase. *Philos Trans R Soc Lond, B, Biol Sci* 359:49–59.

Butland, G., Peregrin-Alvarez, J.M., Li, J., Yang, W., Yang, X., Canadien, V., Starostine, A., Richards, D., Beattie, B., Krogan, N., Davey, M., Parkinson, J., Greenblatt, J., and Emili, A. 2005. Interaction network containing conserved and essential protein complexes in *Escherichia coli*. *Nature* 433:531–537.

Bzymek, M., and Lovett, S.T. 2001. Evidence for two mechanisms of palindrome-stimulated deletion in *Escherichia coli*: single-strand annealing and replication slipped mispairing. *Genetics* 158:527–540.

Capaldo, F.N., and Barbour, S.D. 1975. The role of the *rec* genes in the viability of *Escherichia coli* K12. *Basic Life Sci* 5A:405–418.

Carlini, L.E., and Porter, R.D. 1997. Analysis of *ssb* mutations in vivo implicates SSB protein in two distinct pathways of SOS induction and in recombinational DNA repair. *Mol Microbiol* 24:129–139.

Chase, J.W., and Williams, K.R. 1986. Single-stranded DNA binding proteins required for DNA replication. *Annu Rev Biochem* 55:103–136.

Chaudhury, A.M., and Smith, G.R. 1985. Role of *Escherichia coli* RecBC enzyme in SOS induction. *Mol Gen Genet* 201:525–528.

Chen, Z., Yang, H., and Pavletich, N. 2008. Mechanism of homologous recombination from the RecA–ssDNA/dsDNA structures. *Nature* 453:489–484.

Chiu, S.K., Low, K.B., Yuan, A., and Radding, C.M. 1997. Resolution of an early RecA-recombination intermediate by a junction-specific endonuclease. *Proc Natl Acad Sci USA* 94:6079–6083.

Chow, S.A., Honigberg, S.M., Bainton, R.J., and Radding, C.M. 1986. Patterns of nuclease protection during strand exchange. *recA* protein forms heteroduplex DNA by binding to strands of the same polarity. *J Biol Chem* 261:6961–6971.

Chrysogelos, S., and Griffith, J. 1982. *Escherichia coli* single-strand binding protein organizes single-stranded DNA in nucleosome-like units. *Proc Natl Acad Sci USA* 79:5803–5807.

Clark, A.J. 1971. Toward a metabolic interpretation of genetic recombination of *E. coli* and its phages. *Annu Rev Microbiol* 25:437–464.

Clark, A.J. 1973. Recombination deficient mutants of *E. coli* and other bacteria. *Annu Rev Genet* 7:67–86.

Clark, A.J. 1991. rec genes and homologous recombination proteins in *Escherichia coli*. *Biochimie* 73:523–532.

Clark, A.J., and Margulies, A.D. 1965. Isolation and characterization of recombination-deficient nutants of *Escherichia coli* K12. *Proc Natl Acad Sci USA* 53:451–459.

Clark, A.J., and Sandler, S.J. 1994. Homologous genetic recombination: the pieces begin to fall into place. *Crit Rev Microbiol* 20:125–142.

Cohen, A., and Laban, A. 1983. Plasmidic recombination in *Escherichia coli* K-12: the role of *recF* gene function. *Mol Gen Genet* 189:471–474.

Connelly, J.C., and Leach, D.R. 1996. The *sbcC* and *sbcD* genes of *Escherichia coli* encode a nuclease involved in palindrome inviability and genetic recombination. *Genes Cells* 1:285–291.

Connelly, J.C., Kirkham, L.A., and Leach, D.R. 1998. The SbcCD nuclease of *Escherichia coli* is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. *Proc Natl Acad Sci USA* 95:7969–7974.

Connelly, J.C., de Leau, E.S., and Leach, D.R. 1999. DNA cleavage and degradation by the SbcCD protein complex from *Escherichia coli*. *Nucleic Acids Res* 27:1039–1046.

Connelly, J.C., de Leau, E.S., and Leach, D.R. 2003. Nucleolytic processing of a protein-bound DNA end by the *E. coli* SbcCD (MR) complex. *DNA Repair (Amst)* 2:795–807.

Corrette-Bennett, S.E., and Lovett, S.T. 1995. Enhancement of RecA strand-transfer activity by the RecJ exonuclease of *Escherichia coli*. *J Biol Chem* 270:6881–6885.

Courcelle, C.T., Chow, K.H., Casey, A., and Courcelle, J. 2006. Nascent DNA processing by RecJ favors lesion repair over translesion synthesis at arrested replication forks in *Escherichia coli*. *Proc Natl Acad Sci USA* 103:9154–9159.

Courcelle, J., and Hanawalt, P.C. 1999. RecQ and RecJ process blocked replication forks prior to the resumption of replication in UV-irradiated *Escherichia coli*. *Mol Gen Genet* 262:543–551.

Cox, M.M. 2001. Recombinational DNA repair of damaged replication forks in *Escherichia coli*: questions. *Annu Rev Genet* 35:53–82.

Cox, M.M. 2007a. Motoring along with the bacterial RecA protein. *Nat Rev Mol Cell Biol* 8:127–138.

Cox, M.M. 2007b. Regulation of bacterial RecA protein function. *Crit Rev Biochem Mol Biol* 42:41–63.

Cromie, G.A., and Leach, D.R. 2001. Recombinational repair of chromosomal DNA double-strand breaks generated by a restriction endonuclease. *Mol Microbiol* 41:873–883.

Cromie, G.A., Millar, C.B., Schmidt, K.H., and Leach, D.R. 2000. Palindromes as substrates for multiple pathways of recombination in *Escherichia coli*. *Genetics* 154:513–522.

Cromie, G.A., Connelly, J.C., and Leach, D.R. 2001. Recombination at double-strand breaks and DNA ends: conserved mechanisms from phage to humans. *Mol Cell* 8:1163–1174.

Cunningham, R.P., Wu, A.M., Shibata, T., DasGupta, C., and Radding, C.M. 1981. Homologous pairing and topological linkage of DNA molecules by combined action of *E. coli* RecA protein and topoisomerase I. *Cell* 24:213–223.

DasGupta, C., Shibata, T., Cunningham, R.P., and Radding, C.M. 1980. The topology of homologous pairing promoted by RecA protein. *Cell* 22:437–446.

Dillingham, M.S., Webb, M.R., and Kowalczykowski, S.C. 2005. Bipolar DNA translocation contributes to highly processive DNA unwinding by RecBCD enzyme. *J Biol Chem* 280:37069–37077.

Dixon, D.A., and Kowalczykowski, S.C. 1991. Homologous pairing in vitro stimulated by the recombination hotspot, Chi. *Cell* 66:361–371.

Dixon, D.A., and Kowalczykowski, S.C. 1993. The recombination hotspot chi is a regulatory sequence that acts by attenuating the nuclease activity of the *E. coli* RecBCD enzyme. *Cell* 73:87–96.

Donaldson, J.R., Courcelle, C.T., and Courcelle, J. 2006. RuvABC is required to resolve holliday junctions that accumulate following replication on damaged templates in *Escherichia coli*. *J Biol Chem* 281:28811–28821.

Drees, J.C., Lusetti, S.L., and Cox, M.M. 2004. Inhibition of RecA protein by the *Escherichia coli* RecX protein: modulation by the RecA C terminus and filament functional state. *J Biol Chem* 279:52991–52997.

Dutra, B.E., Sutera, V.A., and Lovett, S.T. 2007. RecA-independent recombination is efficient but limited by exonucleases. *Proc Natl Acad Sci USA* 104:216–221.

Emmerson, P.T., and Howard-Flanders, P. 1967. Cotransduction with *thy* of a gene required for genetic recombination in *Escherichia coli*. *J Bacteriol* 93:1729–1731.

Enquist, L.W., and Skalka, A. 1973. Replication of bacteriophage lambda DNA dependent on the function of host and viral genes. I. Interaction of *red, gam* and *rec*. *J Mol Biol* 75:185–212.

Eykelenboom, J.K., Blackwood, J.K., Okely, E., and Leach, D.R. 2008. SbcCD causes a double-strand break at a DNA palindrome in the *Escherichia coli* chromosome. *Mol Cell* 29:644–651.

Feinstein, S.I., and Low, K.B. 1986. Hyper-recombining recipient strains in bacterial conjugation. *Genetics* 113:13–33.

Felzenszwalb, I., Sargentini, N.J., and Smith, K.C. 1984. Characterization of a new radiation-sensitive mutant, *Escherichia coli* K-12 *radC102*. *Radiat Res* 97:615–625.

Feschenko, V.V., Rajman, L.A., and Lovett, S.T. 2003. Stabilization of perfect and imperfect tandem repeats by single-strand DNA exonucleases. *Proc Natl Acad Sci USA* 100:1134–1139.

Finch, P.W., Chambers, P., and Emmerson, P.T. 1985. Identification of the *Escherichia coli recN* gene product as a major SOS protein. *J Bacteriol* 164:653–658.

Fishel, R.A., and Kolodner, R. 1984. *Escherichia coli* strains containing mutations in the structural gene for topoisomerase I are recombination deficient. *J Bacteriol* 160:1168–1170.

Foti, J.J., Schienda, J., Sutera, V.A., Jr., and Lovett, S.T. 2005. A bacterial G protein-mediated response to replication arrest. *Mol Cell* 17:549–560.

Friedman-Ohana, R., and Cohen, A. 1998. Heteroduplex joint formation in *Escherichia coli* recombination is initiated by pairing of a 3′-ending strand. *Proc Natl Acad Sci USA*, 95:6909–6914.

Friedman-Ohana, R., Karunker, I., and Cohen, A. 1999. A RecG-independent nonconservative branch migration mechanism in *Escherichia coli* recombination. *J Bacteriol* 181:7199–7205.

Galitski, T., and Roth, J.R. 1997. Pathways for homologous recombination between chromosomal direct repeats in *Salmonella typhimurium*. *Genetics* 146:751–767.

Galletto, R., Amitani, I., Baskin, R.J., and Kowalczykowski, S.C. 2006. Direct observation of individual RecA filaments assembling on single DNA molecules. *Nature* 443:875–878.

Gangloff, S., McDonald, J.P., Bendixen, C., Arthur, L., and Rothstein, R. 1994. The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. *Mol Cell Biol* 14:8391–8398.

Gibson, F.P., Leach, D.R., and Lloyd, R.G. 1992. Identification of *sbcD* mutations as cosuppressors of *recBC* that allow propagation of DNA palindromes in *Escherichia coli* K-12. *J Bacteriol* 174:1222–1228.

Gillen, J.R., Willis, D.K., and Clark, A.J. 1981. Genetic analysis of the RecE pathway of genetic recombination in *Escherichia coli* K-12. *J Bacteriol* 145:521–532.

Glassberg, J., Meyer, R.R., and Kornberg, A. 1979. Mutant single-strand binding protein of *Escherichia coli*: genetic and physiological characterization. *J Bacteriol* 140:14–19.

Goldfless, S.J., Morag, A.S., Belisle, K.A., Sutera, V.A., and Lovett, S.T. 2006. DNA repeat rearrangements mediated by DnaK-dependent replication fork repair. *Mol Cell* 21:595–604.

Goodman, M.F., and Tippin, B. 2000. Sloppier copier DNA polymerases involved in genome repair. *Curr Opin Genet Dev* 10:162–168.

Grompone, G., Sanchez, N., Dusko Ehrlich, S., and Michel, B. 2004. Requirement for RecFOR-mediated recombination in *priA* mutant. *Mol Microbiol* 52:551–562.

Haber, J.E. 1998. Mating-type gene switching in *Saccharomyces cerevisiae*. *Annu Rev Genet* 32:561–599.

Han, E.S., Cooper, D.L., Persky, N.S., Sutera, V.A., Jr., Whitaker, R.D., Montello, M.L., and Lovett, S.T. 2006. RecJ exonuclease: substrates, products and interaction with SSB. *Nucleic Acids Res* 34:1084–1091.

Harmon, F.G., and Kowalczykowski, S.C. 2001. Biochemical characterization of the DNA helicase activity of the *Escherichia coli* RecQ helicase. *J Biol Chem* 276:232–243.

Harmon, F.G., DiGate, R.J., and Kowalczykowski, S.C. 1999. RecQ helicase and topoisomerase III comprise a novel DNA strand passage function: a conserved mechanism for control of DNA recombination. *Mol Cell* 3:611–620.

Heller, R.C., and Marians, K.J. 2005. The disposition of nascent strands at stalled replication forks dictates the pathway of replisome loading during restart. *Mol Cell* 17:733–743.

Hirano, T. 2005. SMC proteins and chromosome mechanics: from bacteria to humans. *Philos Trans R Soc Lond, B, Biol Sci* 360:507–514.

Hobbs, M.D., Sakai, A., and Cox, M.M. 2007. SSB protein limits RecOR binding onto single-stranded DNA. *J Biol Chem* 282:11058–11067.

Horii, Z., and Clark, A.J. 1973. Genetic analysis of the recF pathway to genetic recombination in *Escherichia coli* K12: isolation and characterization of mutants. *J Mol Biol* 80:327–344.

Howard-Flanders, P., and Theriot, L. 1966. Mutants of *Escherichia coli* K-12 defective in DNA repair and in genetic recombination. *Genetics* 53:1137–1150.

Joo, C., McKinney, S.A., Nakamura, M., Rasnik, I., Myong, S., and Ha, T. 2006. Real-time observation of RecA filament dynamics with single monomer resolution. *Cell* 126:515–527.

Kang, J., and Blaser, M.J. 2006. UvrD helicase suppresses recombination and DNA damage-induced deletions. *J Bacteriol* 188:5450–5459.

Kantake, N., Madiraju, M.V., Sugiyama, T., and Kowalczykowski, S.C. 2002. *Escherichia coli* RecO protein anneals ssDNA complexed with its cognate ssDNA-binding protein: A common step in genetic recombination. *Proc Natl Acad Sci USA* 99:15327–15332.

Kaplan, D.L., and O'Donnell, M. 2006. RuvA is a sliding collar that protects Holliday junctions from unwinding while promoting branch migration. *J Mol Biol* 355:473–490.

Kidane, D., and Graumann, P.L. 2005. Intracellular protein and DNA dynamics in competent *Bacillus subtilis* cells. *Cell* 122:73–84.

Kidane, D., Sanchez, H., Alonso, J.C., and Graumann, P.L. 2004. Visualization of DNA double-strand break repair in live bacteria reveals dynamic recruitment of Bacillus subtilis RecF, RecO and RecN proteins to distinct sites on the nucleoids. *Mol Microbiol* 52:1627–1639.

Kogoma, T. 1996. Recombination by replication. *Cell* 85:625–627.

Kogoma, T., Cadwell, G.W., Barnard, K.G., and Asai, T. 1996. The DNA replication priming protein, PriA, is required for homologous recombination and double-strand break repair. *J Bacteriol* 178:1258–1264.

Kolodner, R., Fishel, R.A., and Howard, M. 1985. Genetic recombination of bacterial plasmid DNA: effect of RecF pathway mutations on plasmid recombination in *Escherichia coli*. *J Bacteriol* 163:1060–1066.

Konforti, B.B., and Davis, R.W. 1992. ATP hydrolysis and the displaced strand are two factors that determine the polarity of RecA-promoted DNA strand exchange. *J Mol Biol* 227:38–53.

Kornberg, A., and Baker, T.A. 1991. *DNA replication*, 2nd edn W. H. Freeman, New York, NY.

Kosa, J.L., Zdraveski, Z.Z., Currier, S., Marinus, M.G., and Essigmann, J.M. 2004. RecN and RecG are required for *Escherichia coli* survival of Bleomycin-induced damage. *Mutat Res* 554:149–157.

Kouzminova, E.A., and Kuzminov, A. 2004. Chromosomal fragmentation in dUTPase-deficient mutants of *Escherichia coli* and its recombinational repair. *Mol Microbiol* 51:1279–1295.

Kouzminova, E.A., Rotman, E., Macomber, L., Zhang, J., and Kuzminov, A. 2004. RecA-dependent mutants in *Escherichia coli* reveal strategies to avoid chromosomal fragmentation. *Proc Natl Acad Sci USA* 101:16262–16267.

Kowalczykowski, S.C. 2000. Initiation of genetic recombination and recombination-dependent replication. *Trends Biochem Sci* 25:156–165.

Kowalczykowski, S.C., and Krupp, R.A. 1987. Effects of *Escherichia coli* SSB protein on the single-stranded DNA-dependent ATPase activity of *Escherichia coli* RecA protein. Evidence that SSB protein facilitates the binding of RecA protein to regions of secondary structure within single-stranded DNA. *J Mol Biol* 193:97–113.

Kowalczykowski, S.C., Clow, J., Somani, R., and Varghese, A. 1987. Effects of the *Escherichia coli* SSB protein on the binding of *Escherichia coli* RecA protein to single-stranded DNA. Demonstration of competitive binding and the lack of a specific protein-protein interaction. *J Mol Biol* 193:81–95.

Kreuzer, K.N. 2005. Interplay between DNA replication and recombination in prokaryotes. *Annu Rev Microbiol* 59:43–67.

Kushner, S.R., Nagaishi, H., Templin, A., and Clark, A.J. 1971. Genetic recombination in *Escherichia coli*: the role of exonuclease I. *Proc Natl Acad Sci USA* 68:824–827.

Kuzminov, A. 1999. Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage lambda. *Microbiol Mol Biol Rev* 63:751–813.

Kuzminov, A., and Stahl, F.W., Editor N.P. Higgins. 2005. Overview of homologous recombination and repair machines. In *The Bacterial Chromosome*, 2nd edition: 349–368. Washington, DC: American Society of Microbiology Press.

Lam, S.T., Stahl, M.M., McMilin, K.D., and Stahl, F.W. 1974. Rec-mediated recombinational hot spot activity in bacteriophage lambda. II. A mutation which causes hot spot activity. *Genetics* 77:425–433.

Lavery, P.E., and Kowalczykowski, S.C. 1992. A postsynaptic role for single-stranded DNA-binding protein in *recA* protein-promoted DNA strand exchange. *J Biol Chem* 267:9315–9320.

Lehman, I.R., and Nussbaum, A.L. 1964. The deoxyribonucleases of *Escherichia coli*. V. On the specificity of Exonuclease I (phosphodiesterase). *J Biol Chem* 239:2628–2636.

Lim, D.S., and Hasty, P. 1996. A mutation in mouse *rad51* results in an early embryonic lethal that is suppressed by a mutation in p53. *Mol Cell Biol* 16:7133–7143.

Lin, Z., Kong, H., Nei, M., and Ma, H. 2006. Origins and evolution of the *recA*/*RAD51* gene family: evidence for ancient gene duplication and endosymbiotic gene transfer. *Proc Natl Acad Sci USA* 103:10328–10333.

Lindsley, J.E., and Cox, M.M. 1990. Assembly and disassembly of RecA protein filaments occur at opposite filament ends. Relationship to DNA strand exchange. *J Biol Chem* 265:9043–9054.

Linn, S., and Imlay, J.A. 1987. Toxicity, mutagenesis and stress responses induced in *Escherichia coli* by hydrogen peroxide. *J Cell Sci Suppl* 6:289–301.

Lloyd, R.G. 1991. Conjugational recombination in resolvase-deficient *ruvC* mutants of *Escherichia coli* K-12 depends on *recG*. *J Bacteriol* 173:5414–5418.

Lloyd, R.G., and Buckman, C. 1985. Identification and genetic analysis of *sbcC* mutations in commonly used *recBC sbcB* strains of *Escherichia coli* K-12. *J Bacteriol* 164:836–844.

Lloyd, R.G., and Buckman, C. 1995. Conjugational recombination in *Escherichia coli*: genetic analysis of recombinant formation in Hfr x F- crosses. *Genetics* 139:1123–1148.

Lloyd, R.G., Benson, F.E., and Shurvinton, C.E. 1984. Effect of *ruv* mutations on recombination and DNA repair in *Escherichia coli* K12. *Mol Gen Genet* 194:303–309.

Lohman, T.M., and Ferrari, M.E. 1994. *Escherichia coli* single-stranded DNA-binding protein: multiple DNA-binding modes and cooperativities. *Annu Rev Biochem* 63:527–570.

Lopez, C.R., Yang, S., Deibler, R.W., Ray, S.A., Pennington, J.M., Digate, R.J., Hastings, P.J., Rosenberg, S.M., and Zechiedrich, E.L. 2005. A role for topoisomerase III in a recombination pathway alternative to RuvABC. *Mol Microbiol* 58:80–101.

Lovett, S.T. 2003. Connecting replication and recombination. *Mol Cell* 11:554–556.

Lovett, S.T. 2006. Replication arrest-stimulated recombination: Dependence on the RecA paralog, RadA/Sms and translesion polymerase, DinB. *DNA Repair (Amst)* 5:1421–1427.

Lovett, S.T., and Clark, A.J. 1984. Genetic analysis of the *recJ* gene of *Escherichia coli* K-12. *J Bacteriol* 157:190–196.

Lovett, S.T., and Feschenko, V.V. 1996. Stabilization of diverged tandem repeats by mismatch repair: evidence for deletion formation via a misaligned replication intermediate. *Proc Natl Acad Sci USA* 93:7120–7124.

Lovett, S.T., and Kolodner, R.D. 1989. Identification and purification of a single-stranded-DNA-specific exonuclease encoded by the *recJ* gene of *Escherichia coli*. *Proc Natl Acad Sci USA* 86:2627–2631.

Lovett, S.T., and Sutera, V.A. 1995. Suppression of *recJ* exonuclease mutants of *Escherichia coli* by alterations in DNA helicases II (*uvrD*) and IV (*helD*). *Genetics* 140:27–45.

Lovett, S.T., Drapkin, P.T., Sutera, V.A., and Gluckman-Peskind, T.J. 1993. A sister-strand exchange mechanism for *recA*-independent deletion of repeated DNA sequences in *Escherichia coli*. *Genetics* 135:631–642.

Lovett, S.T., Hurley, R.L., Sutera, V.A., Jr., Aubuchon, R.H., and Lebedeva, M.A. 2002. Crossing over between regions of limited homology in *Escherichia coli*. RecA-dependent and RecA-independent pathways. *Genetics* 160:851–859.

Luisi-DeLuca, C. 1995. Homologous pairing of single-stranded DNA and superhelical double-stranded DNA catalyzed by RecO protein from *Escherichia coli*. *J Bacteriol* 177:566–572.

Luisi-DeLuca, C., and Kolodner, R. 1994. Purification and characterization of the *Escherichia coli* RecO protein. Renaturation of complementary single-stranded DNA molecules catalyzed by the RecO protein. *J Mol Biol* 236:124–138.

Lusetti, S.L., Voloshin, O.N., Inman, R.B., Camerini-Otero, R.D., and Cox, M.M. 2004a. The DinI protein stabilizes RecA protein filaments. *J Biol Chem* 279:30037–30046.

Lusetti, S.L., Drees, J.C., Stohl, E.A., Seifert, H.S., and Cox, M.M. 2004b. The DinI and RecX proteins are competing modulators of RecA function. *J Biol Chem* 279:55073–55079.

Lusetti, S.L., Hobbs, M.D., Stohl, E.A., Chitteni-Pattu, S., Inman, R.B., Seifert, H.S., and Cox, M.M. 2006. The RecF protein antagonizes RecX function via direct interaction. *Mol Cell* 21:41–50.

Madiraju, M.V., Templin, A., and Clark, A.J. 1988. Properties of a mutant *recA*-encoded protein reveal a possible role for *Escherichia coli recF*-encoded protein in genetic recombination. *Proc Natl Acad Sci USA* 85:6592–6596.

Magner, D.B., Blankschien, M.D., Lee, J.A., Pennington, J.M., Lupski, J.R., and Rosenberg, S.M. 2007. RecQ promotes toxic recombination in cells lacking recombination intermediate-removal proteins. *Mol Cell* 26:273–286.

Mahdi, A.A., and Lloyd, R.G. 1989. Identification of the *recR* locus of *Escherichia coli* K-12 and analysis of its role in recombination and DNA repair. *Mol Gen Genet* 216:503–510.

Mahdi, A.A., Sharples, G.J., Mandal, T.N., and Lloyd, R.G. 1996. Holliday junction resolvases encoded by homologous *rusA* genes in *Escherichia coli* K-12 and phage 82. *J Mol Biol* 257:561–573.

Mandal, T.N., Mahdi, A.A., Sharples, G.J., and Lloyd, R.G. 1993. Resolution of Holliday intermediates in recombination and DNA repair: indirect suppression of *ruvA*, *ruvB*, and *ruvC* mutations. *J Bacteriol* 175:4325–4334.

Marians, K.J. 2004. Mechanisms of replication fork restart in *Escherichia coli*. *Philos Trans R Soc Lond, B, Biol Sci* 359:71–77.

Mazin, A.V., and Kowalczykowski, S.C. 1998. The function of the secondary DNA-binding site of RecA protein during DNA strand exchange. *EMBO J* 17:1161–1168.

McEachern, M.J., and Haber, J.E. 2006. Break-induced replication and recombinational telomere elongation in yeast. *Annu Rev Biochem* 75:111–135.

McGlynn, P., and Lloyd, R.G. 2002. Genome stability and the processing of damaged replication forks by RecG. *Trends Genet* 18:413–419.

McGrew, D.A., and Knight, K.L. 2003. Molecular design and functional organization of the RecA protein. *Crit Rev Biochem Mol Biol* 38:385–432.

Meddows, T.R., Savory, A.P., and Lloyd, R.G. 2004. RecG helicase promotes DNA double-strand break repair. *Mol Microbiol* 52:119–132.

Meddows, T.R., Savory, A.P., Grove, J.I., Moore, T., and Lloyd, R.G. 2005. RecN protein and transcription factor DksA combine to promote faithful recombinational repair of DNA double-strand breaks. *Mol Microbiol* 57:97–110.

Menetski, J.P., and Kowalczykowski, S.C. 1985. Interaction of *recA* protein with single-stranded DNA. Quantitative aspects of binding affinity modulation by nucleotide cofactors. *J Mol Biol* 181:281–295.

Meyer, R.R., and Laine, P.S. 1990. The single-stranded DNA-binding protein of *Escherichia coli*. *Microbiol Rev* 54:342–380.

Michel, B., Boubakri, H., Baharoglu, Z., LeMasson, M., and Lestini, R. 2007. Recombination proteins and rescue of arrested replication forks. *DNA Repair (Amst)*, 6:967–980.

Molineux, I.J., Friedman, S., and Gefter, M.L. 1974. Purification and properties of the *Escherichia coli* deoxyribonucleic acid-unwinding protein. Effects on deoxyribonucleic acid synthesis in vitro. *J Biol Chem* 249:6090–6098.

Molineux, I.J., and Gefter, M.L. 1975. Properties of the *Escherichia coli* DNA-binding (unwinding) protein interaction with nucleolytic enzymes and DNA. *J Mol Biol* 98:811–825.

Moreau, P.L. 1988. Overproduction of single-stranded-DNA-binding protein specifically inhibits recombination of UV-irradiated bacteriophage DNA in *Escherichia coli*. *J Bacteriol* 170:2493–2500.

Morel, P., Hejna, J.A., Ehrlich, S.D., and Cassuto, E. 1993. Antipairing and strand transferase activities of *E. coli* helicase II (UvrD). *Nucleic Acids Res* 21:3205–3209.

Morel, P., Stasiak, A., Ehrlich, S.D., and Cassuto, E. 1994. Effect of length and location of heterologous sequences on RecA-mediated strand exchange. *J Biol Chem* 269:19830–19835.

Morimatsu, K., and Kowalczykowski, S.C. 2003. RecFOR proteins load RecA protein onto gapped DNA to accelerate DNA strand exchange: a universal step of recombinational repair. *Mol Cell* 11:1337–1347.

Muniyappa, K., Shaner, S.L., Tsang, S.S., and Radding, C.M. 1984. Mechanism of the concerted action of *recA* protein and helix-destabilizing proteins in homologous recombination. *Proc Natl Acad Sci USA*, 81:2757–2761.

Nagashima, K., Kubota, Y., Shibata, T., Sakaguchi, C., Shinagawa, H., and Hishida, T. 2006. Degradation of *Escherichia coli* RecN aggregates by ClpXP protease and its implications for DNA damage tolerance. *J Biol Chem* 281:30941–30946.

Nakayama, H., Nakayama, K., Nakayama, R., Irino, N., Nakayama, Y., and Hanawalt, P.C. 1984. Isolation and genetic characterization of a thymineless death-resistant mutant of *Escherichia coli* K12: identification of a new mutation (recQ1) that blocks the RecF recombination pathway. *Mol Gen Genet* 195:474–480.

Nassif, N., Penney, J., Pal, S., Engels, W.R., and Gloor, G.B. 1994. Efficient copying of nonhomologous sequences from ectopic sites via P-element-induced gap repair. *Mol Cell Biol* 14:1613–1625.

Neuwald, A.F., Berg, D.E., and Stauffer, G.V. 1992. Mutational analysis of the *Escherichia coli serB* promoter region reveals transcriptional linkage to a downstream gene. *Gene* 120:1–9.

Nowosielska, A., Calmann, M.A., Zdraveski, Z., Essigmann, J.M., and Marinus, M.G. 2004. Spontaneous and cisplatin-induced recombination in *Escherichia coli*. *DNA Repair (Amst)*, 3:719–728.

Nowosielska, A., Smith, S.A., Engelward, B.P., and Marinus, M.G. 2006. Homologous recombination prevents methylation-induced toxicity in *Escherichia coli*. *Nucleic Acids Res*, 34:2258–2268.

Petit, M.A., Dimpfl, J., Radman, M., and Echols, H. 1991. Control of large chromosomal duplications in *Escherichia coli* by the mismatch repair system. *Genetics* 129:327–332.

Pham, P., Rangarajan, S., Woodgate, R., and Goodman, M.F. 2001. Roles of DNA polymerases V and II in SOS-induced error-prone and error-free repair in *Escherichia coli*. *Proc Natl Acad Sci USA* 98:8350–8354.

Picksley, S.M., Attfield, P.V., and Lloyd, R.G. 1984. Repair of DNA double-strand breaks in *Escherichia coli* K12 requires a functional recN product. *Mol Gen Genet* 195:267–274.

Ponder, R.G., Fonville, N.C., and Rosenberg, S.M. 2005. A switch from high-fidelity to error-prone DNA double-strand break repair underlies stress-induced mutation. *Mol Cell* 19:791–804.

Prell, A., and Wackernagel, W. 1980. Degradation of linear and circular DNA with gaps by the *recBC* enzyme of *Escherichia coli*. Effects of gap length and the presence of cell-free extracts. *Eur J Biochem* 105:109–116.

Quinones, A., and Piechocki, R. 1987. Differential suppressor effects of the *ssb-1* and *ssb-113* alleles on *uvrD* mutator of *Escherichia coli* in DNA repair and mutagenesis. *J Basic Microbiol* 27:263–273.

Raghunathan, S., Ricard, C.S., Lohman, T.M., and Waksman, G. 1997. Crystal structure of the homo-tetrameric DNA binding domain of *Escherichia coli* single-stranded DNA-binding protein determined by multiwavelength x-ray diffraction on the selenomethionyl protein at 2.9-A resolution. *Proc Natl Acad Sci USA*, 94:6652–6657.

Rayssiguier, C., Thaler, D.S., and Radman, M. 1989. The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. *Nature* 342:396–401.

Register, J.C., and Griffith, J. 1985. The direction of RecA protein assembly onto single strand DNA is the same as the direction of strand assimilation during strand exchange. *J Biol Chem* 260:12308–12312.

Renzette, N., Gumlaw, N., and Sandler, S.J. 2007. DinI and RecX modulate RecA-DNA structures in *Escherichia coli* K-12. *Mol Microbiol* 63:103–115.

Roberts, D., Hoopes, B.C., McClure, W.R., and Kleckner, N. 1985. IS10 transposition is regulated by DNA adenine methylation. *Cell* 43:117–130.

Rupp, W.D., and Howard-Flanders, P. 1968. Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. *J Mol Biol* 31:291–304.

Rupp, W.D., Wilde, C.E., Reno, D.L., and Howard-Flanders, P. 1971. Exchanges between DNA strands in ultraviolet-irradiated *Escherichia coli*. *J Mol Biol* 61:25–44.

Sanchez, H., Cardenas, P.P., Yoshimura, S.H., Takeyasu, K., and Alonso, J.C. 2008. Dynamic structures of Bacillus subtilis RecN-DNA complexes. *Nucleic Acids Res* 36:110–120.

Sandigursky, M., and Franklin, W.A. 1994. *Escherichia coli* single-stranded DNA binding protein stimulates the DNA deoxyribophosphodiesterase activity of exonuclease I. *Nucleic Acids Res* 22:247–250.

Sandler, S.J., and Clark, A.J. 1994. RecOR suppression of recF mutant phenotypes in *Escherichia coli* K-12. *J Bacteriol* 176:3661–3672.

Sandler, S.J., and Marians, K.J. 2000. Role of PriA in replication fork reactivation in *Escherichia coli*. *J Bacteriol* 182:9–13.

Sandler, S.J., Marians, K.J., Zavitz, K.H., Coutu, J., Parent, M.A., and Clark, A.J. 1999. *dnaC* mutations suppress defects in DNA replication- and recombination-associated functions in *priB* and *priC* double mutants in *Escherichia coli* K-12. *Mol Microbiol* 34:91–101.

Sano, Y. 1993. Role of the *recA*-related gene adjacent to the *recA* gene in *Pseudomonas aeruginosa*. *J Bacteriol* 175:2451–2454.

Sargentini, N.J., and Smith, K.C. 1986. Quantitation of the involvement of the *recA, recB, recC, recF, recJ, recN, lexA, radA, radB, uvrD,* and *umuC* genes in the repair of X-ray-induced DNA double-strand breaks in *Escherichia coli*. *Radiat Res* 107:58–72.

Saveson, C.J., and Lovett, S.T. 1999. Tandem repeat recombination induced by replication fork defects in *Escherichia coli* requires a novel factor, RadC. *Genetics* 152:5–13.

Savvides, S.N., Raghunathan, S., Fütterer, K., Kozlov, A.G., Lohman, T.M., and Waksman, G. 2004. The C-terminal domain of full-length *E. coli* SSB is disordered even when bound to DNA. *Protein Sci* 13:1942–1947.

Sawitzke, J.A., and Stahl, F.W. 1992. Phage lambda has an analog of *Escherichia coli recO, recR* and *recF* genes. *Genetics* 130:7–16.

Schwacha, A., and Kleckner, N. 1995. Identification of double Holliday junctions as intermediates in meiotic recombination. *Cell* 83:783–791.

Shan, Q., Bork, J.M., Webb, B.L., Inman, R.B., and Cox, M.M. 1997. RecA protein filaments: end-dependent dissociation from ssDNA and stabilization by RecO and RecR proteins. *J Mol Biol* 265:519–540.

Sharma, R.C., and Smith, K.C. 1987. Role of DNA polymerase I in postreplication repair: a reexamination with *Escherichia coli* delta *polA*. *J Bacteriol* 169:4559–4564.

Sharples, G.J., Chan, S.N., Mahdi, A.A., Whitby, M.C., and Lloyd, R.G. 1994. Processing of intermediates in recombination and DNA repair: identification of a new endonuclease that specifically cleaves Holliday junctions. *EMBO J* 13:6133–6142.

Shen, P., and Huang, H.V. 1986. Homologous recombination in *Escherichia coli*: dependence on substrate length and homology. *Genetics* 112:441–457.

Shereda, R.D., Bernstein, D.A., and Keck, J.L. 2007. A central role for SSB in *Escherichia coli* RecQ DNA helicase function. *J Biol Chem* 282:19247–19258.

Sigal, N., Delius, H., Kornberg, T., Gefter, M.L., and Alberts, B. 1972. A DNA-unwinding protein isolated from *Escherichia coli*: its interaction with DNA and with DNA polymerases. *Proc Natl Acad Sci USA* 69:3537–3541.

Singleton, M.R., Dillingham, M.S., Gaudier, M., Kowalczykowski, S.C., and Wigley, D.B. 2004. Crystal structure of RecBCD enzyme reveals a machine for processing DNA breaks. *Nature* 432:187–193.

Smith, G.R. 1987. Mechanism and control of homologous recombination in *Escherichia coli*. *Annu Rev Genet* 21:179–201.

Smith, G.R. 1991. Conjugational recombination in *E. coli*: myths and mechanisms. *Cell* 64:19–27.

Smith, G.R. 2001. Homologous recombination near and far from DNA breaks: alternative roles and contrasting views. *Annu Rev Genet* 35:243–274.

Smith, K.C. 2004. Recombinational DNA repair: the ignored repair systems. *Bioessays* 26:1322–1326.

Smith, K.C., and Wang, T.C. 1989. *recA*-dependent DNA repair processes. *Bioessays* 10:12–16.

Song, Y., and Sargentini, N.J. 1996. *Escherichia coli* DNA repair genes *radA* and *sms* are the same gene. *J Bacteriol* 178:5045–5048.

Spies, M. and Kowalczykowski, S.C. 2005. Homologous recombination by the RecBCD and RecF pathways, pp. 389–403. In Higgins, N.P., ed. *The Bacterial Chromosome*, 2nd edition. Washington, DC: ASM Press.

Spies, M., and Kowalczykowski, S.C. 2006. The RecA binding locus of RecBCD is a general domain for recruitment of DNA strand exchange proteins. *Mol Cell* 21:573–580.

Spies, M., Dillingham, M.S., and Kowalczykowski, S.C. 2005. Translocation by the RecB motor is an absolute requirement for {chi}-recognition and RecA protein loading by RecBCD enzyme. *J Biol Chem* 280:37078–37087.

Spies, M., Amitani, I., Baskin, R.J., and Kowalczykowski, S.C. 2007. RecBCD enzyme switches lead motor subunits in response to chi recognition. *Cell* 131:694–705.

Srivenugopal, K.S., and Morris, D.R. 1986. Modulation of the relaxing activity of *Escherichia coli* topoisomerase I by single-stranded DNA binding proteins. *Biochem Biophys Res Commun* 137:795–800.

Steiner, W.W., and Kuempel, P.L. 1998. Sister chromatid exchange frequencies in *Escherichia coli* analyzed by recombination at the dif resolvase site. *J Bacteriol* 180:6269–6275.

Stohl, E.A., Brockman, J.P., Burkle, K.L., Morimatsu, K., Kowalczykowski, S.C., and Seifert, H.S. 2003. *Escherichia coli* RecX inhibits RecA recombinase and coprotease activities in vitro and in vivo. *J Biol Chem* 278:2278–2285.

Stracker, T.H., Theunissen, J.W., Morales, M., and Petrini, J.H. 2004. The Mre11 complex and the metabolism of chromosome breaks: the importance of communicating and holding things together. *DNA Repair (Amst)* 3:845–854.

Strunnikov, A.V. 2006. SMC complexes in bacterial chromosome condensation and segregation. *Plasmid* 55:135–144.

Suski, C., and Marians, K.J. 2008. Resolution of converging replication forks by RecQ and topoisomerase III. *Mol Cell* 30:779–789.

Symington, L.S. 2002. Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. *Microbiol Mol Biol Rev* 66:630–670, table of contents.

Szostak, J.W., Orr-Weaver, T.L., Rothstein, R.J., and Stahl, F.W. 1983. The double-strand-break repair model for recombination. *Cell* 33:25–35.

Taylor, A.F., and Smith, G.R. 1985. Substrate specificity of the DNA unwinding activity of the RecBC enzyme of *Escherichia coli*. *J Mol Biol* 185:431–443.

Taylor, A.F., and Smith, G.R. 2003. RecBCD enzyme is a DNA helicase with fast and slow motors of opposite polarity. *Nature* 423:889–893.

Thoms, B., and Wackernagel, W. 1998. Interaction of RecBCD enzyme with DNA at double-strand breaks produced in UV-irradiated *Escherichia coli*: requirement for DNA end processing. *J Bacteriol* 180:5639–5645.

Thresher, R.J., Christiansen, G., and Griffith, J.D. 1988. Assembly of presynaptic filaments. Factors affecting the assembly of RecA protein onto single-stranded DNA. *J Mol Biol* 201:101–113.

Tippin, B., Pham, P., and Goodman, M.F. 2004. Error-prone replication for better or worse. *Trends Microbiol* 12:288–295.

Tomer, G., Cohen-Fix, O., O'Donnell, M., Goodman, M., and Livneh, Z. 1996. Reconstitution of repair-gap UV mutagenesis with purified proteins from *Escherichia coli*: a role for DNA polymerases III and II. *Proc Natl Acad Sci USA* 93:1376–1380.

Tsuzuki, T., Fujii, Y., Sakumi, K., Tominaga, Y., Nakao, K., Sekiguchi, M., Matsushiro, A., Yoshimura, Y., and MoritaT, 1996. Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. *Proc Natl Acad Sci USA* 93:6236–6240.

Umezu, K., and Kolodner, R.D. 1994. Protein interactions in genetic recombination in *Escherichia coli*. Interactions involving RecO and RecR overcome the inhibition of RecA by single-stranded DNA-binding protein. *J Biol Chem* 269:30005–30013.

Umezu, K., and Nakayama, H. 1993. RecQ DNA helicase of *Escherichia coli*. Characterization of the helix-unwinding activity with emphasis on the effect of single-stranded DNA-binding protein. *J Mol Biol* 230:1145–1150.

Umezu, K., Nakayama, K., and Nakayama, H. 1990. *Escherichia coli* RecQ protein is a DNA helicase. *Proc Natl Acad Sci USA* 87:5363–5367.

Umezu, K., Chi, N.W., and Kolodner, R.D. 1993. Biochemical interaction of the *Escherichia coli* RecF, RecO, and RecR proteins with RecA protein and single-stranded DNA binding protein. *Proc Natl Acad Sci USA*, 90:3875–3879.

van der Heijden, T., Modesti, M., Hage, S., Kanaar, R., Wyman, C., and Dekker, C. 2008. Homologous recombination in real time: DNA strand exchange by RecA. *Mol Cell* 30:530–538.

Veaute, X., Delmas, S., Selva, M., Jeusset, J., Le Cam, E., Matic, I., Fabre, F., and Petit, M.A. 2005. UvrD helicase, unlike Rep helicase, dismantles RecA nucleoprotein filaments in *Escherichia coli*. *EMBO J* 24:180–189.

Viswanathan, M., and Lovett, S.T. 1998. Single-strand DNA-specific exonucleases in *Escherichia coli*. Roles in repair and mutation avoidance. *Genetics* 149:7–16.

Volkert, M.R., and Hartke, M.A. 1984. Suppression of *Escherichia coli recF* mutations by *recA*-linked *srfA* mutations. *J Bacteriol* 157:498–506.

Voloshin, O.N., Ramirez, B.E., Bax, A., and Camerini-Otero, R.D. 2001. A model for the abrogation of the SOS response by an SOS protein: a negatively charged helix in DinI mimics DNA in its interaction with RecA. *Genes Dev*, 15:415–427.

Walker, G.C. 1987. The SOS response of *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology. pp. 1346–1357. In Neidhardt, F.C., ed. *Cellular and Molecular Biology*, Vol. 2. Washington, DC: American Society for Microbiology.

Wang, T.C., and Smith, K.C. 1982. Effects of the *ssb-1* and *ssb-113* mutations on survival and DNA repair in UV-irradiated delta *uvrB* strains of *Escherichia coli* K-12. *J Bacteriol* 151:186–192.

Wang, T.V., and Smith, K.C. 1984. *recF*-dependent and *recF recB*-independent DNA gap-filling repair processes transfer dimer-containing parental strands to daughter strands in *Escherichia coli* K-12 uvrB. *J Bacteriol* 158:727–729.

Wang, T.C., and Smith, K.C. 1986. recA (Srf) suppression of *recF* deficiency in the postreplication repair of UV-irradiated *Escherichia coli* K-12. *J Bacteriol* 168:940–946.

Webb, B.L., Cox, M.M., and Inman, R.B. 1997. Recombinational DNA repair: the RecF and RecR proteins limit the extension of RecA filaments beyond single-strand DNA gaps. *Cell* 91:347–356.

West, S.C. 1997. Processing of recombination intermediates by the RuvABC proteins. *Annu Rev Genet* 31:213–244.

Whitby, M.C., Ryder, L., and Lloyd, R.G. 1993. Reverse branch migration of Holliday junctions by RecG protein: a new mechanism for resolution of intermediates in recombination and DNA repair. *Cell* 75:341–350.

Worth, L., Clark, S., Radman, M., and Modrich, P. 1994. Mismatch repair proteins MutS and MutL inhibit RecA-catalyzed strand transfer between diverged DNAs. *Proc Natl Acad Sci USA* 91:3238–3241.

Wu, L., and Hickson, I.D. 2003. The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature* 426:870–874.

Wyman, C., Ristic, D., and Kanaar, R. 2004. Homologous recombination-mediated double-strand break repair. *DNA Repair (Amst)* 3:827–833.

Xu, L., and Marians, K.J. 2003. PriA mediates DNA replication pathway choice at recombination intermediates. *Mol Cell* 11:817–826.

Yasuda, T., Morimatsu, K., Horii, T., Nagata, T., and Ohmori, H. 1998. Inhibition of *Escherichia coli* RecA coprotease activities by DinI. *Embo J* 17:3207–3216.

Yin, J.C., Krebs, M.P., and Reznikoff, W.S. 1988. Effect of dam methylation on Tn5 transposition. *J Mol Biol* 199:35–45.

Yu, X., VanLoock, M.S., Yang, S., Reese, J.T., and Egelman, E.H. 2004. What is the structure of the RecA-DNA filament? *Curr Protein Pept Sci* 5:73–79.

Zahradka, K., Slade, D., Bailone, A., Sommer, S., Averbeck, D., Petranovic, M., Lindner, A.B., and Radman, M. 2006. Reassembly of shattered chromosomes in *Deinococcus radiodurans*. *Nature* 443:569–573.

Zieg, J., Maples, V.F., and Kushner, S.R. 1978. Recombinant levels of *Escherichia coli* K-12 mutants deficient in various replication, recombination, or repair genes. *J Bacteriol* 134:958–966.

Editor: William S. Reznikoff