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The Translocating RecBCD Enzyme Stimulates Recombination by Directing RecA Protein onto ssDNA in a χ-Regulated Manner

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Summary

Double-stranded DNA break repair and homologous recombination in E. coli are initiated by the RecBCD enzyme, which unwinds and simultaneously degrades DNA from a double-stranded DNA end. This process is stimulated by *cis*-acting DNA elements, known as χ sites. Using both in vitro pairing and nuclease protection assays, we demonstrate that the translocating RecBCD enzyme, which has been activated by χ, coordinates the preferential loading of the homologous pairing protein, RecA, onto the resultant single-stranded DNA downstream of χ. This facilitated loading of RecA protein results in a substantial increase in both the efficiency and rate of in vitro recombination reactions and offers an explanation for stimulation of recombination and repair in vivo by χ.

Introduction

The repair of double-stranded DNA (dsDNA) breaks is a fundamentally important facet of genomic maintenance. These potentially fatal lesions arise frequently during DNA replication (Kuzminov, 1995; Michel et al., 1997) or as a result of high energy irradiation (for review, see Friedberg et al., 1995). In Escherichia coli, repair of dsDNA breaks occurs by homologous recombination between the damaged DNA and the chromosome (for review, see Kogoma, 1996). Two enzymes that are essential for this process are the RecA protein and the heterotrimeric RecBCD enzyme. Null mutations in either the *recA*, *recB*, or *recC* genes completely eliminate dsDNA break repair (Sargentini and Smith, 1986) and reduce homologous recombination to approximately 0.1% of wild-type levels (Clark and Margulies, 1965; Howard-Flanders and Theriot, 1966; Emmerson, 1968).

The pairing and exchange of homologous DNA substrates is an essential step in both dsDNA break repair and recombination. In E. coli, this activity is catalyzed by the ubiquitous RecA protein (for review, see Kowalczykowski and Eggleston, 1994). One requirement for RecA protein-promoted pairing is that at least one of the homologous DNA substrates must contain ssDNA. The RecA protein nucleates randomly on ssDNA, after which cooperative binding leads to a 5′→3′ extension of the RecA protein filament (Register and Griffith, 1985). While extension of the RecA protein filament is quite efficient, the initial nucleation of RecA protein onto ssDNA is relatively slow (Chabbert et al., 1987). The slow association of RecA protein onto ssDNA leads to inefficient binding in the presence of competitor ssDNA-binding proteins such as E. coli single-stranded DNA-binding (SSB) protein (Kowalczykowski and Krupp, 1987; Kowalczykowski et al., 1987; Kowalczykowski, 1991). This limitation raises the question of whether there is a mechanism by which RecA protein is targeted to DNA breaks in order to achieve efficient repair.

Repair of dsDNA breaks in E. coli is initiated by the RecBCD enzyme (for review, see Kowalczykowski et al., 1994). The RecBCD enzyme binds the dsDNA breaks with a high affinity and then proceeds to unwind while simultaneously degrading the DNA (Telander-Muskavitch and Linn, 1981; Taylor and Smith, 1985). This degradation is asymmetric, with the 3′-terminal strand at the entry site for RecBCD enzyme being degraded much more extensively than the 5′-terminal strand (Dixon and Kowalczykowski, 1993). One interesting aspect of this recombination pathway is that it is stimulated by the presence of DNA sequence elements known as χ sites (Lam et al., 1974; Stahl et al., 1975; Smith et al., 1981). The χ site (5′-GCTGGTGG-3′) stimulates recombination 5- to 10-fold in its vicinity, and this stimulation is detectable up to 10 kb downstream of the χ site (Stahl et al., 1980; Ennis et al., 1987; Cheng and Smith, 1989; Myers et al., 1995). Upon encountering a properly oriented χ site, the polarity of RecBCD enzyme DNA degradation is switched from 3′→5′ to 5′→3′ (Anderson and Kowalczykowski, 1997). The continued unwinding and 5′→3′ degradation of the DNA downstream of χ leads to the production of ssDNA with a 3′ overhang terminating with the χ sequence.

The initial steps of homologous recombination can be reconstituted in vitro in a coupled reaction requiring RecA protein, RecBCD enzyme, SSB protein, and homologous DNA (Roman et al., 1990; Dixon and Kowalczykowski, 1991). Processing of a linear dsDNA substrate by RecBCD enzyme produces ssDNA that invades a homologous supercoiled DNA counterpart in a RecA- and SSB protein-promoted reaction. A hallmark of this reaction is the dramatic stimulation of joint molecule formation when the linear dsDNA substrate contains a χ site (Dixon and Kowalczykowski, 1991, 1995). In these reactions, the χ-specific ssDNA fragment downstream from χ is far more invasive than ssDNA that does not contain χ (Dixon and Kowalczykowski, 1991, 1995).

Here we demonstrate that the RecBCD enzyme stimulates the preferential use of χ-containing DNA by RecA protein. In a reaction in where RecA, SSB, and RecBCD proteins are present at the same time, the RecBCD enzyme coordinates the loading of RecA protein onto the χ-containing DNA strand. The facilitated loading of RecA protein occurs only on the χ-containing strand and is dependent upon the simultaneous action of both the RecA and RecBCD proteins. Through facilitated loading of RecA protein by RecBCD enzyme, the inhibitory effects of SSB protein are alleviated. This unexpected activity demonstrates a new level of coordination during the initiation of dsDNA break repair and recombination

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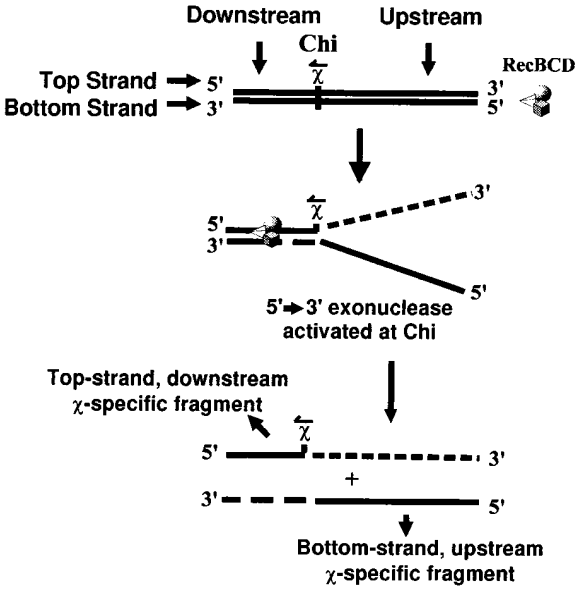

Figure 1. DNA Fragments Produced by the Action of RecBCD Enzyme on χ-Containing dsDNA

The strand of DNA that terminates 3′ at the entry site of RecBCD enzyme is termed the "top-strand"; the complementary strand is termed the "bottom-strand." The region of dsDNA between χ and the entry site of RecBCD enzyme is referred to as the "upstream" region, and the region between χ and the opposite end is termed the "downstream" region. The arrow above the χ site indicates the direction that RecBCD enzyme must travel in order to recognize χ. Unwinding and 3′→5′ exonuclease activity upstream of χ, followed by both attenuation and a switch in the polarity of exonuclease degradation to 5′→3′ upon χ recognition, lead to the production of both a bottom-strand, upstream χ-specific fragment and top-strand, downstream χ-specific fragment. Adapted from Anderson et al. (1997).

in E. coli and is another means by which χ acts to regulate this process.

Results

Formation of χ-Dependent Joint Molecules Is Much More Efficient in Coupled RecABCD Reactions

The processing of linear dsDNA containing χ by the RecBCD enzyme produces three distinct ssDNA products. For clarity, we define the region between the χ site and the end of the DNA at which the RecBCD enzyme initiated unwinding to be the "upstream" region and the region between the χ site and the opposite end of the DNA to be the "downstream" region (Figure 1). In addition, the strand of dsDNA that terminates 3′ at the entry point of RecBCD enzyme is defined as the "top-strand"; the DNA strand opposite the top strand is defined as the "bottom-strand." Thus, the three discrete ssDNA products are: (1) full-length ssDNA, produced from the bottom-strand when χ is not recognized; (2) top-strand, downstream χ-specific fragment; and (3) bottom-strand, upstream χ-specific fragment (Figure 1) (Anderson and Kowalczykowski, 1997). Each of these ssDNA fragments is a potential substrate for RecA protein-promoted joint molecule formation.

We define the efficiency of joint molecule formation for any given ssDNA species to be the amount of ssDNA incorporated into a joint molecule divided by the total amount of that ssDNA produced. Dixon and Kowalczykowski (1995) demonstrated that the top-strand, downstream χ-specific fragment is incorporated into joint molecules most efficiently in reconstituted RecABCD reactions. To investigate whether enhanced χ-specific joint molecule formation is due to the presence of the χ sequence on the 3′ end of that DNA fragment (Tracy and Kowalczykowski, 1996) or to an activity of RecBCD enzyme occurring during translocation, coupled RecABCD reactions were compared to uncoupled reactions. In a coupled reaction, RecA and SSB proteins are present together with the linear and supercoiled DNA when DNA unwinding and processing is commenced by addition of RecBCD enzyme. In the uncoupled reaction, the dsDNA is completely acted upon by RecBCD enzyme first (either in the presence or absence of SSB protein), and then RecA protein is added to begin the pairing phase of the reaction. If the translocating RecBCD enzyme does not play a role in the stimulation of joint molecule formation by χ, then the efficiency of joint molecule formation in both coupled and uncoupled reactions should be the same (though the absolute yield of products may differ). On the other hand, if RecA protein and translocating RecBCD enzyme in some way cooperate, then the coupled reaction should be more efficient than the uncoupled reaction.

Figure 2A shows a comparison of coupled and uncoupled RecABCD reactions. In these reactions, 5′ end-labeled linear dsDNA containing χ (HindIII χ^+F) and homologous supercoiled DNA was incubated with either RecA and SSB proteins (coupled) or just SSB protein (uncoupled), and then RecBCD enzyme was added; in the uncoupled reaction, RecA protein was added after complete unwinding (4 min). For the coupled reaction, the starting linear DNA substrate was completely processed after 2 min into the three major ssDNA fragments. Of these, the downstream χ-specific fragment was incorporated into a joint molecule product at a 12-fold higher efficiency than the full-length ssDNA after 2 min (Figure 2B). The efficiency of the downstream, χ-specific joint molecules plateaus after ~4 min, while the efficiency of χ-independent joint molecules (incorporating full-length ssDNA) continues to increase over the 12 min course of the reaction. Only negligible amounts of joint molecules were created using the bottom-strand, upstream χ-specific fragment, showing that this χ-specific fragment did not pair preferentially; because of the low yield of both joint molecules and of this species under these conditions (Anderson et al., 1997) quantitation was not performed.

In the uncoupled reaction, the same three major

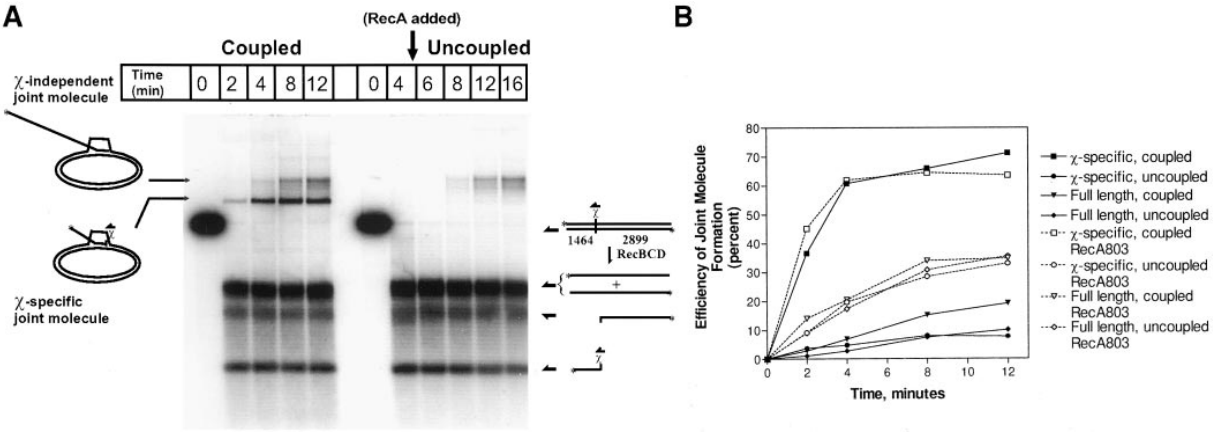

Figure 2. Joint Molecule Formation Involving the Downstream χ-Specific ssDNA Occurs with a High Efficiency in Coupled RecABCD Reactions

The linear χ-containing dsDNA substrate was created by restriction of pBR322 χ^+F with HindIII and labeled at the 5′-end with ^{32}P. The location of the χ site is given in nucleotides. The DNA was treated with RecBCD enzyme in the presence of saturating SSB protein and RecA protein (coupled), or saturating SSB protein followed by the addition of RecA protein after 4 min (uncoupled). Zero represents the time of RecBCD enzyme addition. Full-length ssDNA and the χ-specific fragments, together with the downstream χ-specific joint molecule and the χ-independent joint molecule are indicated. The migration of bands corresponding to the χ-specific and χ-independent joint molecules has been determined previously (Dixon and Kowalczykowski, 1991); the χ-independent joint molecule is the species with the lowest mobility. (A) shows a standard RecABCD reaction with 20 μM (nucleotides) supercoiled DNA. (B) shows quantitation of a RecABCD reaction with 80 μM (nucleotides) supercoiled DNA and either wild-type RecA protein or RecA803 protein. The efficiency of joint molecules formed is defined relative to ssDNA of each type produced by RecBCD enzyme. The zero time point for the uncoupled data shown in (B) represents time after addition of RecA protein.

ssDNA species are formed after 4 min of processing by RecBCD enzyme (Figure 2A), at which point RecA protein is added (this represents zero time in Figure 2B). The formation of both downstream χ-specific and χ-independent joint molecules occurs with identical efficiencies (Figure 2B). This efficiency of χ-independent joint molecule formation is nearly the same as for the coupled reaction, but joint molecule formation by the downstream χ-specific fragment is dramatically reduced in uncoupled reactions. The rate of incorporation of the downstream χ-specific fragment into a joint molecule is approximately 12-fold lower in the uncoupled reaction than in the coupled reaction. The observation that only the downstream χ-specific fragment has enhanced efficiency of joint molecule formation in coupled reactions argues that this stimulation is mediated by the χ-activated, translocating RecBCD enzyme.

To show that the effects observed in the coupled reaction are specific to χ, coupled and uncoupled reactions were repeated using dsDNA devoid of χ (Figure 3). The starting DNA was almost completely unwound after 2 min, resulting in only one major ssDNA product: full-length ssDNA. The full-length ssDNA produced in either reaction was incorporated into joint molecules at the same rate (Figure 3B), which was the same rate as for full-length ssDNA in both reactions using χ-containing DNA (Figure 2B). These results further verify that the coupled reaction shows a unique dependence on χ for efficient joint molecule formation.

One explanation for the enhanced efficiency of joint molecule formation in coupled, χ-specific reactions is that the RecBCD enzyme itself is needed to facilitate loading of RecA protein onto the χ-containing strand, thereby eliminating inhibition by SSB protein. To examine whether complete coating of ssDNA with RecA protein could mimic the observed increase in efficiency, we examined the effects of SSB protein on RecA protein–promoted joint molecule formation. In these reactions, 5′ end-labeled Hind III χ^0 DNA was heat denatured and then used as an ssDNA substrate for joint molecule formation (Figure 4). These reactions were initiated in three different ways: (1) RecA protein was preincubated with the ssDNA for 4 min and then the reaction was started by the addition of SSB protein and supercoiled DNA; (2) RecA and SSB proteins were added as a mixture; and (3) SSB protein was preincubated with the supercoiled and ssDNA for 4 min, and then the reaction was started by the addition of RecA protein. As can be seen in Figure 4, preincubation with RecA protein led to a dramatic increase in joint molecule formation that mimics the efficiency seen in χ-dependent coupled reactions. Addition of RecA and SSB proteins as a mixture and preincubation with SSB protein before the addition of RecA protein were equally inefficient, an observation consistent with examination of uncoupled reactions.

The inhibition of pairing by SSB protein competition was also observed in uncoupled reactions. Uncoupled RecABCD reactions were repeated with χ^+F DNA but started in the absence of any additional protein except RecBCD enzyme. After 4 min, a mix of SSB and RecA proteins was added (Figure 3B); again, this uncoupled reaction was much less efficient in downstream χ-specific joint molecule formation than the coupled reaction. In fact, this reaction was as inefficient as the previous uncoupled reactions in which the DNA was unwound in the presence of SSB protein (Figure 2B) and the reactions using DNA without a χ site. This is consistent with the observation that pairing reactions initiated by a mixture of RecA and SSB proteins and reactions that are allowed to pre-incubate with SSB protein before RecA protein addition are equally inefficient (Figure 4).

Finally, all of these reactions were repeated using

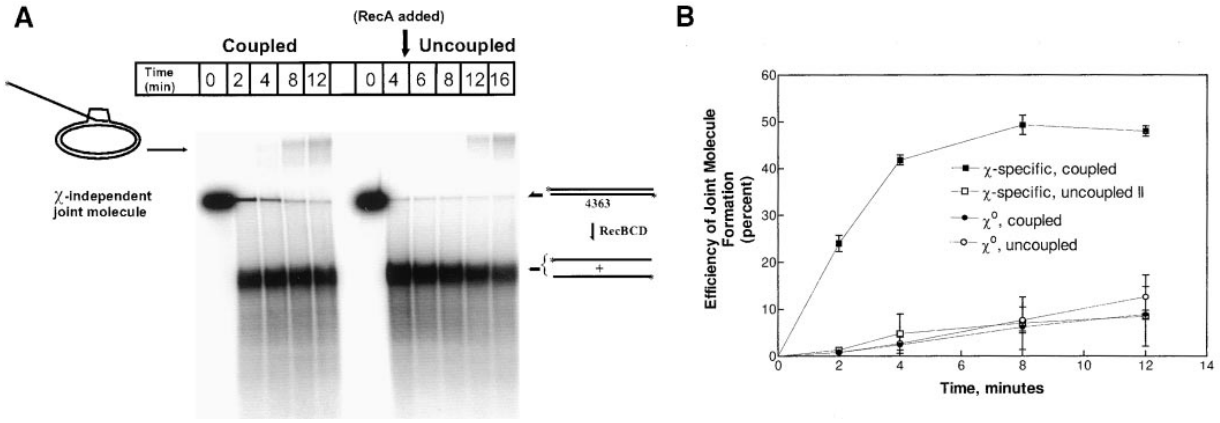

Figure 3. The Efficiency of χ-Independent Joint Molecule Formation Is the Same in Coupled and Uncoupled RecABCD Reactions

(A) The linear dsDNA substrate was created by restriction of pBR322 χ^0 with HindIII labeled at the 5′ end with ^{32}P. This DNA does not contain a χ site. The DNA (40 μM) was treated with RecBCD enzyme in the presence of 20 μM (nucleotides) supercoiled DNA, saturating SSB protein, and RecA protein (coupled), or saturating SSB protein followed by the addition of RecA protein after 4 min (uncoupled). Full-length ssDNA and the χ-independent joint molecule are indicated.

(B) Quantitation reveals that both coupled (closed circles) and uncoupled joint molecules (open circles) are formed at the same efficiency in the absence of χ. For comparison, (B) shows χ-specific joint molecule formation in an uncoupled reaction (open square, uncoupled reaction II) in which pBR322 χ^+F was treated with RecBCD enzyme in the absence of SSB protein, and then RecA and SSB proteins were added as a mix to start the reaction. As a reference, quantitation of the χ-specific, coupled, joint molecule formation data from Figure 2A are also shown (closed squares). Reactions were performed in triplicate to generate the standard deviation error bars shown.

RecA803 protein. The mutant RecA803 protein is more effective at competing with SSB protein for ssDNA binding than the wild-type RecA protein (Madiraju et al., 1992). As expected, in both coupled and uncoupled RecA803 protein–RecBCD enzyme reactions, the incorporation of full-length ssDNA into joint molecules was more efficient relative to reactions catalyzed by the wild-type RecA protein, while the formation of coupled, χ-specific joint molecules was not stimulated (Figure 2B). Furthermore, the efficiency of joint molecule formation using heat-denatured DNA was enhanced about 2-fold, relative to the wild-type protein, when RecA803 protein was added simultaneously with or after the addition of SSB protein. Collectively, these and the previous experiments argue that a consequence of dsDNA unwinding by RecBCD enzyme is the preferential loading of RecA, rather than SSB protein, onto the χ-containing ssDNA exclusively.

The enhancement of downstream, χ-specific joint molecule formation is not specific to pBR322 χ^+F (Smith et al., 1981). These reactions were repeated with another χ-containing substrate (pBR322 χ^+F,H; Dixon and Kowalczykowski, 1993), and joint molecule formation involving either of the downstream, χ-specific fragments was much more efficient in coupled reactions (Dixon and Kowalczykowski, 1995; data not shown).

The Translocating RecBCD Enzyme Facilitates Loading of RecA Protein onto the Downstream χ-Specific Fragment

The previous experiments revealed that the simultaneous presence of RecA protein and the translocating RecBCD enzyme are required for enhanced incorporation of the downstream χ-specific ssDNA fragment into a joint molecule. Dixon and Kowalczykowski (1991) showed that in coupled RecABCD reactions, homology is only necessary at the 3′ end of the downstream χ-specific fragment for formation of plectonemic joint molecules. Thus, it is possible that the translocating RecBCD enzyme stimulates formation of χ-dependent joint molecules by facilitating the loading of RecA protein, in preference to SSB protein, onto the 3′-end of

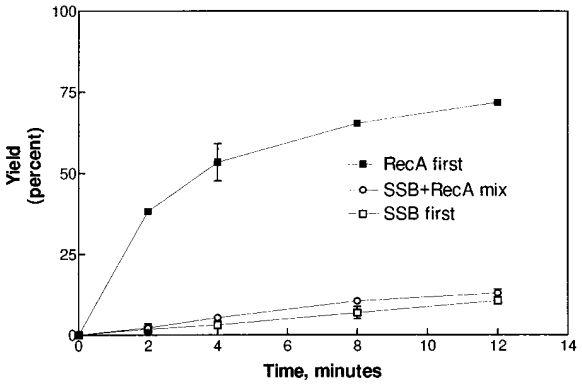

Figure 4. Prebinding of RecA Protein to ssDNA Results in a High Effciency of Joint Molecule Formation in the Absence of χ

The linear ssDNA substrate was created by heat denaturation of HindIII linearized, 5′ end-labeled pBR322 χ^0. The reactions were performed as follows: (1) (closed squares) incubated with RecA protein for 4 min and started by the addition of supercoiled DNA and SSB protein, (2) (open circles) started by the addition of a mixture of RecA and SSB proteins, and (3) (closed squares) incubated with SSB protein for 4 min and started by the addition of RecA protein. The reactions were performed in duplicate to generate the range shown.

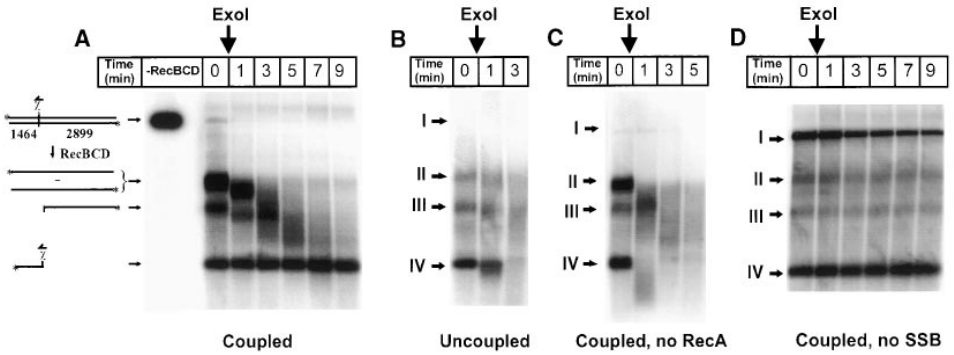

Figure 5. The Downstream, χ-Specific Fragment Is Protected from ExoI Degradation in Coupled but Not Uncoupled Reactions

The protocol described in the legend to Figure 2 was followed, except that supercoiled DNA was omitted and, after 2 min, excess M13 ssDNA and ATPγ-S were added. This was allowed to incubate for 1 min and then ExoI was added. The zero time point was taken just prior to ExoI addition. The coupled reaction is shown in (A), while the uncoupled reaction is in (B). The starting substrate dsDNA substrate (I), is shown before addition of RecBCD enzyme in the lane labeled -RecBCD. Full-length ssDNA (II), the bottom-strand upstream χ-specific fragment (III), and the top-strand downstream χ-specific fragment (IV) are indicated. As further controls, the reaction was performed in the absence of RecA protein (C) or in the absence of SSB protein (D). The dominant ssDNA product produced by RecBCD enzyme in the absence of SSB protein is the downstream χ-specific ssDNA fragment. This is due to increased degradation by the RecBCD enzyme when unwinding occurs in the absence of SSB protein (D. G. A. and S. C. K, unpublished data).

the resultant downstream, χ-specific fragment. To test this possibility, we determined the state of the 3′ ends of the three defined ssDNA fragments by assaying the ability of RecA protein to protect each end from degradation by Exonuclease I (ExoI) in both coupled and uncoupled reactions. The strategy used was to allow RecA and SSB proteins to bind to ssDNA produced by RecBCD enzyme, freeze the already bound RecA protein in place by addition of the nonhydrolyzable ATP analog, ATPγ-S, and then test for susceptibility to ExoI, which is a 3′→5′ ssDNA exonuclease whose activity is stimulated by SSB protein (Molineux and Gefter, 1975).

Coupled reactions using 5′ end-labeled linear χ^+ dsDNA, RecA protein, and SSB protein, but without homologous supercoiled DNA, were started by the addition of the RecBCD enzyme (Figure 5A). After 2 min, the DNA was completely processed into the three major ssDNA species; at this point, an excess of M13 ssDNA and ATPγ-S was added. The ATPγ-S stabilizes all RecA protein bound to ssDNA (Menetski et al., 1988), and the excess unlabeled M13 ssDNA titrates all of the free SSB protein and free RecA protein. After 1 min, a saturating amount of ExoI was added. If the 3′ ends of the ssDNA fragments are coated with RecA protein, they will be resistant to ExoI degradation. Figure 5A shows that in a coupled reaction, protection of the downstream χ-specific fragment is 100%. This is in contrast to the other ssDNA species, which are nearly completely degraded by ExoI (Figure 5A).

To determine whether protection of the downstream χ-specific fragment in coupled RecABCD reactions is dependent upon the coordinated interplay of RecA protein and RecBCD enzyme, protection in uncoupled reactions was examined (Figure 5B). In this reaction, linear χ^+ dsDNA was incubated with RecBCD enzyme for 4 min to unwind the DNA completely (Figure 2A). RecA and SSB proteins, at the same concentrations used in the coupled reactions, were then added as a mix to the resultant ssDNA and incubated for 2 min, followed by addition of excess M13 ssDNA, ATPγ-S, and ExoI. After addition of ExoI, all of the ssDNA products disappeared, including the downstream χ-specific fragment (Figure 5B). These fragments were degraded at nearly the same rate as the full-length ssDNA in the coupled reaction.

In the coupled reaction, the only ssDNA product that is protected from ExoI is the downstream χ-specific fragment. To show that protection of this fragment is dependent upon RecA protein but not on SSB protein, coupled reactions were repeated in the absence of either RecA protein (Figure 5C) or SSB protein (Figure 5D). These experiments revealed that the χ-specific fragment is completely degraded in the absence of RecA protein (Figure 5C), while it and all other ssDNA species are completely protected in the absence of SSB protein (Figure 5D). We conclude that it is the loading of RecA protein at the 3′ end of the downstream χ-specific fragment that is responsible for protection from ExoI degradation. Furthermore, the fact that the protection of this fragment occurs only in coupled RecABCD reactions shows that RecBCD enzyme must be translocating through the DNA at the same time that RecA protein is present in order to promote RecA protein loading.

Discussion

The repair of dsDNA breaks in E. coli requires the coordination of a number of recombination proteins at the site of this lesion. The first step is the unwinding and degradation of the DNA from this end by the RecBCD enzyme (Taylor and Smith, 1985; Dixon and Kowalczykowski, 1991, 1993). During this process, recognition of the recombination hot spot χ results in the formation of a 3′-ssDNA overhang with the χ sequence at the 3′ end (Ponticelli et al., 1985; Taylor et al., 1985; Anderson and Kowalczykowski, 1997), thereby providing a potential ssDNA substrate that is optimal for the homologous pairing protein, RecA (Konforti and Davis, 1987, 1990; Tracy and Kowalczykowski, 1996). However, the role of the RecBCD enzyme during the initiation of recombination is not limited to production of an ssDNA substrate

suitable for RecA protein action. Here we demonstrate that the RecBCD enzyme stimulates recombination by facilitating the preferential loading of the RecA protein, to the exclusion of SSB protein, onto the invasive DNA strand in a χ-regulated manner. This conclusion stems from in vitro recombination reactions using RecA protein, RecBCD enzyme, and SSB protein, and from direct nuclease protection assays.

In the pairing reactions, the RecBCD enzyme greatly stimulates both the rate and extent at which ssDNA is used by the RecA protein to form heteroduplex DNA (Figure 2). Moreover, this stimulation is only observed in the simultaneous presence of RecA protein and RecBCD enzyme (defined as the coupled reaction) and is specific to the ssDNA downstream of the χ site. This behavior seems to be unique to the RecBCD enzyme, since coupled reactions using a different processive helicase, the RecQ protein, do not display this unusual enhancement (F. G. Harmon and S. C. Kowalczykowski, unpublished data). Full-length ssDNA, produced by unwinding events where χ is not recognized, is incorporated by RecA protein into joint molecules at the same inefficient level in both coupled and uncoupled reactions (Figures 2 and 3). Furthermore, in uncoupled reactions the χ-specific fragment is utilized with the same low efficiency as full-length ssDNA. Thus, stimulation of joint molecule formation in vitro is regulated by the χ sequence, as is recombination in vivo.

Since efficient joint molecule formation requires RecA protein to coat the 3′ end of ssDNA (Register and Griffith, 1985; Konforti and Davis, 1987, 1990), we sought physical evidence for preferential assembly of RecA protein onto the χ-specific ssDNA. We showed directly that in coupled reactions the ssDNA downstream of χ is coated with RecA protein whereas the other ssDNA species are not. This is demonstrated by the complete resistance of the ssDNA downstream of χ to exonucleolytic degradation by ExoI (Figure 5). However, the same χ-specific ssDNA is thoroughly degraded in uncoupled reactions, as is the full length ssDNA in both coupled and uncoupled reactions. Thus, the protection of DNA in these reactions is dependent on the simultaneous presence of RecA and RecBCD proteins and on χ, as is stimulation of pairing. We therefore conclude that the translocating RecBCD enzyme facilitates the loading of RecA protein onto the recombinogenic DNA strand in a χ-regulated manner.

The rationale for the existence of a facilitated loading mechanism stems from the physical attributes and limitations of RecA protein. As previously mentioned, the RecA protein binds randomly to ssDNA; in addition, nucleation of the nascent RecA filament is slow and rate-limiting (Chabbert et al., 1987). This slow initial binding of RecA protein makes it a poor competitor with other ssDNA-binding proteins such as SSB (Kowalczykowski and Krupp, 1987; Kowalczykowski et al., 1987; Kowalczykowski, 1991). These considerations are manifest as poor joint molecule formation, in the absence of RecBCD enzyme, when SSB protein is allowed to bind ssDNA prior to or simultaneously with RecA protein (Figure 4). In contrast, when initiated by RecBCD enzyme, the potential block to presynaptic complex formation by SSB protein is overcome. In this case, the efficiency of pairing is the same as that observed when RecA protein is allowed to prebind ssDNA. Thus, RecBCD enzyme and χ act to direct the assembly of RecA protein onto the χ-containing ssDNA, mimicking the reaction where RecA protein is the primary ssDNA-binding protein. Consequently, the χ-activated, translocating RecBCD enzyme must possess a means to promote the binding of RecA protein and/or exclude the binding of SSB protein.

The conclusion that the RecBCD enzyme coordinates the loading of RecA protein in preference to SSB protein is supported by genetic analyses of homologous recombination (Kuzminov, 1996). In *recBC* mutant backgrounds, the reduction in recombination proficiency can be suppressed by either *sbcA*, *sbcB*, or *sbcCD* mutations. *SbcB* mutations inactivate the exonuclease activity of ExoI (Sunshine and Kelly, 1971) while the *sbcCD* mutations inactivate a dsDNA exonuclease (Kowalczykowski et al., 1994). Although both of these sets of suppressor mutations completely restore recombination proficiency, they introduce a new set of genetic requirements, three of which are a dependence upon the *recF*, *recO*, and *recR* genes (Clark and Sandler, 1994). Genetic and biochemical evidence suggest that the products of these genes facilitate the loading of RecA protein onto ssDNA by destabilizing the SSB protein–ssDNA complex (Umezu et al., 1993; Kowalczykowski et al., 1994; Umezu and Kolodner, 1994). The requirement for *recF*, *recO*, and *recR* genes when the RecBCD enzyme is inactive, therefore, suggests that an alternative mechanism is needed to remove or destabilize the SSB protein–ssDNA complex and is consistent with the finding that when the RecBCD enzyme is active, their functions are not essential owing to the direct facilitated loading of RecA protein onto ssDNA.

The biochemical means by which the RecBCD enzyme facilitates the loading of RecA protein is unclear. Although no direct interaction between the RecBCD enzyme and RecA protein has been shown to date, it could explain how the RecBCD enzyme coordinates the loading of RecA protein on DNA downstream of χ. Direct interaction between the RecBCD enzyme and RecA protein is supported by the observation that interspecies complementation of recombination in vivo is achieved only when both RecBCD enzyme and RecA protein are from the same species (Rinken et al., 1991; de Vries and Wackernagel, 1992). We have not detected a direct interaction between RecA protein and RecBCD enzyme using either coimmunoprecipitation or an IAsys biosensor instrument (unpublished data). However, it is possible that interaction is transient and requires translocation after χ, thereby precluding easy detection by conventional means. Another possibility, however, is that χ-activated RecBCD enzyme induces a DNA structure to which RecA protein preferentially binds. The exact mechanism by which RecBCD enzyme catalyzes the loading of RecA protein remains to be determined.

Based on our observations, we propose a model for how RecBCD enzyme and RecA protein initiate homologous recombination at χ (Figure 6). Unwinding and degradation of DNA is initiated by the RecBCD enzyme from a dsDNA break during which a loop of ssDNA is extruded from the top strand (Figure 6B). Electron microscopic analysis of RecBCD enzyme-promoted unwinding of DNA revealed the formation of "loop-tail" structures as intermediates of DNA unwinding (Figure

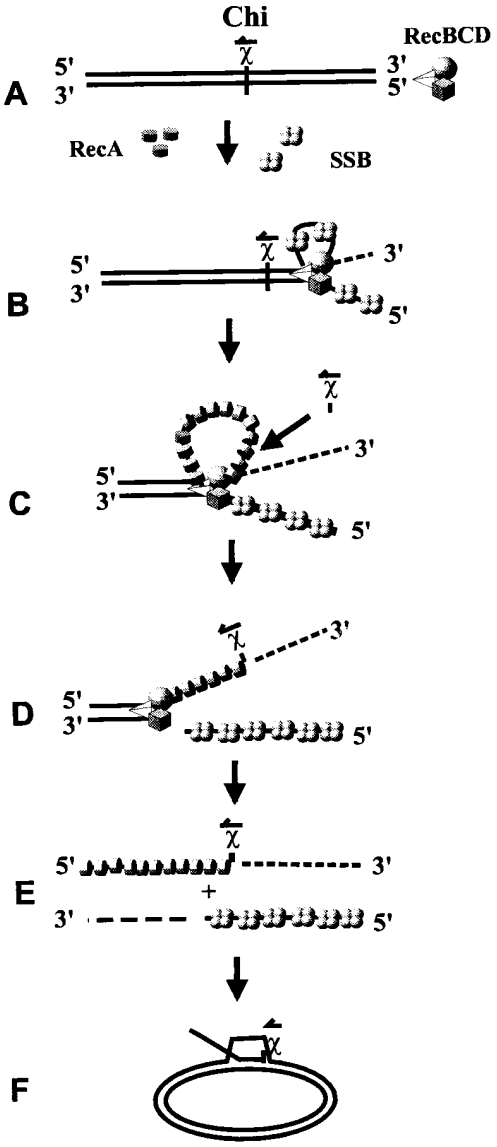

Figure 6. Biochemical Events That Occur during the Initiation of Genetic Recombination

Details are discussed in the text.

(A) Recombination is initiated by binding of RecBCD enzyme to a dsDNA end.

(B) During the process of unwinding and degradation, the top strand is extruded as a loop of ssDNA. The resultant ssDNA from the bottom strand is primarily bound by SSB protein.

(C) Degradation of the DNA with a 3′→5′ polarity continues until a χ site is recognized, at which time RecBCD enzyme pauses, the 3′→5′ exonuclease activity is attenuated, and RecA protein is loaded onto the top DNA strand within the loop.

(D) The nuclease polarity is then switched, with continued degradation occurring 5′→3′, leading to the production of a 3′-ssDNA overhang, which is coated with RecA protein.

(E and F) The 3′-ssDNA overhang is coated with RecA protein and can invade a homologous supercoiled DNA substrate, forming a joint molecule.

6) (Taylor and Smith, 1980; Telander-Muskavitch and Linn, 1980). The strand of DNA that forms the loop corresponds to the strand of DNA that would contain the potential downstream χ-specific fragment (Braedt and Smith, 1989). Recent work from Bianco and Kowalczykowski (1997) suggests that χ is in a single-stranded form when recognized by the RecBCD enzyme. This, together with other unpublished data (D.G.A. and S.C.K), suggests that recognition of χ occurs on the top strand at the back of the loop (Figure 6C). Thus, when the RecBCD enzyme recognizes a χ site, the 3′ end of the eventual downstream χ-specific fragment is likely to be single-stranded.

During the course of unwinding, ssDNA is primarily bound by SSB protein. Degradation of the DNA continues until a χ site is recognized, at which time the RecBCD enzyme pauses, and the 3′→5′ exonuclease activity is attenuated (Dixon and Kowalczykowski, 1993). We propose that when the RecBCD enzyme is paused at χ, the RecBCD enzyme serves as a nucleator, either directly or indirectly, for the binding of RecA protein to the top strand loop (Figure 6C). The polarity of RecA protein filamentation is 5′→3′. Thus, nucleation would begin at the 5′ end of the loop and extend to the 3′ end where χ is located, displacing the resident SSB protein. At this point the loop is released, and the 5′→3′ exonuclease activity of the opposite polarity is up-regulated (Figure 6D). While the processive helicase action of RecBCD enzyme leads to the complete unwinding of the small, pBR322-based plasmids used in vitro, the result of the 5′→3′ exonuclease activity on longer DNA substrates (i.e., chromosomal or phage lambda DNA) would be production of a 3′-overhanging ssDNA end on the remaining intact dsDNA. This 3′-ssDNA overhang is now coated with RecA protein and can invade a homologous supercoiled DNA substrate, forming a joint molecule (Figure 6F). This intermediate is then processed into recombinant molecules (Durnderdale and West, 1994; Shinagawa and Iwasaki, 1996).

In summary, the role of the RecBCD enzyme in the initiation of dsDNA break repair and recombination is not limited to producing ssDNA substrates for RecA protein. We demonstrated that the RecBCD enzyme plays an additional role by coordinating the loading of RecA protein onto a specific strand of DNA in a χ-regulated manner. These data illustrate the uniquely multifunctional nature of the RecBCD enzyme and demonstrate a new level of functional interaction between the two major homologous recombination proteins of E. coli. Recent results indicate that enzyme-mediated loading of a DNA strand-exchange protein onto ssDNA is not unique to E. coli. The binding of the S. cerevisiae DNA strand exchange protein, Rad51 (Sung, 1994; Sung and Robberson, 1995), to ssDNA is both competitive with and limited by the yeast ssDNA-binding protein, Replication protein-A (RPA) (Brill and Stillman, 1989; Alani et al., 1992; Sugiyama et al., 1997). As in the E. coli system, there are yeast proteins, the Rad55 and Rad57 complex, that facilitate the loading of Rad51 protein onto ssDNA in preference to RPA, thereby alleviating the inhibitory effects and stimulating homologous pairing of DNA (Sung, 1997). Thus, the preferential loading of strand-exchange protein may represent a universal aspect of initiation of homologous recombination and DNA repair.

Experimental Procedures

Enzymes

RecBCD enzyme was purified as described (Roman and Kowalczykowski, 1989) with further purification by an additional FPLC Mono-Q

step. Protein concentration was determined using an extinction coefficient of 4.0×10^5 M^{-1} cm^{-1} at 280 nm (Roman and Kowalczykowski, 1989). The enzyme was determined to be 55% functional by helicase assays (Roman and Kowalczykowski, 1989). SSB protein was isolated from strain RLM727 and purified according to LeBowitz (1985). Protein concentration was determined using an extinction coefficient of 3.0×10^4 at 280 nm (Ruyechan and Wetmur, 1975).

RecA protein was purified using a procedure based on spermidine precipitation (Griffith and Shores, 1985; S. C. K., unpublished data). RecA803 protein was purified according to Madiraju et al. (1992). Protein concentration was determined using an extinction coefficient of 2.7×10^4 M^{-1} cm^{-1} at 280 nm.

All restriction endonucleases and DNA-modifying enzymes were purchased from New England BioLabs, Bethesda Research Laboratories, or United States Biochemical. The enzymes were used according to Sambrook et al. (1989) or as indicated by the specific vendor.

DNA Substrates

The plasmids pBR322 χ^0 (wild type) and pBR322 χ^+F225 (Smith et al., 1981) were prepared from strains S819 and S818, respectively, provided by G. R. Smith and A. F. Taylor. All plasmid DNAs were purified by cesium chloride density gradient centrifugation (Sambrook et al., 1989). The molar concentration of the dsDNA in nucleotides was determined using an extinction coefficient of 6290 M^{-1} cm^{-1} at 260 nm. Plasmid DNA was linearized with HindIII and radioactively labeled at the 5′ end by sequential reactions with shrimp alkaline phosphatase followed by T4 polynucleotide kinase and [γ-^{32}P]ATP (NEN), using methods given by the vendor or Sambrook et al. (1989). The DNA was further purified by passage through an S-200 MicroSpin column (Pharmacia Biotech).

Reaction Conditions

The standard RecABCD reaction mixture (Dixon and Kowalczykowski, 1991) consisted of 25 mM Tris-acetate (pH 7.5), 8 mM magnesium acetate, 5 mM ATP, 1 mM dithiothreitol, 1 mM phosphoenolpyruvate, 4 U/ml pyruvate kinase, 40 μM (nucleotides) linear dsDNA, 20 μM or 80 μM nucleotides supercoiled DNA, 20 μM RecA protein, 4 μM SSB protein, 1.34 nM total RecBCD enzyme (0.74 nM functional RecBCD enzyme, corresponding to 0.083 functional RecBCD enzyme molecules per linear dsDNA end). Assays were performed at 37°C. Reactions using RecA803 protein were the same as the standard reaction, except that 80 μM (nucleotides) supercoiled DNA was used in all reactions.

Coupled pairing reactions were started by the addition of RecBCD enzyme after preincubation of all other standard components for 2 min. Uncoupled pairing reactions were performed by the addition of RecBCD enzyme after preincubation of all components except for RecA protein. After 4 min, RecA protein was added to a concentration of 20 μM.

Conditions for pairing reactions without RecBCD enzyme were the same as the standard conditions, except that the DNA was heat denatured and that 80 μM (nucleotides) supercoiled DNA was used. Components were added in the order described in the results.

Coupled exonuclease protection assays were started by the addition of RecBCD protein after preincubation of all standard components, except supercoiled DNA, for 2 min. After 2 min, a mix of M13 ssDNA and ATPγ-S was added to a final concentration of 200 μM and 5 mM, respectively. After 1 min of incubation, ExoI was added to a concentration of 100 U/ml. Uncoupled exonuclease protection assays were performed by incubation of all standard components, except supercoiled DNA, RecA, and SSB proteins. After 2 min, RecBCD protein was added to a final total concentration of 1.34 nM. Four minutes later, a mixture of RecA and SSB proteins was added to 20 μM and 4 mM, respectively. After 2 min of incubation with RecA and SSB proteins, a mix of M13 ssDNA and ATPγ-S was added to a final concentration of 200 μM and 5 mM, respectively. After 1 min, ExoI was added to a concentration of 100 U/ml.

Analysis of Reaction Products

Aliquots of the reaction mixture (30 μl) were taken at the indicated time points and added to 10 μl of stop buffer (0.1 M EDTA, 2.5% SDS, 10% ficol, 0.125% bromophenol blue, and 0.125% xylene cylanol) to halt the reaction and to deproteinize the sample. For clarity, all zero time points are one-third the volume of the remaining time points. Samples were electrophoresed in 1% agarose gels for approximately 15 hr at 1.4 V/cm in TAE (40 mM Tris-acetate [pH 8.0], 2 mM EDTA). The gels were dried and analyzed on a Molecular Dynamics Storm 840 phosphoimager using Image-QuaNT software. Data was graphed using GraphPad Prism version 2.0 software.

The efficiency of joint molecule formation was calculated by dividing the amount of joint molecule formed, under any particular set of reaction conditions, by the total amount of ssDNA produced for each particular fragment.

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References

Alani, E., Thresher, R., Griffith, J.D., and Kolodner, R.D. (1992). Characterization of DNA-binding and strand-exchange stimulation properties of y-RPA, a yeast single-strand-DNA-binding protein. J. Mol. Biol. *227*, 54–71.

Anderson, D.G., and Kowalczykowski, S.C. (1997). The recombination hot spot, Chi, is a regulatory element that switches the polarity of DNA degradation by the RecBCD enzyme. Genes Dev. *11*, 571–581.

Anderson, D.G., Churchill, J.J., and Kowalczykowski, S.C. (1997). Chi-activated RecBCD enzyme possesses 5′→3′ nucleolytic activity, but RecBC enzyme does not: evidence suggesting that the alteration induced by Chi is not simply ejection of the RecD subunit. Genes to Cells *2*, 117–128.

Bianco, P.R., and Kowalczykowski, S.C. (1997). The recombination hotspot Chi is recognized by the translocating RecBCD enzyme as the single strand of DNA containing the sequence 5′-GCTGGTGG-3′. Proc. Natl. Acad. Sci. USA, in press.

Braedt, G., and Smith, G.R. (1989). Strand specificity of DNA unwinding by recBCD enzyme. Proc. Natl. Acad. Sci. USA *86*, 871–875.

Brill, S.J., and Stillman, B. (1989). Yeast replication factor-A functions in the unwinding of the SV40 origin of DNA replication. Nature *342*, 92–95.

Chabbert, M., Cazenave, C., and Helene, C. (1987). Kinetic studies of recA protein binding to a fluorescent single-stranded polynucleotide. Biochemistry *26*, 2218–2225.

Cheng, K.C., and Smith, G.R. (1989). Distribution of Chi-stimulated recombinational exchanges and heteroduplex endpoints in phage lambda. Genetics *123*, 5–17.

Clark, A.J., and Margulies, A.D. (1965). Isolation and characterization of recombination-deficient mutants 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.

de Vries, J., and Wackernagel, W. (1992). Recombination and UV resistance of *Escherichia coli* with the cloned *recA* and *recBCD* genes of *Serratia marcescens* and *Proteus mirabilis*: evidence for an advantage of intraspecies combination of *P. mirabilis* RecA protein and RecBCD enzyme. J. Gen. Microbiol. *138*, 31–38.

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 Escherichia coli recBCD enzyme. Cell *73*, 87–96.

Dixon, D.A., and Kowalczykowski, S.C. (1995). Role of the Escherichia coli recombination hotspot, chi, in RecABCD-dependent homologous pairing. J. Biol. Chem. *270*, 16360–16370.

Dunderdale, H.J., and West, S.C. (1994). Recombination genes and proteins. Curr. Opin. Genet. Dev. *4*, 221–228.

Emmerson, P.T. (1968). Recombination deficient mutants of *Escherichia coli* K12 that map between *thyA* and *argA*. Genetics *60*, 19–30.

Ennis, D.G., Amundsen, S.K., and Smith, G.R. (1987). Genetic functions promoting homologous recombination in Escherichia coli: a study of inversions in phage lambda. Genetics *115*, 11–24.

Friedberg, E.C., Walker, G.C., and Siede, W. (1995). DNA Repair and Mutagenesis (Washington, D.C.: ASM Press).

Griffith, J., and Shores, C.G. (1985). RecA protein rapidly crystallizes in the presence of spermidine: a valuable step in its purification and physical characterization. Biochemistry *24*, 158–162.

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.

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

Konforti, B.B., and Davis, R.W. (1987). 3′ homologous free ends are required for stable joint molecule formation by the RecA and single-stranded binding proteins of Escherichia coli. Proc. Natl. Acad. Sci. USA *84*, 690–694.

Konforti, B.B., and Davis, R.W. (1990). The preference for a 3′ homologous end is intrinsic to RecA-promoted strand exchange. J. Biol. Chem. *265*, 6916–6920.

Kowalczykowski, S.C. (1991). Biochemical and biological function of Escherichia coli recA protein: behavior of mutant recA proteins. Biochimie *73*, 289–304.

Kowalczykowski, S.C., and Eggleston, A.K. (1994). Homologous pairing and DNA strand-exchange proteins. Annu. Rev. Biochem. *63*, 991–1043.

Kowalczykowski, S.C., and Krupp, R.A. (1987). Effects of the 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.C., 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.

Kowalczykowski, S.C., Dixon, D.A., Eggleston, A.K., Lauder, S.D., and Rehrauer, W.M. (1994). Biochemistry of homologous recombination in *Escherichia coli*. Microbiol. Rev. *58*, 401–465.

Kuzminov, A. (1995). Collapse and repair of replication forks in Escherichia coli. Mol. Microbiol. *16*, 373–384.

Kuzminov, A. (1996). Molecular Biology Intelligence Unit: Recombinational Repair of DNA Damage (Austin, Texas: R. G. Landes Company), pp. 135–136.

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.

LeBowitz, J. (1985). Biochemical mechanism of strand initiation in bacteriophage lambda DNA replication. PhD thesis, Johns Hopkins University, Baltimore, Maryland.

Madiraju, M.V.V.S., Lavery, P.E., Kowalczykowski, S.C., and Clark, A.J. (1992). Enzymatic properties of the RecA803 protein, a partial suppressor of *recF* mutations. Biochemistry *31*, 10529–10535.

Michel, B., Ehrlich, S.D., and Uzest, M. (1997). DNA double-strand breaks caused by replication arrest. EMBO J. *16*, 430–438

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.

Myers, R.S., Stahl, M.M., and Stahl, F.W. (1995). Chi recombination activity in phage lambda decays as a function of genetic distance. Genetics *141*, 805–812.

Ponticelli, A.S., Schultz, D.W., Taylor, A.F., and Smith, G.R. (1985). Chi-dependent DNA strand cleavage by recBC enzyme. Cell *41*, 145–151.

Register, J.C., III, 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.

Rinken, R., de Vries, J., Weichenhan, D., and Wackernagel, W. (1991). The recA-recBCD dependent recombination pathways of Serratia marcescens and Proteus mirabilis in Escherichia coli: functions of hybrid enzymes and hybrid pathways. Biochimie *73*, 375–384.

Roman, L.J., and Kowalczykowski, S.C. (1989). Characterization of the helicase activity of the Escherichia coli recBCD enzyme using a novel helicase assay. Biochemistry *28*, 2863–2873.

Roman, L.J., Dixon, D.A., and Kowalczykowski, S.C. (1990). RecBCD-dependent joint molecule formation promoted by the Escherichia coli RecA and SSB proteins. Proc. Natl. Acad. Sci. USA *88*, 3367–3371.

Ruyechan, W.T., and Wetmur, J.G. (1975). Studies on the cooperative binding of the Escherichia coli DNA unwinding protein to single-stranded DNA. Biochemistry *14*, 5529–5534.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd Ed. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 1.33–1.52.

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.

Shinagawa H., and Iwasaki, H. (1996). Processing the Holliday junction in homologous recombination. Trends Biochem. Sci. *21*, 107–111

Smith, G.R., Kunes, S.M., Schultz, D.W., Taylor, A., and Triman, K.L. (1981). Structure of Chi hotspots of generalized recombination. Cell *24*, 429–436.

Stahl, F.W., Crasemann, J.M., and Stahl, M.M. (1975). Rec-mediated recombinational hot spot activity in bacteriophage lambda. III. Chi mutations are site-mutations stimulating rec-mediated recombination. J. Mol. Biol. *94*, 203–212.

Stahl, F.W., Stahl, M.M., Malone, R.E., and Crasemann, J.M. (1980). Directionality and nonreciprocality of Chi-stimulated recombination in phage lambda. Genetics *94*, 235–248.

Sugiyama, T., Zaitseva, E.M., Kowalczykowski, S.C. (1997). A single-stranded DNA binding protein is needed for efficienct presynaptic complex formation by the *Saccharomyces cerevisiae* Rad51 protein. J. Biol. Chem. *272*, 7940–7945.

Sung, P. (1994). Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. Science *265*, 1241–1243.

Sung, P. (1997). Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51. Genes Dev. *11*, 1111–1122.

Sung, P., and Robberson, D.L. (1995). DNA strand exchange mediated by a RAD51-ssDNA nucleoprotein filament with polarity opposite to that of RecA. Cell *82*, 453–461.

Sunshine, M.G., and Kelly, B. (1971). Extent of host deletions associated with bacteriophage P2-mediated education. J. Bacteriol. *108*, 695–704.

Taylor, A., and Smith, G.R. (1980). Unwinding and rewinding of DNA by the recBC enzyme. Cell *22*, 447–457.

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., Schultz, D.W., Ponticelli, A.S., and Smith, G.R. (1985). RecBC enzyme nicking at Chi sites during DNA unwinding: location and orientation-dependence of the cutting. Cell *41*, 153–163.

Telander-Muskavitch, K.M., and Linn, S. (1980). Electron microscopy

of *E. coli* recBC enzyme reaction intermediates. In Mechanistic Studies of DNA Replication and Genetic Recombination, B. Alberts, ed. (New York: Academic Press), pp. 901–918.

Telander-Muskavitch, K.M., and Linn, S. (1981). RecBC-like enzymes: Exonuclease V deoxyribonucleases. In The Enzymes, P.D. Boyer, ed. (New York: Academic Press), pp. 233–250.

Tracy, R.B., and Kowalczykowski, S.C. (1996). In vitro selection of preferred DNA pairing sequences by the Escherichia coli RecA protein. Genes Dev. *10*, 1890–1903.

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., 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.