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RecBCD is required to Complete Chromosomal Replication: Implications for Double-Strand Break Frequencies and Repair Mechanisms

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ABSTRACT

Several aspects of the mechanism of homologous double strand break repair remain unclear. Although intensive efforts have focused on how recombination reactions initiate, far less is known about the molecular events that follow. Based upon biochemical studies, current models propose that RecBCD processes double strand ends and loads RecA to initiate recombinational repair. However, recent studies have shown that RecBCD plays a critical role in completing replication events on the chromosome through a mechanism that does not involve RecA or recombination. Here, we examine several studies, both early and recent, that suggest RecBCD also operates late in the recombination process- after initiation, strand invasion, and crossover resolution have occurred. Similar to its role in completing replication, we propose a model in which RecBCD is required to resect and resolve the DNA synthesis associated with homologous recombination at the point where the missing sequences on the broken molecule have been restored. We explain how the impaired ability to complete chromosome replication in *recBC* and *recD* mutants is likely to account for the loss of viability and genome instability in these mutants, and conclude that spontaneous double strand breaks and replication fork collapse occur far less frequently than previously speculated.

Key Words: double-strand break repair, homologous recombination, replication completion, RecBCD

1. Double-Strand Break Repair in *Escherichia coli*

In *Escherichia coli*, the major pathway for repairing double-strand breaks requires RecBCD, an enzymatic complex that current models suggest serves to process and recruit RecA to DNA ends, where it promotes strand invasion with an intact homologous duplex molecule [1, 2]. Models propose that once this occurs, the sequences between the opposing strands are replicated and joined using the second molecule as a template (Figure 1). While there has been extensive consideration of how the recombination process initiates, of equal importance is how the cell senses, recognizes, and completes the repair replication step to the precise nucleotide at which two intact DNA molecules have been restored. There is little experimental evidence for many of the proposed molecular intermediates and events associated with the steps following initiation. However, as we describe below, these later events bear a striking structural similarity to the process that must occur whenever two replication forks converge, and so it may be catalyzed by similar enzymes.

recB and *recC* mutants were originally isolated as genes that were required for the formation of recombinant genomes during the sexual cycle of conjugation, and additionally rendered asexually replicating cells hypersensitive to several DNA damage-inducing agents, including those that generate double-strand breaks [3-5]. Biochemical work demonstrated that

these gene products interact with the product from *recD* to form a helicase-nuclease complex [3, 4, 6-9]. Biochemically, RecBCD binds to double-strand DNA ends, then unwinds and degrades the DNA until it encounters a Chi sequence, 5'-GCTGGTGG-3', where the enzyme complex then recruits and loads RecA at a 3'-end created by the RecB nuclease subunit [10-12]. Loading of RecA by RecBCD onto single-stranded DNA is thought to initiate the recombination or repair reaction [13, 14]. Mutations that inactivate either RecB or RecC result in loss of both nuclease and helicase activities, whereas mutations in RecD inactivate nuclease activity and Chi recognition, but not the helicase activity [15, 16].

Based on these biochemical characterizations, current recombination models all propose that RecBCD functions to initiate recombination. However, a range of cellular and genetic observations associated with RecBCD suggest that this enzyme has a broader, more fundamental role in the normal replication cycle. These observations provide insight and prompt us to reconsider the fundamental role of RecBCD in repairing double-strand breaks.

2. Phenotypic Enigmas of RecBCD

recA mutants are deficient in homologous recombination, and all known recombination events that depend on RecBCD also require RecA [3, 17, 18]. However, *recBC* and *recD* mutants exhibit a range of phenotypes that are distinct from those of *recA* mutants, and which are difficult to explain using the current double strand break repair models.

Compared to wild-type cells, *recBC* mutants grow poorly, form small colonies on plates, and contain elevated levels of nonviable cells in culture [19-22]. Curiously however, *recA* mutants, which are completely defective in homologous recombination, and far more sensitive to DNA damage than *recBC* mutants, grow comparatively well and do not exhibit severe viability or growth problems [20-22] (Figure 2A). If the growth abnormalities of *recBC* mutants were simply due to defects in homologous recombination or double-strand break repair, one would predict that the *recA* mutants' phenotypes would be similar or even more severe.

recBC and *recD* mutants also exhibit abnormalities in their ability to replicate plasmids. Plasmids are unstable and rapidly lost when grown in *recD* mutants [22-26]. The small size of the plasmids (~5 kb) argues strongly against the idea that double-strand breaks arise so frequently that they could account for this observed instability. Furthermore, when one examines the fate of replicating plasmids in *recD* mutants, one finds that the plasmid instability arises due to the replication machinery continuing through the doubling point. This produces large quantities of multimeric circles, as well as long linear multimeric plasmids (Figure 2B). These multimeric circles in *recD* mutants are unique in that they contain both odd- and even-numbered multimeric products as though the mechanism for counting molecules in pairs has been inactivated [22, 23, 25]. In *recBC* mutants, plasmid replication tends to produce elevated levels of dimer molecules, but can also lead to over-replication and plasmid loss when second site mutations arise in these strains [22, 24, 25, 27]. In contrast to *recBC* and *recD* cells, plasmids replicate normally and remain stable in *recA* mutants [23, 28, 29]. The stability of plasmids in *recA* mutants is highlighted by the fact that many of the strains adopted by biotech companies to maintain and propagate plasmids are *recA* mutants [30-32]. The stability of plasmids in *recA* mutants, which are completely defective in double-strand break repair, strongly implies that double-strand breaks cannot account for the plasmid instability in *recBC* and *recD* mutants. Furthermore, if plasmid instability arose due to an inability of *recBC* and *recD* mutants to

process double-strand breaks, one would expect that linearized, broken plasmid intermediates would accumulate in these mutants. Yet, despite the presence of several abnormal plasmid species in *recBC* and *recD* strains, the one intermediate that is diminished or missing in these cells, relative to wild-type or *recA* mutants, is the linear broken molecule (Figure 2B) [22].

3. A Role for RecBCD in Completing Replication Events

The impaired growth, lack of broken intermediates, and plasmid over-replication that occurs in *recBC* and *recD* mutants, but not in *recA* mutants, are phenotypes that are each inconsistent with the model in which the only role of RecBCD is to initiate recombination by RecA. Yet, many genes involved in the processes of replication initiation or elongation were initially isolated through screens for mutants that exhibited impaired growth [33-39], or based on their inability to stably maintain plasmids [40-43]. These phenotypes closely resemble those seen in *recBC* and *recD* mutants, and suggest a function for RecBCD in the normal replication cycle of *E. coli*.

When one examines replication occurring on the *E. coli* chromosome, a general defect in the normal replication cycle of these mutants does indeed become apparent. However, rather than a defect in initiation or elongation, the defect in *recBC* or *RecD* mutants specifically arises in the step of completing DNA replication. The completion of replication in *E. coli* occurs in a defined region of the genome, which is located opposite to its single, bidirectional origin of replication, *oriC*. Most completion events can be further localized to one of six termination (*ter*) sequences within the 400-kb terminus region due to the action of the Tus protein. Tus has been shown to bind to *ter* sequences and inhibit replication fork progression in an orientation-dependent manner, in effect stalling one replication fork at this site until the second fork arrives [44-48] (Figure 3A). Because some *ter* sites are located further away from the terminus region, the majority of completion events can be expected to occur between the two most terminal *ter* sites under normal conditions [48]. Although Tus may determine where termination occurs, the protein does not appear to be directly involved in the replication completion reaction. *E. coli* strains deficient in *tus* do not have an observable phenotype and termination appears to occur normally in these mutants [44, 48]. Furthermore, plasmids and bacteriophage lacking *ter*-like sequences are maintained and propagated normally in *E. coli*.

A single origin of replication in *E. coli* means that in an asynchronous population of replicating cells, the copy number of sequences surrounding the bidirectional origin is higher than those in regions further away from the origin. The copy number of sequences can be seen to decrease gradually until it reaches the terminus where the replication events meet and complete as shown for wild-type cells (Figure 3B). In *recBC* mutants, which lack both the helicase and nuclease activities of the RecBCD complex, a marked decrease is observed in the copy number of sequences specifically in the terminus region where the replication forks meet, indicating that these cells are impaired in their ability to maintain the DNA in this region. Conversely, in *recD* mutants, which retain the helicase activity but lack the nuclease function of the RecBCD complex, an over-replication of genomic DNA is observed within the region surrounding the two most distal *ter* sequences that otherwise prevent the replication forks from progressing further. These observations are consistent with those seen on replicating plasmids in these mutants. The reciprocal under- and over-replication of the region where replication forks converge argues strongly that RecBCD function is required to allow the efficient and accurate

completion of chromosomal replication. We infer from the observed over-replication of the terminus region in *recD* mutants that the replication forks transiently pass each other before these sequences are resected back to the doubling point. Similarly, the absence of sequences at the terminus region in *recBC* mutants implies that RecBCD is needed to resolve and join the convergent forks at the doubling point. In their absence, the DNA ends of the converging forks presumably remain unjoined and are subject to exonucleolytic attack.

Our inference that the chromosomal abnormalities in *recBC* and *recD* mutants are independent of their role in double-strand break repair comes from the observation that such defects or abnormalities are not seen on the chromosomes of *recA* mutants. This should not necessarily be interpreted to indicate that RecA plays no role in the completion reaction in wild-type cells. Indeed, the extensive runaway replication that is seen on plasmids in *recD* and other mutants depends on the presence of RecA [22-26]. However, it does imply that the completion reaction can occur efficiently in the absence of RecA. To the best of our knowledge, there are no recombination models for repairing collapsed forks that do not involve RecA, nor do any known recombinational processes require RecBC, but not RecA. Thus, the lack of DNA in the terminus region of *recBC* mutants is inconsistent with the idea that the intermediates are associated with a recombination defect or collapsed replication forks occurring at these sites.

4. A Revised Model for RecBCD function in Double-Strand Break Repair

The sequence profiles of replicating genomes, taken together with previous studies on plasmids, argue that RecBCD is directly involved in completing replication events and functions by degrading over-replicated regions and resolving them at the point where the DNA sequences have doubled (Figure 4A). The process of completing replication appears to be distinct from double-strand break repair in that it occurs efficiently in the absence of RecA or homologous recombination. However, when one considers the mechanism by which double-strand breaks are repaired, it becomes very clear how these two processes may be related. Double-strand break repair in *E. coli* has an absolute requirement for RecA, which is thought to pair the severed strands with intact homologous duplex DNA [49-54]. Once this initiates, the current models propose that the sequences between the opposing strands are replicated and joined using the second molecule as a template. These post-initiation events structurally mimic those that must also occur whenever two replication forks converge. However, in the case of completing replication, the opposing nascent strands have been brought together by the replication forks, rather than by RecA.

Current models propose that RecBCD processes the DNA ends prior to strand exchange, and most biochemical characterizations of RecBCD have exclusively used linear double-stranded substrates to show that the enzyme binds, unwinds, and degrades the molecule up to the Chi site [1, 2, 55]. However, the requirement for RecBCD in completing replication events and forming intact molecules on both the chromosome and on plasmids, independently of RecA, raises the possibility that the enzyme plays a similar role in double-strand break repair. Following strand invasion of homologous duplex DNA to initiate the repair, the proposed reaction events are nearly identical to those that must also occur to complete molecules at the end of replication. This would imply that during recombination, the enzymatic activity of RecBCD acts to unwind, degrade, and resolve the sequences to the point at which the two intact molecules have been restored (Figure 4B). Similar to the completion of replication, the completion of double-strand break repair must have a mechanism to sense, recognize, and resolve these molecules to the point

where the broken ends have been precisely restored, without gaining or losing a nucleotide.

5. Early studies inferred that RecBCD acted late, not early, in recombination

Initial studies characterizing the timing of the recombination reaction led several researchers to propose that RecBC operated late in the recombination process [56-58]. During conjugation between an Hfr donor and an F⁻ recipient, the ability to recover viable recombinants depends upon *recBC* and is reduced 100-1000 fold in mutants [18, 59]. The initial concept that RecBC acted late in recombination came from studies by Wilkins, as well as Hall and Howard-Flanders, in which they found that *recB* and *recC* mutants, but not *recA* mutants, receiving an F' factor could initially transfer their chromosomal genes to another cell at frequencies approaching that of wild type cells, but that this ability rapidly decreased over time [56, 58]. The authors inferred that this meant that the recombination reaction proceeded beyond a point at which the incoming DNA was joined to the chromosome in *recB* and *recC* mutants, but that *recA* mutants were blocked prior to this event. Birge and Low took this a step further when they demonstrated that in crosses between Hfr and F⁻ strains carrying two separate, noncomplementing mutations in *lacZ*, *recB* and *recC* mutants produced levels of β -galactosidase within two fold of those seen in wild type cells, indicating that recombination was able to efficiently proceed up to the point where a transcribable, mutation-free copy of LacZ⁺ was produced [57]. However, although these recombinant intermediates arose normally, the recovery of viable LacZ⁺ recombinant progeny was severely diminished, 100-1000 fold. No such intermediates or viable recombinants could be recovered from *recA* mutants. The observations led the authors to conclude that "*early steps in recombination can proceed efficiently in RecB- and RecC- strains, but that late steps, such as the degradation of excess DNA 'tails', might be defective.*" An observation that bears a striking similarity to its apparent role in completing replication on the chromosome as shown in Figures 3 and 4.

6. RecBCD is more than a Helicase and Nuclease

A role for RecBCD in resecting over-replicated regions and resolving replication events may also help to explain its biochemical properties. RecBCD is the most processive helicase-nuclease complex in *E. coli*, capable of degrading the entire genome when left unchecked [4, 60-63]. Double-strand breaks are a lethal form of DNA damage in part because of the potential for genetic information to be lost. Any degradation of the broken DNA ends represents a serious risk to the integrity of the genome. Thus, given the abundance of other, less aggressive exonucleases in the cell, it seems odd that selective pressures would lead to the evolution of a repair pathway that begins with the degradation of tens of kilobases of genetic information [64]. This appears to take an already dangerous situation for the cell, and make it worse.

However, a processive nuclease, such as that found in RecBCD, may be required during both sexual and asexual cell cycles to degrade and resolve over-replicated regions of the chromosome prior to cell division. A key to maintaining genome stability in all cells, both prokaryotic and eukaryotic, is that each cell inherits an exact copy of the DNA at the time of division. During asexual cell cycles, ample evidence suggests that over-replication is a significant and ever-present problem, and one that requires an enzyme capable of degrading and limiting these events. Converging replisomes have been shown *in vitro* to continue through their meeting point as one replisome displaces the other, resulting in over-replication, or a third copy, of the region where the forks meet [65]. Additionally, the *ter* sites on the chromosome are oriented in a manner that allows up to a tenth of the genome to over-replicate, a fact that is

readily observed in the absence of RecD, and can be exaggerated under conditions of stress or in the presence of other mutations [66-69]. Furthermore, illegitimate initiations of replication are common events occurring at single-strand nicks or gaps, D-loops, and R-loops throughout the genomes of both prokaryotes and eukaryotes [69-74]. Both replication forks bypassing each other and illegitimate initiation events in previously replicated regions would create a third copy of genomic material in this region, leading to the presence of kilobases of DNA that must be degraded and resolved back to the point where each sequence is represented by exactly two copies.

Sexual events in bacteria, such as conjugation, transduction, and transformation, effectively 'inject' large multi-kilobase segments of DNA into the cell, which may or may not contain homology to the chromosome. Prior to division, the cell must deal with the DNA ends as it does any other broken DNA, degrading the sequences that lack a partner and resolving those that do have a partner to ensure that each daughter cell inherits a single, equal copy of the genome. Thus, the observed recombinants can be thought of as the products of the cellular enzymes trying to sense and resolve the ends of DNA sequences to determine which belong in the final pair of genomic copies that will be passed on.

Referring to RecBCD as a helicase/nuclease is somewhat like referring to the replisome as a DNA polymerase. The heterotrimeric RecBCD enzyme is remarkably complex, exhibits multiple activities, and has several unexplained properties that suggest its capabilities go far beyond unwinding and degrading DNA ends at double-strand breaks. The enzyme contains dual ATP-dependent helicases that have opposite polarity and different unwinding rates as well as an exo/endonuclease that can act on either DNA strand, and an ability to recruit and load RecA [14, 75-81] (Figure 5A). Each of these activities is regulated by the nonpalindromic Chi sequence, 5' GCTGGTGG3', which is highly over-represented in the leading-strand template of the *E. coli* genome leading up to the terminus region [82-84] (Figure 5B). On encountering a Chi site, the nucleolytic activity of RecBCD is attenuated, while the helicase activity remains unaffected [10, 16, 85]. The differing speeds of the dual helicases can lead to DNA loops forming as the enzyme progressively tracks along the DNA [6, 86], a phenotype similar to that seen with replisomes and mismatch repair complexes that must track and maintain polarity on DNA over large distances [87-89] (Figure 5C). These features must have arisen under strong selective pressure for specific functions, many of which are not considered in current double-strand break repair models. Yet, cells contain multiple nucleases and helicases with much fewer features. The presence of each of these features argues against the concept that the enzyme acts simply to unwind and degrade linear double-stranded DNA.

Although RecBCD is most often characterized using linear, double-stranded substrates, the enzyme will also unwind and nick cruciform DNA at its junction, and can unwind duplex DNA containing gaps on either strand [90, 91] (Figure 5D). There is also evidence to suggest that the complex may also have other important protein partners, including Polymerase I, and SbcC, another protein involved in the completion of replication that is homologous to human Mre11 [22, 92-94]. Certainly the complexity of this enzyme, its unexplained properties, and its dramatic role in completing replication on the chromosome suggest that potential protein partners and alternative substrates deserve more thorough consideration in future studies.

7. Double-Strand Breaks May Be Infrequent Events

Double strand breaks are proposed to be frequent events that occur due to endogenous damage or other impediments encountered during replication, and are speculated to cause much

of the spontaneous genomic instability and loss of viability that arises in all cells. This viewpoint comes from the observation that several double strand break repair mutants exhibit reduced viability in the absence of DNA damage [20, 21, 95-102]. Similarly, the poor viability and impaired growth of *recBC* mutants has often been taken as an indication that double strand breaks arise frequently in cells, with most estimates ranging between 0.2-1 break events per replication cycle [20, 21, 95-97, 99]. Although several models have been proposed to explain how double strand breaks may frequently arise in cells, experiments have generally failed to support these models or account for the compromised viability of these mutants.

An early concept-based model speculated that double strand breaks might occur frequently if replication forks collapsed upon encountering various impediments to their progression [96, 103]. Several lines of study have revealed that when replication forks are impeded by UV damage, helicase inactivation, thymine starvation, or other impediments, some limited degradation of the nascent replication fork DNA occurs [104-106]. These models imply that if RecBCD is responsible for this degradation, forks collapse and form double strand breaks at these arrest sites. However, multiple studies have shown empirically that *recBC* mutants remain able to restore replication after these challenges, and a series of studies have shown directly that RecBCD plays no role in the degradation of the nascent fork DNA at impeded replication forks [62, 106-109]. In addition, the use of 2D-agarose gels to examine arrested forks at a range of impediments in both prokaryotes and eukaryotes has shown that the replication fork DNA remains stable and intact throughout the recovery process [110-115]. Thus, despite its persistence in the literature [116-122], there is little experimental support to suggest that fork collapse occurs frequently or is a substrate for *recBCD* processing. Another study using a fluorescent RecA as a reporter for double strand breaks found that breaks in the chromosome were observed ~20-100 fold less frequently than would be expected based on these previous estimates and could not account for the poor viability of *recBC* mutants [123].

The impaired ability of *recBC* mutants to complete replication events leads to a dramatic absence of DNA in its terminus, and provides an alternative explanation that can readily account for the reduced viability of these mutants. The completion of replication does not involve recombination or double strand break intermediates, implying that the frequency of spontaneous double strand breaks arising in cells may be significantly over-estimated. Mutations in several eukaryotic double-strand break repair genes also cause reduced viability and genome instabilities, similar to those resulting from RecBCD mutations [98, 124, 125]. Several of these eukaryotic genes have bacterial homologs in *E. coli* that have also been shown to be involved in the completion reaction [22, 70, 70], suggesting the possibility that the underlying cause of the instabilities in these mutants may primarily relate to an impaired ability to complete the events of replication rather than to process double-strand breaks.

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References

- [1] G. R. Smith. How RecBCD enzyme and Chi promote DNA break repair and recombination: a molecular biologist's view. *Microbiol Mol Biol Rev.* 76 (2012) 217-228.
- [2] M. S. Dillingham, S. C. Kowalczykowski. RecBCD enzyme and the repair of double-stranded DNA breaks. *Microbiol Mol Biol Rev.* 72 (2008) 642-71, Table of Contents.
- [3] S. D. Barbour, A. J. Clark. Biochemical and genetic studies of recombination proficiency in *Escherichia coli*. I. Enzymatic activity associated with *recB*⁺ and *recC*⁺ genes. *Proc Natl Acad Sci U S A.* 65 (1970) 955-961.
- [4] D. A. Youngs, I. A. Bernstein. Involvement of the *recB-recC* nuclease (exonuclease V) in the process of x-ray-induced deoxyribonucleic acid degradation in radiosensitive strains of *Escherichia coli* K-12. *J Bacteriol.* 113 (1973) 901-906.
- [5] N. S. Willetts, A. J. Clark, B. Low. Genetic location of certain mutations conferring recombination deficiency in *Escherichia coli*. *J Bacteriol.* 97 (1969) 244-249.
- [6] A. Taylor, G. R. Smith. Unwinding and rewinding of DNA by the RecBC enzyme. *Cell.* 22 (1980) 447-457.
- [7] A. F. Taylor, G. R. Smith. Substrate specificity of the DNA unwinding activity of the RecBC enzyme of *Escherichia coli*. *J Mol Biol.* 185 (1985) 431-443.
- [8] G. Braedt, G. R. Smith. Strand specificity of DNA unwinding by RecBCD enzyme. *Proc Natl Acad Sci U S A.* 86 (1989) 871-875.
- [9] R. Rinken, B. Thomas, W. Wackernagel. Evidence that *recBC*-dependent degradation of duplex DNA in *Escherichia coli recD* mutants involves DNA unwinding. *J Bacteriol.* 174 (1992) 5424-5429.
- [10] A. S. Ponticelli, D. W. Schultz, A. F. Taylor, G. R. Smith. Chi-dependent DNA strand cleavage by RecBC enzyme. *Cell.* 41 (1985) 145-151.
- [11] A. F. Taylor, D. W. Schultz, A. S. Ponticelli, G. R. Smith. RecBC enzyme nicking at Chi sites during DNA unwinding: location and orientation-dependence of the cutting. *Cell.* 41 (1985) 153-163.
- [12] D. A. Dixon, S. C. Kowalczykowski. The recombination hotspot *chi* is a regulatory sequence that acts by attenuating the nuclease activity of the *E. coli* RecBCD enzyme. *Cell.* 73 (1993) 87-96.
- [13] L. J. Roman, D. A. Dixon, S. C. Kowalczykowski. RecBCD-dependent joint molecule formation promoted by the *Escherichia coli* RecA and SSB proteins. *Proc Natl Acad Sci U S A.* 88 (1991) 3367-3371.
- [14] D. A. Arnold, S. C. Kowalczykowski. Facilitated loading of RecA protein is essential to recombination by RecBCD enzyme. *J Biol Chem.* 275 (2000) 12261-12265.
- [15] D. S. Thaler, E. Sampson, I. Siddiqi, S. M. Rosenberg, L. C. Thomason, F. W. Stahl, M. M. Stahl. Recombination of bacteriophage lambda in *recD* mutants of *Escherichia coli*. *Genome.* 31 (1989) 53-67.
- [16] R. S. Myers, A. Kuzminov, F. W. Stahl. The recombination hot spot *chi* activates RecBCD recombination by converting *Escherichia coli* to a *recD* mutant phenocopy. *Proc Natl Acad Sci U S A.* 92 (1995) 6244-6248.
- [17] A. J. Clark, A. D. Margulies. Isolation and Characterization of Recombination-Deficient Mutants of *Escherichia coli* K12. *Proc Natl Acad Sci U S A.* 53 (1965) 451-459.

- [18] N. S. Willetts, A. J. Clark. Characteristics of some multiply recombination-deficient strains of *Escherichia coli*. *J Bacteriol.* 100 (1969) 231-239.
- [19] S. D. Barbour, H. Nagaishi, A. Templin, A. J. Clark. Biochemical and genetic studies of recombination proficiency in *Escherichia coli*. II. Rec⁺ revertants caused by indirect suppression of rec⁻ mutations. *Proc Natl Acad Sci U S A.* 67 (1970) 128-135.
- [20] F. N. Capaldo, S. D. Barbour. The role of the rec genes in the viability of *Escherichia coli* K12. *Basic Life Sci.* 5A (1975) 405-418.
- [21] F. Capaldo-Kimball, S. D. Barbour. Involvement of recombination genes in growth and viability of *Escherichia coli* K-12. *J Bacteriol.* 106 (1971) 204-212.
- [22] B. J. Wendel, C. T. Courcelle, J. Courcelle. The Completion of DNA replication in *Escherichia coli*. *Proc Natl Acad Sci U S A.* (2014)
- [23] D. P. Biek, S. N. Cohen. Identification and characterization of recD, a gene affecting plasmid maintenance and recombination in *Escherichia coli*. *J Bacteriol.* 167 (1986) 594-603.
- [24] A. Cohen, A. J. Clark. Synthesis of linear plasmid multimers in *Escherichia coli* K-12. *J Bacteriol.* 167 (1986) 327-335.
- [25] Z. Silberstein, A. Cohen. Synthesis of linear multimers of OriC and pBR322 derivatives in *Escherichia coli* K-12: role of recombination and replication functions. *J Bacteriol.* 169 (1987) 3131-3137.
- [26] H. Niki, C. Ichinose, T. Ogura, H. Mori, M. Morita, M. Hasegawa, N. Kusakawa, S. Hiraga. Chromosomal genes essential for stable maintenance of the mini-F plasmid in *Escherichia coli*. *J Bacteriol.* 170 (1988) 5272-5278.
- [27] H. Niki, T. Ogura, S. Hiraga. Linear multimer formation of plasmid DNA in *Escherichia coli* hopE (recD) mutants. *Mol Gen Genet.* 224 (1990) 1-9.
- [28] P. E. Berg. Cloning and characterization of the *Escherichia coli* gene coding for alkaline phosphatase. *J Bacteriol.* 146 (1981) 660-667.
- [29] D. K. Summers, D. J. Sherratt. Multimerization of high copy number plasmids causes instability: CoIE1 encodes a determinant essential for plasmid monomerization and stability. *Cell.* 36 (1984) 1097-1103.
- [30] C. Yanisch-Perron, J. Vieira, J. Messing. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene.* 33 (1985) 103-119.
- [31] R. G. Taylor, D. C. Walker, R. R. McInnes. *E. coli* host strains significantly affect the quality of small scale plasmid DNA preparations used for sequencing. *Nucleic Acids Res.* 21 (1993) 1677-1678.
- [32] F. Bolivar, K. Backman. Plasmids of *Escherichia coli* as cloning vectors. *Methods Enzymol.* 68 (1979) 245-267.
- [33] W. L. Fangman, A. Novick. Characterization of two bacterial mutants with temperature-sensitive synthesis of DNA. *Genetics.* 60 (1968) 1-17.
- [34] M. Kohiyama, D. Cousin, A. Ryter, F. Jacob. [Thermosensitive mutants of *Escherichia coli* K 12. I. Isolation and rapid characterization]. *Ann Inst Pasteur (Paris).* 110 (1966) 465-486.

- [35] Y. Hirota, A. Ryter, F. Jacob. Thermosensitive mutants of *E. coli* affected in the processes of DNA synthesis and cellular division. *Cold Spring Harb Symp Quant Biol.* 33 (1968) 677-693.
- [36] J. A. Wechsler, J. D. Gross. *Escherichia coli* mutants temperature-sensitive for DNA synthesis. *Mol Gen Genet.* 113 (1971) 273-284.
- [37] T. Horiuchi, H. Maki, M. Sekiguchi. A new conditional lethal mutator (*dnaQ49*) in *Escherichia coli* K12. *Mol Gen Genet.* 163 (1978) 277-283.
- [38] F. BONHOEFFER, H. SCHALLER. A METHOD FOR SELECTIVE ENRICHMENT OF MUTANTS BASED ON THE HIGH UV SENSITIVITY OF DNA CONTAINING 5-BROMOURACIL. *Biochem Biophys Res Commun.* 20 (1965) 93-97.
- [39] F. Bonhoeffer. DNA transfer and DNA synthesis during bacterial conjugation. *Z Vererbungsl.* 98 (1966) 141-149.
- [40] A. C. Leonard, W. G. Whitford, C. E. Helmstetter. Involvement of DNA superhelicity in minichromosome maintenance in *Escherichia coli*. *J Bacteriol.* 161 (1985) 687-695.
- [41] G. T. Maine, P. Sinha, B. K. Tye. Mutants of *S. cerevisiae* defective in the maintenance of minichromosomes. *Genetics.* 106 (1984) 365-385.
- [42] V. Rodriguez Lemoine, R. J. Rowbury. Instability of the *Flac+* factor in a *dnaC* mutant of *Salmonella typhimurium*. *J Gen Microbiol.* 90 (1975) 360-363.
- [43] H. Nakamura. Plasmid-instability in *acrA* mutants of *Escherichia coli* K12. *J Gen Microbiol.* 84 (1974) 85-93.
- [44] T. M. Hill, M. L. Tecklenburg, A. J. Pelletier, P. L. Kuempel. *tus*, the trans-acting gene required for termination of DNA replication in *Escherichia coli*, encodes a DNA-binding protein. *Proc Natl Acad Sci U S A.* 86 (1989) 1593-1597.
- [45] T. M. Hill, K. J. Mariani. *Escherichia coli* Tus protein acts to arrest the progression of DNA replication forks in vitro. *Proc Natl Acad Sci U S A.* 87 (1990) 2481-2485.
- [46] T. MacAllister, G. S. Khatri, D. Bastia. Sequence-specific and polarized replication termination in vitro: complementation of extracts of *tus- Escherichia coli* by purified Ter protein and analysis of termination intermediates. *Proc Natl Acad Sci U S A.* 87 (1990) 2828-2832.
- [47] T. M. Hill. Arrest of bacterial DNA replication. *Annu Rev Microbiol.* 46 (1992) 603-633.
- [48] T. Kobayashi, M. Hidaka, T. Horiuchi. Evidence of a *ter* specific binding protein essential for the termination reaction of DNA replication in *Escherichia coli*. *Embo J.* 8 (1989) 2435-2441.
- [49] M. M. Cox, I. R. Lehman. Directionality and polarity in *recA* protein-promoted branch migration. *Proc Natl Acad Sci U S A.* 78 (1981) 6018-6022.
- [50] M. M. Cox, I. R. Lehman. *recA* protein of *Escherichia coli* promotes branch migration, a kinetically distinct phase of DNA strand exchange. *Proc Natl Acad Sci U S A.* 78 (1981) 3433-3437.
- [51] M. M. Cox, I. R. Lehman. *recA* protein-promoted DNA strand exchange. Stable complexes of *recA* protein and single-stranded DNA formed in the presence of ATP and single-stranded DNA binding protein. *J Biol Chem.* 257 (1982) 8523-8532.

- [52] R. P. Cunningham, C. DasGupta, T. Shibata, C. M. Radding. Homologous pairing in genetic recombination: recA protein makes joint molecules of gapped circular DNA and closed circular DNA. *Cell*. 20 (1980) 223-235.
- [53] T. Shibata, C. DasGupta, R. P. Cunningham, C. M. Radding. Purified *Escherichia coli* recA protein catalyzes homologous pairing of superhelical DNA and single-stranded fragments. *Proc Natl Acad Sci U S A*. 76 (1979) 1638-1642.
- [54] R. P. Cunningham, T. Shibata, C. DasGupta, C. M. Radding. Single strands induce recA protein to unwind duplex DNA for homologous pairing. *Nature*. 281 (1979) 191-195.
- [55] J. T. Yeeles, M. S. Dillingham. The processing of double-stranded DNA breaks for recombinational repair by helicase-nuclease complexes. *DNA Repair (Amst)*. 9 (2010) 276-285.
- [56] J. D. Hall, P. Howard-Flanders. Recombinant F' factors from *Escherichia coli* K-12 strains carrying recB or recC. *J Bacteriol*. 110 (1972) 578-584.
- [57] E. A. Birge, K. B. Low. Detection of transcribable recombination products following conjugation in rec⁺, recB⁻ and recC⁻ strains of *Escherichia coli* K12. *J Mol Biol*. 83 (1974) 447-457.
- [58] B. M. Wilkins. Chromosome transfer from F-lac⁺ strains of *Escherichia coli* K-12 mutant at recA, recB, or recC. *J Bacteriol*. 98 (1969) 599-604.
- [59] P. T. Emmerson. Recombination deficient mutants of *Escherichia coli* K12 that map between thy A and argA. *Genetics*. 60 (1968) 19-30.
- [60] Z. I. Horii, K. Suzuki. Degradation of the DNA of *Escherichia coli* K12 rec⁻ (JC1569b) after irradiation with ultraviolet light. *Photochem photobiol*. 8 (1968) 93-105.
- [61] S. A. Friedman, J. B. Hays. Selective inhibition of *Escherichia coli* recBC activities by plasmid-encoded GamS function of phage lambda. *Gene*. 43 (1986) 255-263.
- [62] K. H. Chow, J. Courcelle. RecBCD and RecJ/RecQ initiate DNA degradation on distinct substrates in UV-irradiated *Escherichia coli*. *Radiat Res*. 168 (2007) 499-506.
- [63] K. Zahradka, M. Buljubasic, M. Petranovic, D. Zahradka. Roles of ExoI and SbcCD nucleases in "reckless" DNA degradation in recA mutants of *Escherichia coli*. *J Bacteriol*. 191 (2009) 1677-1687.
- [64] L. J. Roman, A. K. Eggleston, S. C. Kowalczykowski. Processivity of the DNA helicase activity of *Escherichia coli* recBCD enzyme. *J Biol Chem*. 267 (1992) 4207-4214.
- [65] H. Hiasa, K. J. Marians. Tus prevents overreplication of oriC plasmid DNA. *J Biol Chem*. 269 (1994) 26959-26968.
- [66] T. R. Magee, T. Asai, D. Malka, T. Kogoma. DNA damage-inducible origins of DNA replication in *Escherichia coli*. *Embo J*. 11 (1992) 4219-4225.
- [67] B. de Massy, J. Patte, J. M. Louarn, J. P. Bouche. oriX: a new replication origin in *E. coli*. *Cell*. 36 (1984) 221-227.
- [68] N. Z. Maduike, A. K. Tehrani, J. D. Wang, K. N. Kreuzer. Replication of the *Escherichia coli* chromosome in RNase HI-deficient cells: multiple initiation regions and fork dynamics. *Mol Microbiol*. 91 (2014) 39-56.
- [69] B. de Massy, O. Fayet, T. Kogoma. Multiple origin usage for DNA replication in sdrA(rnh) mutants of *Escherichia coli* K-12. Initiation in the absence of oriC. *J Mol Biol*. 178 (1984) 227-236.

- [70] C. J. Rudolph, A. L. Upton, A. Stockum, C. A. Nieduszynski, R. G. Lloyd. Avoiding chromosome pathology when replication forks collide. *Nature*. 500 (2013) 608-611.
- [71] T. Asai, D. B. Bates, T. Kogoma. DNA replication triggered by double-stranded breaks in *E. coli*: dependence on homologous recombination functions. *Cell*. 78 (1994) 1051-1061.
- [72] T. Asai, T. Kogoma. D-loops and R-loops: alternative mechanisms for the initiation of chromosome replication in *Escherichia coli*. *J Bacteriol*. 176 (1994) 1807-1812.
- [73] R. A. Donnianni, L. S. Symington. Break-induced replication occurs by conservative DNA synthesis. *Proc Natl Acad Sci U S A*. 110 (2013) 13475-13480.
- [74] S. Hamperl, K. A. Cimprich. The contribution of co-transcriptional RNA:DNA hybrid structures to DNA damage and genome instability. *DNA Repair (Amst)*. 19 (2014) 84-94.
- [75] C. G. Wu, C. Bradford, T. M. Lohman. *Escherichia coli* RecBC helicase has two translocase activities controlled by a single ATPase motor. *Nat Struct Mol Biol*. 17 (2010) 1210-1217.
- [76] A. F. Taylor, S. K. Amundsen, M. Guttman, K. K. Lee, J. Luo, J. Ranish, G. R. Smith. Control of RecBCD Enzyme Activity by DNA Binding- and Chi Hotspot-Dependent Conformational Changes. *J Mol Biol*. 426 (2014) 3479-3499.
- [77] S. K. Amundsen, A. F. Taylor, M. Reddy, G. R. Smith. Intersubunit signaling in RecBCD enzyme, a complex protein machine regulated by Chi hot spots. *Genes Dev*. 21 (2007) 3296-3307.
- [78] M. R. Singleton, M. S. Dillingham, M. Gaudier, S. C. Kowalczykowski, D. B. Wigley. Crystal structure of RecBCD enzyme reveals a machine for processing DNA breaks. *Nature*. 432 (2004) 187-193.
- [79] M. S. Dillingham, M. Spies, S. C. Kowalczykowski. RecBCD enzyme is a bipolar DNA helicase. *Nature*. 423 (2003) 893-897.
- [80] A. F. Taylor, G. R. Smith. RecBCD enzyme is a DNA helicase with fast and slow motors of opposite polarity. *Nature*. 423 (2003) 889-893.
- [81] J. J. Churchill, D. G. Anderson, S. C. Kowalczykowski. The RecBC enzyme loads RecA protein onto ssDNA asymmetrically and independently of chi, resulting in constitutive recombination activation. *Genes Dev*. 13 (1999) 901-911.
- [82] G. R. Smith, S. M. Kunes, D. W. Schultz, A. Taylor, K. L. Triman. Structure of chi hotspots of generalized recombination. *Cell*. 24 (1981) 429-436.
- [83] A. F. Taylor, G. R. Smith. RecBCD enzyme is altered upon cutting DNA at a chi recombination hotspot. *Proc Natl Acad Sci U S A*. 89 (1992) 5226-5230.
- [84] D. G. Anderson, J. J. Churchill, S. C. Kowalczykowski. 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 Cells*. 2 (1997) 117-128.
- [85] K. C. Cheng, G. R. Smith. Cutting of chi-like sequences by the RecBCD enzyme of *Escherichia coli*. *J Mol Biol*. 194 (1987) 747-750.
- [86] J. Rosamond, K. M. Telander, S. Linn. Modulation of the action of the recBC enzyme of *Escherichia coli* K-12 by Ca²⁺. *J Biol Chem*. 254 (1979) 8646-8652.
- [87] J. D. Griffith. Many ways to loop DNA. *J Biol Chem*. 288 (2013) 29724-29735.

- [88] D. J. Allen, A. Makhov, M. Grilley, J. Taylor, R. Thresher, P. Modrich, J. D. Griffith. MutS mediates heteroduplex loop formation by a translocation mechanism. *EMBO J.* 16 (1997) 4467-4476.
- [89] K. Park, Z. Debyser, S. Tabor, C. C. Richardson, J. D. Griffith. Formation of a DNA loop at the replication fork generated by bacteriophage T7 replication proteins. *J Biol Chem.* 273 (1998) 5260-5270.
- [90] A. F. Taylor, G. R. Smith. Action of RecBCD enzyme on cruciform DNA. *J Mol Biol.* 211 (1990) 117-134.
- [91] F. Xie, C. G. Wu, E. Weiland, T. M. Lohman. Asymmetric regulation of bipolar single-stranded DNA translocation by the two motors within *Escherichia coli* RecBCD helicase. *J Biol Chem.* 288 (2013) 1055-1064.
- [92] R. W. Hendler, M. Pereira, R. Scharff. DNA synthesis involving a complexed form of DNA polymerase I in extracts of *Escherichia coli*. *Proc Natl Acad Sci U S A.* 72 (1975) 2099-2103.
- [93] S. K. Kulkarni, F. W. Stahl. Interaction between the *sbcC* gene of *Escherichia coli* and the *gam* gene of phage lambda. *Genetics.* 123 (1989) 249-253.
- [94] G. J. Sharples, D. R. Leach. Structural and functional similarities between the SbcCD proteins of *Escherichia coli* and the RAD50 and MRE11 (RAD32) recombination and repair proteins of yeast. *Mol Microbiol.* 17 (1995) 1215-1217.
- [95] J. D. McCool, E. Long, J. F. Petrosino, H. A. Sandler, S. M. Rosenberg, S. J. Sandler. Measurement of SOS expression in individual *Escherichia coli* K-12 cells using fluorescence microscopy. *Mol Microbiol.* 53 (2004) 1343-1357.
- [96] A. Kuzminov. Instability of inhibited replication forks in *E. coli*. *Bioessays.* 17 (1995) 733-741.
- [97] B. Michel, M. J. Flores, E. Viguera, G. Grompone, M. Seigneur, V. Bidnenko. Rescue of arrested replication forks by homologous recombination. *Proc Natl Acad Sci U S A.* 98 (2001) 8181-8188.
- [98] G. Luo, M. S. Yao, C. F. Bender, M. Mills, A. R. Bladl, A. Bradley, J. H. Petrini. Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. *Proc Natl Acad Sci U S A.* 96 (1999) 7376-7381.
- [99] M. M. Cox, M. F. Goodman, K. N. Kreuzer, D. J. Sherratt, S. J. Sandler, K. J. Marians. The importance of repairing stalled replication forks. *Nature.* 404 (2000) 37-41.
- [100] A. H. Syeda, M. Hawkins, P. McGlynn. Recombination and replication. *Cold Spring Harb Perspect Biol.* 6 (2014) a016550.
- [101] C. Bruhn, Z. W. Zhou, H. Ai, Z. Q. Wang. The essential function of the MRN complex in the resolution of endogenous replication intermediates. *Cell Rep.* 6 (2014) 182-195.
- [102] M. M. Vilenchik, A. G. Knudson. Endogenous DNA double-strand breaks: production, fidelity of repair, and induction of cancer. *Proc Natl Acad Sci U S A.* 100 (2003) 12871-12876.
- [103] A. Kuzminov. Collapse and repair of replication forks in *Escherichia coli*. *Mol Microbiol.* 16 (1995) 373-384.

- [104] G. E. Veomett, P. L. Kuempel. Strand-specific DNA degradation in a mutant of *Escherichia coli*. *Mol Gen Genet*. 123 (1973) 17-28.
- [105] T. R. Breitman, P. B. Maury, J. N. Toal. Loss of deoxyribonucleic acid-thymine during thymine starvation of *Escherichia coli*. *J Bacteriol*. 112 (1972) 646-648.
- [106] J. Courcelle, C. Carswell-Crumpton, P. C. Hanawalt. *recF* and *recR* are required for the resumption of replication at DNA replication forks in *Escherichia coli*. *Proc Natl Acad Sci U S A*. 94 (1997) 3714-3719.
- [107] M. A. Khidhir, S. Casaregola, I. B. Holland. Mechanism of transient inhibition of DNA synthesis in ultraviolet-irradiated *E. coli*: inhibition is independent of *recA* whilst recovery requires *RecA* protein itself and an additional, inducible SOS function. *Mol Gen Genet*. 199 (1985) 133-140.
- [108] J. Courcelle, P. C. Hanawalt. *RecQ* and *RecJ* process blocked replication forks prior to the resumption of replication in UV-irradiated *Escherichia coli*. *Mol Gen Genet*. 262 (1999) 543-551.
- [109] J. J. Belle, A. Casey, C. T. Courcelle, J. Courcelle. Inactivation of the *DnaB* helicase leads to the collapse and degradation of the replication fork: a comparison to UV-induced arrest. *J Bacteriol*. 189 (2007) 5452-5462.
- [110] M. Lopes, C. Cotta-Ramusino, A. Pelliccioli, G. Liberi, P. Plevani, M. Muzi-Falconi, C. S. Newlon, M. Foiani. The DNA replication checkpoint response stabilizes stalled replication forks. *Nature*. 412 (2001) 557-561.
- [111] J. M. Sogo, M. Lopes, M. Foiani. Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects. *Science*. 297 (2002) 599-602.
- [112] M. Lopes, M. Foiani, J. M. Sogo. Multiple mechanisms control chromosome integrity after replication fork uncoupling and restart at irreparable UV lesions. *Mol Cell*. 21 (2006) 15-27.
- [113] J. Courcelle, J. R. Donaldson, K. H. Chow, C. T. Courcelle. DNA Damage-Induced Replication Fork Regression and Processing in *Escherichia coli*. *Science*. 299 (2003) 1064-1067.
- [114] K. H. Chow, J. Courcelle. *RecO* Acts with *RecF* and *RecR* to Protect and Maintain Replication Forks Blocked by UV-induced DNA Damage in *Escherichia coli*. *J Biol Chem*. 279 (2004) 3492-3496.
- [115] H. A. Jeiranian, B. J. Schallow, C. T. Courcelle, J. Courcelle. Fate of the replisome following arrest by UV-induced DNA damage in *Escherichia coli*. *Proc Natl Acad Sci U S A*. 110 (2013) 11421-11426.
- [116] A. D. Fricker, J. E. Peters. Vulnerabilities on the lagging-strand template: opportunities for mobile elements. *Annu Rev Genet*. 48 (2014) 167-186.
- [117] S. Sharma, C. M. Helchowski, C. E. Canman. The roles of DNA polymerase zeta and the Y family DNA polymerases in promoting or preventing genome instability. *Mutat Res*. 743-744 (2013) 97-110.
- [118] A. Gospodinov, Z. Herceg. Chromatin structure in double strand break repair. *DNA Repair (Amst)*. 12 (2013) 800-810.
- [119] R. D. Paulsen, K. A. Cimprich. The ATR pathway: fine-tuning the fork. *DNA Repair (Amst)*. 6 (2007) 953-966.

- [120] M. M. Cox. Recombinational DNA repair of damaged replication forks in *Escherichia coli*: questions. *Annu Rev Genet.* 35 (2001) 53-82.
- [121] A. Mehta, J. E. Haber. Sources of DNA double-strand breaks and models of recombinational DNA repair. *Cold Spring Harb Perspect Biol.* 6 (2014) a016428.
- [122] A. Nowosielska. Bacterial DNA repair genes and their eukaryotic homologues: 5. The role of recombination in DNA repair and genome stability. *Acta Biochim Pol.* 54 (2007) 483-494.
- [123] J. M. Pennington, S. M. Rosenberg. Spontaneous DNA breakage in single living *Escherichia coli* cells. *Nat Genet.* 39 (2007) 797-802.
- [124] T. H. Stracker, J. W. Theunissen, M. Morales, J. H. Petrini. The Mre11 complex and the metabolism of chromosome breaks: the importance of communicating and holding things together. *DNA Repair (Amst).* 3 (2004) 845-854.
- [125] D. D'Amours, S. P. Jackson. The yeast Xrs2 complex functions in S phase checkpoint regulation. *Genes Dev.* 15 (2001) 2238-2249.

FIGURE LEGENDS

Figure 1. Current model for RecBCD in double-strand break repair. RecBCD is proposed to initiate recombination by processing the broken DNA ends before recruiting RecA to these sites. RecA then promotes strand invasion with a homologous duplex molecule. The missing sequence between the breaks is resynthesized, before the crossovers are resolved and the two intact molecules are restored.

Figure 2. Enigmatic phenotypes of *recBC* and *recD* mutants. A) *recA* mutants are far more sensitive than *recBC* mutants to DNA damaging agents, such as UV radiation. However in the absence of DNA damage, it is *recBC* mutants that exhibit poor viability, while *recA* mutants are comparatively healthy. B) Plasmids replicating in *recD* mutants are unstable, producing large amounts of linear and circular multimeric concatamers. *recBC* mutants also have difficulty maintaining the circular form of the monomeric plasmid. Yet plasmids are stably maintained in *recA* mutants. Broken linear intermediates might be expected to accumulate in mutants defective in initiating double strand break repair, yet these intermediates are actually diminished in *recBC* and *recD* mutants. Methods: UV sensitivity- Overnight cultures were applied to a Luria broth plate using a cotton swab. A sheet of aluminum foil, covering the plate, was progressively retracted under a 254 nm germicidal UV lamp, to achieve the indicated exposure before incubation at 37°C overnight. Viability- 10 µl drops of 10 fold serially diluted overnight cultures were plated on Luria broth plates and incubated at 37°C overnight. Plasmid visualization- Genomic DNA was purified from cultures containing the plasmid pBR322, electrophoresed through a 1.0% agarose gel in 0.5x TAE, and probed with 32P-labeled pBR322 by standard Southern analysis. Species are supercoiled unless otherwise indicated. (B) Adapted from [22].

Figure 3. RecBCD is required to complete replication on the chromosome. A) Replication initiates bidirectionally from *oriC* and completes in the terminus region. B) Replication profiles of wildtype *recBC*, *recD*, and *recA* cultures. Genomic DNA from replicating cultures was purified, fragmented, and profiled using high-throughput sequencing. Sequence read frequencies, normalized to stationary-phase cells, are plotted relative to their position on the genome. The

origin and terminus, containing *terD*, -A, -C, and -B, is shown. An 8-kb floating average of the sequence frequency is plotted in red. In replicating wild-type cultures, sequences frequencies are gradually decreases from the origin to the terminus where replication completes. *recBC* mutants fail to complete replicate events, leaving the ends of the replication forks susceptible to nucleolytic attack and degradation. Conversely, *recD* mutants fail to recognize and limit replication at the double point, leading to over-replication of the region on the chromosome where replication forks converge. Importantly, *recA* mutants complete replication normally, indicating that the reaction catalyzed by RecBCD does not involve homologous recombination or double strand break intermediates. Adapted from [22].

Figure 4. A revised model for RecBCD function in completing recombination events. A) *RecBCD is required to complete replication events.* Converging replication forks pass each other leading to transiently over-replicated regions of the chromosome. RecBCD promotes the degradation and resolution of the over-replicated regions at their doubling point. In the absence of RecD, the regions are not degraded leading to extra, odd numbered portions of genomic material [22]. In the absence of RecBC, the regions fail to join and remain susceptible to degradation [22]. B) *RecBCD is required to complete recombination events.* Initiation events can occur efficiently in the absence of RecBCD [56-58]. Following strand invasion of a homologous duplex molecule by RecA, replication resynthesizes the missing sequences, leading to a transiently over-replicated region. RecBCD promotes the degradation and resolution of the over-replicated regions at their doubling point, to restore the broken molecule. The presence of linked recombinant molecules in the absence of RecBCD, suggests that resolution of the cross-over events occurs before, or independent of, RecBCD action [56-58].

Figure 5. RecBCD is a complex enzyme with multiple properties. A) RecBCD is a heterotrimer with separate helicases located within RecB and RecD. RecC function as a molecular switch that upon excountering a *Chi* sequence (5'-GCTGGTGG-3'), inactivates the RecD helicase, alters the nucleolytic activity of RecB, and induces RecA loading [14, 75-81]. B) The orientation of *Chi* sites around the genome is strongly associated with DNA replication. The frequency of *Chi* sites/100 kp is plotted on the leading and lagging strand of the *E. coli* chromosome. C) Products of RecBCD end processing. If $[Mg^{+2}] > [ATP]$, RecBCD degrades the 3' end of double stranded DNA up to a *Chi* sequence, where the endonucleolytic activity switches to degrade the 5' strand. If $[Mg^{+2}] < [ATP]$, RecBCD unwinds DNA, nicking the strand at a *Chi* sequence, then continues to unwind the molecule [11, 12] D) *In vitro* substrates of RecBCD. In addition to unwinding double stranded DNA (i), the complex can translocate through single-stranded gaps on either strand (ii), and will unwind cruciform structures, making an incision (*) at the branch point (iii) [90, 91].

Figure 1

Figure 2

Figure 3

Figure 4

A. RecBCD completes replication

B. RecBCD completes recombination

Figure 5