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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.

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 (Fig.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

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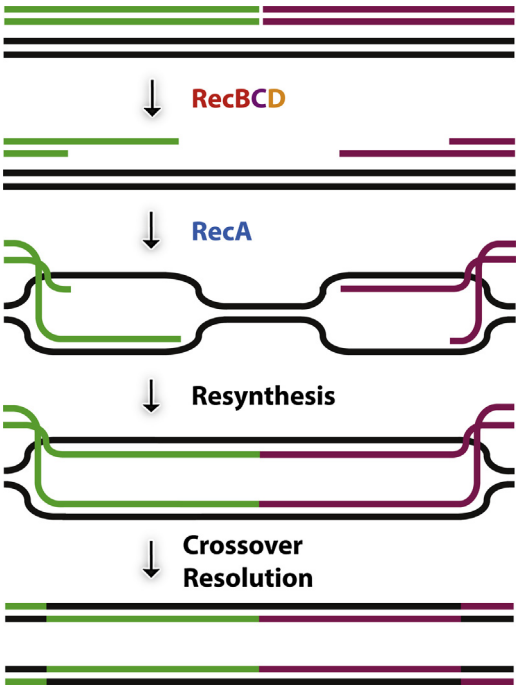

Fig. 1. Current model for the role of RecBCD in double-strand break repair. RecBCD is proposed to initiate recombination by processing the broken DNA ends before recruiting RecA to these sites. RecA then promotes strand invasion with a homologous duplex molecule. The missing sequence between the breaks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

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] (Fig. 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 (Fig. 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 (Fig. 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] (Fig. 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

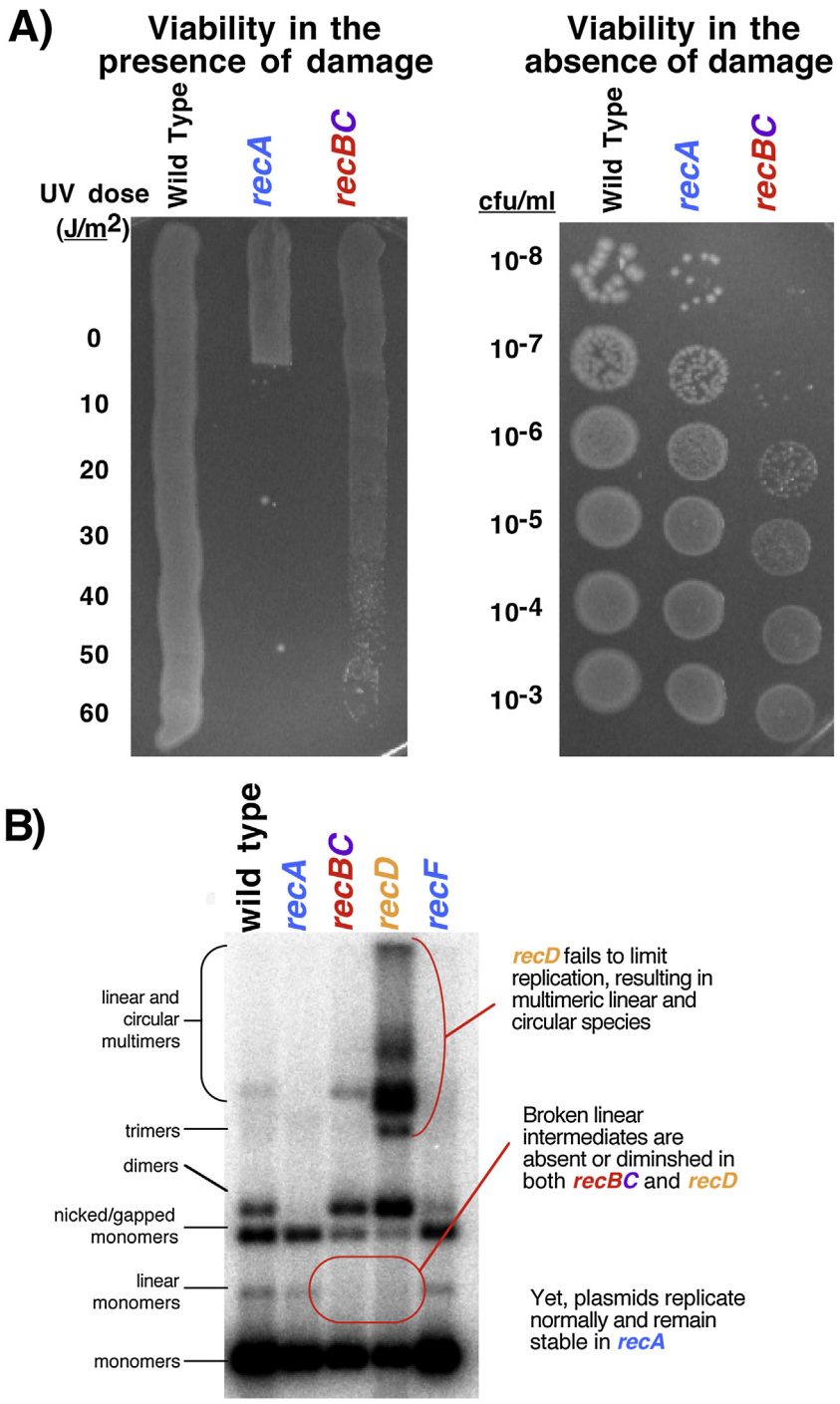

Fig. 2. Enigmatic phenotypes of *recBC* and *recD* mutants.
(A) *recA* mutants are far more sensitive than *recBC* mutants to DNA damaging agents, such UV radiation. However, in the absence of DNA damage, it is *recBC* mutants that exhibit poor viability, while *recA* mutants are comparatively healthy. (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 37C overnight. Viability – 10 μl drops of 10-fold serially diluted overnight cultures were plated on Luria broth plates and incubated at 37C overnight. Plasmid visualizaton – Genomic DNA was purified from cultures containing the plasmid pBR322, electrophoresed through a 1.0% agarose gel in 0.5*x* TAE, and probed with ^{32}P-labeled pBR322 by standard Southern analysis. Species are supercoiled unless otherwise indicated.
Source: (B) Adapted from [22].

terminus where the replication events meet and complete as shown for wild-type cells (Fig. 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

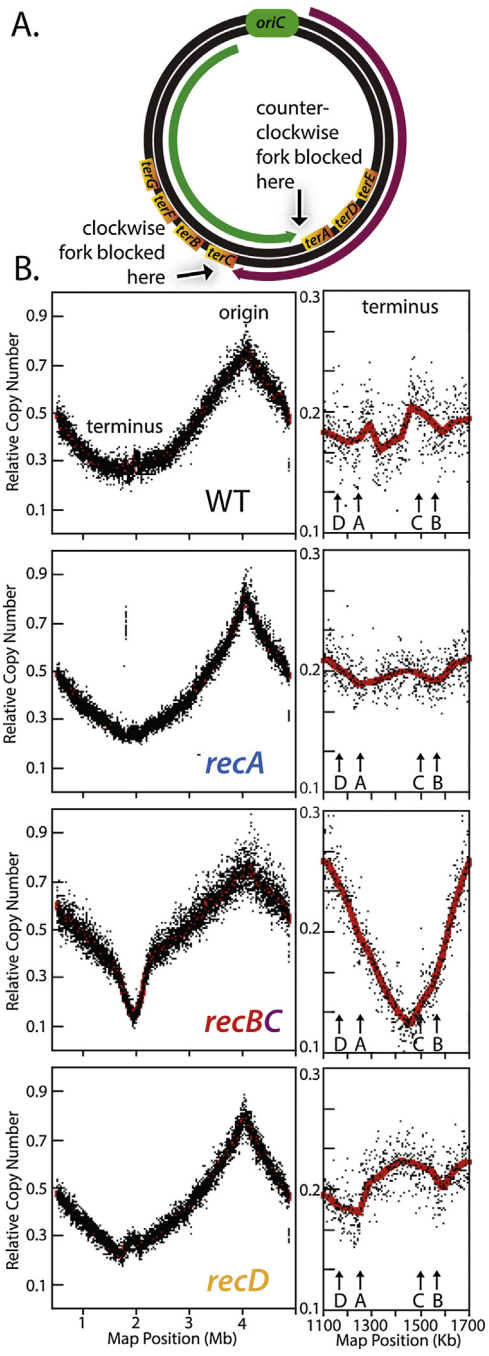

Fig. 3. RecBCD is required to complete replication on the chromosome.
(A) Replication initiates bidirectionally from *oriC* and completes in the terminus region. (B) Replication profiles of wildtype, *recBC*, *recD*, and *recA* cultures. Genomic DNA from replicating cultures was purified, fragmented, 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, sequence frequencies gradually decrease from the origin to the terminus where replication completes. *recBC* mutants fail to complete replication 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 doubling 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.
Source: Adapted from [22].

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 (Fig. 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, 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 (Fig. 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.

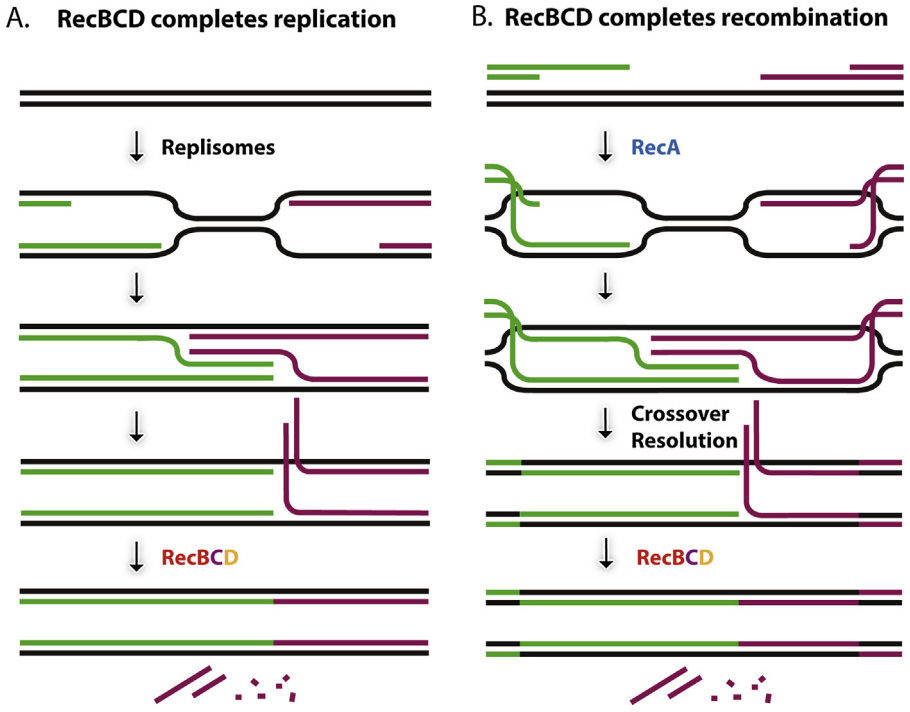

Fig. 4. A revised model for RecBCD function in completing recombination events.
(A) RecBCD is required to complete replication events. Converging replication forks pass each other leading to transiently over-replicated regions of the chromosome. RecBCD promotes the degradation and resolution of the over-replicated 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].

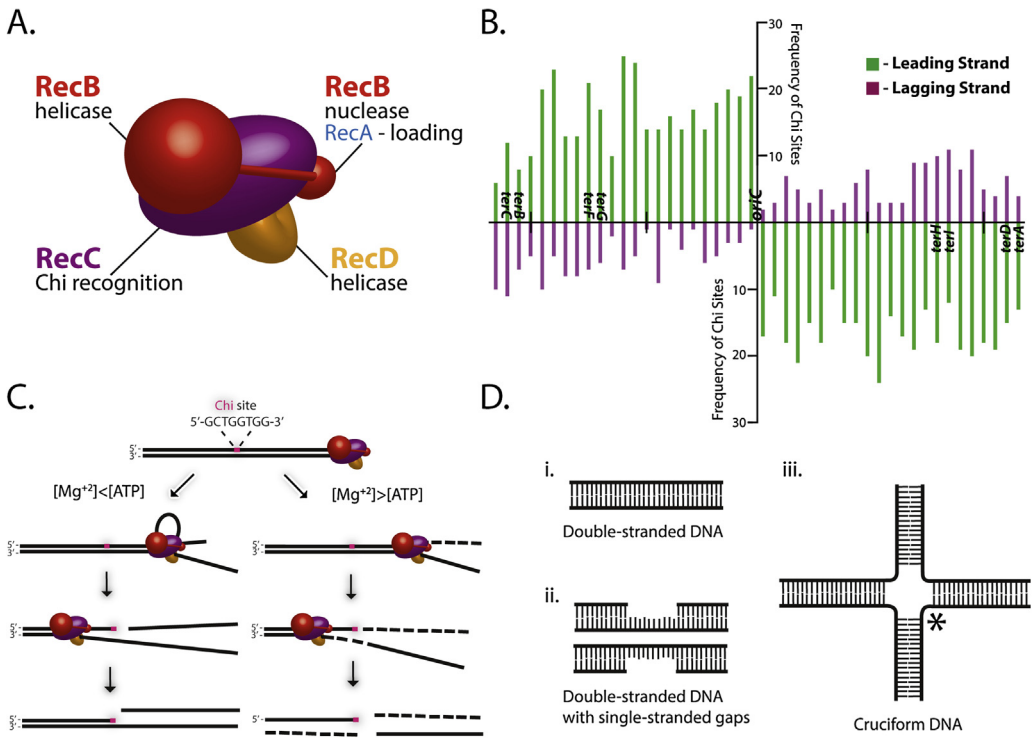

Fig. 5. RecBCD is a complex enzyme with multiple properties.
(A) RecBCD is a heterotrimer with separate helicases located within RecB and RecD. RecC functions as a molecular switch that upon encountering a Chi sequence (5′-GCTGGTGG-3′), inactivates the RecD helicase, alters the nucleolytic activity of 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].

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

Initial studies characterizing the timing of the recombination reaction led several researchers to propose that RecBC operates 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 acts 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 decreases over time [56,58]. The authors inferred that the recombination reaction proceeds beyond a point at which the incoming DNA is joined to the chromosome in *recB* and *recC* mutants, but that *recA* mutants are 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 produce levels of β-galactosidase within two-fold of those seen in wild-type cells, indicating that recombination is able to efficiently proceed up to the point where a transcribable, mutation-free copy of LacZ+ is produced [57]. However, although these recombinant intermediates arise normally, the recovery of viable LacZ+ recombinant progeny is 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 RecBCD's apparent role in completing replication on the chromosome as shown in Figs. 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 beex-aggerated 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 illigitimate 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] (Fig. 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] (Fig. 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] (Fig. 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] (Fig. 5D). There is also evidence to suggest that the complex may also have other important protein partners, including DNA 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 and 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 collapse 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], 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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