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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.

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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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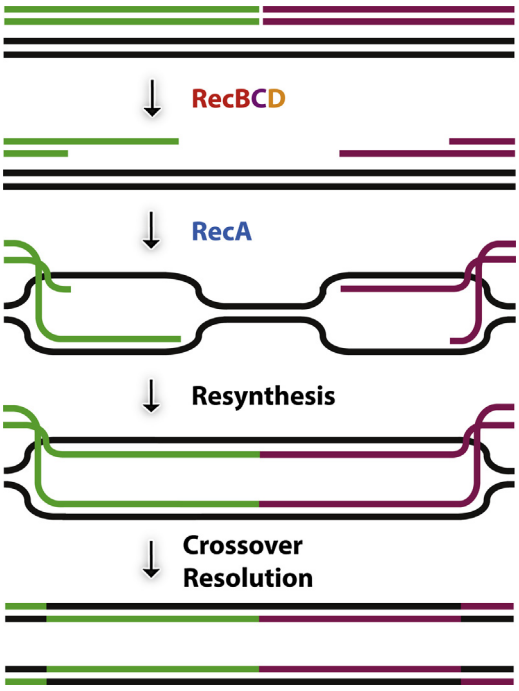

Fig. 1. Current model for the role of RecBCD in double-strand break repair. RecBCD is proposed to initiate recombination by processing the broken DNA ends before recruiting RecA to these sites. RecA then promotes strand invasion with a homologous duplex molecule. The missing sequence between the breaks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

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