Completion of DNA replication in *Escherichia coli*

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The mechanism by which cells recognize and complete replicated regions at their precise doubling point must be remarkably efficient, occurring thousands of times per cell division along the chromosomes of humans. However, this process remains poorly understood. Here we show that, in *Escherichia coli*, the completion of replication involves an enzymatic system that effectively counts pairs and limits cellular replication to its doubling point by allowing converging replication forks to transiently continue through the doubling point before the excess, over-replicated regions are incised, resected, and joined. Completion requires RecBCD and involves several proteins associated with repairing double-strand breaks including, ExoI, SbcDC, and RecG. However, unlike double-strand break repair, completion occurs independently of homologous recombination and RecA. In some bacterial viruses, the completion mechanism is specifically targeted for inactivation to allow over-replication to occur during lytic replication. The results suggest that a primary cause of genomic instabilities in many double-strand-break-repair mutants arises from an impaired ability to complete replication, independent from DNA damage.

replication completion | double-strand break repair | RecBCD | homologous recombination | SbcDC

During chromosomal replication, cells tightly regulate the processes of initiation, elongation, and completion to ensure that each daughter cell inherits an identical copy of the genetic information. Although the mechanisms regulating initiation and elongation have been well characterized (reviewed in refs. 1, 2), the process of how cells recognize replicated regions and complete replication at the precise doubling point remains a fundamental question yet to be addressed. Whether this event occurs once per generation as in *Escherichia coli* or thousands of times per generation as in human cells, the failure to efficiently carry out this function would be expected to result in a loss of genomic stability. Considering the large number of proteins that cells devote to ensuring the fidelity of replication initiation and elongation, it seems highly probable that the final critical step in this process will be also be tightly regulated and controlled enzymatically.

In some aspects, one could argue that the efficiency of completion is likely to be more critical to the faithful duplication of the genome than that of initiation. When replication origins fail to initiate efficiently, elongation of replication forks from neighboring origins is often able to compensate (3, 4), and both prokaryotic and eukaryotic cells are able to tolerate variations in their origin number without severe phenotypic consequences (5–7). However, a failure to accurately limit or join any event where forks converge would be expected to result in duplications, deletions, rearrangements, or a loss of viability depending upon how the DNA ends are resolved at segregation.

A number of studies suggest that an ability to sense when all sequences in the genome have doubled is critical to genomic replication. In vitro, converging replisomes 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 (8). Complicating the process of genomic doubling even further, several studies have suggested that illegitimate initiations of replication frequently occur at single-strand nicks, gaps, D-loops, and R-loops throughout the genomes of both prokaryotes and eukaryotes (9–14). Similar to when replication forks continue through a previously replicated template, each of these events would generate a third copy of the chromosomal region where the event occurs. Thus, over-replication may be inherent and promiscuous during the duplication of genomes. If true, then to ensure that each sequence of the genome replicates once, and only once, per generation, cells must encode an enzymatic system that is essentially able to count in pairs and efficiently degrade odd or over-replicated regions until the two nascent end pairs of replication events can be joined.

The model organism *E. coli* is particularly well-suited to dissect how this fundamental process occurs. In *E. coli*, the completion of replication occurs at a defined region on the genome, opposite to the bidirectional origin of replication (15). 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 Tus, which binds to *ter* and inhibits replication fork progression in an orientation-dependent manner, in effect stalling the replication fork at this site until the second arrives (16, 17). Although Tus confines converging replication forks to a specific region, it does not appear to be directly involved in the completion reaction because *tus* mutants have no phenotype and complete replication normally (18). Furthermore, plasmids and bacteriophage lacking *ter* sequences are maintained stably (19).

Many mutants impaired for either replication initiation or elongation were initially isolated based on their growth defects or an impaired ability to maintain plasmids (20–22). We reasoned that mutants impaired for the ability to complete replication might be expected to exhibit similar phenotypes and initially focused our attention on the properties of *recBC* and *recD* mutants. RecB-C-D forms a helicase–nuclease complex that is required for homologous repair of double-strand breaks in *E. coli* (23, 24). The enzyme uses specific DNA sequences, termed "Chi sites," to initiate recombination between pairs of molecules. Loss of RecB or C inactivates the enzyme complex, whereas loss of RecD inactivates the nuclease and Chi recognition, but retains helicase activity (23, 24). Here, we show that inactivation of RecBCD leads to a failure

Significance

All phases of DNA replication are tightly regulated to ensure that daughter cells inherit a precise copy of the genomic DNA. Although the mechanisms regulating initiation and elongation have been well characterized, the process of how cells recognize replicated regions and complete replication at the precise doubling point remains a fundamental question yet to be addressed. Here we show that the completion of replication involves a transient over-replication of the region where forks converge before the excess regions are incised, resected, and joined. Completion requires several proteins associated with repairing double-strand breaks, but unlike break repair, it occurs independently of homologous recombination and is targeted for inactivation by some bacterial viruses during the transition to lytic replication.

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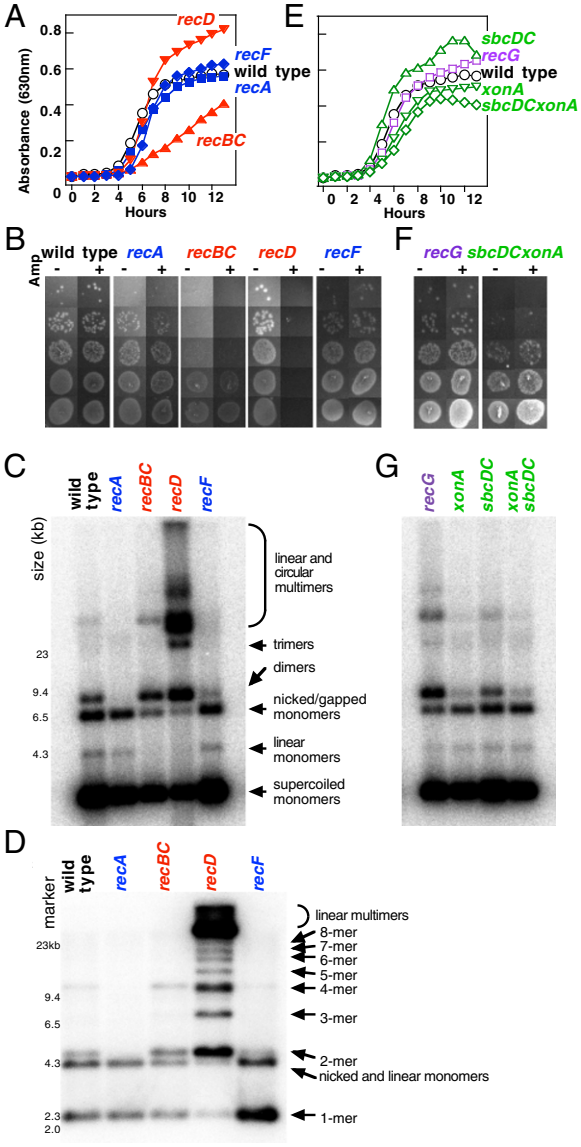

Fig. 1. *recBC* and *recD* mutants exhibit growth abnormalities and an impaired ability to maintain monomeric plasmids. (*A*) The growth of *recBC* mutants is impaired, whereas *recD* mutants grow for a longer period and reach a higher density relative to cultures of wild-type, *recA*, or *recF* mutants. The absorbance at 630 nm of cultures grown at 37 °C is plotted over time. (*B*) *recBC* mutants and *recD* mutants exhibit plasmid instability. Cultures containing the plasmid pBR322 were grown for 30 generations before 10-μL drops of 10-fold serial dilutions were plated with and without ampicillin to determine the fraction of cells that retained the plasmid in each strain. (*C*) Plasmids replicating in *recBC* cultures accumulate dimer plasmids, whereas *recD* cultures accumulate circular and linear multimers. Linear monomers, indicative of double-strand breaks, are reduced in both *recBC* and *recD* mutants relative to wild-type cultures. Total genomic and plasmid DNA was prepared from replicating cultures containing pBR322 and examined by Southern analysis using ^{32}P-labeled pBR322 as a probe. DNA was electrophoresed through a 1.0% agarose gel in Tris base, acetic acid, and EDTA (TAE) at 4 V/cm. (*D*) Unlike other mutants, replication of plasmids in *recD* mutants leads to multimeric circles that contain both odd and even numbers of plasmid copies. Samples were analyzed as in *C* except the DNA was electrophoresed through a 0.5% agarose gel in TAE at 1 V/cm. Resolution under these conditions resolves molecules primarily based on the molecule's size and reduces the impact that shape has on the migration rate of the molecule. (*E*) Growth, (*F*) plasmid stability, and (*G*) plasmid intermediates for *recG*, *xonA*, *sbcDC*, and *xonAsbcDC* mutants were analyzed as in *A*–*C*.

to recognize and join replicating molecules at their doubling point. Although the completion process requires RecBCD, it is distinct from double-strand break repair and does not involve a double-strand break intermediate, homologous recombination, or RecA.

Results

Similar to other mutants that are involved in replication initiation or elongation, *recBC* and *recD* mutants each exhibit growth abnormalities and plasmid instabilities. These phenotypes are unique compared to those of other recombination mutants, and suggest that these mutants have a broader, more fundamental function in replicating cells. Relative to wild-type cultures, *recBC* cultures grow poorly and produce large numbers of small, nonviable cells, whereas *recD* cultures grow for a longer time period and reach a higher cell density (Fig. 1*A*) (25–28). By comparison, cultures lacking either RecF or RecA, which is essential for all homologous recombination and RecBCD-mediated double-strand break repair, grow comparatively well, arguing that some function of RecBCD is unique from homologous repair and DNA damage.

Mutations inactivating RecBC or RecD also affect the stability of plasmid minichromosomes, a feature that is again distinct from other recombination mutants (Fig. 1*B*) (28, 29). Plasmids

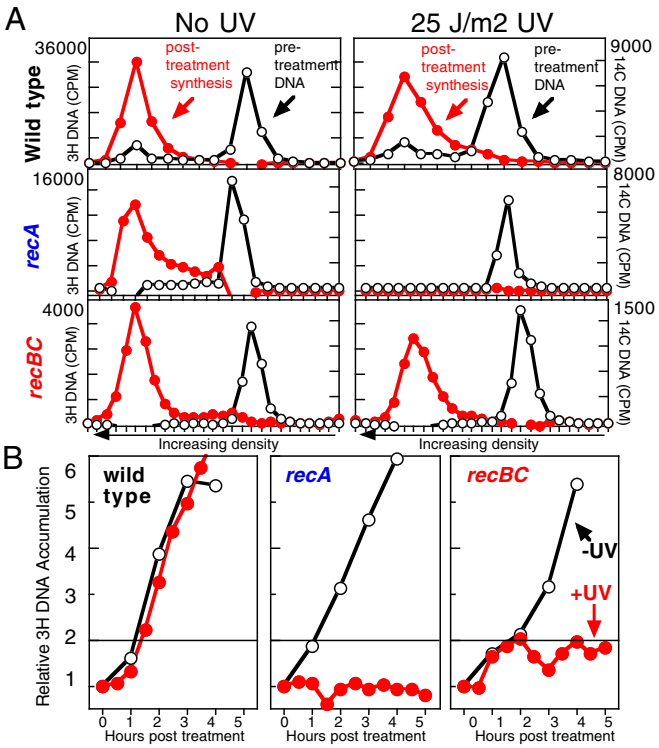

Fig. 2. Following UV irradiation, *recBC* mutants initially recover replication, but then replication arrests after an approximate doubling of their genomic material. (*A*) *recBC* mutants initially recover replication. [^{14}C]Thymine-prelabeled cultures were UV-irradiated or mock-treated and resuspended in media containing [^{3}H]5-bromodeoxyuridine for 1 h to density-label the replication occurring during this period. The denser-replicated DNA was then separated in alkaline CsCl density gradients and quantified. Both wild-type and *recBC* mutants restore replication equally well during the first hour after UV treatment. *recA* mutants do not recover. (*B*) Replication arrests in *recBC* mutants after an approximate doubling of the DNA. Cultures growing in [^{3}H]thymine were UV-irradiated or mock-treated and sampled at various times to determine the total amount of [^{3}H]DNA accumulated. Wild-type cells recover replication and continue to grow following irradiation. *recA* mutants do not recover replication. *recBC* initially recover replication, but then arrest replication once the DNA has approximately doubled. Initial [^{3}H] DNA counts were between 1,057 and 2,610 cpm for all experiments. Plots represent the average of duplicate samples.

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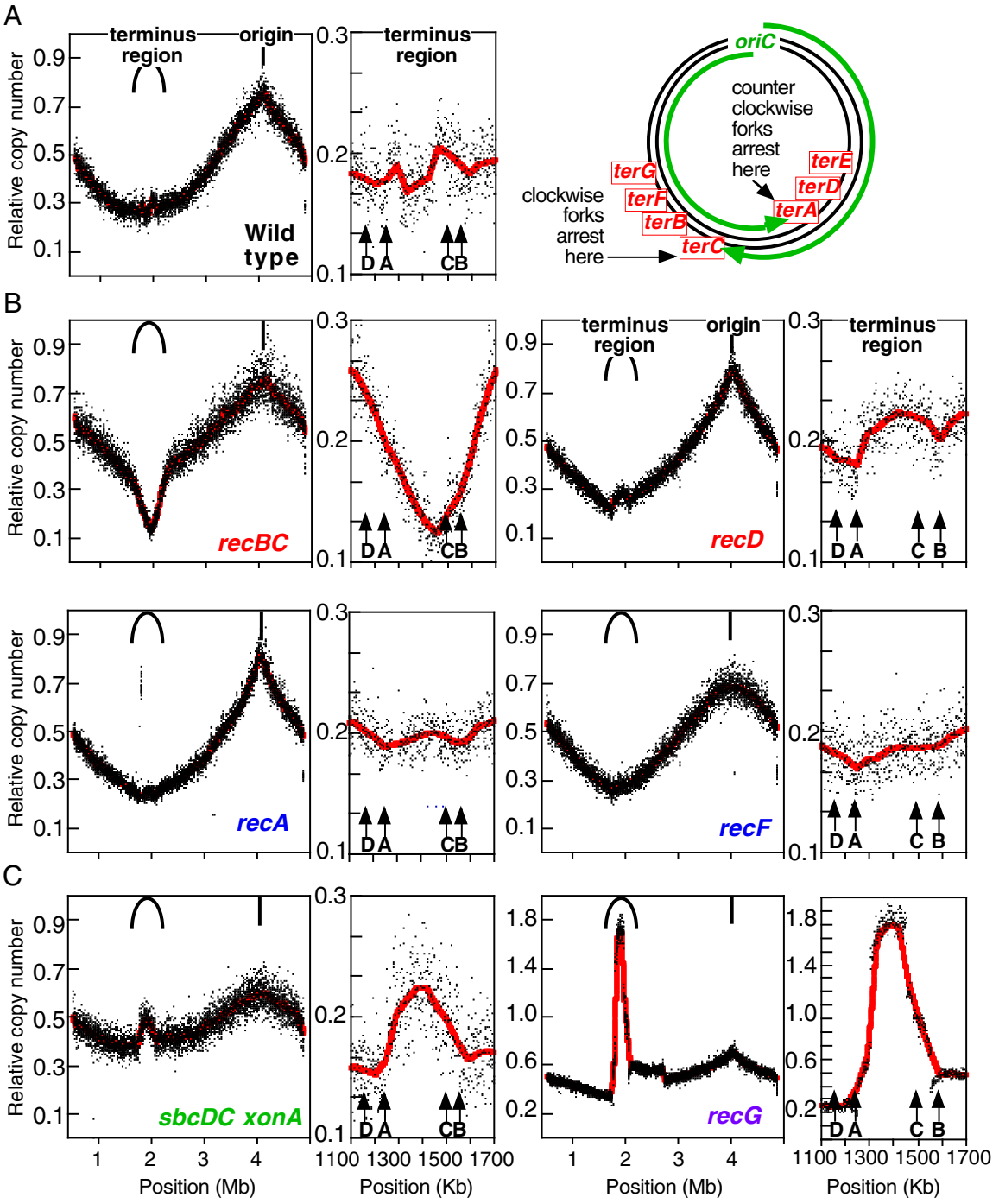

Fig. 3. RecBCD resolves and completes replication at the doubling point on the chromosome, independently of homologous recombination. (*A*) In wild-type cultures, replication proceeds bidirectionally from the origin and completes in the terminus region. Genomic DNA from replicating cultures was purified, 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 terminus region of the chromosome, containing *terD*, *-A*, *-C*, and *-B*, is shown next to each plot. An 8-kb floating average of the sequence frequency is plotted in red. (*B*) *recBC* mutants fail to complete replication, leading to degradation of the terminus region. *recD* mutants fail to resect and limit replication to the doubling point, leading to over-replicated regions in the terminus. Completion occurs normally in *recF* and *recA* mutants. (*C*) Over-replicated regions persist in *sbcDC xonA* mutants. Illegitimate reinitiations of replication occur in *recG*. Note the different scale for *recG*.

grown in *recD* mutants continue to replicate past the doubling point, producing large quantities of multimeric circles as well as long linear multimers (Fig. 1*C*). The over-replicated products observed in *recD* mutants are distinct in that they contain both odd- and even-numbered multimeric products (Fig. 1*D*). By contrast, in other recombination mutants or in wild-type cultures, the few multimeric products that are detected occur as paired or even-numbered multimers. *recBC* mutants are also less able to retain plasmids relative to wild-type cultures, although overall cell viability is similarly reduced (Fig. 1*B*). The unstable phenotype in *recBC* is distinct from *recD* mutants and involves an elevated level of gapped molecules and dimer plasmids, rather than extensive over-replication (Fig. 1*C*). We interpret these observations to suggest that during plasmid replication, the RecD and

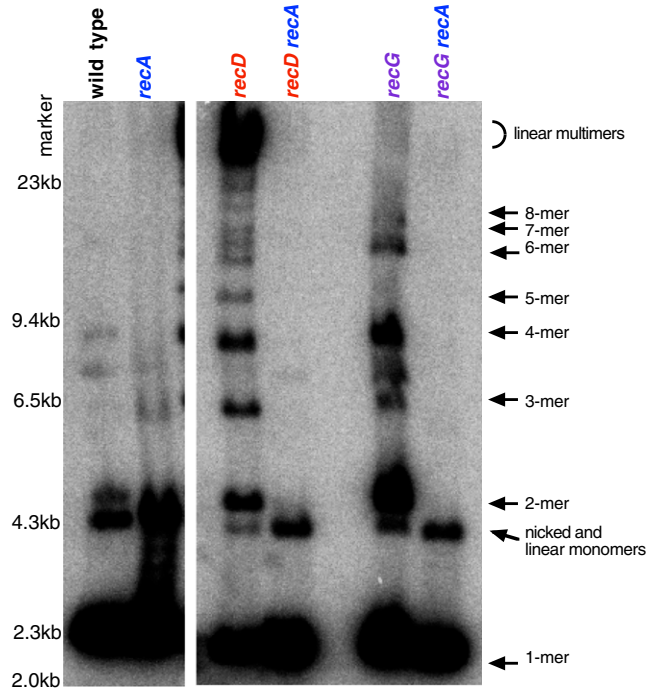

Fig. 4. DNA ends from unresolved completion events lead to distinct recombination-dependent, over-replication intermediates on plasmids in *recD* and *recG* mutants. *recD* mutants accumulate long linear multimers, as well as both odd- and even-numbered multimeric circles. In *recG* mutants, the over-replicated products consist of predominantly even-numbered, multimeric circles. In both *recD* and *recG* mutants, the illegitimate reinitiations of replication depend on RecA. DNA was analyzed as in Fig. 1*D*.

RecBC subunits of the enzyme are required for cells to recognize and resolve those ends at the doubling point, respectively.

If the plasmid instability in *recBC* and *recD* mutants arose from an inability to process double-strand breaks, these mutants would be expected to accumulate broken intermediates. However, as Fig. 1 *C* and *F* demonstrate, the proportion of broken, linear plasmids is actually lower in *recBC* or *recD* cultures relative to wild-type or other recombination mutants. Additionally, double-strand breaks are estimated to arise in vivo at frequencies ranging from 0.01 to 1 break per 4.5 Mb of replicated genome (30), making it unlikely that these account for the instability of a 4.5-kb plasmid. Finally, plasmids remain stable and replicate normally in *recA* mutants, which are defective in all homologous recombination and RecBCD-mediated double-strand break repair (Fig. 1 *B* and *C*). Taken together, these observations argue strongly against the idea that the growth and minichromosome abnormalities in *recBC* and *recD* mutants arise from defective processing of double-strand breaks. However, these phenotypes are all consistent with those expected of mutants that have an impaired ability to recognize and complete replication.

Other phenotypes associated with *recBC* mutants also suggest that the gene products play a role at the end of the cell cycle. Following UV irradiation, many hypersensitive recombination mutants, including *recA* and *recF*, cease DNA synthesis immediately after replication encounters the DNA damage (31, 32). However, *recBC* mutants are unusual in that they initially recover and continue to replicate similar to wild-type cells. The replication continues normally for a short period before DNA synthesis ceases (31) (Fig. 2), indicating that the defect in *recBC* mutants is distinct from RecA and arises at the final stages of replication. Consistent with this interpretation, *ter* sequences are hot spots for RecBCD-mediated recombination (33, 34), implying that the region where replication completes contains substrates frequently recognized by RecBCD in vivo.

To directly examine whether RecBCD functions in completing replication on the chromosome, we profiled the genomes of replicating wild-type and mutant cultures using high-throughput sequencing. In replicating wild-type cultures, the copy number of sequences is highest surrounding the bidirectional origin and then gradually decreases until it reaches the terminus where replication completes (Fig. 3*A*). In mutants lacking RecBC, there is a marked decrease in the copy number of sequences specifically in the terminus region. The terminus sequences in *recBC* mutants are underrepresented by up to twofold, relative to wild-type cultures. Assuming that more than half of the sequence reads correspond to parental DNA, one can infer that the majority of cells in the population have difficulty replicating or maintaining sequences in this region. Conversely, an increase in the copy number of sequences within the terminus region is observed in *recD* mutants, which inactivates the exonuclease activity of the enzyme complex (Fig. 3*B*). Consistent with the observations on plasmids, the results indicate that the RecBCD complex is required to allow the efficient and accurate completion of replication on the chromosome. The presence of the over-replicated intermediate inside the boundary of the *ter* sites in *recD* mutants implies that converging forks transiently pass each other before the nuclease activity of RecBCD resects these over-replicated intermediates back to the doubling point. The lack of sequences at the termination region in *recBC* mutants reveals that the enzyme complex is required to resolve and join the convergent forks at the doubling point. In its absence, the DNA ends of the converging forks remain subject to nucleolytic attack and are degraded.

Importantly, the completion of replication on the chromosome occurs normally in *recF* and *recA* mutants, indicating that the completion reaction catalyzed by RecBCD does not require homologous recombination or involve the repair of double-strand breaks (Fig. 3*B*). We are not aware of any 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 the terminus region DNA in *recBC* mutants is inconsistent with the idea that the intermediates are associated with a recombination defect or collapsed replication forks occurring in this region. We infer that the impaired ability to complete replication in *recBC* mutants is independent from RecBC's role in double-strand break repair and likely accounts for the poor growth of these cells relative to *recA* or other recombination mutants.

Additional genes associated with double-strand break repair are also involved in completion. SbcDC, a structure-specific helicase–nuclease, and ExoI, a prominent 3′–5′ exonuclease, suppress the growth defects of *recBC* mutants when mutated and lead to

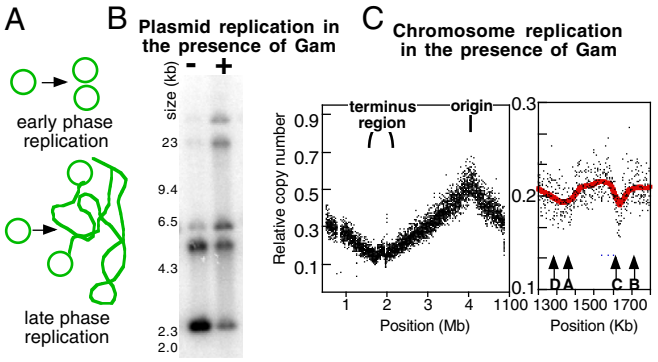

Fig. 5. Induction of the bacteriophage *gam* gene inactivates the cellular mechanism that limits replication to the doubling point. (*A*) Lambda late-phase replication requires *gam* induction. (*B*) *gam* induction leads to over-replication on plasmids and (*C*) on the chromosome. Cultures containing a plasmid with an arabinose-inducible *gam* gene were grown with 0.4% glucose (–) or 0.4% arabinose (+) and prepared as in Fig. 1 *B* and *C*.

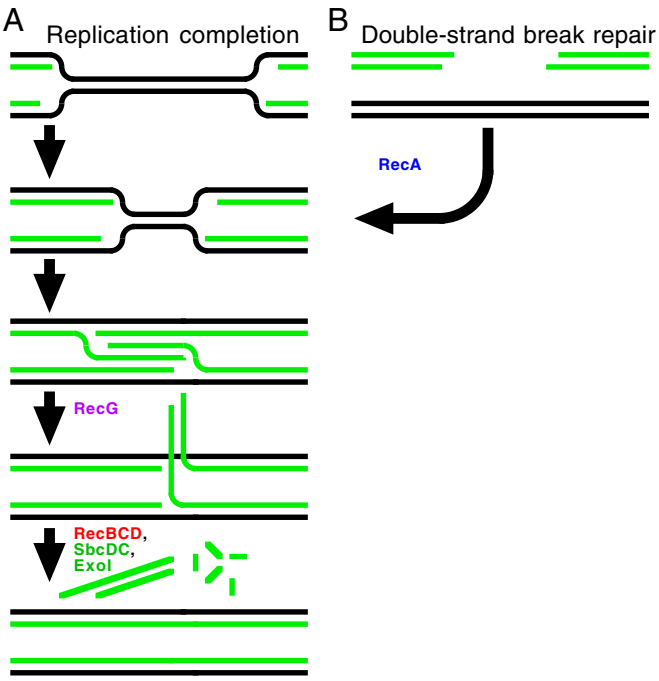

Fig. 6. Model for the completion of replication and its relationship to homologous double-strand break repair. (*A*) Converging replication forks pass each other, leading to a transient over-replicated intermediate. RecG facilitates unwinding of the over-replicated intermediates to reduce reinitiation events and illegitimate replication. RecBCD promotes the degradation and resolution of the over-replicated regions at the doubling point. SbcDC and ExoI also participate in the degradation of the over-replicated regions to limit replication. (*B*) RecA initiates homologous double-strand break repair by pairing DNA ends with a homologous double-stranded template, generating an intermediate that can be repaired by completing the replication of the intervening sequences.

plasmid instability similar to *recD* (25, 35, 36). Mutations in human homologs of these proteins are associated with genetic instabilities and impaired double-strand break repair (37). In replicating *sbcDC xonA* mutants, a similar over-replication of the terminus region is observed (Fig. 3*C*), indicating that these genes play a role in processing or resolving the transient over-replicated regions. Over-replication was less pronounced in the single mutants (Fig. S1), suggesting either functional redundancy or cooperativity between these gene products.

A recent study has shown that mutants lacking RecG, a translocase important for dissolving mis-primed events after DNA damage, also over-replicates its terminus region (Fig. 3*C*) (38). In both *recD* and *recG* mutants, the DNA ends from unresolved completion events lead to over-replication that can also be observed on plasmids. However, as shown in Fig. 4, the over-replication that occurs in these mutants is distinct in several aspects. The aberrant long linear multimeric intermediates that accumulate in *recD* mutants do not appear in *recG* mutants. In addition, *recD* mutants are unique in that they contain prominent odd-numbered circular plasmid multimers, suggesting that RecD contributes to efficient pair recognition before resolution. In contrast, the over-replicated species in *recG* mutants predominately consist of even-numbered circular multimers (Fig. 4), suggesting that these mutants retain the ability to recognize and resolve molecules as pairs. We interpret these results to suggest that although RecG plays a role in preventing illegitimate reinitiations from occurring, it is not directly involved in recognition or joining of the linear DNA ends at the doubling point. Consistent with this interpretation, *recG* mutants grow normally and plasmids are stably maintained (Fig. 1 *D* and *E*). *recG* mutants are also constitutively induced for SOS expression (39), which may contribute to the over-replication that occurs on plasmids and the chromosome in these strains (10, 11). Interestingly, the over-replication that occurs in both *recD* and *recG* mutants depends on RecA (Fig. 4), demonstrating that recombination can lead to aberrant reinitiation events when the efficiency of the completion reaction is compromised.

Many lytic viruses, including bacteriophage lambda, have two modes of replication: an early phase in which its genome doubles similarly to the bacterial chromosome and a late phase in which the viral genome is amplified before packaging and release from the cell (40). Late-phase replication in phage lambda requires expression of the phage Gam protein, which targets and inactivates RecD and SbcDC in the host (41, 42). Similar to the amplification of phage and plasmid DNA (36), we observed that *gam* expression results in an over-replication of the terminus region (Fig. 5). Thus, to initiate genomic amplification during lytic infection, the phage targets and inactivates the cellular mechanism that limits replication to the doubling point, allowing over-replication to occur.

Discussion

Taken together, the plasmid and chromosomal data presented here indicate that RecBCD is directly involved in limiting replication events and resolving them at points where sequences have doubled. This process is distinct from double-strand break repair and occurs efficiently in the absence of RecA or homologous recombination on both plasmids and the chromosome. However, when one considers the mechanism by which double-strand breaks are repaired, it becomes clear how these two processes may be related (Fig. 6). Double-strand break repair in *E. coli* requires both RecA and RecBCD function. RecA is believed to pair the severed strands with intact homologous duplex DNA (23, 24). Once this occurs, the sequences between the opposing strands are replicated and joined using the second molecule as a template. A structurally similar process must also occur whenever two replication forks converge. However, in the case of completion, the opposing nascent strands have been brought together by replication forks and should be independent of RecA.

During double-strand break repair, RecBCD is proposed to process the DNA ends before strand invasion. In most models, this processing is restricted to the early stages of the reaction (23, 24). However, in vivo experiments have suggested that strand invasion can occur in the absence of RecBCD, but that its function is still required if viable recombinants are to be recovered (43, 44), arguing that RecBCD enzyme function acts late in the recombination process, perhaps by actively resolving the rereplicated regions at the doubling point.

Considering the chromosomal phenotypes of *recBC* and *recD* mutants, it is tempting to speculate that monomeric linear plasmid species, which are diminished or absent in these strains, represent an incised intermediate of over-replicated products (Fig. 1*D*). However, the precise substrates RecBCD, SbcDC, ExoI, and RecG act upon in the completion process remains to be determined, as does the presumed role that a polymerase and ligase must play in joining the DNA ends.

Several observations favor a mechanism involving a transient over-replication when forks converge. The location of *ter* sequences on the chromosome is positioned to allow overreplication of the terminus region to occur before replication is blocked by the action of the Tus protein (15). A number of early studies found that, under various stress conditions, the copy number of sequences surrounding the *ter* regions increased and speculated that these represented cryptic origins of replication, termed *oriX*, *oriK*, or *oriM* (9, 11, 45). However, it is also reasonable to consider that these alternative "origins" actually represent replication continuing through the terminus because both events would result in elevated copy numbers in this region. Consistent with this, chromosomal over-replication is generally observed to occur under the same conditions as when it is seen on plasmids (Figs. 1 and 3), arguing against the idea of cryptic origins in the terminus. In vitro, converging replisomes continue through their meeting point as one replisome displaces the other, resulting in over-replication at the region where the forks meet (8).

Finally, transient over-replication has the intuitive advantage of buffering against any potential loss of genetic information and may prevent the loss of genetic information should cell division occur prematurely.

Mutations in several human double-strand break repair genes also exhibit growth defects and genetic instabilities in the absence of exogenous DNA damage, suggesting that some of these mutants may have an impaired ability to complete replication.

Materials and Methods

Bacteria. All strains are derived from SR108 (Table S1) (46).

Plasmid stability, genomic and plasmid DNA extractions, Southern analysis, density-labeled CsCl analysis, and DNA accumulation assays have been described previously (29, 31, 46). Detailed descriptions can be found in *SI Materials and Methods*.

Copy-Number Analysis. Overnight cultures were diluted 1:250 in LB supplemented with thymine (LBthy) media and grown at 37 °C to an OD_{600} of 0.4. Following DNA purification, library preparation and sequencing were performed using NexteraXT and HiSeq2000 (Illumina). All strains were aligned using SR108 parent as reference. Aligned read numbers were determined using Perl scripts and normalized to values obtained for stationary-phase cultures. Further details are provided in *SI Materials and Methods*.

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Supporting Information

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SI Materials and Methods

Bacteria and Plasmids. Strains and plasmids used in this study are presented in Table S1.

Growth Rates. Fresh overnight cultures were 10-fold serially diluted in LB medium supplemented with 10 μg/mL thymine (LBthy), and 0.2-mL aliquots then were plated in duplicate into the wells of a sterile 96-well microtiter dish. The microtiter cultures were then agitated at 37 °C, and the absorbance at 630 nm for each culture was measured over time using a BIO-Whittaker ELx808 plate reader. The number of viable colonies per milliliter in each overnight culture was determined at the start of every experiment.

Plasmid Stability. Cultures containing the plasmid pBR322 were grown for 30 generations in LBthy medium at 37 °C with aeration. Ten-microliter aliquots of serial 10-fold dilutions were then spotted on LBthy plates in the presence or absence of 100 μg/mL ampicillin. Viable colonies were counted following overnight incubation at 37 °C.

Total Genomic and Plasmid DNA Extraction. Two hundred microliters of a fresh overnight culture grown in LBthy medium supplemented with 100 μg/mL ampicillin was pelleted and used to inoculate 20 mL of LBthy medium. Cultures were grown without ampicillin selection in a shaking incubator at 37 °C to an OD_{600} of 0.5 (~5 × 10^8 cells/mL). Samples (0.75-mL) were then placed into 0.75 mL of cold 2× NET (100 mM NaCl, 10 mM Tris, pH 8.0, 10 mM EDTA). Each sample was pelleted, resuspended in 150 μL of 1 mg/mL lysozyme and 0.2 mg/mL RNaseA in TE (10 mM Tris, pH 8.0, 1 mM EDTA), and lysed at 37 °C for 20 min. At this time, proteinase K (10 μL, 10 mg/mL) and Sarkosyl [10 μL, 20% (wt/wt)] were added and incubated at 37 °C for 1 h. Samples were then extracted with 4 vol of phenol/chloroform (1/1) and dialyzed for 1 h on 47 mm Whatman 0.05-μm pore disks (Whatman #VMWP04700) floating on a 250-mL beaker of TE.

Southern Analysis of Plasmid Replication Intermediates. Total genomic DNA samples were digested with Sac II (New England Biolabs), which is not found in pBR322, and extracted with chloroform, and equal volumes were loaded onto a 1.0% agarose gel containing 1× TAE (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8) and electrophoresed at 4 V/cm. Alternatively, to resolve plasmid sizes, samples were electrophoresed in a 0.5% agarose gel containing 1× TAE at 1 V/cm. Gels were transferred to Hybond N+ nylon membranes and probed with pBR322 that had been labeled with ^{32}P by nick translation according to the protocol supplied by Roche using [α-^{32}P]dCTP (PerkinElmer). Radioactivity was visualized using a Storm 840 and its associated ImageQuant Software (Molecular Dynamics).

Sequencing, Assembly, and Copy-Number Analysis. Fresh overnight cultures were diluted 1:250 in LBthy media and grown at 37 °C with aeration to an OD_{600} of 0.4. Total genomic DNA was extracted as described above. Stationary-phase cultures were grown for 36 h before genomic DNA extraction. Library preparation and sequencing of the genomic DNA samples were performed using NexteraXT and Illumina HiSeq2000 (Illumina). The SR108 parent strain sequence was determined using single-end, 51-bp, bar-coded reads to assemble contigs with the Velvet 1.2.10 de Novo assembler with a k-mer value of 31 and a minimum coverage depth >34 (1). Contigs were then aligned to *E. coli* K12 W3110, as a scaffold, using CONTIGuator 2.7.3 (2). The original Illumina sequence reads for all subsequent strains were then aligned and assembled using Bowtie 1.0.0 (3) with the SR108 parent as reference. The aligned reads were then analyzed for nucleotide frequencies at each position, and the copy number of sequences per kilobase was determined using Perl scripts. Relative copy-number values for each strain were normalized to values obtained for stationary-phase cultures to correct for any differences in read depth and then plotted against their location on the genome (4).

Density Labeling and CsCl Analysis. Fresh overnight cultures were diluted 1:100 in 20 mL of Davis medium (2.0 g KH_2PO_4, 7.0 g K_2HPO_4, 0.5 g $Na_3C_6H_5O_7$, 0.1 g $MgSO_4$, 1.0 g $(NH_4)_2SO_4$ per liter, pH 7.0) supplemented with 0.4% glucose, 0.2% cas-amino acids, and 10 μg/mL thymine (DGCthy) supplemented with 0.1 μCi/mL of [^{14}C]thymine (53 mCi/mmol) and were grown to an OD_{600} of 0.5 at 37 °C with aeration. At this time, half the culture was UV-irradiated with 25 J/m^2 and the other half was mock-irradiated. Cultures were then filtered onto FisherBrand general filtration 0.45-μm membranes, washed with NET buffer, resuspended in 10 mL DGC medium supplemented with 20 μg/mL 5-bromouracil in place of thymine and 0.5 μCi/mL [^3H]thymine (60.5 Ci/ mmol), and allowed to recover for a period of 1 h at 37 °C with aeration. Two volumes of ice-cold NET buffer were added to the 10-mL cultures, and the cells were then pelleted, resuspended in 150 μL TE (10 mm Tris, 1 mm EDTA, pH 8.0), and lysed in 170 μL of 0.5 M H_2KPO_4/KOH, pH 12.5, and 1.25% (wt/wt) Sarkosyl. Isopycnic alkali CsCl gradients composed of 0.3 g of a DNA lysate solution, 2.23 g CsCl, and 3.31 g of a 0.1 M H_2KPO_4/KOH, pH 12.5, solution (refractive index 1.4055) were centrifuged to equilibrium at 80,000 × *g* for 96 h at 20 °C. Gradient fractions were collected onto Whatman no. 17 paper, washed in 5% (wt/vol) TCA, and then washed in 95% (vol/vol) ethanol. The quantity of ^3H and ^{14}C in each fraction was determined by liquid scintillation counting.

Total DNA Accumulation. Fresh overnight cultures were diluted 1:100 in 40 mL DGCthy medium supplemented with 0.1 μCi/mL [^3H]thymine (60.5 Ci/mmol) and grown to an OD_{600} of 0.4 at 37 °C with aeration. At this time, half the culture was UV-irradiated with 25 J/m^2 and the other half was mock-irradiated. At the indicated times, duplicate 200-μL aliquots were precipitated in 5 mL of 5% (wt/vol) TCA and filtered onto Fisherbrand glass fiber filters. The amount of ^3H-labeled DNA on each filter was determined by liquid scintillation counting.

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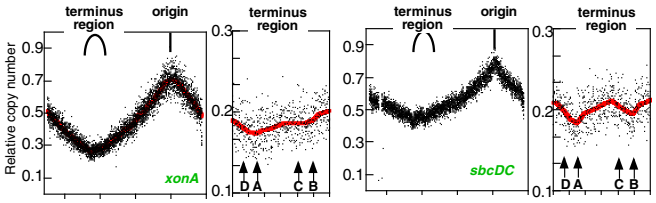

Fig. S1. Over-replication of the terminus region is less pronounced in *xonA* and *sbcDC* mutants relative to the *xonA sbcDC* double mutant. Genomic DNA from replicating cultures was purified, fragmented, and profiled using high-throughput sequencing. Sequence read frequencies, normalized to stationary-phase cells, are plotted relative to their position on the genome. The terminus region of the chromosome, containing *terD*, *-A*, *-C*, and *-B*, is shown next to each plot. An 8-kb floating average of the sequence frequency is plotted in red.

Table S1. *E. coli* strains and plasmids used in this study

Strain	Genotype	Source or construction
SR108	*λ-, thyA, deo, IN(rrnD-rrnE)*	(1)
HL921	SR108 D(*srlR-recA*)306::*Tn*10	(2)
HL922	SR108 *recB21C22 argA81*::*Tn*10	(2)
HL923	SR108 *recD1011 argA81*::*Tn*10	(2)
HL946	SR108 *recF332*::*Tn*3	(3)
CL008	SR108 *recG258*::*Tn*5	(4)
HL1034	SR108 *xonA*::*Cat300*	(5)
CL826	D(*lacU169*) *nadA*::*Tn*10, *gal490*, Lambda *cI857*, D(*cro-bioA*), *sbcCD*::*Cat*	Recombineering strain DY329 (6) was transformed with primers 5′TCCTGCTGAATAGTTATTTCACTGCAAACGTACTTTCCAGCTTTCGAATTTCTGCCATTC 5′AGGGAACCGTTATGCGCATCCTTCACACCTCAGACTGGCAATGAGACGTTGATCGGCAC to replace codon 10 of *sbcD* through codon 1040 of *sbcC* with a chloramphenicol resistance cassette.
CL835	SR108 *sbcCD1040*::*cat*	P1 transduction of *sbcCD1040*::*cat* from CL826 parent into SR108 recipient
CL2357	SR108 *xonA*::*Cat300 sbcCD*::*Gm*	P1 transduction of *sbcCD*::Gm from KM135 (7) into recipient HL1034
pBADGam	Arabinose-inducible expression plasmid, ampicillin resistance, ColE1 origin	Phage Lambda *gam* ORF cloned into the multiple cloning site of pBAD/Myc-HisA (Invitrogen)
pBR322	Ampicillin, tetracycline resistance, ColE1 origin	(8)

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