Supporting Information

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SI Materials and Methods

Bacteria. All strains used in this work are derived from SR108, a *thyA deoC* derivative of W3110, and listed in Table S1.

Growth Rates. Equal numbers of viable cells were grown in 0.1-mL cultures in LB medium supplemented with 10 μg/mL thymine (LBthy) at 37 °C with agitation in a 96-well microtiter dish. Absorbance at 630 nm was measured over time, using a BIO-Whittaker ELx808 plate reader (1).

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 after overnight incubation at 37 °C (1).

Total Genomic DNA Extraction. Then, 0.75-mL cultures grown in LBthy medium supplemented with 100 μg/mL ampicillin were taken and placed into 0.75-mL cold 2× NET (100 mM NaCl, 10 mM Tris at pH 8.0, 10 mM EDTA). Each sample was pelleted, resuspended in 140 μL of 1 mg/mL lysozyme and 0.2 mg/mL RNaseA in TE (10 mM Tris at pH 8.0, 1 mM EDTA), and lysed at 37 °C for 30 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 30 min. Samples were then extracted with 4 vol phenol/chloroform (1/1) and dialyzed for 1 h on 47 mm Whatman 0.05-μm pore disks (#VMWP04700; Whatman) floating on a 250-mL beaker of TE(1 mM Tris at pH 8.0, 1 mM EDTA) (1).

Southern Analysis of Plasmid Replication Intermediates. Total genomic DNA samples were digested with Sac II (New England Biolabs), pBR322 lacks Sac II restriction sites, and extracted with chloroform. Equal volumes were loaded onto a 0.5% and 1.0% agarose gel containing 0.5× TBE (220 mM Tris, 180 mM Borate, 5 mM EDTA at pH 8.3) and electrophoresed at 1 and 2.5 V/cm, respectively. Gels were transferred to Hybond N+ nylon membranes and probed with pBR322 that had been labeled with 32P by nick translation according to the protocol supplied by Roche using [α-32P]dCTP (PerkinElmer). Radioactivity was visualized using a Storm 840 and its associated ImageQuant Software (Molecular Dynamics) (1).

Sequencing, Assembly, and Replication Profiling. Fresh overnight cultures were diluted 1:250 in LBthy media and grown at 37 °C with aeration to an OD_{600} of 0.4. Stationary phase cultures were grown for 36 h. Total genomic DNA was prepared from cultures. Sequencing of the genomic DNA samples was performed using NexteraXT and Illumina HiSeq2000 (Illumina), using single-end, 51-bp, bar-coded reads according to the manufacturer's instructions. SR108 parent sequence was determined using *breseq* to identify structural variations between SR108 and its W3110 parent genome, and differences were hand annotated to generate the SR108 reference genome (2). The original Illumina sequence reads for all subsequent strains were then aligned using Bowtie 1.0.0 (3), using SR108 as reference. Aligned reads were then analyzed for nucleotide frequencies at each position, and the copy number of sequences per kilobase was determined using custom Perl scripts. Copy number values were normalized to those of stationary phase cells to determine replication-specific copy number frequencies and eliminate any sequencing bias. These relative copy number values were then plotted against their location along the genome to generate replication profiles for each strain of interest.

1. Wendel BM, Courcelle CT, Courcelle J (2014) Completion of DNA replication in Escherichia coli. *Proc Natl Acad Sci USA* 111:16454–16459.
2. Barrick JE, et al. (2014) Identifying structural variation in haploid microbial genomes from short-read resequencing data using breseq. *BMC Genomics* 15:1039.
3. Langmead B (2010) Aligning short sequencing reads with Bowtie. Curr Protoc Bioinformatics Chapter 11:Unit 11.7.
4. Mellon I, Hanawalt PC (1989) Induction of the Escherichia coli lactose operon selectively increases repair of its transcribed DNA strand. *Nature* 342:95–98.
5. Courcelle J, Carswell-Crumpton C, Hanawalt PC (1997) recF and recR are required for the resumption of replication at DNA replication forks in Escherichia coli. *Proc Natl Acad Sci USA* 94:3714–3719.
6. Chow KH, Courcelle J (2007) RecBCD and RecJ/RecQ initiate DNA degradation on distinct substrates in UV-irradiated Escherichia coli. *Radiat Res* 168:499–506.
7. Maloy SR, Nunn WD (1981) Selection for loss of tetracycline resistance by Escherichia coli. *J Bacteriol* 145:1110–1111.
8. Courcelle J, Hanawalt PC (1999) RecQ and RecJ process blocked replication forks prior to the resumption of replication in UV-irradiated Escherichia coli. *Mol Gen Genet* 262:543–551.
9. Poteete AR, Rosadini C, St Pierre C (2006) Gentamicin and other cassettes for chromosomal gene replacement in Escherichia coli. *Biotechniques* 41:261–262, 264.

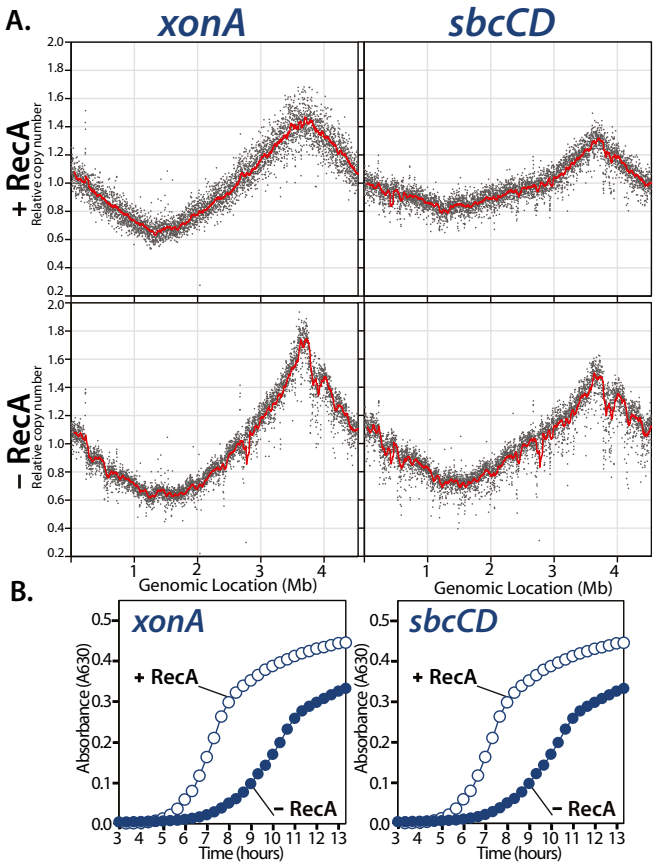

Fig. S1. *xonA* and *sbcCD* mutations are additive in their effect on overreplication in the terminus and RecA-dependent growth. (*A*) Replication profiles for *xonA* and *sbcCD* in the presence of absence of RecA are plotted as in Fig. 1. (*B*) Growth rates for *xonA* and *sbcCD* in the presence or absence of RecA are plotted as in Fig. 1.

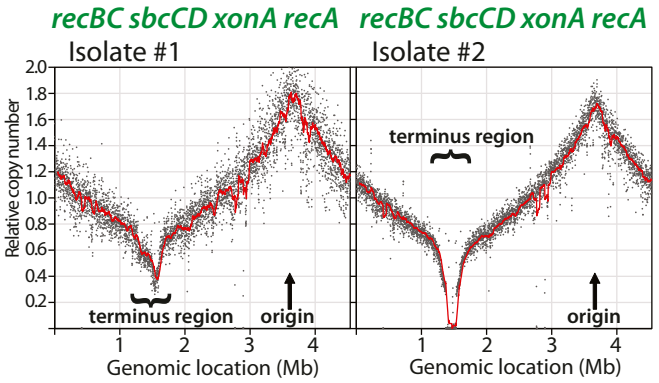

Fig. S2. When the normal mechanism of completing replication is prevented, maintaining the region where forks converge becomes dependent on RecA and recombination. Replication profiles from two separate isolates of *recBC sbcCD xonA* mutants are shown. Both fail to maintain the region where replication forks converge. In the second isolate, the terminus region was deleted entirely. Replication profiles were performed as in Fig. 1. Isolates correspond to strains CL2576 and CL2575, respectively.

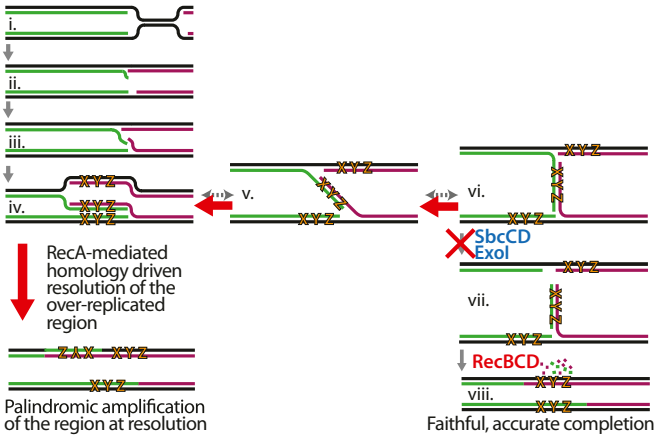

Fig. S3. When the normal mechanism of completing replication is prevented, maintaining the region where forks converge becomes dependent on RecA and recombination. The model is shown as in Fig. 5. When processing of the overreplicated regions where forks converge is impaired, such as occurs in the absence of SbcCD ExoI, resolution of the joint molecules occurs through an aberrant form of RecA-mediated recombination. Resolution in this manner is termed aberrant because it produces chromosome amplifications at loci where forks converge. In addition, when the normal completion reaction cannot occur, cells fail to grow and these loci are degraded in the absence of RecA.

Table S1. Strains used in this study

Strain	Relevant genotype	Source, reference, and/or construction
SR108	λ - *thyA deo IN(rrnD-rrnE)*	(4)
HL922	SR108 *recB21C22 argA81::*Tn*10*	(5)
HL923	SR108 *recD1011 argA81::*Tn*10*	(5)
CL542	SR108 *recA::cam*	(6)
CL1056	SR108 D(*recC ptr recB recD*)::*cam*	P1 transduction of D(*recC ptr recB recD*)::*cam* from KM78 (gift from K. C. Murphy, Department of Molecular Genetics and Microbiology University of Massachusetts Medical School, Worcester, MA)
CL2357	SR108 *xonA::Cat300 sbcCD::Gm*	(1)
CL3539	SR108 *xonA::Cat300 sbcCD::Gm D(srlR-recA)306::Tn10*	P1 transduction of *D(srlR-recA)306::*Tn*10* from HL921 (5) into CL2359
CL851	SR108 *recB21C22 argA81::*Tn*10 recA::cam*	(6)
CL726	SR108 *recD1011 argA81::*Tn*10 recA::cam*	(6)
CL2542	SR108 *xonA::Cat300 sbcCD::Gm recB21C22 argA81::*Tn*10*	P1 transduction of *recB21C22 argA81::*Tn*10* from HL922 (5) into CL2357
CL2539	SR108 *xonA::Cat300 sbcCD::Gm recD1011 argA81::*Tn*10*	P1 transduction of *recD1011 argA81::*Tn*10* from HL923 (5) into CL2357
CL2575	SR108 *xonA::Cat300 sbcCD::Gm recB21C22 argA81 D(srlR-recA)306::*Tn*10*	CL2542 was first cured of Tn*10* (7). This was followed by P1 transduction of *D(srlR-recA)306::*Tn*10* from HL921 (5) into CL2542.
CL2576	SR108 *xonA::Cat300 sbcCD::Gm recB21C22 argA81 D(srlR-recA)306::*Tn*10*	CL2542 was first cured of Tn*10* (7). This was followed by P1 transduction of *D(srlR-recA)306::*Tn*10* from HL921 (5) into CL2542 (separate isolate of 2575).
CL2577	SR108 *xonA::Cat300 sbcCD::Gm recD1011 argA81 D(srlR-recA)306::*Tn*10*	CL2539 was first cured of Tn*10* (7). This was followed by P1 transduction of *D(srlR-recA)306::*Tn*10* from HL921 (5) into CL2539.
HL1034	SR108 *xonA::Cat300*	(8)
CL2344	SR108 *sbcCD::Gm*	P1 transduction of *sbcCD::Gm* from KM137 (9) into SR108
CL718	SR108 *xonA::Cat300 D(srlR-recA)306::*Tn*10*	P1 transduction of *D(srlR-recA)306::*Tn*10* from HL921 (5) into HL1034
CL3535	SR108 *sbcCD::Gm D(srlR-recA)306::*Tn*10*	P1 transduction of *D(srlR-recA)306::*Tn*10* from HL921 (5) into CL2344