SUPPORTING ONLINE MATERIAL

Materials and methods

Strains and plasmids. SR108 is a *thyA36 deoC2* derivative of W3110. HL952 (SR108 *uvrA*::Tn*10*), HL1034 (SR108 *recA*::Tn*10*), HL924 (SR108 *recJ284*::Tn*10*), and HL922 (SR108 *recB21recC22 argA81*::Tn*10*) have been reported previously (*2, 9, 10*). CL579 (SR108 *recF6206*::tet857) was constructed by P1 transduction of the *recF6206*::tet857 allele from TP577 (*S1*) into SR108. CL544 (SR108 *recR6212*::cat883*)* was constructed by P1 transduction of the *recR6212*::cat883 allele from TP647 (*S1*) into SR108. CL581 (SR108 *recQ6215*::cat883) was constructed by P1 transduction of the *recQ6215*::cat883 allele from TP648 (*S1*) into SR108. CL679 (SR108 *recF6206*::tet857, *recQ6215*::cat883) was constructed by P1 transduction of the *recQ6215*::cat883 allele from TP648 (*S1*) into CL579. CL685 (SR108 *recR6212*::cat883, *recJ284*::*Tn*10*)* was constructed by P1 transduction of the *recJ284*::*Tn*10 allele from JC12123 (*S2*) into CL544. pBR322 is a medium copy number, ColE1 based, 4.4kb plasmid (Promega).

Growth, UV Irradiation, and DNA isolation. 200μl of a fresh overnight culture grown in Davis medium supplemented with 0.4% glucose, 0.2% casamino acids, and 10 μg/ml thymine (DGCthy medium) and 50 μg/ml ampicillin was pelleted and used to inoculate 20 ml of DGCthy medium. Cultures were grown without ampicillin selection in a shaking incubator at 37° C to an OD_{600} of 0.5 (~ 5 x 10^8 cells/ml) and UV irradiated with 50 J/m2 (50 sec) using a 15-watt germicidal lamp. At the indicated time points, 0.75 ml

samples were placed into 0.75 ml cold 2X 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), lysed at 37°C for 20 min. At this time, proteinase K (10μl, 10mg/mg) and sarcosyl (10μl, 20%) was added and incubated at 50°C for 1 hr. Samples were then extracted, twice, with 4 volumes of phenol/chloroform/isoamyl alcohol (25/24/1), once with 4 volumes of chloroform/isoamyl alcohol (24/1), and dialysed for 3 hours on 47mm Whatman 0.05 μm pore disks (Whatman #VMWP04700) floating on a 250 ml beaker of TE. Samples were then digested with PvuII (New England Biolabs), extracted with chloroform/isoamyl alcohol (24/1), and equal volumes were loaded onto the gel. Note: to minimize structural artifacts and DNA shearing, the isolation procedure does not include involve DNA precipitation or concentration of the samples following cell lysis.

T4 Endonuclease V repair assay. The frequency of CPDs remaining in the plasmid pBR322 was determined by an established method (*28, S3*). 10μl of each sample (500ng) was treated or mock treated with 0.2μg of T4 endonuclease V in NET buffer containing bovine serum albumin (1mg/ml) for 40 minutes at 37C. Samples were electrophoresed at 1 V/cm in 1% alkaline agarose gels. DNA was transferred to Hybond N+ membranes probed with 32P-labeled pBR322 as before. The frequency of CPDs per 4.3kb plasmid strand was calculated from the percentage of fragments with no CPDs (zero class), using the Poisson expression (-ln of the zero class = average number of dimers per fragment). By this calculation, an average of 0.5 lesions are produced on each strand of the plasmid

at a dose of 50J/m2 under our irradiation conditions. Graphs represent and average of 2 independent experiments. Error bars represent one standard deviation.

2D gel and southern analysis. 2D gel analysis was modified from (*19*). Restricted genomic DNA samples were run in the 1^{ST} dimension in 0.4%agarose, 1X TBE at 1V/cm. Gel lanes were cut out, recast, and run in the 2^{nd} dimension in 1.0% agarose, 1X TBE at 6.5V/cm. 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 Promega using alpha [32-P]dCTP (ICN). Radioactivity was visualized and quantitated using a Storm 820 and its associated ImageQuant Software (Molecular Dynamics). All plots represent an average of at least two independent experiments. Wild type cells represent an average of four independent experiments. Higher order intermediates, such as those that appear in *uvrA* mutants, were not included in the cone region quantitation. All error bars represent one standard deviation.

Time course of replication recovery. Cells were grown in DGCthy medium containing 1.0 μCi of [3H]thymine per ml to an optical density at 600 nm (OD_{600}) of 0.3 (approximately $3 * 10^8$ cells/ml) at which point half of the culture was irradiated while the other half was mock irradiated (time zero). The amount of 3H incorporated into the DNA was measured by averaging results for duplicate, 0.2-ml samples precipitated in 5% cold trichloroacetic acid and then collected on Whatman glass fiber filters. Graphs represent and average of 2 independent experiments. Error bars represent one standard

deviation.

DNA degradation following UV irradiation. A 100 μl aliquot of a fresh overnight culture was used to inoculate 10 ml of DGCthy medium containing 0.2 μCi of [14C]thymine/ml. Cells were grown in a shaking water bath at 37°C to an OD600 of 0.4 (approximately 4 * 10^8 cells/ml). At this time, 1 μCi of [3H]thymidine/ml was added to the culture. After 10 s, the cells were filtered through a Whatman HA filter (4.5 cm diameter, 0.45 μm pore size). The cells were then washed with 5 ml, and then 2 ml of NET buffer. Washing times varied between 10 and 30 s depending on the cell line. The cells were then resuspended in nonradioactive DGCthy medium and irradiated with a dose of 25 J/m2. The elapsed time between transfer of the cells into nonradioactive medium and irradiation was approximately 10 s. The 14C and 3H remaining in the DNA was measured by averaging duplicate, 0.2-ml samples precipitated in 5% cold trichloroacetic acid and filtered onto Whatman glass fiber filters. All zero time points were taken in triplicate rather than duplicate. The increase in 3H-labeled DNA after 40 min in wildtype cells is due to the re-incorporation of the remaining intracellular pools of [3H]thymidine when replication resumes (*2, 10*). Graphs represent and average of 2 independent experiments. Error bars represent one standard deviation.

Figure S1

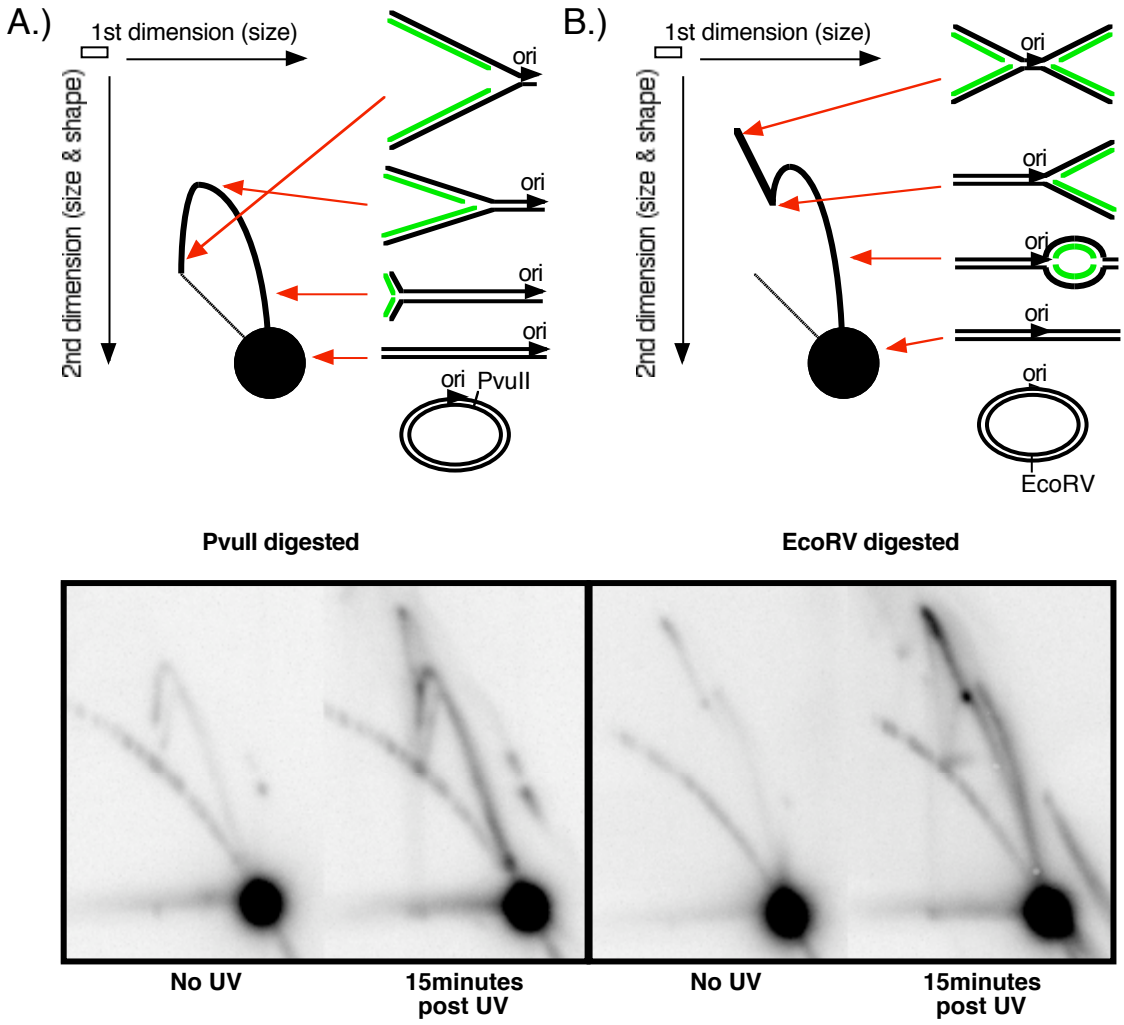

Figure S1. The migration pattern of replicating pBR322 plasmids following restriction with either PvuII or EcoRV. A.) The migration pattern of nonirradiated pBR322 following restriction with PvuII. PvuII linearizes pBR322 slightly downstream from its unidirectional origin of replication. Therefore, replicating fragments approximate a simple Y-shaped arc that extends out from the linear fragment. B.) The migration pattern of nonirradiated pBR322 following restriction with EcoRV. EcoRV linearizes pBR322 at

a point opposite to the unidirectional origin of replication. Therefore, replicating fragments form a bubble-arc that extends out from the linear fragment and runs slightly above a simple Y-shaped arc. Once replication extends past the EcoRV site, the bubble is converted to a double-Y or X-shaped molecule as replication runs to completion. The UV-induced intermediates in the PvuII digest, although absent in preirradiated samples, migrate above the normal Y-shaped replication arc, in region where X-shaped molecules migrate.

Figure S2

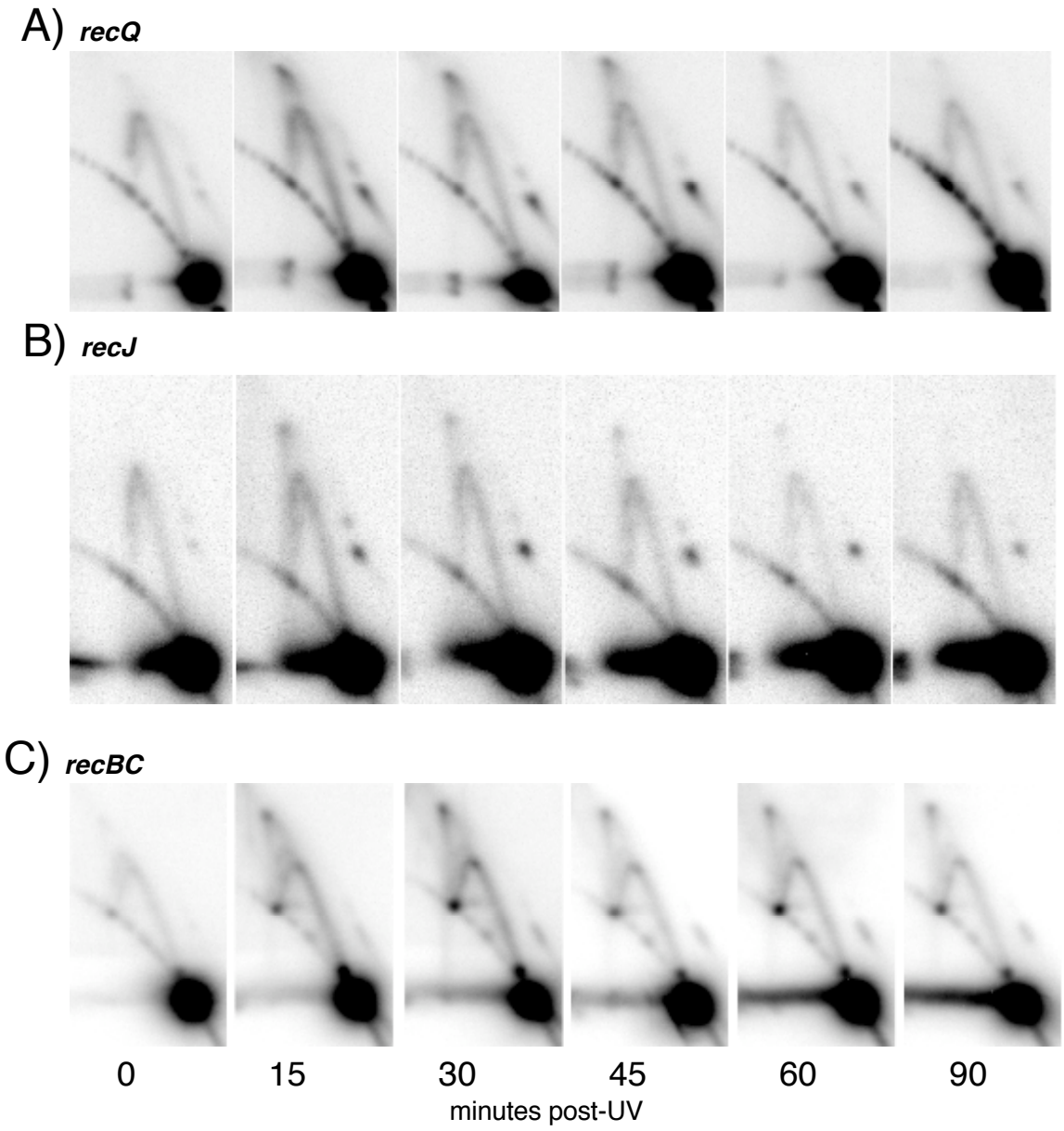

Figure S2. UV-induced DNA replication intermediates observed during the recovery of replication in A) *recQ* mutants, B) *recJ* mutants , and C) *recBC* mutants. In the absence of either RecJ or RecQ, replication recovers normally (not shown) and the migration

pattern of replication intermediates in the 2D gel analysis looks similar to wild type cells. Previous studies have shown that *recBC* and *recD* mutants recover replication normally and, unlike *recJ* or *recQ*, are not involved in the nascent DNA processing that occurs following UV irradiation (*9*, 10, *S4*). However, it is important to note that *recBC* and *recD* mutants fail to stably maintain plasmid molecules and that replication occurs abnormally in these mutants even in the absence of DNA damage, complicating the interpretation of a 2D gel analysis. Nevertheless, the absence of *recBC* clearly does not prevent the X-shaped intermediates from forming, although they do persist slightly longer than is observed in wild type cells and they are detectable even in the absence of UV irradiation.

Supplemental References

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S3. G. Spivak, P. Hanawalt, *METHODS: A Companion to Methods in Enzymology* **7**, 147 (1995).

S4 M.A. Khidir, S. Casaregola, I.B. Holland, *Mol. Gen. Genet.* **199**, 133 (1985).