Supporting Information

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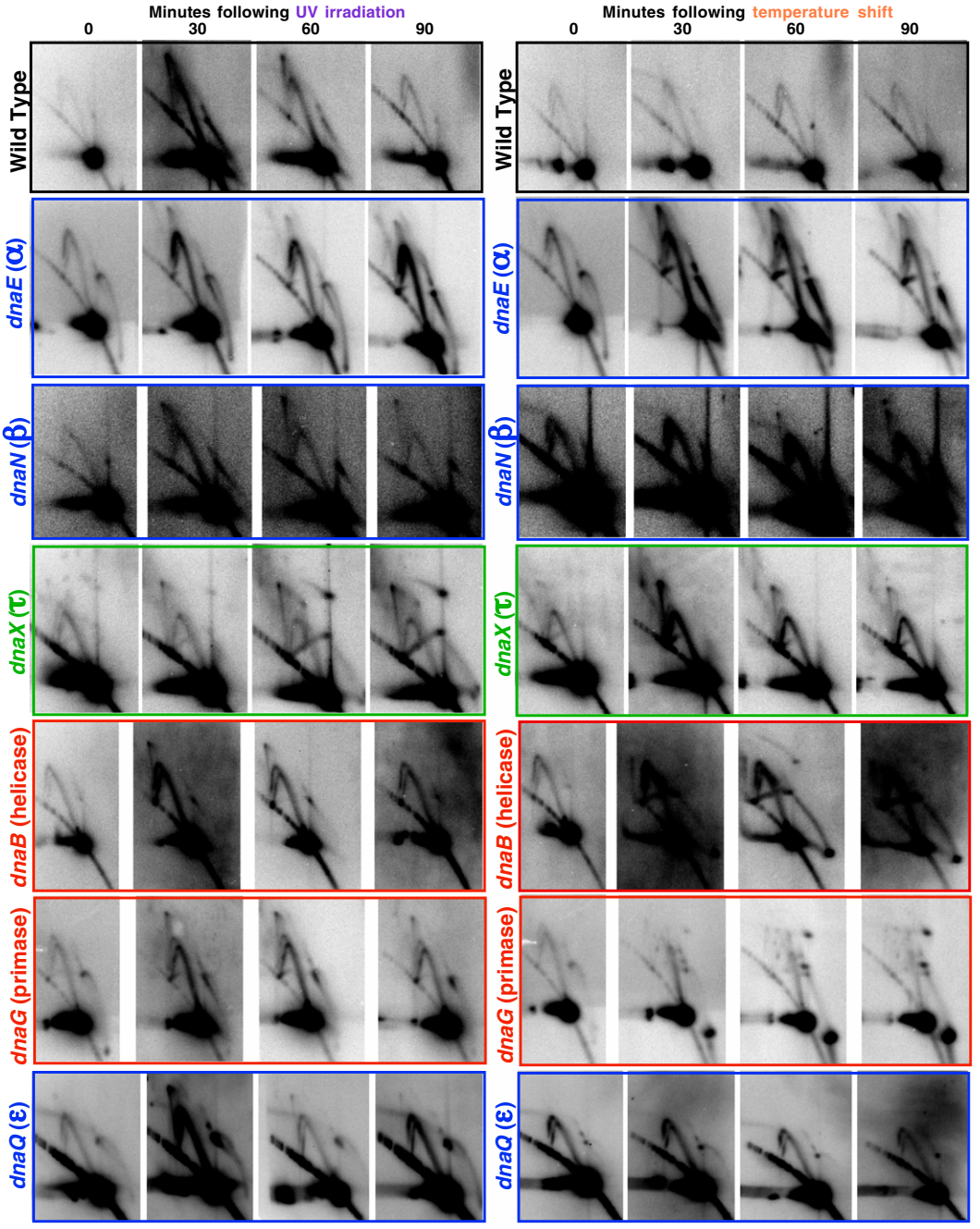


Fig. S1. A comparison of the replication intermediates observed by 2D agarose gel analysis following UV irradiation or temperature shift over time is shown for wild type and each of the temperature-sensitive replication mutants used in this study. Strains containing plasmid pBR322 were UV-irradiated with 50 J/m^2 or filtered and placed in prewarmed media at 42 °C. Genomic and plasmid DNA was then purified, digested with PvuII, and analyzed by 2D agarose gel analysis at 0, 30, 60, and 90 min following UV-irradiation or temperature shift, as indicated.

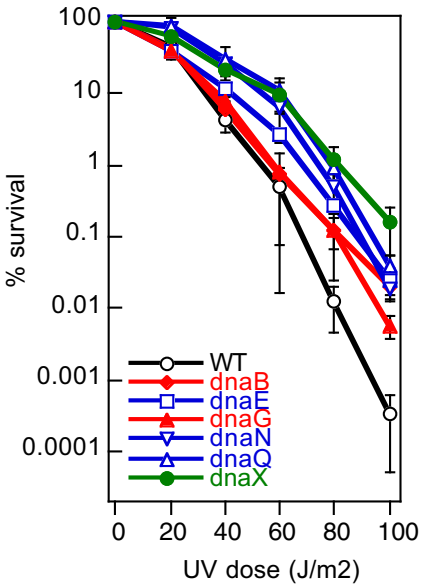


Fig. S2. The survival of each strain when grown at 30 °C is plotted at the indicated dose relative to unirradiated cultures. At the permissive temperature, all of the temperature sensitive mutants used in this study were as resistant as wild-type cells to UV irradiation. The modestly elevated resistance of the temperature-sensitive mutants to UV irradiation is likely to be due to the slower growth rate of these mutants relative to the wild-type strain at the permissive temperature, which allows more time for repair to occur before the potentially lethal act of replication. However, differences between strain backgrounds also cannot be ruled out. Error bars represent 1 SD of three independent experiments.

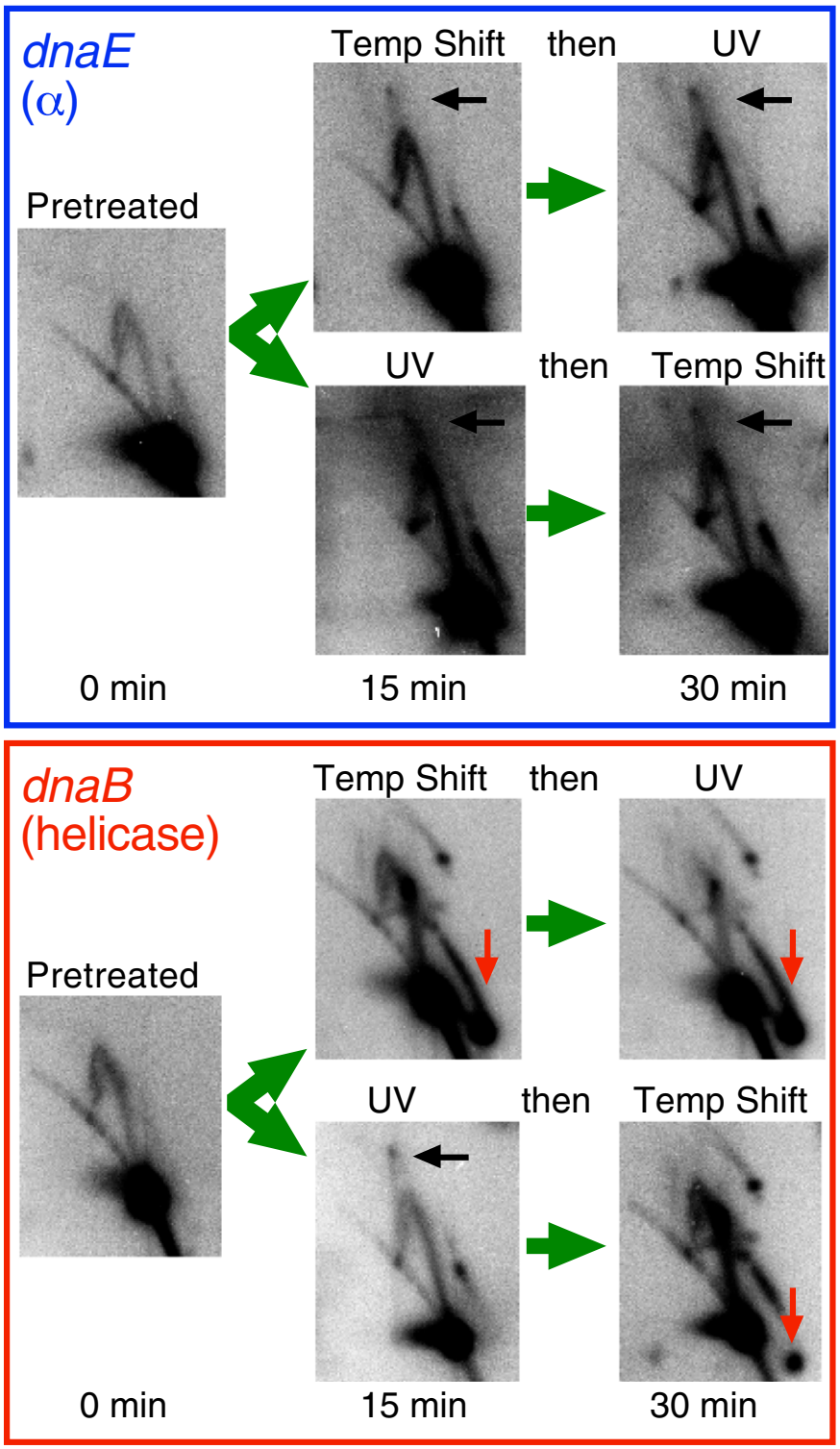
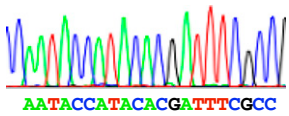


Fig. S3. Replication fork processing induced by polymerase inactivation or UV-induced damage remains unchanged when compounded by the second form of challenge, consistent with the idea that the UV-induced intermediates are similar to those that occur when the polymerase dissociates. In contrast, inactivation of the helicase prevents the formation of UV-induced processing intermediates and also destroys any UV-induced processing intermediates that are present at the time of inactivation, consistent with the idea that the fork loses integrity upon helicase inactivation and that UV-induced processing requires the integrity of the fork to remain intact. Strains containing plasmid pBR322 were split and then either UV-irradiated with 50 J/m2 or shifted to 42 °C and incubated for 15 min. At this time, the UV irradiated half of the culture was shifted to 42 °C, whereas the 42 °C half of the culture was UV irradiated with 50 J/m2. Incubation was then continued for another 15 min. Aliquots of the culture were taken before treatment began, at 15 min, and at 30 min following the initial treatment. The genomic and plasmid DNA was purified, digested with PvuII, and analyzed by 2D agarose gel analysis as described in Fig. 2.



wild type *dnaE* sequence:

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5′ AATACCATACACGATTTCGCC 3′ TS
3′ TTATGGTATGTGCTAAAGCGG 5′ NTS
C-  I  G  Y  V  I  E  G  -N Protein
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Fig. S4. Strain KH1366 retains a wild type copy of the *dnaE* gene. The lack of phenotype in KH1366 (*dnaQ49ts*) is not due to the acquisition of an *spq*-2 suppressor mutation. These compensatory mutations in DNA Polymerase III alpha, which convert valine at position 832 to a glycine, have been shown to frequently appear and can suppress phenotypes associated with *dnaQ* mutants (1).

1. Slater SC, Lifsics MR, O'Donnell M, Maurer R (1994) *holE*, the gene coding for the theta subunit of DNA polymerase III of *Escherichia coli*: Characterization of a *holE* mutant and comparison with a *dnaQ* (epsilon-subunit) mutant. *J Bacteriol* 176(3):815–821.

Table S1. *Escherichia coli* strains and plasmids used

Strain or plasmid	Genotype	Source or Construction
SR108	*λ-, thyA, deo, IN(rrnD-rrnE)*	(1)
AB1157	*λ-, thr, ara-14, leu, (gpt-proA)62, lacY1, tsx-33, supE44, galK2, hisG4, rfbD1, mgl-51, rpsL, kdgK51, xyl-5, mtl-1, argE3, thi*	(2)
CRT266	*dnaB266(ts), λ-, thr, leu, met, thyA, deo, supE, tonA*	(3)
E486	*dnaE486(ts), λ-, thr, leu, met, thi, thyA, deo, lac, rpsL, tonA*	(4)
PC3	*dnaG3(ts), λ-, leu, thyA, deo, rpsL*	(5)
MS101	*dnaN159(ts), λ-, thr, araD, (gpt-proA)62, lacY1, tsx-33 supE, galK2, hisG4, rpsL, xyl-5, mtl-1, argE3, thi, sulA, tnaA300::Tn10*	(6)
KH1366	*dnaQ49(ts), λ-, met, (cod-lacI), tsx-7, srl-8, relA, spoT*	(7)
AX727	*dnaX2016(ts), λ-, lac, rpsL, thi*	(8)
CL756	*dnaB266(ts), λ-, thr, leu, met, thyA, deo, supE, tonA recF6206*::tet^R	(9)
CL069	*dnaE486(ts), λ-, thr, leu, met, thi, thyA, deo, lac, rpsL, tonA recF349 tna300*::*Tn*10	E486 × P1 from HL 919 (10)
CL583	*λ-, thyA, deo, IN(rrnD-rrnE), recF6206*::tet^R	(11)
Plasmid pBR322		(12)

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