Fate of the replisome following arrest by UV-induced DNA damage in *Escherichia coli*

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Accurate replication in the presence of DNA damage is essential to genome stability and viability in all cells. In *Escherichia coli*, DNA replication forks blocked by UV-induced damage undergo a partial resection and RecF-catalyzed regression before synthesis resumes. These processing events generate distinct structural intermediates on the DNA that can be visualized in vivo using 2D agarose gels. However, the fate and behavior of the stalled replisome remains a central uncharacterized question. Here, we use thermosensitive mutants to show that the replisome's polymerases uncouple and transiently dissociate from the DNA in vivo. Inactivation of α, β, or τ subunits within the replisome is sufficient to signal and induce the RecF-mediated processing events observed following UV damage. By contrast, the helicase–primase complex (DnaB and DnaG) remains critically associated with the fork, leading to a loss of fork integrity, degradation, and aberrant intermediates when disrupted. The results reveal a dynamic replisome, capable of partial disassembly to allow access to the obstruction, while retaining subunits that maintain fork licensing and direct reassembly to the appropriate location after processing has occurred.

replication fork processing | RecF pathway

The replisome consists of several, multisubunit protein complexes and is responsible for duplicating the genome. In *Escherichia coli*, it is comprised of three DNA polymerase complexes tethered to the DNA template by dimeric processivity factors, a τ complex that couples leading and lagging strand synthesis, and a helicase–primase complex that separates the duplex DNA and primes lagging strand synthesis (1–3).

When the replisome encounters DNA damage that blocks its progression, the potential for mutagenesis, rearrangements, and lethality increases significantly. Replication in the presence of DNA damage can generate mutations if the wrong base is incorporated, rearrangements if it resumes from the wrong site, or lethality if the obstructing lesion cannot be overcome. Following the arrest of replication at UV-induced damage, the nascent lagging strand is partially resected by the combined action of the RecQ helicase and RecJ nuclease (4, 5). RecF-O-R, along with RecA, limit this degradation and promote a transient regression of the DNA branch point, which is thought to be important for restoring the damaged region to a form that can be acted on by repair enzymes or translesion DNA polymerases (4–10). These processing events generate distinct structural intermediates on the DNA that can be readily visualized using 2D agarose gel analysis, a technique that allows one to identify the shape and structure of DNA molecules (5, 11).

Although the processing that occurs on the DNA is well characterized, little is known about the behavior or composition of the replisome itself during these events. If the replisome remains bound to the arresting lesion, it may sterically obstruct repair or bypass from occurring. Conversely, complete dissociation of the replisome would likely abolish the licensing for the replication fork and expose DNA ends that have the potential to recombine, generating deletions, duplications, or rearrangements on the chromosome. Recent studies in vitro have suggested that dynamic interactions between replisome components may play a role in

allowing the machinery to overcome specific challenges such as collisions with the transcription apparatus or DNA-bound proteins (1, 12, 13). In this study, we used thermosensitive replication mutants to characterize how the composition of the replisome changes following encounters with UV-induced photoproducts, a biologically relevant lesion that is known to block the progression of the replisome when located in the leading strand template (6, 14–16). The results demonstrate that the DNA polymerases can dissociate from the replisome in a modular manner without compromising the integrity of the replication fork. Dissociation of the DNA polymerase from the replisome is sufficient and can serve to initiate the processing of the replication fork DNA via the RecF pathway, similar to that seen when replication is arrested by UV-induced damage. By comparison, the helicase complex remains associated with the replication fork throughout the recovery process. If the helicase is disrupted, aberrant intermediates, degradation, and loss of fork integrity ensue. We propose that the retention of the helicase is needed to maintain licensing for the replication fork and direct reassembly to the appropriate location after processing has occurred.

Results

Dissociation of the Polymerase from the Replisome Is Sufficient to Induce Processing Events Similar to Those Observed After UV-Induced Arrest. A schematic of each of the components of the replisome tested in this study and their function is presented in Fig. 1*A*. Temperature-sensitive mutants exist in subunits from each of replisome's complexes for which viability or functionality is supported at 30 °C, but not at 42 °C (Fig. 1*B*). Although replication proceeds normally at the permissive temperature, it rapidly decreases following inactivation of the thermosensitive protein at the restrictive temperature, similar to that seen after UV irradiation (Fig. 1*C*). The exception to this is in the proofreading subunit ε, encoded by *dnaQ*ᵗˢ, which is mutagenic at the restrictive temperature, but is not essential for viability or replication (17).

To determine how the replisome behaves or is modified following encounters with DNA damage in vivo, these thermosensitive mutants were used to deliberately disrupt the specific components of the replisome. We then compared the effect that the loss of that component had on replication processing to that seen when replication encounters DNA damage. Replication and processing intermediates were visualized in vivo on replicating fragments of the plasmid pBR322 (Fig. 2). This plasmid replicates using the host's replication machinery, it can be linearized at its single origin of replication, and it maintains a moderate copy number, making it useful to detect rare events such as replication through a defined fragment (18). In this type of analysis, actively
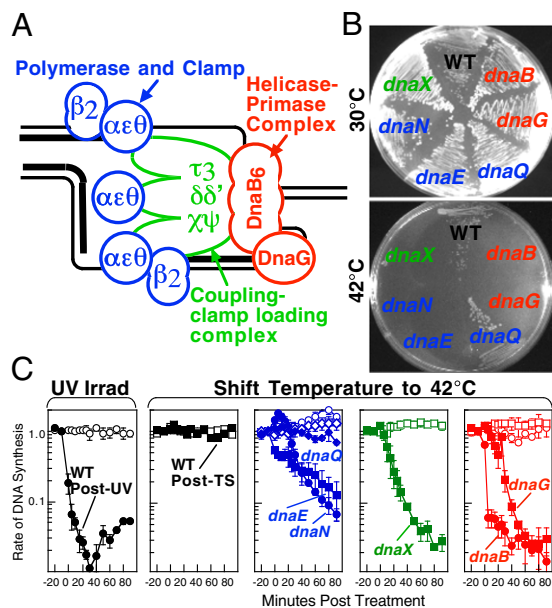
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Fig. 1. Replication is disrupted by UV-induced damage or following inactivation of the DNA polymerase, τ complex, or helicase–primase complex. (*A*) A diagram of the replisome, indicating the subunits of each protein complex. (*B*) Thermosensitive mutants that inactivate the polymerase core, τ complex, or helicase complex are viable at 30 °C but fail to grow at the restrictive temperature of 42 °C following overnight incubation. (*C*) The rate of DNA synthesis is inhibited following UV-induced damage or inactivation of the replisome's essential subunits. Wild-type or mutant cultures, grown at 30 °C were pulse-labeled with 1 μCi per 10 μg/mL [³H]thymidine for 2 min at the indicated times following mock treatment (open symbols), 50 J/m² UV irradiation (filled symbols), or a shift to 42 °C (filled symbols). The amount of radioactivity incorporated into the DNA, relative to pretreated cultures is plotted. Error bars represent SE of two experiments.

replicating molecules appear as "Y"-shaped structures that migrate in an arc that extends out from the prominent spot of non-replicating linear molecules. Following the arrest of replication in irradiated wild-type cultures, replication forks undergo a transient regression and other processing events that generate intermediates having two branch points (18). These intermediates, form double-Y or "X"-shaped molecules that migrate more slowly due to their nonlinear shape, forming a cone region above the arc of replicating molecules that can be clearly distinguished from the normal replicating molecules (Fig. 2). Importantly, no processing intermediates or aberrant DNA structures are observed in wild-type cultures shifted to 42 °C.

To characterize how the composition of the replisome changes or is modified during these processing events, cultures of thermosensitive replication mutants were grown at the permissive temperature, split in half, and then either UV-irradiated with 50 J/m² or shifted to the restrictive temperature at 42 °C. Aliquots of each culture were taken at various times and the DNA was prepared and examined by 2D agarose gel analysis. In all mutants, only normal Y-shaped replication intermediates were observed before treatment, and normal damage-induced processing of the replication fork occurred following UV-induced damage, as evidenced by their resistance to UV, and the timely appearance of cone region intermediates in each mutant (Fig. 2 and Figs. S1 and S2).

Following inactivation of either the catalytic subunit of the DNA polymerase, α (DnaE), or the processivity factor that tethers the DNA polymerase to the DNA template, β (DnaN), processing events were induced at the replication fork DNA that appeared similar to those seen after UV irradiation. Both α and

β are required to maintain DNA polymerase binding to the DNA template (19, 20). The processing intermediates were induced specifically by the disruption of the DNA polymerase III subunits, because no processing intermediates were induced in wild-type cells at the restrictive temperature or following inactivation of the nonessential proofreading subunit, ε (DnaQ) (Fig. 2 and Fig. S1).

Similar to the DNA polymerase subunits, UV-like processing intermediates were also induced following inactivation of τ (DnaX), which is responsible for coupling the polymerases to the replisome, coordinating leading and lagging strand synthesis, and repetitive cycling of the processivity factor onto the lagging strand template (21–25). The observed induction of UV-like intermediates following inactivation of the DNA polymerase or coupling factor indicates that the UV-induced processing of replication forks is likely to involve the transient dissociation of the polymerase from the DNA.

The helicase–primase complex interacts with τ and the holoenzyme and tracks along the lagging strand template, serving to unwind and prime it during replication (26–28). In contrast to α, β, or τ, inactivation of either the helicase (DnaB) or primase (DnaG) led to the production of aberrant structural intermediates that were unlike any of those that are observed during the processing of UV-induced damage (Fig. 2 and Fig. S1), arguing that disruption of the helicase complex does not normally occur during the recovery of replication after UV-induced arrest.

Similar to UV, the Integrity of the Replication Fork DNA Is Maintained After Polymerase Dissociation, and Replication Fork Processing Is Catalyzed by RecF. The above results are consistent with the idea that the DNA polymerases dissociate from the replisome upon encounters with UV-induced damage whereas the helicase complex remains associated with the DNA. Following UV irradiation, the integrity of the arrested replication fork is normally maintained and the nascent DNA undergoes only limited degradation before the resumption of replication (5). To further characterize how the dissociation of the DNA polymerase or helicase affect replication fork integrity, we monitored the fate of the nascent DNA after disruption of these components and compared it to that at UV-arrested replication forks. To this end, wild-type, *dnaE*ᵗˢ, and *dnaB*ᵗˢ cultures, grown at 30 °C in the presence of [¹⁴C]thymine, were pulse-labeled with [³H]thymidine for 20 s and immediately placed in fresh nonradioactive media before either UV-irradiating or shifting the culture to 42 °C. In wild-type cells after UV irradiation, no degradation was detected in the [¹⁴C]-labeled genomic DNA and only ~10% of the [³H]-labeled nascent DNA at the arrested fork was degraded following DNA damage (Fig. 3). Similarly, following inactivation of the DNA polymerase, both the genomic and nascent DNA remained protected and was maintained to a similar extent as that seen after UV-induced arrest. The results strongly suggest that DNA polymerase dissociation can occur without compromising the integrity of the replication fork. By contrast and distinct from UV-arrested replication forks, extensive degradation of the nascent DNA was observed following disruption of the helicase, DnaB (Fig. 3), suggesting that the aberrant structural intermediates are associated with the collapse and degradation of the replication fork. The observed degradation was targeted specifically to the DNA at the replication fork because no degradation was detected in the overall genomic DNA. Neither the abnormal intermediates nor the extensive degradation of the fork are observed during the recovery of replication after UV-induced damage, arguing that the helicase complex normally remains associated with the DNA during the recovery process and is needed to maintain the integrity of the replication fork.

Consistent with these interpretations, the replication fork processing induced by either polymerase inactivation or UV-induced damage remains unchanged when the second form of
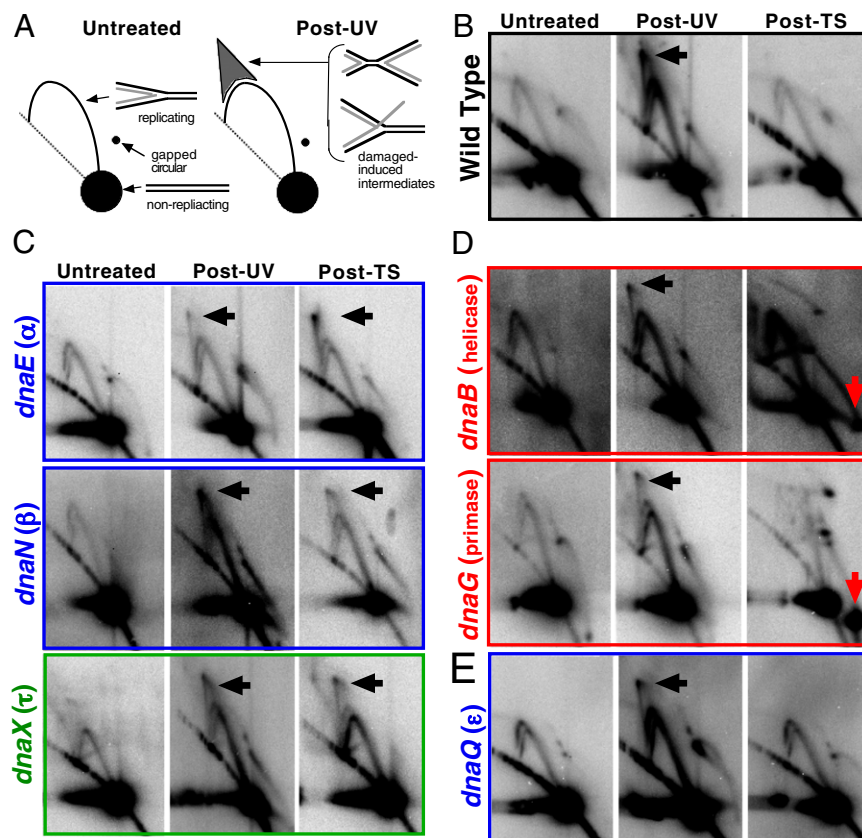
Fig. 2. Inactivation of the DNA polymerase or τ complex, but not the helicase complex, induces replication fork processing intermediates similar to UV-induced damage. (*A*) Diagram depicting the migration pattern of replicating DNA fragments and intermediates associated with processing forks disrupted by UV-induced damage in 2D agarose gels. Strains containing plasmid pBR322 were UV-irradiated with 50 J/m² 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 15 min following UV-irradiation and 90 min following temperature shift. (*B*) In wild-type cultures, replication fork processing intermediates are observed after disruption by UV-irradiation but not after a shift to 42 °C. (*C*) Disruption of the DNA polymerase core (*dnaE*ts), processivity factor (*dnaN*ts) or τ (*dnaX*ts) is sufficient to induce replication fork processing similar to that seen after UV-induced damage. (*D*) Abnormal intermediates, distinct from any of those associated with processing UV-induced damage, arise after disruption of the helicase (*dnaB*ts) or primase (*dnaG*ts). (*E*) No atypical intermediates are observed following disruption of the nonessential proofreading subunit of the DNA polymerase (*dnaQ*ts).

challenge is administered (Fig. S3). By contrast, disruption of the helicase by thermal inactivation prevents the UV-induced processing from occurring and destroys any processing intermediates that exist at the time of inactivation.

We have previously shown that the formation of the UV-induced processing intermediates is catalyzed by the RecF pathway gene products, depends on active replication, and resolves at a time that correlates with when the lesions are repaired and replication resumes (5). In the absence of RecF, the transient regression and processing intermediates fail to form at the replication fork after UV-induced damage (Fig. 4). To determine if the DNA processing events induced by DNA polymerase dissociation are identical to those occurring after UV-induced damage, we examined whether the intermediates formed by DNA polymerase dissociation also depend on RecF. Wild-type, *dnaE*ts, and *dnaB*ts cultures containing an intact *recF* gene or a *recF* deletion were grown at 30 °C, UV irradiated or shifted to the nonpermissive temperature, and then examined by 2D agarose gel analysis, as before. Similar to UV treatment, the formation of the replication fork intermediates induced following temperature shift in *dnaE* mutants were dependent on RecF, strongly arguing that the processing intermediates observed after polymerase dissociation are identical to those occurring following the arrest of replication at UV-induced damage (Fig. 4). By contrast, the aberrant intermediates observed following disruption of the helicase

complex appeared irrespective of whether RecF was present or absent, further supporting the idea that these intermediates and processing events are distinct from those that occur during the recovery of replication at UV damage.

Discussion

Following encounters with DNA damage that blocks the progression of replication, the DNA at the arrest site undergoes a partial resection and transient regression to allow the lesion to be repaired or bypassed before DNA synthesis resumes. How the replisome allows repair enzymes to access the lesion without compromising the replication fork's integrity or license to resume replication has remained a challenging question. These data indicate that, in vivo, dissociation of the replisome's polymerases from the DNA is sufficient and can serve as a signal to initiate the RecF-mediated processing that occurs following arrest by a UV-induced adduct. The DNA polymerase dissociation occurs without compromising the integrity of the fork DNA, which remains protected even after the polymerase dissociates or uncouples from the replisome. By contrast, the helicase complex remains associated with the DNA, and is necessary to maintain the integrity of the replication fork. We propose that uncoupling of the polymerase from the replisome may be required to allow repair enzymes or translesion DNA polymerases to gain access to and effect repair (Fig. 5). Retention of the helicase complex at
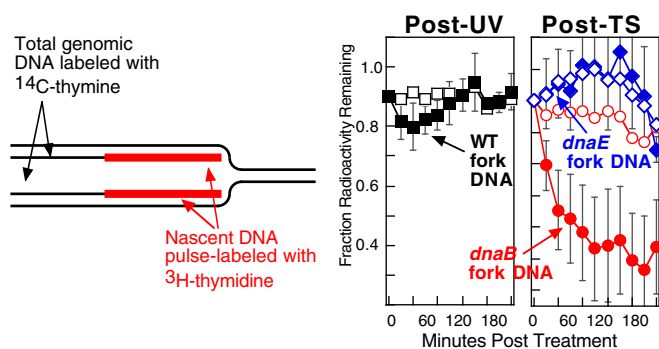
Fig. 3. Similar to after UV, the integrity of the replication fork DNA is maintained after DNA polymerase dissociation. The integrity of the replication fork is maintained following arrest by UV irradiation or polymerase dissociation, but collapses after disruption of the helicase. [^{14}C]thymine-prelabeled cultures were pulse-labeled with [^{3}H]thymidine for 20 s before being filtered and placed in nonradioactive media and either UV-irradiated with 50 J/m^2 or shifted to 42 °C as indicated. The amount of radioactivity remaining in the total genomic DNA (open symbols) and nascent DNA at the replication fork (filled symbols) is plotted over time. Error bars represent SE of two independent experiments.

the arrest site maintains licensing for the replication fork and may serve to signal and recruit replisome reassembly to the correct site after the lesion has been processed.

*dnaE*486 contains a S885P mutation that falls within the domains necessary for interactions with β (29, 30). *dnaX*2016 contains a G118D mutation that disrupts the protein's ATPase domain (31). *dnaN*159 contains two amino acid substitutions, G66E and G174A, which impair its interactions with α (32). Considering that α, β, and τ are all necessary to maintain polymerase binding to the DNA and replisome, and that all three temperature-sensitive mutants induce a similar phenotype, it seems reasonable to suggest that the temperature shift is likely to result in polymerase dissociation, rather than having it remain bound but inactive within the replisome. *dnaQ*49 contains a V96G mutation that decreases the ability of ε to interact with α and θ subunits (33, 34). We confirmed that our strain did not contain *spq*-2, a suppressor mutation (Fig. S4), which can frequently arise in *dnaQ* mutants (35).

*dnaG*3 contains a G247D mutation within the catalytic core domain (36). *dnaB*266 is an amber mutation that requires coexpression of suppressor tRNAs (38, 39). It has been characterized to be phenotypically similar to *dnaB*8, an A130V mutation in the hinge region that affects ATPase activity, primer synthesis, and helicase activity at 42 °C (40, 41). The accessibility of the fork DNA to nucleases and its loss of structural integrity suggests that thermal inactivation in this case is likely to result in helicase dissociation in vivo.

We would emphasize that the assays involving thermal inactivation are likely to reflect the events that occur upon the initial arrest of replication. Because thermal inactivation is presumably irreversible, this type of analysis does not permit inferences to be made about the mechanism by which replication resumes, which is almost certain to involve all of the proteins of the replisome.

The idea that the DNA polymerase can function as a modular unit within the replisome and dissociate without disrupting the integrity of the replication fork is consistent with a number of observations from previous studies. Functional replisomes can be reconstituted in vitro that contain one, two, or three polymerases (2, 42), arguing that the presence of all three polymerases is not essential to maintain replication fork integrity (12). Increasing the concentrations of DNA polymerase II or DNA polymerase IV can reduce the speed of the replisome and its helicase in vitro and reduces the rate of DNA synthesis in vivo (43). These observations imply that the DNA polymerases have the capability to swap into a functional replisome, replacing DNA polymerase III during synthesis. A similar form of polymerase dynamics is also observed in phage T4 and T7, in which the polymerases transiently release and rebind the DNA template without losing contact or compromising replisome integrity (44, 45). In eukaryotes, two subunits of the replicative lagging strand polymerase, δ, are also components of translesion DNA polymerase ζ and are required for translesion synthesis in vivo (46–48). Although it remains unclear whether translesion DNA synthesis is occurring within the active replisome or at DNA gaps following replication, the observations are suggestive that eukaryotic DNA polymerases can also function in a modular fashion in some instances.

Replication is differentially affected by lesions in the leading strand and lagging strand template. Both in vitro and in vivo approaches suggest that lesions in the leading strand arrest the overall progression of the fork, whereas the priming activity associated with lagging strand synthesis allows replication to continue past lesions in this template, leaving a gap in the DNA (15, 16, 28, 49). A number of studies have shown that, when replication encounters a leading strand lesion in vivo, nucleotide excision repair plays a predominant role in allowing DNA synthesis to resume following arrest (7, 50). Translesion DNA synthesis affects the timing and kinetics of the recovery only when nascent strand processing or repair cannot occur (7, 50, 51). The observed dissociation of the DNA polymerase seems likely to be required to allow repair enzymes to gain access to and effect repair of the lesions at the arrest site. A similar displacement of RNA polymerase is necessary before nucleotide excision repair can act when transcription is arrested by UV-induced damage (52–54).

In vivo, we observed that RecF-mediated processing was triggered following dissociation of the DNA polymerase from the replisome. Consistent with this observation, in vitro approaches have shown that RecF pathway proteins can displace a DNA polymerase arrested at a leading strand block (28). Although it
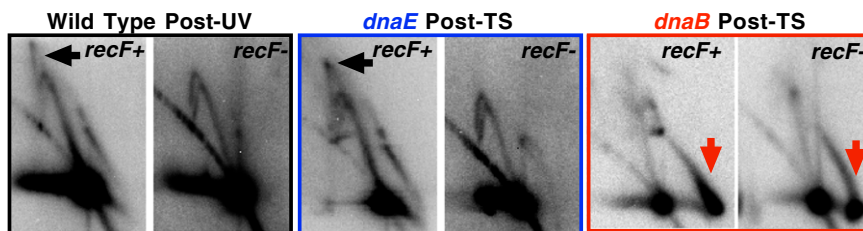
Fig. 4. RecF mediates the formation of replication fork intermediates following DNA polymerase dissociation. The formation and processing of the replication intermediates after DNA polymerase dissociation are mediated by RecF, similar to UV. The formation of the abnormal intermediates that arise following helicase disruption is not dependent on RecF. Isogenic wild-type, *dnaB*^{ts}, and *dnaE*^{ts} cultures containing a normal or mutated copy of the *recF* gene were UV-irradiated or temperature shifted and analyzed by 2D agarose gel analysis as in Fig. 2.
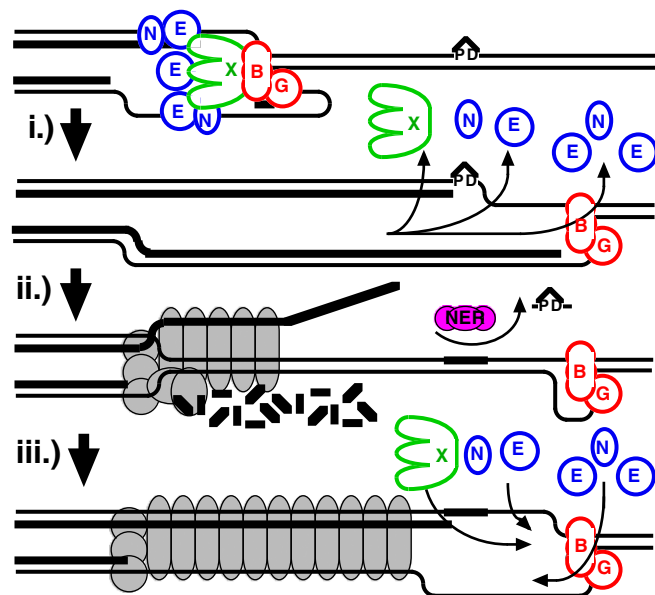
Fig. 5. Model of replisome at UV-induced damage. Upon encountering an arresting lesion (PD, pyrimidine dimer) (*i*), DNA synthesis becomes uncoupled and the polymerases transiently dissociate. (*ii*) This serves as a signal to initiate the replication fork DNA processing by the RecF-pathway gene products (gray circles) allowing repair enzymes (NER) or translesion polymerases to access the lesion. (*iii*) The helicase–primase complex remains bound to the template DNA and serves to maintain the licensing and integrity of the replication fork, directing replisome reassembly to the correct location once the lesion has been processed.

remains unclear from these studies whether the dissociation of the DNA polymerase in vivo requires RecF or occurs spontaneously and serves to trigger the RecF-mediated processing.

Biochemical studies have led to different models for how the helicase may behave when replication is blocked. In vitro, purified DnaB was shown to be capable of loading onto synthetic fork substrates when combinations of the primosomal proteins, PriA, -B, and -C, or Rep are present, leading some to speculate that the helicase may dissociate and reassemble as a mechanism to bypass lesions or other obstructions on the DNA (55). However, other in vitro studies have shown that DnaB remains stably associated with the DNA when replication encounters various impediments or is arrested by a leading strand block, suggesting the helicase remains associated until the obstruction can be processed (15, 56). The observations reported in this study show that disruption of the helicase in vivo leads to aberrant replication intermediates and a loss of fork integrity. Considering that these events are not observed following encounters with UV-induced damage, the observations imply that the helicase complex is normally retained at the site of arrest and is likely to be necessary to maintain the integrity and licensing of the replication forks.

Both prokaryotes and eukaryotes tightly regulate helicase loading onto DNA as a mechanism to control where replication initiates and license regions of DNA for replication to prevent rereplication (57, 58). In eukaryotes, origins are prelicensed for initiation in G1 by loading the DNA helicase, and no new helicase loading is thought to occur once S phase has begun (59–61). Following activation, the remaining components of the replisome are recruited to the helicase and replication begins (61). Thus, in eukaryotic systems, it is generally thought that disruption of the helicase abolishes the ability of the fork to resume replication. A

similar mechanism of helicase loading and activation regulates replisome assembly and initiation at origins in prokaryotes (58, 62). The observed retention of the helicase at UV-arrested replication forks suggests a mechanism by which the replication fork may retain licensing to resume replication. In addition, the presence of the helicase at the replication fork may serve as a signal to recruit the replisome components to reassemble at the correct site once the lesion has been processed, similar to the process that occurs at origins.

The mechanisms of replication and origin initiation are highly conserved among both prokaryotes and eukaryotes organisms, making it likely that the observed replisome modularity and importance of helicase retention will extend broadly among evolutionarily diverse organisms.

Materials and Methods

Strains and Plasmids. Strains used in this study are presented in Table S1. All *recF* strains were made isogenic with their parents for this study. Mutants were constructed using standard P1 transduction and verified according to their temperature- and UV-sensitive phenotypes, as appropriate. The strains containing temperature sensitive alleles, in many cases, are related and have been used comparatively in the literature, but are not strictly isogenic. Thus, in each case, comparisons of the temperature-shift response to the UV response were made within each strain, rather than between strains. In this way, each strain effectively served as its own parental control for the analysis. In addition, temperature-sensitive alleles were verified to have a normal response to UV irradiation as measured by survival, replication recovery, and UV-induced processing intermediates.

Rate of DNA Synthesis. Overnight cultures were diluted 1:100 and grown in Davis medium supplemented with 0.4% glucose, 0.2% casamino acid, and 10 μg/mL thymine (DGCthy) to an OD_{600} of 0.4 in a 30 °C shaking water bath. For UV, cultures were split in half and either UV irradiated with 50 J/m^2 (Sylvania 15-W germicidal lamp, 254 nm, 0.9 J/m^2 per s incident dose) or mock irradiated. For temperature shift, cultures were filtered and resuspended in fresh prewarmed media at either 30 °C or 42 °C. At the indicated times, duplicate 0.5-mL aliquots of the culture were pulse-labeled with [3H] thymidine (1.0 μCi/10 μg/mL) for 2 min before cells were lysed, and DNA was precipitated by the addition of 5 mL of ice-cold 5% (wt/wt) trichloroacetic acid. The precipitate was collected and the amount of radioactivity was determined as described (63).

Two-Dimensional Agarose Gel Analysis. Overnight cultures containing the plasmid pBR322 grown in 100 μg/mL ampicillin were pelleted, diluted 1:100 in DGCthy without antibiotic, and grown to an OD_{600} of 0.4 in a 30 °C shaking water bath. Cultures were split in half and either UV irradiated with 50 J/m^2 or filtered, and resuspended in prewarmed 42 °C media. At the indicated times, DNA was prepared from 0.75-mL aliquots of the cultures and then analyzed by 2D agarose gel electrophoresis as described (63).

Nascent DNA Degradation and Fork Integrity. Overnight cultures were diluted 1:100 and grown in DGCthy and [14C]thymine (0.1 μCi/10 μg/mL) to an OD_{600} of 0.4 in a 30 °C shaking water bath. Cultures were then pulse labeled with [3H]thymidine (1 μCi/10 μg/mL) for 20 s, filtered on Whatman 0.4-μm membrane filters, and washed twice with 3 mL of cold NET buffer (100 mM NaCl/ 10 mM EDTA, pH 8.0/10 mM Tris, pH 8.0). For UV experiments, the filter was then resuspended in prewarmed nonradioactive DGCthy media, UV irradiated with 50 J/m^2, and incubated at 30 °C. For temperature-shift experiments, the filter was instead immediately resuspended in fresh media that had been prewarmed to 42 °C. At the times indicated, duplicate 200-μL aliquots of cells in culture were lysed, the DNA was precipitated, and the amount of radioactivity was determined as described (63).

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Supporting Information
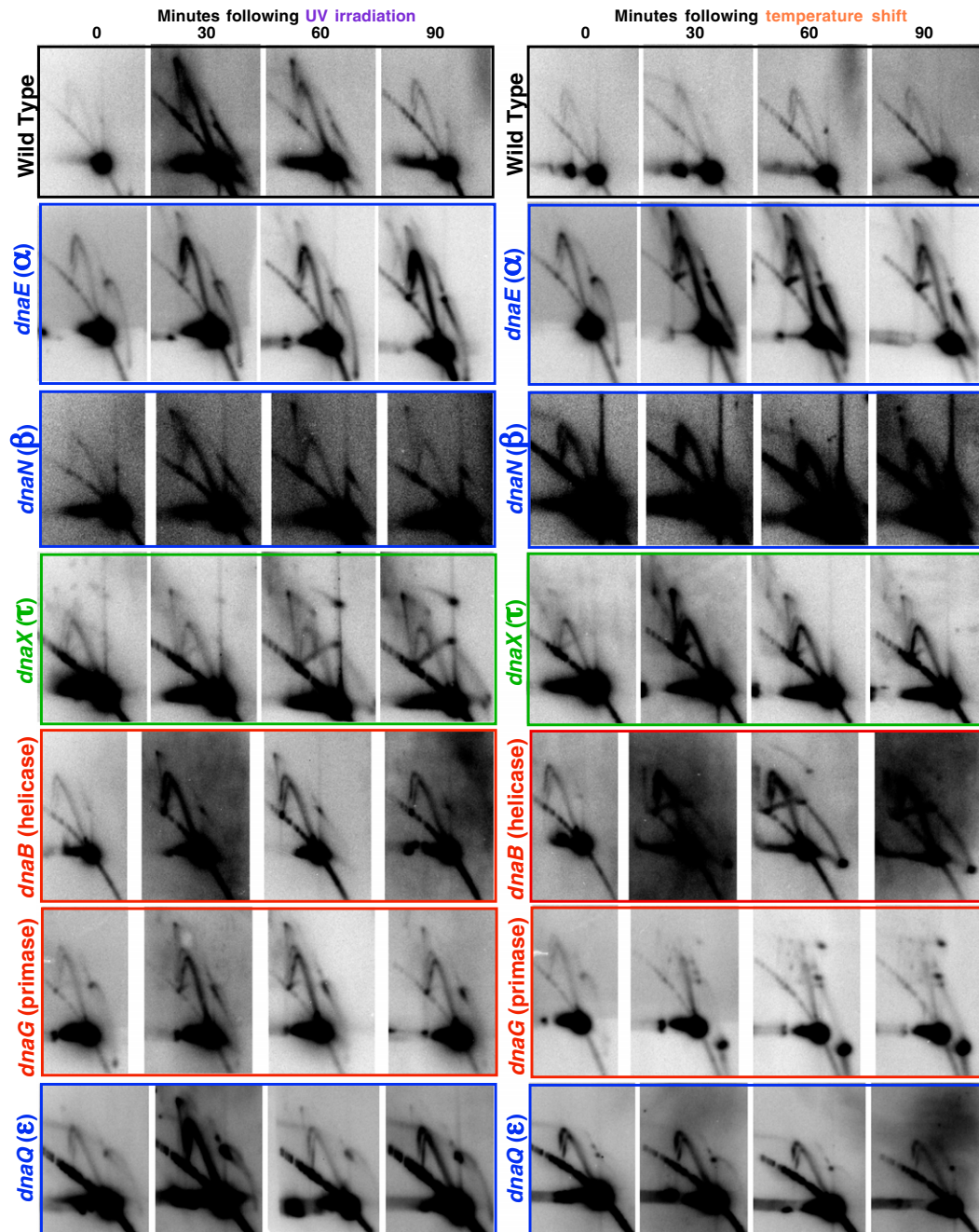
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Fig. S1. A comparison of the replication intermediates observed by 2D agarose gel analysis following UV irradiation or temperature shift over time is shown for wild type and each of the temperature-sensitive replication mutants used in this study. Strains containing plasmid pBR322 were UV-irradiated with 50 J/m² 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.
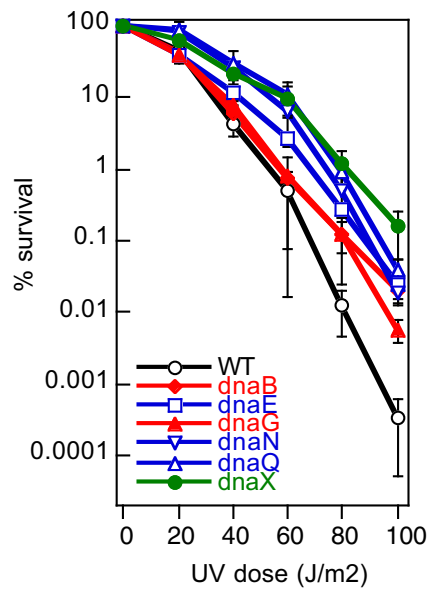
Fig. S2. The survival of each strain when grown at 30 °C is plotted at the indicated dose relative to unirradiated cultures. At the permissive temperature, all of the temperature sensitive mutants used in this study were as resistant as wild-type cells to UV irradiation. The modestly elevated resistance 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.

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wild type dnaE sequence:  5' AATACCATACACGATTTCGCC 3' TS
                          3' TTATGGTATGTGCTAAAGCGG 5' NTS
                          C-  I  G  Y  V  I  E  G  -N Protein
```

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).

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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::Tn10	E486 × P1 from HL 919 (10)
CL583	λ-, thyA, deo, IN(rrnD-rrnE), recF6206::tet^R	(11)
Plasmid pBR322		(12)

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