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UV-induced DNA damage disrupts the coordination between replication initiation, elongation and completion

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Abstract

Replication initiation, elongation and completion are tightly coordinated to ensure that all sequences replicate precisely once each generation. UV-induced DNA damage disrupts replication and delays elongation, which may compromise this coordination leading to genome instability and cell death. Here, we profiled the *Escherichia coli* genome as it recovers from UV irradiation to determine how these replicational processes respond. We show that *oriC* initiations continue to occur, leading to copy number enrichments in this region. At late times, the combination of new *oriC* initiations and delayed elongating forks converging in the terminus appear to stress or impair the completion reaction, leading to a transient over-replication in this region of the chromosome. In mutants impaired for restoring elongation, including *recA*, *recF* and *uvrA*, the genome degrades or remains static, suggesting that cell death occurs early after replication is disrupted, leaving partially duplicated genomes. In mutants impaired for completing replication, including *recBC*, *sbcCD xonA* and *recG*, the recovery of elongation and initiation leads to a bottleneck, where the nonterminus region of the genome is amplified and accumulates, indicating that a delayed cell death occurs in these mutants, likely resulting from mis-segregation of unbalanced or unresolved chromosomes when cells divide.

KEYWORDS

recBCD, recF, replication completion, replication initiation, uvrABC

1 | INTRODUCTION

Duplication of the circular *Escherichia coli* genome normally initiates bidirectionally from a single locus, *oriC* (Kaguni et al., 1982; Meijer et al., 1979). The two replisomes progress until they converge to complete replication at a point localized within a 400-kb terminus region opposite to the origin. The terminus region is flanked by nonpalindromic *ter* sequences, which bind the protein, Tus, and block replication fork progression in an orientation-dependent manner, thereby confining the completion event to this region of the genome (Hill & Marians, 1990; Hill et al., 1989; Kobayashi et al., 1989). While Tus determines the region where completion most frequently occurs, it does not appear to be directly involved in the completion reaction since *tus* mutants have no phenotype and complete replication normally, as do plasmids and bacteriophage that lack *ter* sequences (Roecklein et al., 1991).

The three stages of DNA replication—initiation, elongation and completion—must be tightly coordinated to ensure

that each daughter cell inherits an identical copy of the genetic information. A large number of the cell's gene products are devoted to each of these processes and are required to maintain genome stability and viability. The timing and specificity of initiation is regulated by DnaA, DnaC and a number of regulatory proteins (reviewed in (Costa et al., 2013; Katayama, 2017)). Elongation requires 15 distinct proteins for the replisome core and is maintained by processivity factors such as DnaN, helicases DnaB and Rep, and other accessory factors (reviewed in [Kelman and M. O'Donnell, 1995]). Finally, proteins including RecG, SbcCD, RecBCD and likely others ensure that replication completes accurately at the precise point where replication forks converge and all sequences have doubled (Courcelle et al., 2015; Midgley-Smith et al., 2018, 2019; Wendel et al., 2014, 2018). Excess initiations, incomplete elongations or failed completion events would create over- or under-replicated portions of the genome, leading to copy number imbalances, incomplete chromosomes and heritable changes to the genome or death upon cell division.

DNA damage can compromise the coordination between these three stages of replication, as DNA adducts can block replication fork progression, delaying elongation. A number of studies have shown that excessive DNA ends introduced either directly by the damaging agent or as intermediates in the repair process can lead to illegitimate or recombinational initiation events, producing abnormal copy numbers of chromosome segments, rearrangements and a loss of genome stability (Asai & Kogoma, 1994; Bhatia et al., 2014; Donnianni & Symington, 2013; Hamperl & Cimprich, 2014; Magee et al., 1992; de Massy et al., 1984). To prevent these potential threats, cells encode a number of genes that play critical roles in maintaining the ability of the genome to accurately duplicate in the presence of DNA damage and often render cells hypersensitive to damage when absent.

Two classes of these gene products include those that promote the recovery of replication when it stops prematurely, and those that prevent illegitimate replication or limit replication from going too far. The first class includes genes required for nucleotide excision repair, the *recF* pathway and *recA*. Following the disruption of replication by UV-induced damage, the recovery of replication largely depends on the repair of the lesions by nucleotide excision repair, encoded by *uvrA*, *uvrB* and *uvrC* (Courcelle et al., 1997, 1999; Setlow & Carrier, 1964; Setlow et al., 1963). Cells deficient in nucleotide excision repair are unable to remove UV-induced DNA lesions, are severely impaired in their ability to resume replication and exhibit elevated levels of mutagenesis, rearrangements and cell lethality (Courcelle et al., 1999, 2001; Howard-Flanders et al., 1969; Rothman & Clark, 1977; Rupp et al., 1971; Setlow & Carrier, 1964). In addition, the recovery of replication also depends on several gene products in the RecF pathway which maintain and process blocked replication forks in a manner that allows the blocking lesion to be repaired and replication to resume (Chow & Courcelle, 2004; Courcelle et al., 1997, 1999, 2003; Rothman & Clark, 1977; Rothman et al., 1975). In the absence of RecF or RecA, the DNA at the blocked replication fork is not maintained, replication fails to recover and RecJ-RecQ-mediated exonucleolytic processing of the nascent DNA continues beyond that occurring in wild-type cells (Courcelle et al., 1997, 2003; Courcelle & Hanawalt, 1999). RecF, along with RecO and RecR, facilitates the formation of a RecA filament which restores the region to a form that allows repair to occur. RecA also plays a critical role in protecting DNA ends, including those created by the disrupted fork. In *recA* mutants, the degradation at the fork is much more extensive, involving both nascent DNA and parental DNA strands, resulting in a rapid and complete exonucleolytic digestion of the entire genome (Courcelle et al., 1997; Horii & Suzuki, 1968).

The second class of gene products complete replication events or prevent illegitimate replication from occurring and include enzymes such as RecG, SbcCD, ExoI and RecBCD. RecG is a helicase-branch migration enzyme that functionally disrupts 3′ DNA ends in duplex DNA to prevent replication from continuing or initiating from illegitimate substrates (Courcelle et al., 2015; Hong et al., 1995; Midgley-Smith et al., 2019; Rudolph et al., 2009; Rudolph et al., 2010; Rudolph et al., 2013; Wendel et al., 2014; Wendel et al., 2018). In *recG* mutants, an extreme over-replication is observed specifically in the terminus region of the chromosome (Rudolph et al., 2013; Wendel et al., 2014). By cloning in additional origins into the *E. coli* chromosome, Rudolph's laboratory group has shown that new sites of over-replication can be induced in *recG* mutants anywhere replication forks converge, consistent with the idea that substrates generated by convergent replication forks must be processed by RecG to allow it to complete efficiently and prevent replication from continuing or reinitiating beyond these points (Midgley-Smith et al., 2018). Similarly, the structure-specific nucleases, SbcCD and ExoI, act on a structural intermediate created at the point where forks converge to initiate the resection and degradation of over-replicated DNA segments which arise when replisomes merge (Midgley-Smith et al., 2019; Wendel et al., 2018). In the absence of SbcCD and ExoI nucleases, the over-replicated region persists, leading to genomic instabilities and amplifications at these loci (Chalker et al., 1988; Connelly et al., 1999; Lehman & Nussbaum, 1964; Midgley-Smith et al., 2019; Phillips et al., 1988; Wendel et al., 2018). Following incisions by these enzymes, the RecBCD helicase-nuclease complex processes the over-replicated intermediate and is required to catalyze or recruit enzymes that promote joining of the convergent strands. In vitro, RecB and RecC interact with RecD to form a dual helicase-nuclease complex that unwinds and degrades double-strand DNA ends (Taylor & Smith, 1985, 2003). On the chromosome, in the

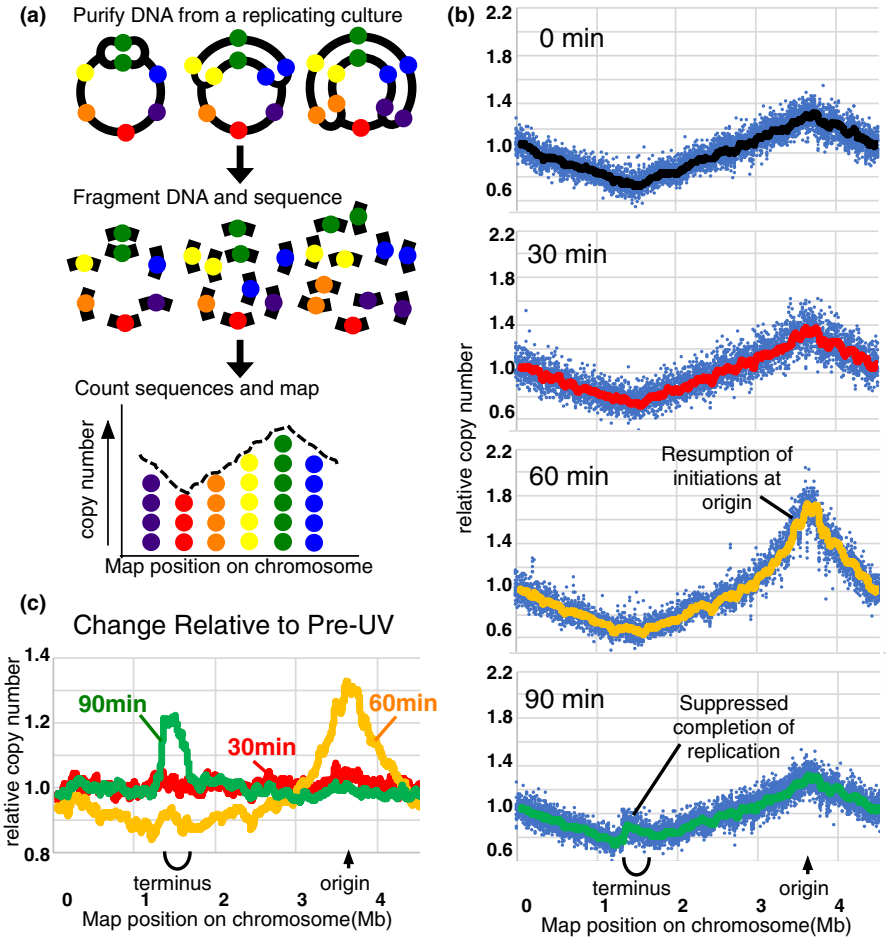

FIGURE 1 Following UV-induced DNA damage, the copy number of the sequences around the genome sequentially remains static, increases at the replication origin and then increases at sites where replication completes on the *E. coli* chromosome. (a) The methodology used to observe the replication profile of a culture is diagramed. (b) The relative copy number of sequences around the chromosome at various times after UV irradiation with 40 J/m^2 is plotted. Cultures were UV-irradiated with 40 J/m^2 and, at the times indicated, the DNA was purified, fragmented and profiled using high-throughput sequencing. Normalized sequence read frequencies are plotted relative to chromosome position along with a 40-kb floating average. The location of the origin and terminus region are indicated. (c) The change in copy number relative to before irradiation is plotted, showing that replication initially arrests and the genome copy number remains static for a period of time (30 min, red). Then, the copy number of sequences around the origin increases and accumulates as replication initiation from the origin resumes even though the repair of blocking lesions has delayed the progression of the previously existing replisomes around the genome (60 min, yellow). At late times, after the lesions have been repaired, the copy number of sequences around the genome returns to preirradiation levels, everywhere except for the region where completion occurs, where an increase in copy number is observed (90 min green). The increase indicates that the completion reaction is either rate limiting or down-regulated during recovery [Colour figure can be viewed at wileyonlinelibrary.com]

absence of RecB or C, the nascent ends of convergent replication forks are not joined, leading to excessive DNA degradation and rendering cells unable to maintain the chromosome region where forks converge (Courcelle et al., 2015; Rudolph et al., 2013; Wendel et al., 2014). The inability to complete replication or maintain this region of the genome severely compromises the viability and growth of *recBC* cultures (Capaldo-Kimball & Barbour, 1971; Courcelle et al., 2015; Wendel et al., 2014).

How the coordination between initiation, elongation and completion is affected by DNA damage, and whether coordination is maintained in mutants of these two enzyme classes is not known. Here, we profiled the genome of replicating cultures following UV-induced damage to understand how these processes are affected in both wild-type cells and mutants that are impaired for recovering or completing replication. We show that the genome of wild-type cultures initially remains static, before progressing through stages where an over-replication of the origin region occurs, followed by an over-replication of the terminus region. We show that while the genome of *recA* mutants is degraded entirely, the genome of other mutants that fail to restore replication remains static, indicating that cell death occurs soon after damage is induced and due to disruption by the DNA lesion. In mutants that are impaired for completing replication, the nonterminus region of the genome is amplified and accumulates, suggesting that

these cells undergo a delayed cell death that results from the inheritance of unresolved chromosomes with copy number imbalances following cell division.

2 | RESULTS

2.1 | UV-induced damage delays elongation, disrupting the coordination between initiation and completion, and leading to copy number imbalances on the chromosome

To examine how the processes of replication initiation, elongation and completion are globally affected by UV-induced DNA damage, we profiled the chromosome of a growing culture during the first 90 min after 40 J/m^2 UV irradiation using high-throughput sequencing. 40 J/m^2 is a dose that generates approximately one lesion every 6 kb and disrupts the progression of replication, reducing the rate of synthesis by ~95%. However, replication recovers and repair is nearly complete within ~40 min, and >90% of parental cells survive the challenge (Courcelle et al., 1997, 2003; Koehler et al., 1996). Under these experimental conditions, the rate of replication remained relatively linear in unirradiated cultures throughout the 90-min time course (Figure S1). To profile replication across the chromosome, genomic DNA is purified from replicating cultures, fragmented and then sequenced using high-throughput sequencing. The replication profile is then determined by counting the number of sequences that align to each segment of the chromosome (Figure 1a). In rapidly growing cultures, sequences surrounding the bidirectional origin replicate first and are observed at higher frequencies relative to chromosome regions that replicate later. In the absence of DNA damage, the frequency of any given sequence decreases inversely with its distance from the origin until reaching the terminus region where the two replication forks converge and replication completes (Figure 1b, time 0). At 30 min after UV exposure, the genome remains unchanged (Figure 1b,c). This correlates with the period during which replication remains blocked by the UV-induced damage and lesion repair is ongoing (Courcelle et al., 1999, 2003, 2005; Koehler et al., 1996). By 60 min postirradiation, the disrupted replication forks have been restored and repair is nearly complete (Courcelle et al., 1999, 2003, 2005; Koehler et al., 1996) and see below). At this time, an increase in copy number is observed that is specific to, and centers on, the origin region. Others have reported that replication from *oriC* is induced in a DnaA-dependent manner, (Rudolph et al., 2007), and using a microarray to examine the transcriptome after UV irradiation, we observed a transient induction of *dnaA* that correlates with the timing of copy number enrichment surrounding the origin region (Figure S2). Other work has demonstrated that replication can also initiate from the *oriC* region in a DnaA-independent manner following DNA damage (Magee et al., 1992). Taken together, we interpret this observation to indicate that in addition to the recovery of disrupted replication forks, replication initiation also resumes or is induced from the *oriC* region during this time.

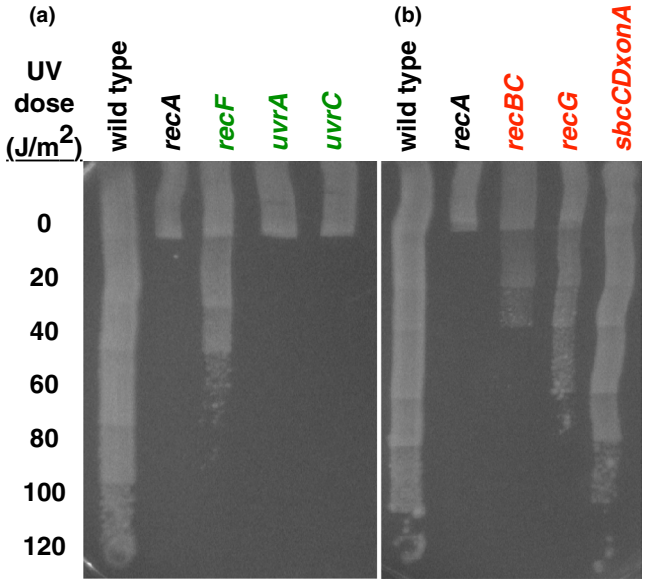

FIGURE 2 The relative sensitivity of (a) mutants known to be impaired for restoring replication following arrest, and (b) mutants impaired in their ability to complete replication are compared to wild-type and *recA* cultures [Colour figure can be viewed at wileyonlinelibrary.com]

At 90 min after irradiation, an over-replication of sequences specifically where forks converge is observed, whereas all other sequences around the genome have returned to their preirradiation levels. The observation implies that the completion reaction is either transiently inhibited or impaired. It is possible that the combined recovery of ongoing replication forks and new initiations at *oriC* exceeds or overwhelms the capacity of the completion enzymes to carry out this reaction, resulting in transient illegitimate initiation events and incomplete degradation of excess sequences in this region.

The results demonstrate that during the recovery from UV-induced damage, genome copy numbers initially remain static, then sequentially become enriched surrounding the origin, before becoming enriched at the site where replication completes.

2.2 | Following disruption, the replicating portions of the genome undergo a rapid and progressive degradation in the absence of RecA

We next examined several mutants involved in either the recovery of replication following disruption, or completing and limiting illegitimate replication to determine how the

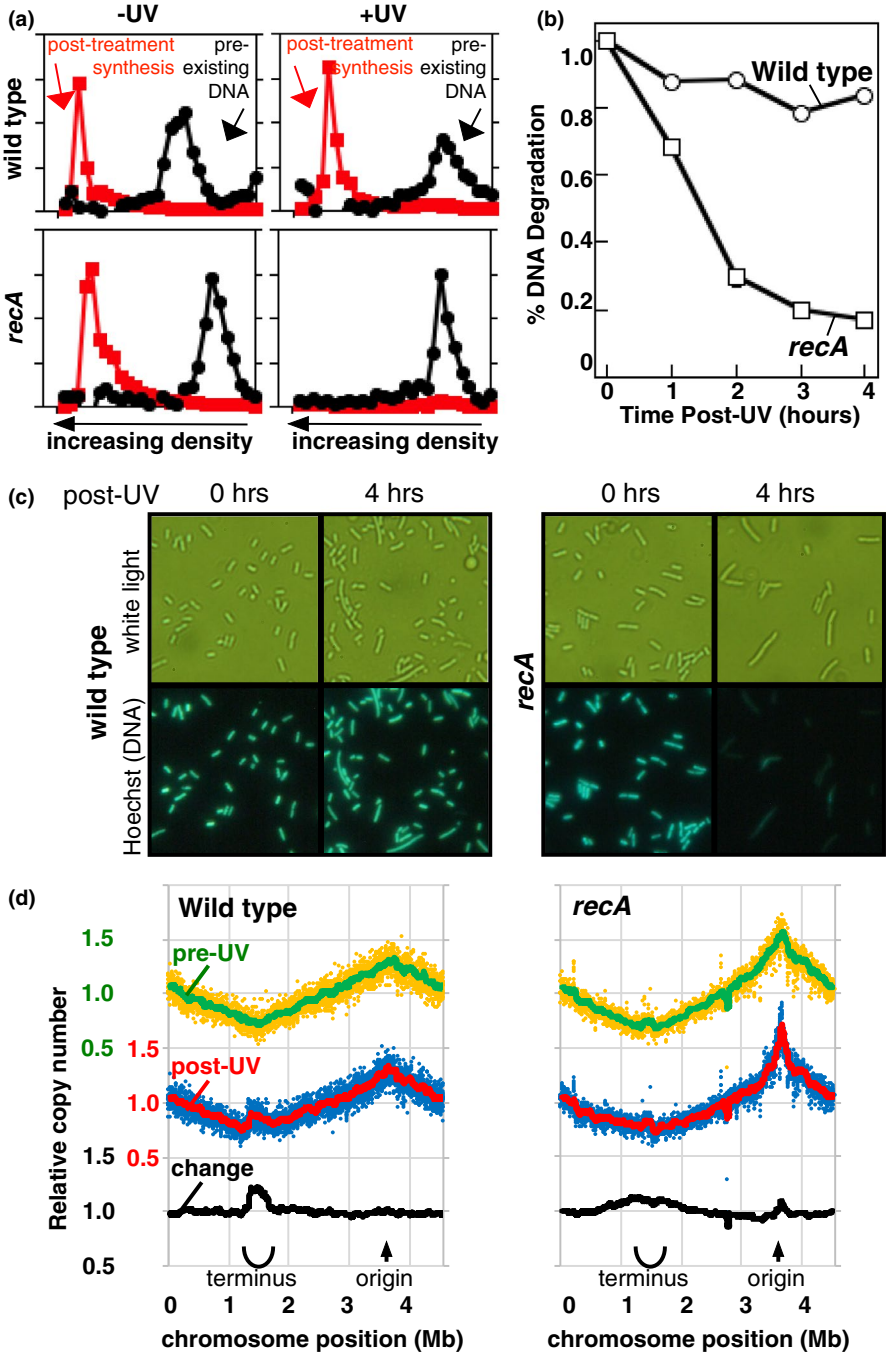

FIGURE 3 In the absence of RecA, replication fails to recover and the genome is progressively degraded at arrested replication forks. (a) *recA* mutants fail to recover replication following UV-induced DNA damage. The amount of replication occurring within 1 hr post-UV irradiation was analyzed by alkaline CsCl density gradients. Cells prelabeled with [^{14}C]thymine were irradiated or not at time 0. Cells then were filtered and grown in media containing 5-bromodeoxyuridine and [^{3}H]thymidine for 1 hr to density label replication occurring after time 0. ^{14}C-prelabeled DNA (circles); post-treatment ^{3}H-DNA synthesis (squares). Plot maximums are 2000–3500 cpm for [^{3}H]; 80–120 cpm for [^{14}C]. [^{3}H]:[^{14}C] maximums are held constant for each strain. (b) Replicating genomes are rapidly degraded in the absence of RecA. Cells prelabeled with [^{3}H]thymidine were filtered and irradiated with 40 J/m^{2} in nonlabeled medium. The fraction of [^{3}H]DNA remaining is plotted over time. Parental (open circles); *recA* (open squares). Plots represent the average of two experiments. Error bars represent one standard error of the mean. (c) *recA* mutant cells lack DNA following UV irradiation. UV-irradiated cells were stained with DNA-binding Hoescht 33342 dye and visualized under white light to observe cells, and a UV filter to observe the DNA. (d) Replication profiles of *recA* mutants before and after UV irradiation. The replication profiles prior to UV (top green), 90 min post-UV (middle red), and the change between these times (bottom black) is plotted. Wild-type data from Figure 1 are shown for comparison [Colour figure can be viewed at wileyonlinelibrary.com]

absence of these gene products affects the ability of cells to maintain the genome (Figure 2). RecA is required for replication to resume following disruption by DNA damage and is necessary to protect DNA ends from degradation (Courcelle et al., 1997; Horii & Suzuki, 1968; Khidhir et al., 1985). A culture's ability to resume replication under the conditions used for our genomic profiles was observed by density-labeling the DNA with 5-bromodeoxyuridine to quantitate the amount of DNA replicated during the first hour after UV irradiation (Figure 3a). Cultures were either left unirradiated or irradiated with 40 J/m^2 and incubated in medium containing 5-bromodeoxyuridine for 1 hr, so that any DNA synthesized during this period would be of a greater density than the pre-existing DNA made prior to treatment. The denser, replicated DNA in each culture was then separated from the rest of the DNA in isopycnic alkaline CsCl gradients and quantitated. In wild-type cells, the amount of replicated DNA in irradiated cultures was nearly equivalent to the amount occurring in unirradiated cultures, indicating that DNA replication had fully recovered by this time. However, in *recA* mutants, no further DNA synthesis was observed. The failure to restore replication, is due in significant part to a failure to protect DNA ends of the disrupted replication forks from exonucleolytic degradation (Figure 3b). Rapidly growing cultures that had been prelabeled with [^{3}H]thymidine were irradiated with 40 J/m^2 and then transferred to nonradioactive medium. In wild-type cells, very little degradation of the overall genomic DNA occurred. By contrast, in *recA* mutants, the genomic DNA undergoes a rapid, progressive degradation following disruption, losing more than 80% of the DNA present prior to irradiation. The inability to protect the genome in *recA* mutants was also observed directly by staining cells with Hoescht 33342 which fluoresces upon binding DNA. Whereas the genome in wild-type cells is clearly maintained, little DNA remains in *recA* mutants 4 hr after irradiation (Figure 3c).

We next profiled the DNA remaining in these cells and compared it to that seen in the parental cells. In parental cells at 90 min following irradiation, the DNA was maintained, replication was restored, and except at sites where replication completes, the genome copy number had been restored to that of preirradiation levels (Figure 3d). In *recA* mutants, approximately half of the genomic DNA was degraded by 90 min (Figure 3b). The profile of the DNA that remains in irradiated *recA* cells is modestly enriched in the distal, non-*oriC*-containing half of the genome relative to times before irradiation (Figure 3d). A modest and sharp enrichment also occurs directly at *oriC*. This loci-dependent enrichment pattern in *recA* cells suggests that genomic degradation initiates at disrupted replication forks and then progressively degrades back toward the origin, leaving the unreplicated portions of the genome partially protected. The observations imply that RecA plays an essential role in protecting the strands of the fork after replication disruption. In its absence, disruption of replication leads to a progressive, nearly complete degradation of the genome, and is likely to be the direct cause of lethality in these cells.

2.3 | Genomes remain static in mutants that fail to recover replication following disruption

We next examined how the genome is affected in the absence of *recF* and nucleotide excision repair, which are required to restore replication following disruption by UV-induced damage (Courcelle et al., 1997, 1999, 2003). Replicating cultures of *recF*, *uvrA* and *uvrC* were UV-irradiated and placed in 5-bromodeoxyuridine for 1 hr to density label the DNA as before. As shown in Figure 4a, all three mutants failed to restore replication, similar to *recA*. However, in contrast to *recA*, the genomic DNA in these mutants primarily remains protected and intact (Figure 4b,c). Previous studies have shown that in the absence of *recF*, some degradation occurs on the nascent lagging strand of DNA catalyzed by the combined action of RecJ and RecQ (Courcelle et al., 1997, 1999, 2003; Courcelle & Hanawalt, 1999). However, this degradation is limited to the nascent DNA and localized specifically at disrupted replication forks. The genomic DNA remains protected in *recF* mutants. In the absence of nucleotide excision repair, no degradation beyond that observed in wild-type cells is detected. Finally, whereas after 4 hr, wild-type cultures had restored cell division with many cells appearing normal in length, *recF*, *uvrA* and *uvrC* mutants remained filamentous, indicating that cell division had not been restored (compare Figure 3c and Figure 4c).

To determine how sequence copy numbers around the genome are affected by UV-induced damage in these mutants, we profiled the replication patterns of these cultures immediately before and 90 min after UV irradiation as described above (Figure 5). With the exception of the DNA immediately proximal to the origin in *recF* and *uvrA* mutants (see below), copy numbers remained unchanged, or static around the genome in each mutant. Taken together, the lack of DNA synthesis, absence of DNA degradation and unchanged replication profile implies that lethality in these cells occurs early after the initial disruption of replication and results from an inability to restore replication forks in the process of duplicating the genome.

Similar to *recA*, the profile of *recF* also shows an enrichment of DNA immediately proximal to the origin of replication. Both *recF* and *recA* fail to recover replication following disruption, but remain proficient for global DNA repair (Courcelle et al., 1999). Thus, the *oriC* copy number enrichment in *recA* and *recF* mutants may simply represent

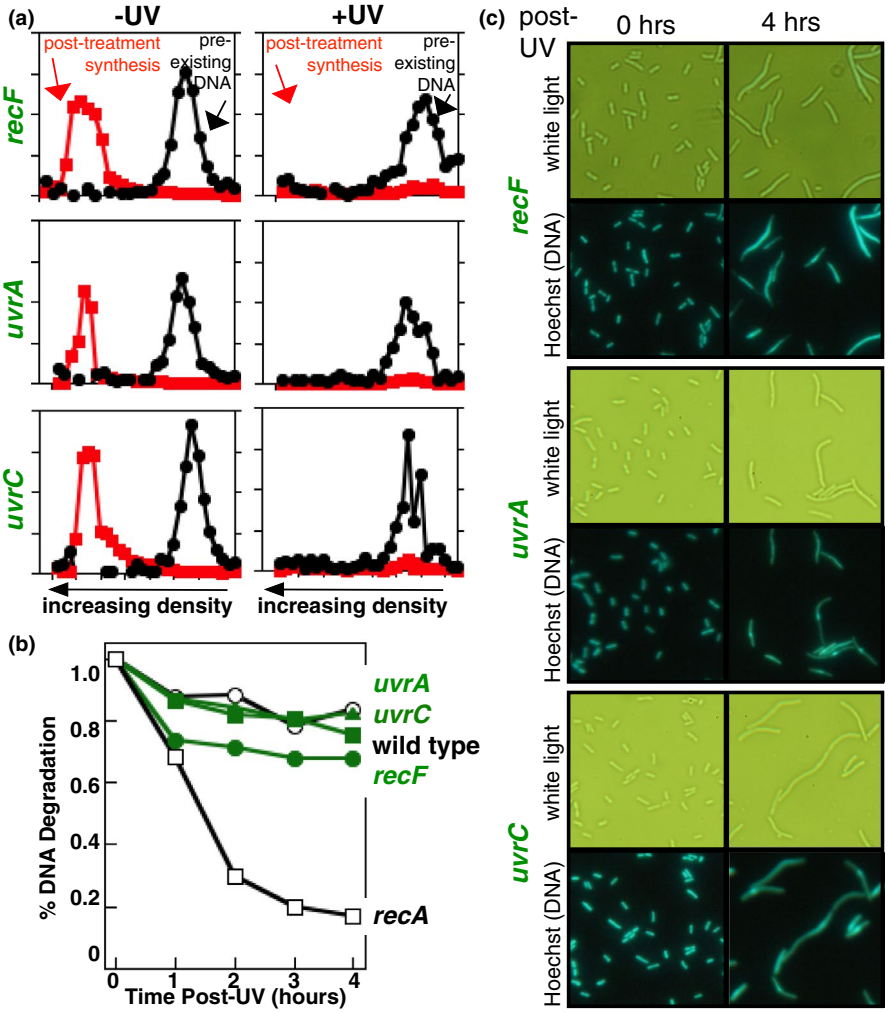

FIGURE 4 *recF* pathway and nucleotide excision repair mutants fail to resume replication following disruption by UV-induced damage, but in contrast to *recA*, do not degrade the genome. (a) *recF*, *uvrA*, and *uvrC* fail to resume replication following disruption. ^{14}C-prelabeled DNA (circles); post-treatment ^{3}H-DNA synthesis (squares). Plot maximums are 1000–2000 cpm for [^{3}H]; 100–200 cpm for [^{14}C]. [^{3}H]:[^{14}C] maximums are held constant for each strain. (b) The genome is not degraded following UV irradiation in replicating *recF*, *uvrA*, and *uvrC* cultures. Parental (open circles); *recA* (open squares); *recF* (filled circle); *uvrA* (filled squares); *uvrC* (filled triangles). Data for wild-type and *recA* mutants are reproduced from Figure 3b for the purposes of comparison and controls. (c) Cells filament, the genomic DNA remains intact, but no further cell divisions are observed in *recF*, *uvrA* or *uvrC* mutants. Replication recovery, DNA degradation, and DNA fluorescent microscopy was measured as described in Figure 3 [Colour figure can be viewed at wileyonlinelibrary.com]

new *oriC* initiations which progress but are then subsequently disrupted by the remaining UV-induced lesions. In *uvrA* or *uvrC*, where no repair occurs, these *oriC*-dependent initiations may be inhibited or are immediately disrupted and do not progress far enough to be detected. We also observed some modest degradation following UV irradiation in the immediate proximity of the origin in the *uvrA* mutant at the 40 J/m^2 dose. This loss of origin DNA was not observed in the *uvrC* mutant. The mechanism or reason for this is not clear.

To examine if the presence of the DNA lesions themselves prevented detection of new synthesis in the *oriC* region, we also examined the profile of a *uvrA* mutant following irradiation with 5 J/m^2 (Figure S3). Irradiation with 40 J/m^2 produces replication-blocking lesions every 6 kb (Courcelle et al., 2003) and prevents any detectable replication after UV irradiation. In contrast, 5 J/m^2 produces lesions, on average, at 48 kb intervals. This distance allows for significant DNA synthesis to occur before replication becomes blocked. As shown in Figure S3, at 90 min post-UV, significant replication has resumed and is detected around *oriC*, but the presence of the lesions prevents these replication forks from reaching the terminus. Importantly, no new synthesis is observed in the terminus region. If the over-replication in the terminus region was caused by new initiation events occurring, then one would expect to observe enrichment in the *oriC* and terminus regions of the *uvrA* mutant at the 5 J/m^2 dose. The absence of over-replication in the terminus region of *uvrA*

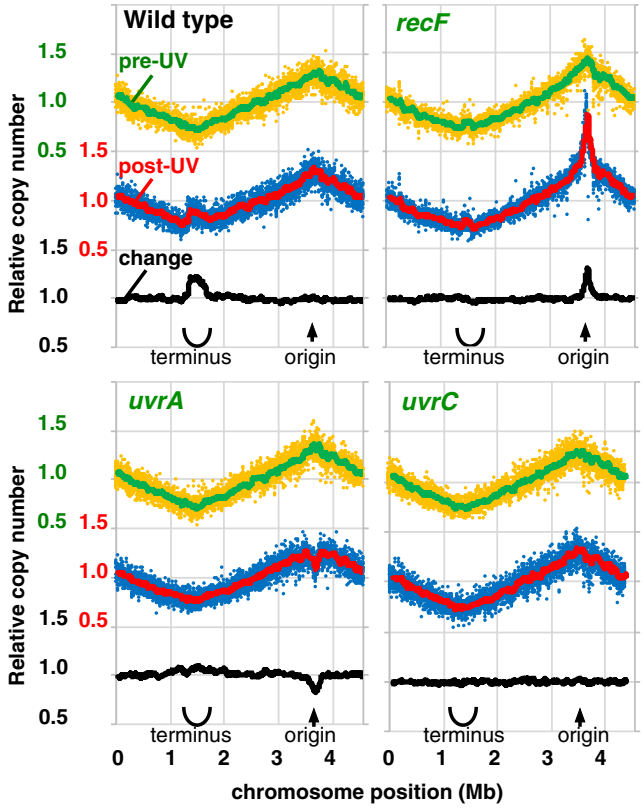

FIGURE 5 In *recF* or nucleotide excision repair mutants, the genome copy numbers remain static following disruption by UV-induced damage. Replication profiles of *recF, uvrA*, and *uvrC* mutants prior to UV (top green), 90 min post-UV (middle red), and the change between these times (bottom black) are plotted. Profiles were analyzed as in Figure 3 [Colour figure can be viewed at wileyonlinelibrary.com]

mutants, when forks initiating from *oriC* are prevented from progressing to the terminus, argues against the idea that new initiations are occurring in the terminus region, but would be consistent with models in which convergent replication forks continuing past their doubling point lead to over-replication of this region.

2.4 | Mutants with an impaired ability to complete replication accumulate and amplify the nonterminus region of the genome

Whereas RecF and nucleotide excision repair are required for replication to resume when it stops short, other gene products are required to complete or limit replication from going too far. The absence of RecBC, SbcCD, ExoI or RecG impairs the cell's ability to complete replication normally (Hamilton et al., 2019; Midgley-Smith et al., 2018; Rudolph et al., 2013; Wendel et al., 2014, 2018). Both *recBC* and *recG* are hypersensitive to UV for reasons that remain unclear. Unlike *recA*, *recF* or nucleotide excision repair mutants, *recBC*, *recG* and *sbcCD xonA* mutants recover replication after UV-induced damage, similar to wild-type cells, and the genomic DNA remains protected (Figure 6a,b). Further several studies have shown that these enzymes do not catalyze fork regression, or process replication forks after disruption (Chow & Courcelle, 2007; Courcelle et al., 1997, 2003; Courcelle & Hanawalt, 1999; Donaldson et al., 2004, 2006). Yet, irradiated *recBC* and *recG* mutants continue to filament 4 hr after irradiation (Figure 6c), despite the recovery of replication. In contrast, in *sbcCD xonA* mutants which are not hypersensitive to UV, many of the cells have restored cell division and appear to be of normal length.

Replication profiles of these mutants were examined before and after UV irradiation, as before, to determine how the copy number of sequences around the genome respond to UV damage. Each mutant displays abnormalities in the region where replication forks converge, even in the absence of DNA damage (Figure 7). *recBC* mutants fail to join the strands of convergent forks leading to extensive degradation of the DNA ends and a failure to maintain that region of the chromosome. The inability to maintain the terminus region of the genome in these mutants, severely compromises viability and growth of these cultures (Capaldo-Kimball & Barbour, 1971; Wendel et al., 2014). In contrast, both *recG* and *sbcCD xonA* mutants are impaired in their ability to resect or degrade the over-replicated regions where forks converge, leading to elevated copy numbers in this region of the genome (Figure 7), although this phenotype does not impair the growth rate or viability of these cultures (Wendel et al., 2014). At 90 min post-UV, the abnormalities in the terminus region of each of these mutants remains the same. However, copy number imbalances and amplifications accumulate throughout the remaining, nonterminus region of the genome. We would interpret this to imply that the recovery of both ongoing replication forks and new initiations from *oriC* that occur following repair, leads to a bottleneck at sites where completion occurs in these mutants. The amplification of the nonterminus region of the genome is most severe in *recBC* mutants, where a fourfold increase in copy number, peaking at the origin, is observed 90 min after UV irradiation. The inability to maintain the region where replication forks converge is also more severely impaired in irradiated *recBC* cells than either of the other two mutants examined. Under normal conditions, *recG* mutants severely over-replicate and amplify the terminus region where forks converge, an observation that is thought to relate to its ability to disrupt illegitimate priming events when polymerases disengage or are disrupted (Dimude et al., 2016; Midgley-Smith et al., 2018; Rudolph, Upton, Harris, et al., 2009; Rudolph et al., 2009; Wendel et al., 2014). Curiously this defect is not exaggerated or worsened by UV, suggesting that the amplifications in the terminus do not directly cause lethality in UV-irradiated *recG* cultures. However, similar to *recBC* mutants, the nonterminus region of the genome becomes amplified at 90 min after UV irradiation in *recG* cells (Figure 7). Taken together, the

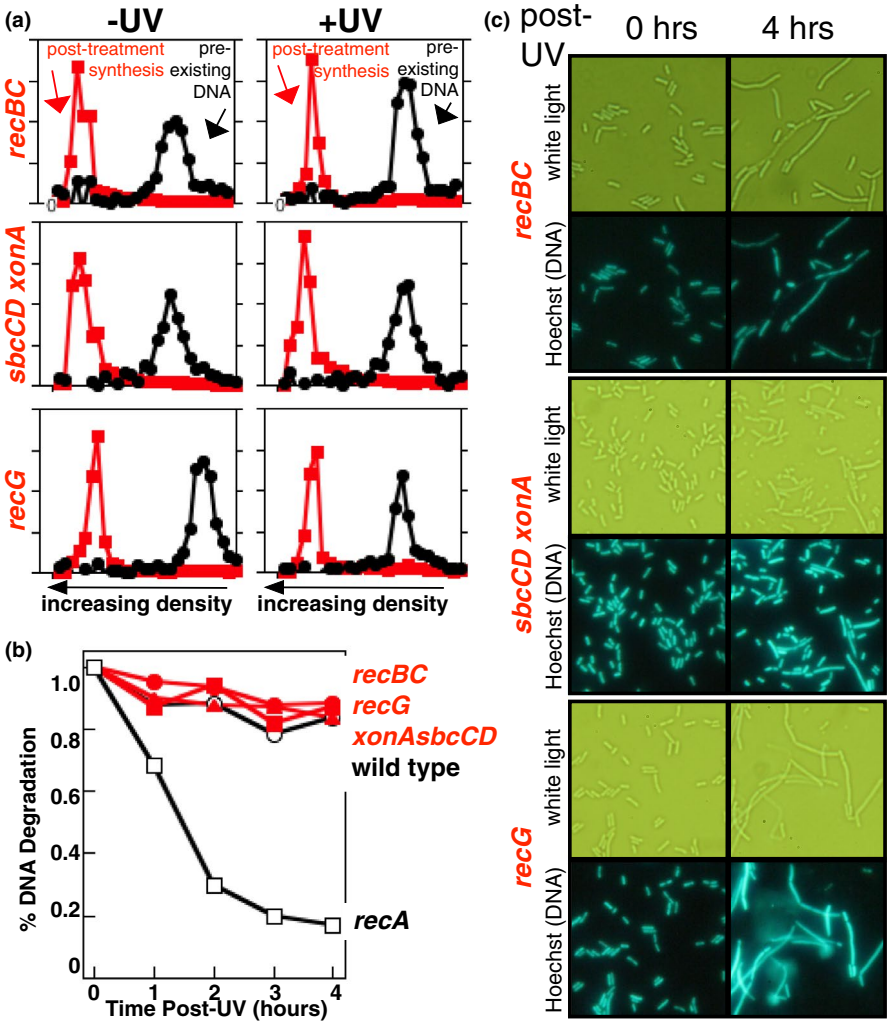

FIGURE 6 *recBC*, *sbcCD xonA* and *recG* mutants resume replication normally following UV-induced DNA damage, but *recBC* and *recG* fail to segregate or partition the noncompleted chromosomes properly upon division. (a) *recBC*, *sbcCD xonA* and *recG* resume replication normally following disruption. ^{14}C-prelabeled DNA (circles); post-treatment ^{3}H-DNA synthesis (squares). Plot maximums are 1500–3200 cpm for [^{3}H]; 100–120 cpm for [^{14}C]. [^{3}H]:[^{14}C] maximums are held constant for each strain. (b) The genome is not degraded following UV irradiation in replicating *recBC*, *sbcCD xonA*, or *recG* cultures. Parental (open circles); *recA* (open squares); *recBC* (filled circle); *sbcCD xonA* (filled squares); *recG* (filled triangles). Data for wild-type and *recA* mutants are reproduced from Figure 3b for the purposes of comparison and controls. (c) *recBC* and *recG* fail to segregate or partition the noncompleted chromosomes properly upon division. Replication recovery, DNA degradation and DNA fluorescent microscopy was measured as described in Figure 3 [Colour figure can be viewed at wileyonlinelibrary.com]

observations suggest that the hypersensitivity of these mutants results from a delayed death that occurs following the mis-segregation of incomplete or unresolved chromosomes when cells divide.

The copy numbers of nonterminus regions are also amplified in *sbcCD xonA* mutants similar to those occurring in *recG*. However, in the case of *sbcCD xonA* mutants, this phenotype is not associated with increased lethality, as cells recover and resume cell division (Figure 6c). In the absence of SbcCD and ExoI completion occurs through an aberrant pathway that is associated with amplifications and genomic instability, but retains viability (Hamilton et al., 2019; Wendel et al., 2018), perhaps accounting for the difference between this mutant and the UV-hypersensitive *recG* and *recBC*. Consistent with this interpretation, whereas *recBC* and *recG* mutant cells remained filamentous for more than 4 hr after irradiation, *sbcCD xonA* mutants appeared filamentous at two hours after irradiation, but returned to normal by 4 hr after irradiation, suggesting that the chromosomal imbalances in this mutant can be resolved in a manner that allows survival (Figure S4). Thus, common to all three mutants having a compromised ability to complete replication results in the amplification of the nonterminus region of the genome after UV irradiation.

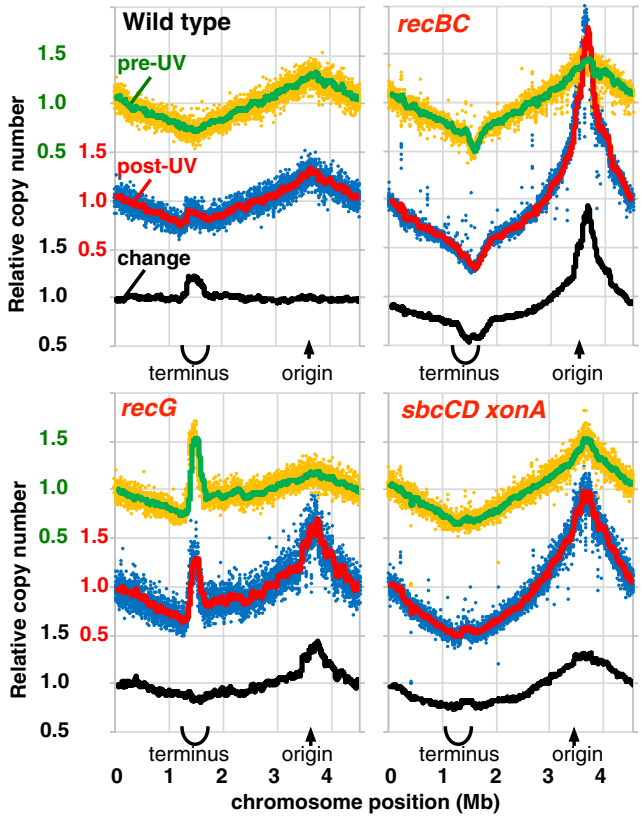

FIGURE 7 Following disruption by UV-induced damage, mutants impaired for their ability to complete replication exhibit copy number imbalances and amplifications in nonterminus regions of the genome. Replication profiles of *recBC*, *sbcCD xonA* and *recG* mutants prior to UV (top green), 90 min post-UV (middle red), and the change between these times (bottom black) are plotted. Profiles were analyzed as in Figure 3 [Colour figure can be viewed at wileyonlinelibrary.com]

3 | DISCUSSION

Here we show that following the disruption of replication by UV-induced DNA damage, the copy number of sequences around the genome initially remains static during the period that correlates with when lesion repair occurs. Following repair and the recovery of ongoing forks, we also observed that new *oriC*-dependent initiations resume, despite the delayed progression of forks initiated prior to DNA damage. This leads to an overall enrichment of the genomic region surrounding the origin and suggests coordination between replication initiation and elongation becomes uncoupled following DNA damage. Using a microscopic approach that employed fluorescent probes to the origin and terminus regions, the Rudolph group similarly found that UV-irradiated cultures continue to initiate from *oriC* in a DnaA-dependent manner, enriching this region of the genome (Rudolph et al., 2007). Quiñones reported that *dnaA*, encoding the *oriC*-specific initiation protein, is up-regulated following DNA damage induced by methyl methanesulfonate and mitomycin C (Quiñones et al., 1991), and several groups have noted that overproduction of DnaA, by itself, is sufficient to induce new *oriC* initiation events (Atlung et al., 1987; Skarstad et al., 1989; Xu & Bremer, 1988). Finally, we observed a transient induction of *dnaA* after UV that correlates with the timing of copy number enrichment surrounding the origin region. Taken together, the observations would be consistent with the idea that *oriC*-dependent initiations continue or are up-regulated following DNA damage. However, we do not exclude the possibility that the initiations originating from *oriC* region are independent of DnaA, as has been demonstrated to occur during inducible stable DNA replication (Magee et al., 1992), discussed below.

At late times during the recovery period, an over-replication of the chromosome region occurs where forks converge. We speculate that the combined recovery of ongoing replication forks and new initiations at *oriC* lead to "congestion" of replisomes at the point of completion, and may stress or overtax the capacity of these enzymes to complete and join converging forks. Previous studies found that the nuclease activity associated with RecBCD, the central enzyme necessary for completing replication, is diminished in UV-irradiated cultures (Thoms & Wackernagel, 1998). Further, the enzyme complex is expressed at low levels, similar to the replisome, has limited turnover and is inactivated following encounters with Chi sequences, which regulate its nuclease and likely other activities (Amundsen et al., 1990; Dixon et al., 1994; Taylor & Smith, 1999; Thoms & Wackernagel, 1998). Plasmids replicated by two replisomes, but not one replisome, over-replicate leading to plasmid instability and loss, consistent with the idea that cells have a limited capacity to enzymatically resolve events where two replisomes converge, and that the excess events created by these moderate copy number plasmids stress or exceed this capacity of the cell to complete replication events (Hamilton et al., 2019).

Similar to the results we report here, a large body of work from Tokio Kogoma's group also observed replication-associated copy number enrichments in the *oriC* and terminus regions of cells after inducing the SOS response through various forms of DNA damage or stress and then adding rifampicin or chloramphenicol to inhibit transcription or protein synthesis, respectively (Magee et al., 1992). They termed this form of synthesis inducible stable DNA replication (iSDR) ([Kogoma & Lark, 1970; Magee et al., 1992; Magee & Kogoma, 1990] and reviewed in [Kogoma, 1997]). Under normal conditions, protein synthesis is required to initiate new rounds of replication. Thus, in the presence of rifampicin or chloramphenicol, replication ceases as the ongoing rounds of replication are completed. However during iSDR, replication would continue for several hours with elevated copy numbers observed at the

origin and terminus, termed *oriM1* and *oriM2* (Kogoma & Lark, 1970; Magee et al., 1992; Magee & Kogoma, 1990). The replication and enrichment surrounding *oriM1* at the origin was shown to be independent of DnaA (Cieśla & Jonczyk, 1980; Magee et al., 1992) and suggest that the UV-induced up-regulation of DnaA we observe is not essential for this enrichment to occur. Replication and enrichment at *oriM2* in the terminus was speculated to be a cryptic origin (Magee et al., 1992; de Massy et al., 1984). However, it is also reasonable to consider that this "origin" arises when replication continues through the terminus without completing. iSDR and the over-replication we observe depend on RecA, and both events would appear identical when analyzed by copy number analysis. Consistent with the idea that impaired completion leads to the over-replication in the terminus, we show that over-replication is observed at the origin but not the terminus in *uvrA* mutants irradiated with low doses that allow replication to progress some distance, but still prevent the forks from reaching or converging at the terminus. If the over-replication represented a new origin, one would predict that enrichments would be observed at both the origin and terminus under these conditions.

Several mutations that inactivate exonucleases or helicases also exhibit an over-replication phenotype in the region where forks converge. Curiously, over-replication by itself, does not impair cell viability or growth rates. *recG* helicase mutants, as well as combinations of *xonA*, *sbcCD*, *xseA*, *recJ* and *recD* exonuclease mutants, each demonstrate persistent over-replication in their terminus region, but grow normally (Midgley-Smith et al., 2018, 2019; Rudolph et al., 2013; Wendel et al., 2014, 2018), although the reason for this remains unclear. By contrast, the ability to join the DNA ends of the convergent forks does correlate with the ability to maintain viability and growth. Mutants lacking RecBCD, which is necessary for joining the strands of convergent forks, are unable to maintain these regions of the chromosome and are severely impaired for growth and viability (Courcelle et al., 2015; Wendel et al., 2014). Similarly, mutants lacking SbcCD and ExoI fail to initiate the normal completion reaction, and in these mutants, the ability to maintain the terminus region and viability depends upon RecA and illegitimate recombination to resolve the structures created when forks converge (Wendel et al., 2018).

The replication profiles employed here were useful for characterizing the cause of lethality in mutants following DNA damage. In *recA, recF* or nucleotide excision repair mutants, which are impaired in their ability to resume replication, the genome either degrades or remains static, suggesting that cell death occurs when replication is initially disrupted by the lesion. In *recBC*, *sbcCD xonA* or *recG* mutants, which are impaired in completing replication, the recovery of both ongoing replication forks and new *oriC*-dependent initiations leads to a bottleneck that creates copy number imbalances and amplifications throughout the nonterminus region of the genome. The UV hypersensitivity of both RecBC and RecG mutants is frequently speculated to be due to defective processing of the disrupted replication forks, either to degrade regressed forks and re-establish replication, or promote their formation, respectively. However, there is extensive evidence demonstrating that these proteins do not function in this capacity. Unlike *recJ* or *recQ* mutants, the absence of RecBCD does not affect the degradation or processing that occurs at the replication fork following disruption (Courcelle & Hanawalt, 1999; Donaldson et al., 2004), arguing that these enzymes do not have access to the replication fork substrates in vivo. In addition, the long-standing observations that RecBCD mutants resume replication similar to wild-type cells after DNA damage strongly argues that its hypersensitivity is not due to a failure to re-establish replication ([Courcelle et al., 1997; Khidhir et al., 1985; Witkin et al., 1987] and Figure 6). Similarly, the absence of RecG does not prevent the progression or regression of disrupted replication forks, and replication resumes normally in its absence, arguing strongly against the idea that its hypersensitivity results from an essential role in regressing and restarting replication forks disrupted by UV (Donaldson et al., 2004, 2006 and Figure 6). By contrast, both of these mutants have copy number abnormalities that localize specifically, and only, at the site on the chromosome where replication forks converge, demonstrating that RecBCD and RecG function at this position and are required for replication to complete normally. As we show here, following UV-induced DNA damage, the defective processing of these completion events leads to the accumulation and amplification of the nonterminus region of the genome. We propose that the recovery of elongation and continuing initiations from *oriC* lead to a bottleneck in the terminus of these mutants, leading to a delayed death that occurs following the mis-segregation of incomplete and unresolved chromosomes when cells divide.

4 | EXPERIMENTAL PROCEDURES

4.1 | Bacteria

SR108 is a thymine-requiring (*thyA deoC*) derivative of W3110 (Mellon & Hanawalt, 1989). HL921 (SR108 del(*srlR-recA*)306::*Tn*10), HL922 (SR108 *recB21C22 argA81*::*Tn*10), CL579 (SR108 *recF6206*::*tet857*), CL008 (SR108 *recG258*::*Tn*5), CL578 (SR108 *ruvAB6204*::*kan*), CL2357 (SR108 *xonA*::*cat300 sbcCD*::*gm*), HL972 (SR108 *uvrA6 zjd*::*Tn*5), and HL925 (SR108 *uvrC*::*Tn*10) have been described previously (Courcelle et al., 1997, 1999, 2003; Donaldson et al., 2004; Wendel et al., 2014).

4.2 | Qualitative survival following UV irradiation

A fresh overnight culture was evenly applied onto a Luria-Bertani medium agar plate with a cotton swab. The plate was covered by a sheet of aluminum foil and placed under a 15-W germicidal lamp (254 nm; 0.8 J/m^2/s). The foil was progressively retracted following 20 J/m^2 exposures, and then incubated at 37°C overnight before being photographed.

4.3 | Density labeling of replicated DNA

20-ml cultures were grown in Davis medium (Davis, 1949), supplemented with 0.4% glucose, 0.2% casamino acids, and 10 μg/ml thymine (DGCthy medium), containing 0.2 μCi of [^{14}C]thymine per ml to an OD_{600} of between 0.4 and 0.5, before cells were harvested by filtration and resuspended in DGCthy medium containing 10 μg/ml 5-bromodeoxyuridine. Half of the culture was UV-irradiated with 40 J/m^2. Both halves received 0.1 μCi/ml [^{3}H]thymidine and were then incubated for 1 hr. Each culture was placed in an equal volume of ice-cold NET buffer (100 mmol/L NaCl, 10 mmol/L Tris [pH 8.0], 10 mmol/L EDTA), pelleted, and lysed in 0.4 ml of 0.5 mol/L K_3PO_4 (pH 12.5) containing 40μl of 10% Sarkosyl. The solution was then subjected to isopycnic alkaline CsCl gradient sedimentation as described previously (Smith et al., 1981). Approximately thirty fractions were collected by puncturing the tube and dripping onto Whatman no. 17 paper. The amounts of ^{14}C and ^{3}H in each fraction were determined by scintillation counting.

4.4 | DNA degradation following UV irradiation

Cells were grown in DGCthy medium containing 0.2 μCi/ml [^{3}H]thymidine to an OD600 of 0.4, before being harvested by filtration, resuspended in nonradioactive DGCthy medium and irradiated with a dose of 40 J/m^2. The amounts of ^{3}H remaining in the DNA were measured by averaging duplicate 0.2-ml samples precipitated in 5% cold trichloroacetic acid, filtered onto Whatman glass fiber filters and determined by scintillation counting.

4.5 | Replication profiling

Cells were grown in DGCthy medium to an OD600 of 0.4 and irradiated with a dose of 40 J/m^2. At the times indicated, genomic DNA was purified by placing 0.75-mL of each culture into 0.75-mL cold 2 × NET (100 mmol/L NaCl, 10 mmol/L Tris at pH 8.0, 10 mmol/L EDTA). Each sample was pelleted, resuspended in 140 μl of 1 mg/ml lysozyme and 0.2 mg/ml RNaseA in TE (10 mmol/L Tris at pH 8.0, 1 mmol/L 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 volumes phenol/chloroform (1/1) and dialyzed for 30 min on 47 mm Whatman 0.05-μm pore disks (#VMWP04700; Whatman) floating on a 250-ml beaker of TE (1 mmol/L Tris at pH 8.0, 1 mmol/L EDTA). 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 (Barrick and Meyer, 2014). The original Illumina sequence reads for all subsequent strains were then aligned using Bowtie 1.0.0 (Langmead, 2010), 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 a custom Python script. Copy number values were normalized to those of a stationary-phase culture, grown for 48 hr, to eliminate biases associated with genomic DNA purification and sequencing. These relative copy number values were then plotted against their location along the genome to generate replication profiles for each strain or time point.

4.6 | Microscopy

Cells were grown in DGCthy medium to an OD_{600} of 0.4 and UV-irradiated with a dose of 40 J/m^2. At the times indicated, 1 ml of cells were pelleted and resuspended in 0.5 ml of distilled water followed by the addition of 0.5 ml ethanol. Cells were then pelleted and resuspended in 0.5 ml 12 μg/ml Hoescht 33342 dye. Slides were prepared by allowing 100 μl of melted 1% agarose to solidify between two slides to create an agar pad. 10 μl of the cell suspension was then placed onto the agar pad with a coverslip and imaged at 40X magnification using an Epi-Fluorescence Microscope (Model M837FLR) fitted with a UV-V filter assembly (A19UV). Images were taken using a Canon EOS Rebel T6 camera fitted with a 2X microscope adaptor (AmScope), using the associated Canon EOS utility software. All fluorescent images were taken at ISO3200 and 0.5 s exposures.

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REFERENCES

Amundsen, S. K., Neiman, A. M., Thibodeaux, S. M., & Smith, G. R. (1990). Genetic dissection of the biochemical activities of RecBCD enzyme. *Genetics*, *126*, 25–40.

Asai, T., & Kogoma, T. (1994). D-loops and R-loops: Alternative mechanisms for the initiation of chromosome replication in Escherichia coli. *Journal of Bacteriology*, *176*, 1807–1812. https://doi.org/10.1128/JB.176.7.1807-1812.1994

Atlung, T., Løbner-Olesen, A., & Hansen, F. G. (1987). Overproduction of DnaA protein stimulates initiation of chromosome and minichromosome replication in Escherichia coli. *Molecular and General Genetics*, *206*, 51–59. https://doi.org/10.1007/BF00326535

Barrick, J. E., Colburn, G., Deatherage, D. E., Traverse, C. C., Strand, M. D., Borges, J. J., Knoester, D. B., Reba, A., & Meyer, A. G. (2014). Identifying structural variation in haploid microbial genomes from short-read resequencing data using breseq. *BMC Genomics*, *15*, 1039. https://doi.org/10.1186/1471-2164-15-1039

Bhatia, V., Barroso, S. I., Garcia-Rubio, M. L., Tumini, E., Herrera-Moyano, E., & Aguilera, A. (2014). BRCA2 prevents R-loop accumulation and associates with TREX-2 mRNA export factor PCID2. *Nature*, *511*, 362–365. https://doi.org/10.1038/nature13374

Capaldo-Kimball, F., & Barbour, S. D. (1971). Involvement of recombination genes in growth and viability of Escherichia coli K-12. *Journal of Bacteriology*, *106*, 204–212. https://doi.org/10.1128/JB.106.1.204-212.1971

Chalker, A. F., Leach, D. R., & Lloyd, R. G. (1988). Escherichia coli sbcC mutants permit stable propagation of DNA replicons containing a long palindrome. *Gene*, *71*, 201–205. https://doi.org/10.1016/0378-1119(88)90092-3

Chow, K. H., & Courcelle, J. (2004). RecO Acts with RecF and RecR to Protect and maintain replication forks blocked by UV-induced DNA damage in Escherichia coli. *Journal of Biological Chemistry*, *279*, 3492–3496.

Chow, K. H., & Courcelle, J. (2007). RecBCD and RecJ/RecQ initiate DNA degradation on distinct substrates in UV-irradiated Escherichia coli. *Radiation Research*, *168*, 499–506. https://doi.org/10.1667/RR1033.1

Cieśla, Z., & Jonczyk, P. (1980). The dnaA gene product is not required during stable chromosome replication in Escherichia coli. *Molecular and General Genetics*, *180*, 617–620. https://doi.org/10.1007/BF00268069

Connelly, J. C., De Leau, E. S., & Leach, D. R. (1999). DNA cleavage and degradation by the SbcCD protein complex from Escherichia coli. *Nucleic Acids Research*, *27*, 1039–1046. https://doi.org/10.1093/nar/27.4.1039

Costa, A., Hood, I. V., & Berger, J. M. (2013). Mechanisms for initiating cellular DNA replication. *Annual Review of Biochemistry*, *82*, 25–54. https://doi.org/10.1146/annurev-biochem-052610-094414

Courcelle, C. T., Belle, J. J., & Courcelle, J. (2005). Nucleotide excision repair or polymerase V-mediated lesion bypass can act to restore UV-arrested replication forks in Escherichia coli. *Journal of Bacteriology*, *187*, 6953–6961. https://doi.org/10.1128/JB.187.20.6953-6961.2005

Courcelle, J., Carswell-Crumpton, C., & Hanawalt, P. C. (1997). recF and recR are required for the resumption of replication at DNA replication forks in Escherichia coli. *Proceedings of the National Academy of Sciences of the USA*, *94*, 3714–3719. https://doi.org/10.1073/pnas.94.8.3714

Courcelle, J., Crowley, D. J., & Hanawalt, P. C. (1999). Recovery of DNA replication in UV-irradiated Escherichia coli requires both excision repair and recF protein function. *Journal of Bacteriology*, *181*, 916–922. https://doi.org/10.1128/JB.181.3.916-922.1999

Courcelle, J., Donaldson, J. R., Chow, K. H., & Courcelle, C. T. (2003). DNA damage-induced replication fork regression and processing in Escherichia coli. *Science*, *299*, 1064–1067. https://doi.org/10.1126/science.1081328

Courcelle, J., Ganesan, A. K., & Hanawalt, P. C. (2001). Therefore, what are recombination proteins there for? *BioEssays*, *23*, 463–470. https://doi.org/10.1002/bies.1065

Courcelle, J., & Hanawalt, P. C. (1999). RecQ and RecJ process blocked replication forks prior to the resumption of replication in UV-irradiated Escherichia coli. *Molecular and General Genetics*, *262*, 543–551. https://doi.org/10.1007/s004380051116

Courcelle, J., Wendel, B. M., Livingstone, D. D., & Courcelle, C. T. (2015). RecBCD is required to complete chromosomal replication: Implications for double-strand break frequencies and repair mechanisms. *DNA Repair (Amst)*, *32*, 86–95. https://doi.org/10.1016/j.dnarep.2015.04.018

Davis, B. D. (1949). The isolation of biochemically deficient mutants of bacteria by means of penicillin. *Proceedings of the National Academy of Sciences of the USA*, *35*, 1–10. https://doi.org/10.1073/pnas.35.1.1

De Massy, B., Fayet, O., & Kogoma, T. (1984). Multiple origin usage for DNA replication in sdrA(rnh) mutants of Escherichia coli K-12. Initiation in the absence of oriC. *Journal of Molecular Biology*, *178*, 227–236. https://doi.org/10.1016/0022-2836(84)90141-4

Dimude, J. U., Midgley-Smith, S. L., Stein, M., & Rudolph, C. J. (2016). Replication termination: Containing fork fusion-mediated pathologies in Escherichia coli. *Genes (Basel)*, *7*(8), 40. https://doi.org/10.3390/genes7080040

Dixon, D. A., Churchill, J. J., & Kowalczykowski, S. C. (1994). Reversible inactivation of the Escherichia coli RecBCD enzyme by the recombination hotspot chi in vitro: Evidence for functional inactivation or loss of the RecD subunit. *Proceedings of the National Academy of Sciences of the USA*, *91*, 2980–2984. https://doi.org/10.1073/pnas.91.8.2980

Donaldson, J. R., Courcelle, C. T., & Courcelle, J. (2004). RuvAB and RecG are not essential for the recovery of DNA synthesis following UV-induced DNA damage in Escherichia coli. *Genetics*, *166*, 1631–1640. https://doi.org/10.1534/genetics.166.4.1631

Donaldson, J. R., Courcelle, C. T., & Courcelle, J. (2006). RuvABC is required to resolve holliday junctions that accumulate following replication on damaged templates in Escherichia coli. *Journal of Biological Chemistry*, *281*, 28811–28821.

Donnianni, R. A., & Symington, L. S. (2013). Break-induced replication occurs by conservative DNA synthesis. *Proceedings of the National Academy of Sciences of the USA*, *110*, 13475–13480. https://doi.org/10.1073/pnas.1309800110

Hamilton, N. A., Wendel, B. M., Weber, E. A., Courcelle, C. T., & Courcelle, J. (2019). RecBCD, SbcCD and ExoI process a substrate created by convergent replisomes to complete DNA replication. *Molecular Microbiology*, *111*, 1638–1651. https://doi.org/10.1111/mmi.14242

Hamperl, S., & Cimprich, K. A. (2014). The contribution of co-transcriptional RNA:DNA hybrid structures to DNA damage and

genome instability. *DNA Repair (Amst)*, *19*, 84–94. https://doi.org/10.1016/j.dnarep.2014.03.023

Hill, T. M., & Marians, K. J. (1990). Escherichia coli Tus protein acts to arrest the progression of DNA replication forks in vitro. *Proceedings of the National Academy of Sciences of the USA*, *87*, 2481–2485. https://doi.org/10.1073/pnas.87.7.2481

Hill, T. M., Tecklenburg, M. L., Pelletier, A. J., & Kuempel, P. L. (1989). tus, the trans-acting gene required for termination of DNA replication in Escherichia coli, encodes a DNA-binding protein. *Proceedings of the National Academy of Sciences of the USA*, *86*, 1593–1597. https://doi.org/10.1073/pnas.86.5.1593

Hong, X., Cadwell, G. W., & Kogoma, T. (1995). Escherichia coli RecG and RecA proteins in R-loop formation. *EMBO Journal*, *14*, 2385–2392. https://doi.org/10.1002/j.1460-2075.1995.tb07233.x

Horii, Z. I., & Suzuki, K. (1968). Degradation of the DNA of Escherichia coli K12 rec- (JC1569b) after irradiation with ultraviolet light. *Photochemistry and Photobiology*, *8*, 93–105.

Howard-Flanders, P., Theriot, L., & Stedeford, J. B. (1969). Some properties of excision-defective recombination-deficient mutants of Escherichia coli K-12. *Journal of Bacteriology*, *97*, 1134–1141. https://doi.org/10.1128/JB.97.3.1134-1141.1969

Kaguni, J. M., Fuller, R. S., & Kornberg, A. (1982). Enzymatic replication of E. coli chromosomal origin is bidirectional. *Nature*, *296*, 623–627. https://doi.org/10.1038/296623a0

Katayama, T. (2017). Initiation of DNA Replication at the Chromosomal Origin of E. coli, oriC. In H. Masai & M. Fioani (Eds.), *DNA Replication: From Old Principles to New Discoveries*, (pp. 79–98). Singapore: Springer Nature.

Kelman, Z., & O'Donnell, M. (1995). DNA polymerase III holoenzyme: Structure and function of a chromosomal replicating machine. *Annual Review of Biochemistry*, *64*, 171–200. https://doi.org/10.1146/annurev.bi.64.070195.001131

Khidhir, M. A., Casaregola, S., & Holland, I. B. (1985). Mechanism of transient inhibition of DNA synthesis in ultraviolet-irradiated E. coli: Inhibition is independent of recA whilst recovery requires RecA protein itself and an additional, inducible SOS function. *Molecular and General Genetics*, *199*, 133–140. https://doi.org/10.1007/BF00327522

Kobayashi, T., Hidaka, M., & Horiuchi, T. (1989). Evidence of a ter specific binding protein essential for the termination reaction of DNA replication in Escherichia coli. *EMBO Journal*, *8*, 2435–2441. https://doi.org/10.1002/j.1460-2075.1989.tb08374.x

Koehler, D. R., Courcelle, J., & Hanawalt, P. C. (1996). Kinetics of pyrimidine(6–4)pyrimidone photoproduct repair in Escherichia coli. *Journal of Bacteriology*, *178*, 1347–1350. https://doi.org/10.1128/JB.178.5.1347-1350.1996

Kogoma, T. (1997). Stable DNA replication: Interplay between DNA replication, homologous recombination, and transcription. *Microbiology and Molecular Biology Reviews*, *61*, 212–238. https://doi.org/10.1128/.61.2.212-238.1997

Kogoma, T., & Lark, K. G. (1970). DNA replication in Escherihia coli: Replication in absence of protein synthesis after replication inhibition. *Journal of Molecular Biology*, *52*, 143–164. https://doi.org/10.1016/0022-2836(70)90022-7

Langmead, B. (2010). Aligning short sequencing reads with Bowtie. *Current Protocols in Bioinformatics*, *11*, Unit 11.7.1–11.7.14. https://doi.org/10.1002/0471250953.bi1107s32

Lehman, I. R., & Nussbaum, A. L. (1964). The deoxyribonucleases of Escherichia coli. V. On the specificity of exonuclease I (phosphodiesterase). *Journal of Biological Chemistry*, *239*, 2628–2636.

Magee, T. R., Asai, T., Malka, D., & Kogoma, T. (1992). DNA damage-inducible origins of DNA replication in Escherichia coli. *EMBO Journal*, *11*, 4219–4225. https://doi.org/10.1002/j.1460-2075.1992.tb05516.x

Magee, T. R., & Kogoma, T. (1990). Requirement of RecBC enzyme and an elevated level of activated RecA for induced stable DNA replication in Escherichia coli. *Journal of Bacteriology*, *172*, 1834–1839. https://doi.org/10.1128/JB.172.4.1834-1839.1990

Meijer, M., Beck, E., Hansen, F. G., Bergmans, H. E., Messer, W., Von Meyenburg, K., & Schaller, H. (1979). Nucleotide sequence of the origin of replication of the Escherichia coli K-12 chromosome. *Proceedings of the National Academy of Sciences of the USA*, *76*, 580–584. https://doi.org/10.1073/pnas.76.2.580

Mellon, I., & Hanawalt, P. C. (1989). Induction of the Escherichia coli lactose operon selectively increases repair of its transcribed DNA strand. *Nature*, *342*, 95–98. https://doi.org/10.1038/342095a0

Midgley-Smith, S. L., Dimude, J. U., & Rudolph, C. J. (2019). A role for 3' exonucleases at the final stages of chromosome duplication in Escherichia coli. *Nucleic Acids Research*, *47*, 1847–1860. https://doi.org/10.1093/nar/gky1253

Midgley-Smith, S. L., Dimude, J. U., Taylor, T., Forrester, N. M., Upton, A. L., Lloyd, R. G., & Rudolph, C. J. (2018). Chromosomal over-replication in Escherichia coli recG cells is triggered by replication fork fusion and amplified if replichore symmetry is disturbed. *Nucleic Acids Research*, *46*, 7701–7715. https://doi.org/10.1093/nar/gky566

Phillips, G. J., Prasher, D. C., & Kushner, S. R. (1988). Physical and biochemical characterization of cloned sbcB and xonA mutations from Escherichia coli K-12. *Journal of Bacteriology*, *170*, 2089–2094. https://doi.org/10.1128/JB.170.5.2089-2094.1988

Quiñones, A., Jüterbock, W. R., & Messer, W. (1991). Expression of the dnaA gene of Escherichia coli is inducible by DNA damage. *Molecular and General Genetics*, *227*, 9–16. https://doi.org/10.1007/BF00260699

Roecklein, B., Pelletier, A., & Kuempel, P. (1991). The tus gene of Escherichia coli: Autoregulation, analysis of flanking sequences and identification of a complementary system in Salmonella typhimurium. *Research in Microbiology*, *142*, 169–175. https://doi.org/10.1016/0923-2508(91)90026-7

Rothman, R. H., & Clark, A. J. (1977). The dependence of postreplication repair on uvrB in a recF mutant of Escherichia coli K-12. *Molecular and General Genetics*, *155*, 279–286. https://doi.org/10.1007/BF00272806

Rothman, R. H., Kato, T., & Clark, A. J. (1975). The beginning of an investigation of the role of recF in the pathways of metabolism of ultraviolet-irradiated DNA in Escherichia coli. *Basic Life Sciences*, *5A*, 283–291.

Rudolph, C. J., Mahdi, A. A., Upton, A. L., & Lloyd, R. G. (2010). RecG protein and single-strand DNA exonucleases avoid cell lethality associated with PriA helicase activity in Escherichia coli. *Genetics*, *186*, 473–492. https://doi.org/10.1534/genetics.110.120691

Rudolph, C. J., Upton, A. L., Harris, L., & Lloyd, R. G. (2009). Pathological replication in cells lacking RecG DNA translocase. *Molecular Microbiology*, *73*, 352–366. https://doi.org/10.1111/j.1365-2958.2009.06773.x

Rudolph, C. J., Upton, A. L., & Lloyd, R. G. (2007). Replication fork stalling and cell cycle arrest in UV-irradiated Escherichia coli. *Genes & Development*, *21*, 668–681. https://doi.org/10.1101/gad.417607

Rudolph, C. J., Upton, A. L., & Lloyd, R. G. (2009). Replication fork collisions cause pathological chromosomal amplification in cells

lacking RecG DNA translocase. *Molecular Microbiology*, *74*, 940–955. https://doi.org/10.1111/j.1365-2958.2009.06909.x

Rudolph, C. J., Upton, A. L., Stockum, A., Nieduszynski, C. A., & Lloyd, R. G. (2013). Avoiding chromosome pathology when replication forks collide. *Nature*, *500*, 608–611. https://doi.org/10.1038/nature12312

Rupp, W. D., Wilde, C. E., Reno, D. L., & Howard-Flanders, P. (1971). Exchanges between DNA strands in ultraviolet-irradiated Escherichia coli. *Journal of Molecular Biology*, *61*, 25–44. https://doi.org/10.1016/0022-2836(71)90204-X

Setlow, R. B., & Carrier, W. L. (1964). The disappearance of thymine dimers from DNA: An error-correcting mechanism. *Proceedings of the National Academy of Sciences of the USA*, *51*, 226–231. https://doi.org/10.1073/pnas.51.2.226

Setlow, R. B., Swenson, P. A., & Carrier, W. L. (1963). Thymine dimers and inhibition of DNA synthesis by ultraviolet irradiation of cells. *Science*, *142*, 1464–1466. https://doi.org/10.1126/science.142.3598.1464

Skarstad, K., Løbner-Olesen, A., Atlung, T., Von Meyenburg, K., & Boye, E. (1989). Initiation of DNA replication in Escherichia coli after overproduction of the DnaA protein. *Molecular and General Genetics*, *218*, 50–56. https://doi.org/10.1007/BF00330564

Smith, C. A., Cooper, P. K., & Hanawalt, P. C. (1981). DNA repair a manual of research procedures. In E. C. Friedberg, & P. C. Hanawalt (Eds.). *Measurement of Repair Replication by Equilibrium Sedimentation* (pp. 289–305). Marcel Dekker Inc.

Taylor, A. F., & Smith, G. R. (1985). Substrate specificity of the DNA unwinding activity of the RecBC enzyme of Escherichia coli. *Journal of Molecular Biology*, *185*, 431–443. https://doi.org/10.1016/0022-2836(85)90414-0

Taylor, A. F., & Smith, G. R. (1999). Regulation of homologous recombination: Chi inactivates RecBCD enzyme by disassembly of the three subunits. *Genes & Development*, *13*, 890–900. https://doi.org/10.1101/gad.13.7.890

Taylor, A. F., & Smith, G. R. (2003). RecBCD enzyme is a DNA helicase with fast and slow motors of opposite polarity. *Nature*, *423*, 889–893. https://doi.org/10.1038/nature01674

Thoms, B., & Wackernagel, W. (1998). Interaction of RecBCD enzyme with DNA at double-strand breaks produced in UV-irradiated Escherichia coli: Requirement for DNA end processing. *Journal of Bacteriology*, *180*, 5639–5645. https://doi.org/10.1128/JB.180.21.5639-5645.1998

Wendel, B. M., Cole, J. M., Courcelle, C. T., & Courcelle, J. (2018). SbcC-SbcD and ExoI process convergent forks to complete chromosome replication. *Proceedings of the National Academy of Sciences of the USA*, *115*, 349–354. https://doi.org/10.1073/pnas.1715960114

Wendel, B. M., Courcelle, C. T., & Courcelle, J. (2014). Completion of DNA replication in Escherichia coli. *Proceedings of the National Academy of Sciences of the USA*, *111*, 16454–16459.

Witkin, E. M., Roegner-Maniscalco, V., Sweasy, J. B., & Mccall, J. O. (1987). Recovery from ultraviolet light-induced inhibition of DNA synthesis requires umuDC gene products in recA718 mutant strains but not in recA+ strains of Escherichia coli. *Proceedings of the National Academy of Sciences of the USA*, *84*, 6805–6809. https://doi.org/10.1073/pnas.84.19.6805

Xu, Y. C., & Bremer, H. (1988). Chromosome replication in Escherichia coli induced by oversupply of DnaA. *Molecular and General Genetics*, *211*, 138–142. https://doi.org/10.1007/BF00338404

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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