

## Article

# Ligase A and RNase HI Participate in Completing Replication on the Chromosome in *Escherichia coli*

Brian M. Wendel<sup>1</sup>, Adrian J. Hernandez<sup>2</sup>, Charmain T. Courcelle<sup>2</sup> and Justin Courcelle<sup>2,\*</sup><sup>1</sup> Department of Microbiology, Cornell University, Ithaca, NY 14853, USA; bmw233@cornell.edu<sup>2</sup> Department of Biology, Portland State University, Portland, OR 97201, USA; adhern2@pdx.edu (A.J.H.); charmc@pdx.edu (C.T.C.)

\* Correspondence: justc@pdx.edu

**Abstract:** In *Escherichia coli*, several enzymes have been identified that participate in completing replication on the chromosome, including RecG, SbcCD, ExoI, and RecBCD. However, other enzymes are likely to be involved and the precise enzymatic mechanism by which this reaction occurs remains unknown. Two steps predicted to be necessary to complete replication are removal of Okazaki RNA fragments and ligation of the nascent strands at convergent replication forks. *E. coli* encodes two RNases that remove RNA-DNA hybrids, *rnhA* and *rnhB*, as well as two ligases, *ligA* and *ligB*. Here, we used replication profiling to show that *rnhA* and *ligA*, encoding RNase HI and Ligase A, participate in the completion reaction. Deletion of *rnhA* impaired the ability to complete replication and resulted in over-replication in the terminus region. It additionally suppressed initiation events from *oriC*, suggesting a role for the enzyme in *oriC*-dependent initiation, as has been suggested previously. We also show that a temperature-sensitive mutation in Ligase A led to over-replication at sites where replication completes, and that degradation at these sites occurred upon shifting to the nonpermissive temperature. Deletion of *rnhB* or *ligB* did not affect the growth or profile of replication on the genome.

**Keywords:** ligase; RNase H; completion of DNA replication

**Citation:** Wendel, B.M.; Hernandez, A.J.; Courcelle, C.T.; Courcelle, J. Ligase A and RNase HI Participate in Completing Replication on the Chromosome in *Escherichia coli*. *DNA* **2021**, *1*, 13–25.  
<https://doi.org/10.3390/dna1010003>

Academic Editors: Rodrigo Bermejo and Darren Griffin

Received: 1 July 2021

Accepted: 30 August 2021

Published: 3 September 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Cells must tightly regulate all phases of DNA replication to ensure that genomic stability is maintained and each daughter cell inherits an identical copy of the genetic information. Cellular genomes devote large numbers of proteins to limit replication initiation events to specific times and loci (reviewed in [1]). Similarly, several proteins ensure that DNA elongation remains processive (reviewed in [2]). Whereas these two phases of replication have been well characterized, the process by which cells complete replication has, until recently, remained largely unknown. To complete replication accurately, cells must have an enzymatic system that limits replication events to a precise doubling, joining the convergent strands at the point where every nucleoside in the parental strands have replicated once, and only once. In human cells, completion occurs thousands of times per cell division at loci all along the chromosomes where replication forks converge, and it must therefore proceed with remarkable efficiency. With respect to genome stability, one could argue that it is more critical to efficiently complete replication events than it is to efficiently initiate them. Both prokaryotic and eukaryotic cells tolerate variations in their origin number without severe phenotypic consequences, as elongation of replication forks from neighboring origins can compensate [3–7]. However, a failure to complete any single replication event would result in mutation, copy number variations, or cell lethality if the ends cannot be joined. Given this critical role, it is reasonable to infer that this final step, though far less understood, is also tightly regulated and controlled enzymatically.

Over-replication appears to be an inherent and promiscuous problem during genome duplication. Early reconstitution experiments found that converging replisomes continue

past their meeting point, resulting in over-replication of the region where the forks meet [8]. Over-replication also occurs in vivo on the chromosome of cells lacking the helicases or nucleases required to disrupt and degrade these events [9–17]. In addition, illegitimate initiations of replication can occur at single-strand nicks, gaps, D-loops and R-loops created by repair or transcriptional processes [13,15,18–22]. Each of the events described above would likely result in excess copies of the chromosome region where replication continues or initiates. Thus, it is extremely likely that cells will encode enzymes to specifically limit, degrade, and join these events when they occur each cell cycle.

The completion reaction has thus far been challenging to characterize in eukaryotic cells, where multiple origins initiate with varying efficiencies and timing, making the location where forks meet highly variable [4,5]. By comparison, *Escherichia coli* is well suited to dissect this reaction since the replication completes within a single ~400 kb region of the chromosome, opposite to its bidirectional origin of replication (reviewed in [23]). This region is flanked by *ter* sequences which bind the protein Tus, blocking replication forks in an orientation-specific manner, further narrowing the location where most completion events occur [24]. Although *ter* sequences ensure that completion occurs within this region, they do not appear to be directly involved in the reaction, as chromosomes lacking *ter* replicate normally and are stably maintained [25–27].

Current models suggest that converging replisomes transiently bypass each other at the point where they converge, creating an over-replicated region that contains three copies of the genetic information. The RecG helicase appears to play a critical role in disrupting the bypassed replication forks, limiting the extent to which over-replication occurs [9,28,29]. Subsequent incisions by the structure-specific nucleases SbcCD and ExoI are thought to cleave the intermediate created by these events [17,30–32]. In the absence of the SbcCD and ExoI nucleases, the over-replicated region persists, leading to genomic instabilities and amplifications at these loci [16,17,28]. The absence of other exonucleases, RecJ and Exo VII, can also exacerbate this effect [12]. Following incision, 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 [16,33]. In vitro, RecB and RecC interact with RecD to form a dual helicase–nuclease complex that unwinds and degrades double-strand DNA ends [34–38]. Loss of RecB or RecC inactivates the enzyme complex, whereas loss of RecD inactivates the nuclease, but retains the helicase activity and recombination proficiency of the complex [37,39,40]. On the chromosome, in the absence of RecB or RecC, the nascent ends of convergent replication forks are not joined, leading to excessive degradation and rendering cells unable to maintain the chromosome region where forks converge [16,28,33]. The inability to complete replication or maintain these regions of the genome severely compromises the viability and growth of *recBCD* cultures [16,33,41]. In the absence of RecD, degradation of the excess sequence is impaired, however, joining appears to occur normally and viability is not compromised [16,17,33].

Importantly, the completion reaction occurs normally in the absence of RecA or recombination [16,33]. However, when completion is impaired or prevented from occurring normally, viability and growth become dependent on an aberrant form of recombination that leads to genetic instabilities and amplifications at these loci [17].

The precise mechanism by which these enzymes catalyze the completion reaction and several key enzymatic steps remain unknown. Two events that are likely to be required during this reaction are the removal of Okazaki RNA fragments where the leading strand polymerase encounters the 5' end of the convergent replisome's lagging strand and the subsequent joining or ligation of the convergent nascent strands. *E. coli* has two known RNases (HI and HII) that remove RNA-DNA hybrids, encoded by *rnhA* and *rnhB*, as well as two Ligases (A and B), encoded by *ligA* and *ligB* [42,43].

In vitro, both RNase HI and HII can incise RNA-DNA hybrids that are analogous to Okazaki fragments formed during DNA replication [44,45]. However, they each recognize different substrates and have unique functions in the cell. RNase HI will cleave 5'-ended RNA hybridized to DNA at multiple sites when multiple RNA bases are present in the sub-

strate and is capable of incising 3' to the RNA base at the RNA-DNA junction [44,45]. RNase HII can also incise these substrates, but makes incisions 5' to the final RNA base at the RNA-DNA junction [45–47]. Mutants lacking RNase HI grow poorly but suppressor mutations rapidly accumulate that relieve this phenotype [48–50]. The enzyme's primary function has been proposed to be for the removal of Okazaki primers generated during replication elongation, along with polymerase I [44,48]. Additionally, RNase HI suppresses illegitimate initiation of replication from sites other than the chromosomal origin, *oriC* [49–52], and may be required for, or contribute to the efficiency of initiation at *oriC* [50,52]. RNase HII is the primary cellular activity that removes ribonucleotides misincorporated by DNA polymerases during replication through ribonucleotide excision repair [46,53]. It has also been suggested that RNase HII may play a minor role in Okazaki fragment removal [43].

Ligase A is an NAD<sup>+</sup>-dependent enzyme that catalyzes joining between 5'-phosphate and 3'-hydroxyl termini at nicks in duplex DNA [54–57]. It is essential for viability and replication, although temperature-sensitive mutants exist [58–61]. It functions in the joining of Okazaki fragments on the lagging strand during replication elongation and is required during nucleotide excision repair, base excision repair, mismatch repair, and double-strand break repair [58,60,62–66]. Ligase B was identified based upon sequence similarity to Ligase A and possesses a similar catalytic activity, but is nonessential [67]. A report recently suggested Ligase B may contribute to resistance during oxidative stress, but otherwise its cellular function presently remains unknown [68]. Whether Ligase A or B participates in the completion of replication has not yet been examined. Here, we used replication profiling to assess the ability of these mutants to complete replication and found that RNase HI and Ligase A participate in the reaction.

## 2. Materials and Methods

### 2.1. Bacteria

Strains utilized in these experiments are derived from SR108, a W3110 derivative that contains *thyA deoC* mutations [69] (see Table 1).

**Table 1.** Strains used and constructed for this study.

Strain	Relevant Genotype	Source or Construction
GR501	<i>ligA251(ts)</i>	[70]
JW0204	<i>rnhA::kan</i>	[71]
JW0178	<i>rnhB::kan</i>	[71]
JW3622	<i>ligB::kan</i>	[71]
DY329	W3110 <i>Del(lacU169) nadA::Tn10, gal490, Lambda cI857, Del(cro-bioA)</i>	[72]
CL1180	DY329 ( <i>nupC-yfeA intergenic region</i> ):: <i>cat</i>	primers 5' GTTACGGGTGTGACAAGCGGAAAGAGATTGCG TCTTGTTCGATGAGACGTTGATCGGCAC, 5' TCCTTTTCGACGATTCTCGCTGAGCAGTCGGGT TTTACTGCTTTCGAATTTCTGCCATTC to amplify <i>cat</i> , transformed into recombineering strain DY329. <i>cat</i> inserts within <i>nupC-yfeA</i> intergenic region.
CL1834	GR501 ( <i>nupC-yfeA intergenic region</i> ):: <i>cat</i>	P1 transduction of ( <i>nupC-yfeA intergenic region</i> ):: <i>cat</i> from CL1180 into GR501
SR108	$\Delta$ - <i>thyA deoC IN(rrnD-rrnE)</i>	[69]
CL1056	SR108 $\Delta$ ( <i>recC ptr recB recD</i> ):: <i>cam</i>	[17]
CL2357	SR108 <i>xonA::Cat300 sbcCD::Gm</i>	[16]
CL3362	SR108 <i>rnhA::kan</i>	P1 transduction of <i>rnhA::kan</i> from JW0204 into SR108
CL3360	SR108 <i>rnhB::kan</i>	P1 transduction of <i>rnhB::kan</i> from JW0178 into SR108
CL3912	SR108 <i>ligA251(ts) (nupC-yfeA intergenic region)</i> ):: <i>cat</i>	P1 transduction of <i>ligA251(ts) (nupC-yfeA intergenic region)</i> ):: <i>cat</i> from CL1834 into SR108
CL3909	SR108 <i>ligB753::kan</i>	P1 transduction of <i>ligB753::kan</i> from JW3622 into SR108

## 2.2. Growth Rates

Equivalent viable cells were inoculated into 0.1 mL cultures of LB supplemented with 10 µg/mL thymine (LBthy medium) and placed into a 96-well microtiter dish. Cultures were then grown at 37 °C with agitation and the absorbance at 630 nm was measured at 20-min intervals, using a BIO-Whittaker ELx808 plate reader (BioTek Instruments, Winooski, VT, USA) [16].

## 2.3. Replication Profiling

Cultures grown overnight were diluted 1:250 in LBthy media. All cultures were grown at 37 °C with aeration, unless otherwise indicated. To normalize profiles, stationary phase cultures were grown for 36 h before harvesting. When cultures reached an OD<sub>600</sub> of 0.4, genomic DNA was purified by placing 0.75-mL of culture into 0.75-mL ice cold 2× NET buffer (100 mM NaCl, 10 mM Tris at pH 8.0, 10 mM EDTA). All samples were then pelleted by centrifugation, resuspended in a solution containing 140 µL of 1 mg/mL lysozyme and 0.2 mg/mL RNaseA in TE (10 mM Tris at pH 8.0, 1 mM EDTA), and incubated at 37 °C for 30 min to lyse cells. Subsequently, Sarkosyl [10 µL, 20% (*wt/wt*)] and proteinase K (10 µL, 10 mg/mL) were added and the incubation was continued at 37 °C for an additional 30 min. The samples were then further purified by extracting the DNA with 4 vol phenol/chloroform (1/1) followed by dialysis for 1 h using 47 mm MF-Millipore 0.05-µm pore disks (#VMWP04700; Merck Millipore, Darmstadt, Germany) to float the samples on a 250-mL beaker of TE buffer (1 mM Tris at pH 8.0, 1 mM EDTA) [16]. A minimum of two replication profiles were obtained for each strain and representative plots are shown.

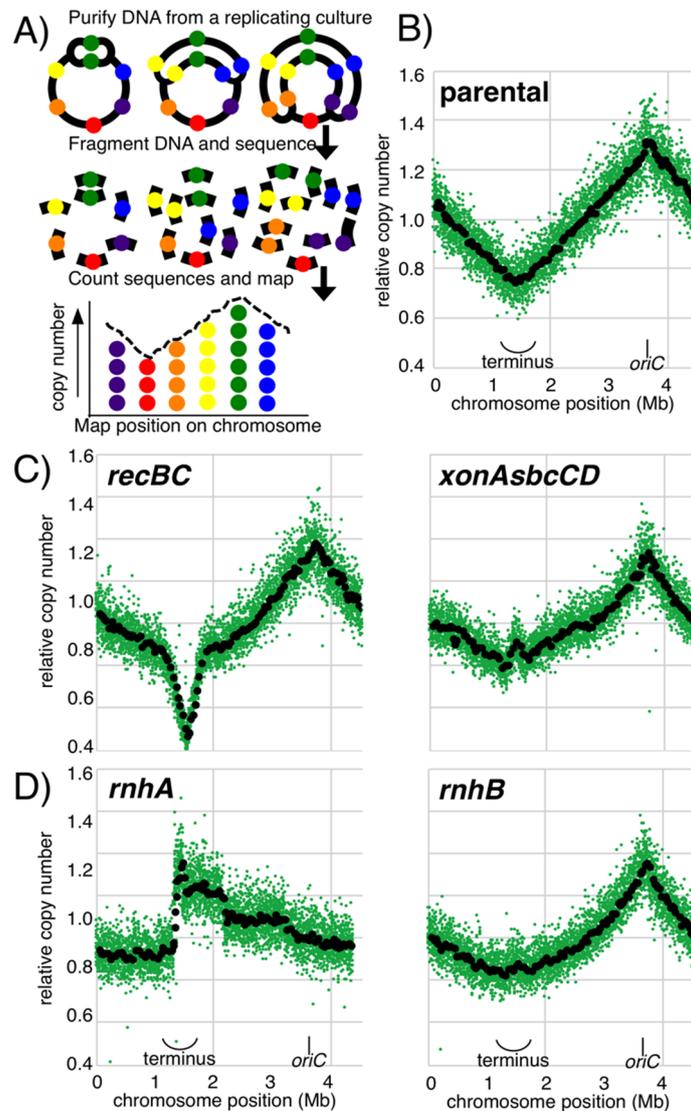
Genomic DNA samples were sequenced using single-end, 51-bp, bar-coded reads prepared and run on NexteraXT and Illumina HiSeq2000 (Illumina, San Diego, CA, USA) following the manufacturer's instructions. To determine the SR108 parent sequence, structural variations between SR108 and its W3110 parent genome were identified using the program Breseq, and the differences were then annotated by hand to generate the reference genome for SR108 [73]. For all subsequent strains, the original Illumina sequence reads were aligned to the SR108 reference genome using the program Bowtie 1.0.0 [74]. All aligned reads were then characterized to determine the nucleotide frequency at each position. The number of sequences per kilobase was determined and plotted using a custom Python script. To prevent sequencing bias caused by the purification or sequencing, the copy number for each strain was normalized to a stationary phase culture of SR108. Plots represent these relative copy number values at each genomic location in 1 kb bins, and depict the replication profile of each strain.

## 3. Results and Discussion

### 3.1. RNase HI, but Not RNase HII, Participates in Completing Replication on the Chromosome

To examine potential contributions of RNase H to replication completion on the chromosome, we compared the replication profiles of isogenic mutants deleted for *rnhA* or *rnhB* to that of the parental strain. For the purposes of controls, we also examined *recBC* and *sbcCD xonA* mutants which have been shown previously to be impaired in their ability to complete replication. To this end, genomic DNA was purified from replicating cultures and fragmented, prior to high-throughput sequencing. The replication profile was then determined by counting the number of sequences that align to each segment of the chromosome. In rapidly growing cultures, sequences proximal to the origin replicate early and are observed at higher frequencies relative to chromosome regions near the terminus, which replicate later (Figure 1A). Overall, we observed that the frequency of a given sequence in our parental cells decreases inversely with its distance from the origin until reaching the terminus region where the two replication forks converge and replication completes (Figure 1B). By comparison, *recBC* mutants fail to join the strands of convergent replication forks, leading to extensive degradation and an inability to maintain this region of the chromosome ([16,28] and Figure 1C). The loss of chromosome integrity severely

compromises growth and viability in these cultures [16,17]. In contrast, SbcCD and ExoI nucleases are required to initiate the processing of convergent forks. In the absence of SbcCD and ExoI, the over-replicated regions at loci where forks converge persist, leading to amplifications in this region ([16,17,30] and Figure 1C).



**Figure 1.** RNase HI, but not RNase HII, affects the ability to complete replication normally on the chromosome. (A) A diagram of method employed to profile replication on the *Escherichia coli* chromosome is shown. Genomic DNA from replicating cultures is purified, fragmented, and profiled using high-throughput sequencing. (B) In wild-type cultures, replication proceeds bidirectionally from the origin and completes in the terminus region. (C) *recBCD* mutants fail to join the strands of the convergent forks, leading to degradation of the terminus region. *sbcCDxonA* mutants fail to incise the intermediate formed where replication forks converge, allowing these over-replicated regions to persist and creating amplifications in the region where forks converge. (D) In *rnhA* mutants, a severe over-replication of the terminus region is observed, suggesting that in the absence of RNase HI, the completion reaction is impaired and does not occur normally. A suppression of *oriC* initiation is also observed in these mutants. By contrast, the completion of replication occurs normally in the absence of *rnhB*. Sequence read frequencies are normalized to stationary-phase cells and are plotted relative to their position on the genome. The position of the origin and terminus region is shown. A 50-kb floating average of the sequence frequency is plotted in black.

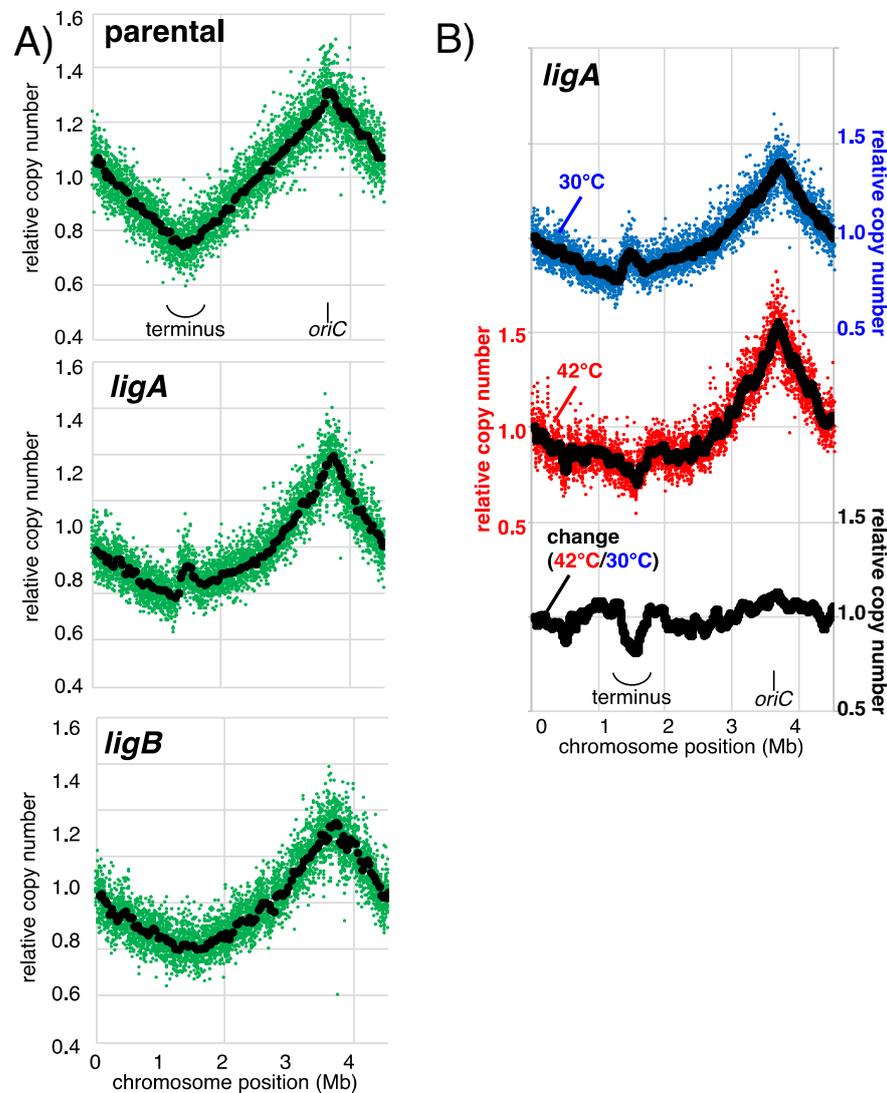
We next examined mutants lacking *rnhA* and *rnhB*, encoding RNase HI and HII, respectively. Whereas the profile of *rnhB* mutants looked similar to that of the parental cultures, the profile of *rnhA* mutants was clearly abnormal, both in the terminus region as well as across the genome (Figure 1D). In the absence of RNase HI, the terminus region was amplified suggesting that the efficiency of the completion reaction has been compromised or impaired. Additionally, an inhibition or severe suppression of replication initiation from *oriC* also occurred. This produced an inversion of the overall replication profile, with the predominant site of replication initiation appearing to occur in the terminus region where replication forks normally converge. Suppression of *oriC* initiation in *rnhA* mutants has been reported by others [10,52]. In these previous studies, the investigators also noted a similar amplification of DNA in the terminus region where replication forks converge and complete. However, neither group observed an inhibition of initiation from *oriC* and inversion of the total profile, unless either Tus or DnaA, which binds and is required for *oriC* initiation, was also inactivated. Tus binds to *ter* sequences in the terminus region and inhibits replication fork progression in an orientation-dependent manner, which helps limit the over-replication to this region [24,75]. DnaA binds to sequences proximal to *oriC* and is required to initiate replication from this locus [76]. Unlike these previous studies, our parental strain retains wild-type copies of both *tus* and *dnaA*. We observed steep declines in read frequencies as replication proceeded out of the terminus region in this strain, suggesting that the replication stalled at the Tus/*ter* sequences for significant periods of time. Our *rnhA* strain did acquire an insertion element-mediated deletion between *abgT-ydeN* during selection or out-growth. It is possible that differences in our conditions, strains, or a gene or sequence in this deletion region is responsible for this phenotype. Several other mutations and conditions have been reported that also bypass the DnaA requirement for initiating replication, including *topA*, *rpoB*, *rpoA*, *rnhA* heat stress, and DNA damage [77–82].

Suppression of *dnaA* by *rnhA* could be mediated by R-loops that serve to open and/or prime initiation at this locus in the absence of DnaA. Several lines of evidence support the idea that transcriptional RNA-DNA hybrids contribute to *oriC* initiation efficiency. Transcription from the genes proximal to *oriC*, allele-specific mutations in RNA polymerase, and deletion of *rnhA* have all been shown to promote *oriC* initiation and would be expected to enhance RNA-DNA hybrid formation in this region [50,83–85]. A similar form of R-loop-mediated replication initiation is also utilized by Cole1 type plasmids [86,87]. R-loops have also been suggested to allow replication initiation from multiple sites around the chromosome [13,49,52]. However, as shown in Figure 1C and consistent with the results reported by others, the predominant point of replication initiation in *rnhA* mutants appears to occur in the terminus where replication forks converge [10,52]. This would imply that when the completion reaction is impaired, DNA ends from the converging replication forks persist, allowing replication to resume or re-initiate from these sites. Consistent with this view, *recG* mutants similarly impair the ability to complete replication, leading to a prominent peak of DNA initiation at the terminus region, and bypassing the requirement for DnaA and *oriC* when specific allelic mutations of RNA polymerase or Tus are also present, similar to *rnhA* [10,88].

### 3.2. Ligase A, but Not Ligase B, Participates in Completing Replication on the Chromosome

Completing replication is also likely to involve ligation of 5'- and 3'-strand ends where forks converge. To determine whether Ligase A or B participates in the reaction, we examined the profiles of a temperature-sensitive *ligA* allele, as well as a deletion mutant of *ligB*. The profile of the *ligA*ts mutant was determined by growing the culture at the permissive temperature of 30 °C. As shown in Figure 2A, the absence of Ligase B did not detectably alter the mutant's profile relative to wild-type cells. By contrast, *ligA*ts mutants exhibited an over-replication in the region where forks converge, even at the permissive temperature. The observation suggests that when Ligase A activity is less than

fully functional, even at 30 °C, the reduced rate of ligation allows DNA ends to initiate or extend replication when forks meet, compromising the completion reaction.

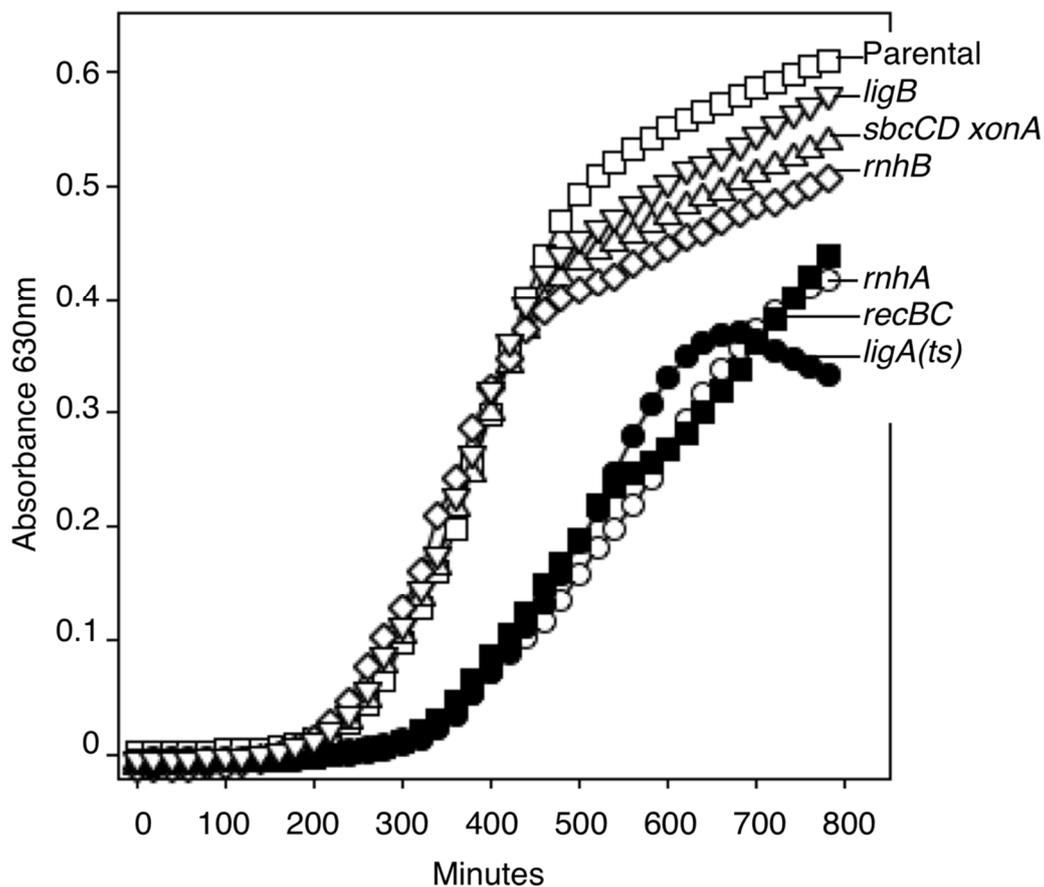


**Figure 2.** Ligase A, but not Ligase B, participates in the normal completion reaction on the chromosome. (A) At the permissive temperature, an over-replication of the terminus region is observed in *ligA(ts)* mutants, suggesting that the completion of replication has been impaired. By comparison, the replication profile and terminus of *ligB* mutants is not detectably different from the parental strain. (B) After 1 h at the non-permissive temperature, extensive degradation in the terminus region is observed in the *ligA(ts)* mutant, suggesting that in the absence of Ligase A, the strands of the convergent replication forks are not joined and remain susceptible to exonucleolytic degradation. Top panel—*ligA(ts)* grown at 30 °C, middle panel—*ligA(ts)* grown at 42 °C for 1 h, bottom panel the difference between these profiles after 1 h at the restrictive, 42 °C temperature; 30 °C *ligA(ts)* profile represents the same experiment. Profiles were determined as in Figure 1. Data for the parental strain is reproduced from Figure 1 and plotted for comparison.

To further characterize the role of Ligase A, we also compared the replication profile of *ligA(ts)* cells at the permissive temperature of 30 °C, to one after the culture had been shifted to the fully restrictive temperature of 42 °C for one hour. As shown in Figure 2B, when Ligase A has been inactivated for a period of one hour, the over-replicated region is lost and the chromosome region where forks converge begins to degrade. The observation indicates that Ligase A participates in the completion reaction and implies that the efficiency or

timing of ligation during completion is critical to maintaining genome stability. When Ligase A activity is reduced, such as occurs at the permissive temperature, 3'-ends may persist and allow replication to reinitiate leading to the amplifications observed. However, if ligation or joining of the convergent forks is completely prevented, after an extended period of time the DNA ends of the replication forks may break and/or succumb to exonucleases, leading to the excessive degradation and loss of this region of the genome.

When we compared the growth rates of these strains at 37 °C, we observed that both *ligA*ts and *rnhA*, but not *ligB* or *rnhB*, impaired growth of cultures, to an extent similar to that of *recBCD* mutants (Figure 3). Both Ligase A and RNase HI have cellular functions other than completing replication, including the processing of Okazaki primers during replication elongation, which would also be expected to reduce growth when these genes are mutated or impaired. Thus, although we cannot specifically attribute the reduced growth rates to an impaired ability to complete replication, the results are consistent with those seen in the replication profiles indicating that Ligase A and RNase HI, but not Ligase B or RNase HII, participate in completing replication on the chromosome.

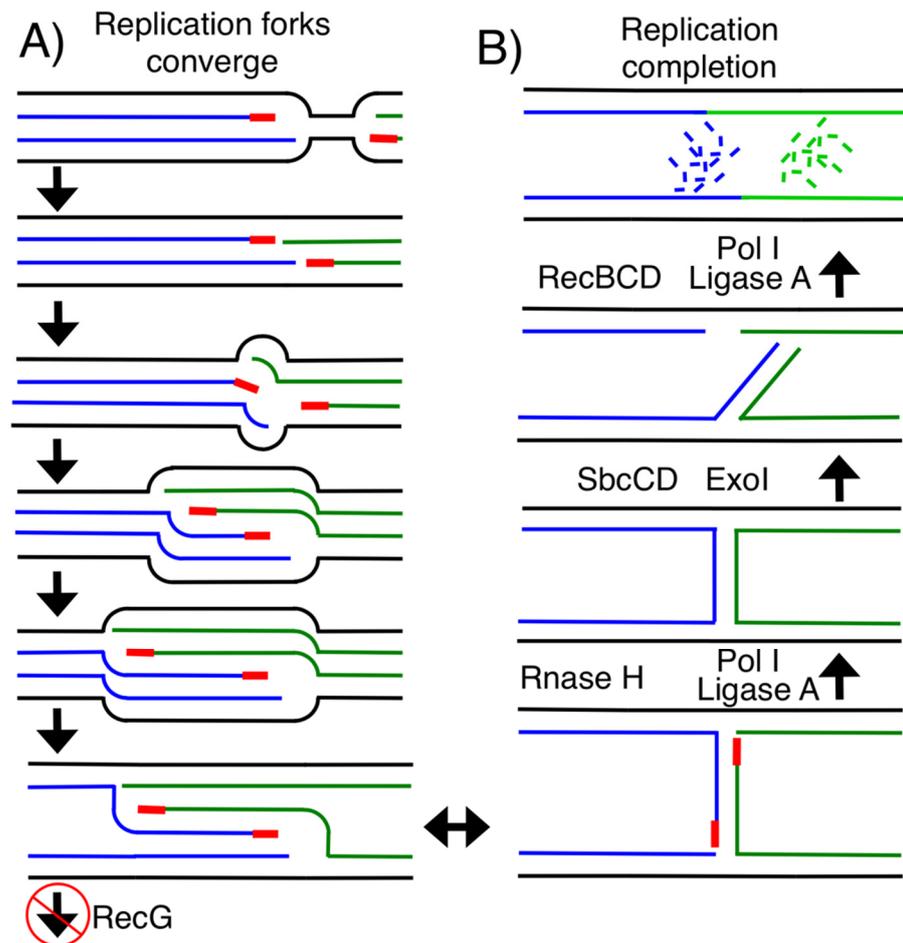


**Figure 3.** Growth is impaired in *rnhA* and *ligA*(ts) mutants, but not in *rnhB* or *ligB* mutants. Absorbance (630 nm) of cultures grown at 37 °C is plotted over time. Parental (open squares); *recBC* (open circles); *sbcCD xonA* (open triangles); *rnhA* (closed squares); *rnhB* (open diamonds); *ligA*(ts) (closed circles); *ligB* (open inverted triangles).

### 3.3. Efficient Removal of Okazaki Primers and Joining of DNA Ends Is Important to Accurately Complete Replication on the Chromosome

RNase HI plays a prominent role, along with the 5'-3' exonuclease activity of Polymerase I, in removing Okazaki primers during replication elongation [48,89], and it is likely to be similarly required to allow replication on the chromosome to complete (Figure 4). Consistent with this interpretation, a similar over-replication in the terminus region is observed in *polA* mutants lacking the 5'-3' exonuclease required for RNA primer removal [11].

Persistent RNA primers at convergent replication forks would prevent joining of 5'- and 3'-ends, leaving the 3'-ends free to prime and initiate or resume illegitimate replication at these sites. This could explain the observed over-replication that is observed at sites of convergent forks in these mutants. Similarly, mutations that inactivate other helicases, nucleases, or topoisomerases which generate excessive 3-DNA ends, appear to lead to over-replication on the chromosome [12,22].



**Figure 4.** Model for the completion of DNA replication on the chromosome. (A) Convergent replication forks continue past their meeting point, creating a partially over-replicated substrate that contains three copies of the genetic information. The RecG helicase disrupts replication, limiting the over-replicated region. (B) SbcCD and ExoI recognize and cleave this hairpin-like, over-replicated substrate, creating a DNA substrate that can be resected and processed by RecBCD. Following resection, RecBCD promotes or recruits enzymes that join the convergent strands at the doubling point. The proposed roles for RNase HI in removing RNA primers (red) at the convergent strands so that Ligase A can join these substrates is indicated.

Reducing the efficiency of Ligase A by the temperature-sensitive mutation would also be expected to allow 3'-ends to persist longer than normal and similarly leads to a modest over-replication in the region where forks converge. Curiously, when inactivated at the restrictive temperature, this leads to loss and degradation of this region of the chromosome. The results imply that if joining of the 5'- and 3'-ends of converging forks is prevented entirely, they remain susceptible to exonucleolytic attack and the region is degraded. A similar degradation is observed in *recBCD* mutants, an observation that is consistent with that idea that this enzyme is required to recruit ligase, either directly or indirectly to these sites to promote joining of the DNA ends, as has been speculated previously [16,17,30,33].

It will be of interest in future work to determine how the progression of this reaction occurs, and whether these proteins interact and function as a complex, ‘completosome’, or sequentially.

**Author Contributions:** Conceptualization, B.M.W., C.T.C. and J.C.; Acquisition, analysis, and interpretation of data B.M.W., A.J.H., C.T.C. and J.C.; writing—original draft preparation, B.M.W. and J.C.; writing—review and editing, B.M.W., C.T.C. and J.C. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the National Science Foundation (NSF), grant number MCB1916625.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data generated or analyzed during this study are included in this published article. All strains used in these experiments are available upon request.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Costa, A.; Hood, I.V.; Berger, J.M. Mechanisms for initiating cellular DNA replication. *Annu. Rev. Biochem.* **2013**, *82*, 25–54. [[CrossRef](#)]
2. O'Donnell, M.; Langston, L.; Stillman, B. Principles and concepts of DNA replication in bacteria, archaea, and eukarya. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, a010108. [[CrossRef](#)] [[PubMed](#)]
3. Dimude, J.U.; Stein, M.; Andrzejewska, E.E.; Khalifa, M.S.; Gajdosova, A.; Retkute, R.; Skovgaard, O.; Rudolph, C.J. Origins Left, Right, and Centre: Increasing the Number of Initiation Sites in the *Escherichia coli* Chromosome. *Genes* **2018**, *9*, 376. [[CrossRef](#)] [[PubMed](#)]
4. Heichinger, C.; Penkett, C.J.; Bahler, J.; Nurse, P. Genome-wide characterization of fission yeast DNA replication origins. *EMBO J.* **2006**, *25*, 5171–5179. [[CrossRef](#)] [[PubMed](#)]
5. Wu, P.Y.; Nurse, P. Establishing the program of origin firing during S phase in fission Yeast. *Cell* **2009**, *136*, 852–864. [[CrossRef](#)]
6. Wang, X.; Lesterlin, C.; Reyes-Lamothe, R.; Ball, G.; Sherratt, D.J. Replication and segregation of an *Escherichia coli* chromosome with two replication origins. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, E243–E250. [[CrossRef](#)]
7. Ivanova, D.; Taylor, T.; Smith, S.L.; Dimude, J.U.; Upton, A.L.; Mehrjouy, M.M.; Skovgaard, O.; Sherratt, D.J.; Retkute, R.; Rudolph, C.J. Shaping the landscape of the *Escherichia coli* chromosome: Replication-transcription encounters in cells with an ectopic replication origin. *Nucleic Acids Res.* **2015**, *43*, 7865–7877. [[CrossRef](#)]
8. Hiasa, H.; Marians, K.J. Tus prevents overreplication of oriC plasmid DNA. *J. Biol. Chem.* **1994**, *269*, 26959–26968. [[CrossRef](#)]
9. Rudolph, C.J.; Upton, A.L.; Harris, L.; Lloyd, R.G. Pathological replication in cells lacking RecG DNA translocase. *Mol. Microbiol.* **2009**, *73*, 352–366. [[CrossRef](#)]
10. Dimude, J.U.; Stockum, A.; Midgley-Smith, S.L.; Upton, A.L.; Foster, H.A.; Khan, A.; Saunders, N.J.; Retkute, R.; Rudolph, C.J. The Consequences of Replicating in the Wrong Orientation: Bacterial Chromosome Duplication without an Active Replication Origin. *MBio* **2015**, *6*, e01294-15. [[CrossRef](#)]
11. Midgley-Smith, S.L.; Dimude, J.U.; Taylor, T.; Forrester, N.M.; Upton, A.L.; Lloyd, R.G.; Rudolph, C.J. Chromosomal over-replication in *Escherichia coli* recG cells is triggered by replication fork fusion and amplified if replicore symmetry is disturbed. *Nucleic Acids Res.* **2018**, *46*, 7701–7715. [[CrossRef](#)]
12. Midgley-Smith, S.L.; Dimude, J.U.; Rudolph, C.J. A role for 3' exonucleases at the final stages of chromosome duplication in *Escherichia coli*. *Nucleic Acids Res.* **2019**, *47*, 1847–1860. [[CrossRef](#)] [[PubMed](#)]
13. de Massy, B.; Fayet, O.; Kogoma, T. Multiple origin usage for DNA replication in sdrA(rnh) mutants of *Escherichia coli* K-12. Initiation in the absence of oriC. *J. Mol. Biol.* **1984**, *178*, 227–236. [[CrossRef](#)]
14. de Massy, B.; Patte, J.; Louarn, J.M.; Bouche, J.P. oriX: A new replication origin in *E. coli*. *Cell* **1984**, *36*, 221–227. [[CrossRef](#)]
15. Asai, T.; Kogoma, T. D-loops and R-loops: Alternative mechanisms for the initiation of chromosome replication in *Escherichia coli*. *J. Bacteriol.* **1994**, *176*, 1807–1812. [[CrossRef](#)] [[PubMed](#)]
16. Wendel, B.M.; Courcelle, C.T.; Courcelle, J. Completion of DNA replication in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 16454–16459. [[CrossRef](#)] [[PubMed](#)]
17. Wendel, B.M.; Cole, J.M.; Courcelle, C.T.; Courcelle, J. SbcC-SbcD and ExoI process convergent forks to complete chromosome replication. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 349–354. [[CrossRef](#)] [[PubMed](#)]
18. Hamperl, S.; Cimprich, K.A. The contribution of co-transcriptional RNA:DNA hybrid structures to DNA damage and genome instability. *DNA Repair* **2014**, *19*, 84–94. [[CrossRef](#)] [[PubMed](#)]
19. Bhatia, V.; Barroso, S.I.; Garcia-Rubio, M.L.; Tumini, E.; Herrera-Moyano, E.; Aguilera, A. BRCA2 prevents R-loop accumulation and associates with TREX-2 mRNA export factor PCID2. *Nature* **2014**, *511*, 362–365. [[CrossRef](#)]

20. Donnianni, R.A.; Symington, L.S. Break-induced replication occurs by conservative DNA synthesis. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 13475–13480. [[CrossRef](#)] [[PubMed](#)]
21. Magee, T.R.; Asai, T.; Malka, D.; Kogoma, T. DNA damage-inducible origins of DNA replication in *Escherichia coli*. *EMBO J.* **1992**, *11*, 4219–4225. [[CrossRef](#)]
22. Brochu, J.; Vlachos-Breton, É.; Sutherland, S.; Martel, M.; Drolet, M. Topoisomerases I and III inhibit R-loop formation to prevent unregulated replication in the chromosomal Ter region of *Escherichia coli*. *PLoS Genet.* **2018**, *14*, e1007668. [[CrossRef](#)]
23. Hill, T.M. Arrest of bacterial DNA replication. *Annu. Rev. Microbiol.* **1992**, *46*, 603–633. [[CrossRef](#)] [[PubMed](#)]
24. Kobayashi, T.; Hidaka, M.; Horiuchi, T. Evidence of a ter specific binding protein essential for the termination reaction of DNA replication in *Escherichia coli*. *EMBO J.* **1989**, *8*, 2435–2441. [[CrossRef](#)]
25. Roecklein, B.; Pelletier, A.; Kuempel, P. The tus gene of *Escherichia coli*: Autoregulation, analysis of flanking sequences and identification of a complementary system in *Salmonella typhimurium*. *Res. Microbiol.* **1991**, *142*, 169–175. [[CrossRef](#)]
26. Duggin, I.G.; Wake, R.G.; Bell, S.D.; Hill, T.M. The replication fork trap and termination of chromosome replication. *Mol. Microbiol.* **2008**, *70*, 1323–1333. [[CrossRef](#)] [[PubMed](#)]
27. Duggin, I.G.; Bell, S.D. Termination structures in the *Escherichia coli* chromosome replication fork trap. *J. Mol. Biol.* **2009**, *387*, 532–539. [[CrossRef](#)]
28. Rudolph, C.J.; Upton, A.L.; Stockum, A.; Nieduszynski, C.A.; Lloyd, R.G. Avoiding chromosome pathology when replication forks collide. *Nature* **2013**, *500*, 608–611. [[CrossRef](#)]
29. McGlynn, P.; Lloyd, R.G. RecG helicase activity at three- and four-strand DNA structures. *Nucleic Acids Res.* **1999**, *27*, 3049–3056.
30. Hamilton, N.A.; Wendel, B.M.; Weber, E.A.; Courcelle, C.T.; Courcelle, J. RecBCD, SbcCD and ExoI process a substrate created by convergent replisomes to complete DNA replication. *Mol. Microbiol.* **2019**, *111*, 1638–1651. [[CrossRef](#)] [[PubMed](#)]
31. Connelly, J.C.; de Leau, E.S.; Okely, E.A.; Leach, D.R. Overexpression, purification, and characterization of the SbcCD protein from *Escherichia coli*. *J. Biol. Chem.* **1997**, *272*, 19819–19826. [[CrossRef](#)]
32. Cromie, G.A.; Millar, C.B.; Schmidt, K.H.; Leach, D.R. Palindromes as substrates for multiple pathways of recombination in *Escherichia coli*. *Genetics* **2000**, *154*, 513–522. [[CrossRef](#)] [[PubMed](#)]
33. Courcelle, J.; Wendel, B.M.; Livingstone, D.D.; Courcelle, C.T. RecBCD is required to complete chromosomal replication: Implications for double-strand break frequencies and repair mechanisms. *DNA Repair* **2015**, *32*, 86–95. [[CrossRef](#)]
34. Taylor, A.; Smith, G.R. Unwinding and rewinding of DNA by the RecBC enzyme. *Cell* **1980**, *22*, 447–457. [[CrossRef](#)]
35. Taylor, A.F.; Schultz, D.W.; Ponticelli, A.S.; Smith, G.R. RecBC enzyme nicking at Chi sites during DNA unwinding: Location and orientation-dependence of the cutting. *Cell* **1985**, *41*, 153–163. [[CrossRef](#)]
36. Taylor, A.F.; Smith, G.R. Substrate specificity of the DNA unwinding activity of the RecBC enzyme of *Escherichia coli*. *J. Mol. Biol.* **1985**, *185*, 431–443. [[CrossRef](#)]
37. Amundsen, S.K.; Taylor, A.F.; Chaudhury, A.M.; Smith, G.R. recD: The gene for an essential third subunit of exonuclease V. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 5558–5562. [[CrossRef](#)]
38. Taylor, A.F.; Smith, G.R. RecBCD enzyme is a DNA helicase with fast and slow motors of opposite polarity. *Nature* **2003**, *423*, 889–893. [[CrossRef](#)]
39. Ponticelli, A.S.; Schultz, D.W.; Taylor, A.F.; Smith, G.R. Chi-dependent DNA strand cleavage by RecBC enzyme. *Cell* **1985**, *41*, 145–151. [[CrossRef](#)]
40. Amundsen, S.K.; Taylor, A.F.; Smith, G.R. The RecD subunit of the *Escherichia coli* RecBCD enzyme inhibits RecA loading, homologous recombination, and DNA repair. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 7399–7404. [[CrossRef](#)]
41. Capaldo, F.N.; Ramsey, G.; Barbour, S.D. Analysis of the growth of recombination-deficient strains of *Escherichia coli* K-12. *J. Bacteriol.* **1974**, *118*, 242–249. [[CrossRef](#)] [[PubMed](#)]
42. Carl, P.L.; Bloom, L.; Crouch, R.J. Isolation and mapping of a mutation in *Escherichia coli* with altered levels of ribonuclease H. *J. Bacteriol.* **1980**, *144*, 28–35. [[CrossRef](#)] [[PubMed](#)]
43. Itaya, M. Isolation and characterization of a second RNase H (RNase HII) of *Escherichia coli* K-12 encoded by the rnhB gene. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 8587–8591. [[CrossRef](#)]
44. Miller, H.I.; Riggs, A.D.; Gill, G.N. Ribonuclease H (hybrid) in *Escherichia coli*. Identification and characterization. *J. Biol. Chem.* **1973**, *248*, 2621–2624. [[CrossRef](#)]
45. Tannous, E.; Kanaya, E.; Kanaya, S. Role of RNase H1 in DNA repair: Removal of single ribonucleotide misincorporated into DNA in collaboration with RNase H2. *Sci. Rep.* **2015**, *5*, 9969. [[CrossRef](#)] [[PubMed](#)]
46. Haruki, M.; Tsunaka, Y.; Morikawa, M.; Kanaya, S. Cleavage of a DNA-RNA-DNA/DNA chimeric substrate containing a single ribonucleotide at the DNA-RNA junction with prokaryotic RNases HII. *FEBS Lett.* **2002**, *531*, 204–208. [[CrossRef](#)]
47. Ohtani, N.; Haruki, M.; Morikawa, M.; Crouch, R.J.; Itaya, M.; Kanaya, S. Identification of the genes encoding Mn<sup>2+</sup>-dependent RNase HII and Mg<sup>2+</sup>-dependent RNase HIII from *Bacillus subtilis*: Classification of RNases H into three families. *Biochemistry* **1999**, *38*, 605–618. [[CrossRef](#)]
48. Ogawa, T.; Okazaki, T. Function of RNase H in DNA replication revealed by RNase H defective mutants of *Escherichia coli*. *Mol. Genet. Genom.* **1984**, *193*, 231–237. [[CrossRef](#)]
49. Kogoma, T.; Subia, N.L.; von Meyenburg, K. Function of ribonuclease H in initiation of DNA replication in *Escherichia coli* K-12. *Mol. Genet. Genom.* **1985**, *200*, 103–109. [[CrossRef](#)]

50. Ogawa, T.; Pickett, G.G.; Kogoma, T.; Kornberg, A. RNase H confers specificity in the *dnaA*-dependent initiation of replication at the unique origin of the *Escherichia coli* chromosome in vivo and in vitro. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 1040–1044. [[CrossRef](#)]
51. Horiuchi, T.; Maki, H.; Sekiguchi, M. RNase H-defective mutants of *Escherichia coli*: A possible discriminatory role of RNase H in initiation of DNA replication. *Mol. Genet. Genom.* **1984**, *195*, 17–22. [[CrossRef](#)] [[PubMed](#)]
52. Maduiké, N.Z.; Tehrani, A.K.; Wang, J.D.; Kreuzer, K.N. Replication of the *Escherichia coli* chromosome in RNase HI-deficient cells: Multiple initiation regions and fork dynamics. *Mol. Microbiol.* **2014**, *91*, 39–56. [[CrossRef](#)] [[PubMed](#)]
53. Vaisman, A.; McDonald, J.P.; Huston, D.; Kuban, W.; Liu, L.; Van Houten, B.; Woodgate, R. Removal of misincorporated ribonucleotides from prokaryotic genomes: An unexpected role for nucleotide excision repair. *PLoS Genet.* **2013**, *9*, e1003878. [[CrossRef](#)]
54. Gellert, M. Formation of covalent circles of lambda DNA by *E. coli* extracts. *Proc. Natl. Acad. Sci. USA* **1967**, *57*, 148–155. [[CrossRef](#)] [[PubMed](#)]
55. Zimmerman, S.B.; Little, J.W.; Oshinsky, C.K.; Gellert, M. Enzymatic joining of DNA strands: A novel reaction of diphosphopyridine nucleotide. *Proc. Natl. Acad. Sci. USA* **1967**, *57*, 1841–1848. [[CrossRef](#)]
56. Olivera, B.M.; Lehman, I.R. Diphosphopyridine nucleotide: A cofactor for the polynucleotide-joining enzyme from *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **1967**, *57*, 1700–1704. [[CrossRef](#)]
57. Olivera, B.M.; Lehman, I.R. Linkage of polynucleotides through phosphodiester bonds by an enzyme from *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **1967**, *57*, 1426–1433. [[CrossRef](#)]
58. Pauling, C.; Hamm, L. Properties of a temperature-sensitive radiation-sensitive mutant of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **1968**, *60*, 1495–1502. [[CrossRef](#)] [[PubMed](#)]
59. Modrich, P.; Lehman, I.R. Enzymatic characterization of a mutant of *Escherichia coli* with an altered DNA ligase. *Proc. Natl. Acad. Sci. USA* **1971**, *68*, 1002–1005. [[CrossRef](#)]
60. Konrad, E.B.; Modrich, P.; Lehman, I.R. Genetic and enzymatic characterization of a conditional lethal mutant of *Escherichia coli* K12 with a temperature-sensitive DNA ligase. *J. Mol. Biol.* **1973**, *77*, 519–529. [[CrossRef](#)]
61. Gottesman, M.M.; Hicks, M.L.; Gellert, M. Genetics and function of DNA ligase in *Escherichia coli*. *J. Mol. Biol.* **1973**, *77*, 531–547. [[CrossRef](#)]
62. Pauling, C.; Hamm, L. Properties of a temperature-sensitive, radiation-sensitive mutant of *Escherichia coli*. II. DNA replication. *Proc. Natl. Acad. Sci. USA* **1969**, *64*, 1195–1202. [[CrossRef](#)]
63. Dean, C.; Pauling, C. Properties of a deoxyribonucleic acid ligase mutant of *Escherichia coli*: X-ray sensitivity. *J. Bacteriol.* **1970**, *102*, 588–589. [[CrossRef](#)] [[PubMed](#)]
64. Verly, W.G.; Gossard, F.; Crine, P. In vitro repair of apurinic sites in DNA. *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 2273–2275. [[CrossRef](#)] [[PubMed](#)]
65. Robertson, A.B.; Matson, S.W. Reconstitution of the very short patch repair pathway from *Escherichia coli*. *J. Biol. Chem.* **2012**, *287*, 32953–32966. [[CrossRef](#)] [[PubMed](#)]
66. Lahue, R.S.; Au, K.G.; Modrich, P. DNA mismatch correction in a defined system. *Science* **1989**, *245*, 160–164. [[CrossRef](#)]
67. Sriskanda, V.; Shuman, S. A second NAD<sup>+</sup>-dependent DNA ligase (LigB) in *Escherichia coli*. *Nucleic Acids Res.* **2001**, *29*, 4930–4934. [[CrossRef](#)]
68. Bodine, T.J.; Evangelista, M.A.; Chang, H.T.; Ayoub, C.A.; Samuel, B.S.; Sugang, R.; Zechiedrich, L. *Escherichia coli* DNA ligase B may mitigate damage from oxidative stress. *PLoS ONE* **2017**, *12*, e0180800. [[CrossRef](#)]
69. Mellon, I.; Hanawalt, P.C. Induction of the *Escherichia coli* lactose operon selectively increases repair of its transcribed DNA strand. *Nature* **1989**, *342*, 95–98. [[CrossRef](#)]
70. Dermody, J.J.; Robinson, G.T.; Sternglanz, R. Conditional-lethal deoxyribonucleic acid ligase mutant of *Escherichia coli*. *J. Bacteriol.* **1979**, *139*, 701–704. [[CrossRef](#)]
71. Baba, T.; Ara, T.; Hasegawa, M.; Takai, Y.; Okumura, Y.; Baba, M.; Datsenko, K.A.; Tomita, M.; Wanner, B.L.; Mori, H. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: The Keio collection. *Mol. Syst. Biol.* **2006**, *2*, 2006. [[CrossRef](#)] [[PubMed](#)]
72. Yu, D.; Ellis, H.M.; Lee, E.C.; Jenkins, N.A.; Copeland, N.G.; Court, D.L. An efficient recombination system for chromosome engineering in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 5978–5983. [[CrossRef](#)]
73. 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. Identifying structural variation in haploid microbial genomes from short-read resequencing data using breseq. *BMC Genom.* **2014**, *15*, 1039. [[CrossRef](#)] [[PubMed](#)]
74. Langmead, B. Aligning short sequencing reads with Bowtie. *Curr. Protoc. Bioinform.* **2010**, *32*, 11.7.1–11.7.14. [[CrossRef](#)]
75. Hill, T.M.; Tecklenburg, M.L.; Pelletier, A.J.; Kuempel, P.L. *tus*, the trans-acting gene required for termination of DNA replication in *Escherichia coli*, encodes a DNA-binding protein. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 1593–1597. [[CrossRef](#)]
76. Fuller, R.S.; Kaguni, J.M.; Kornberg, A. Enzymatic replication of the origin of the *Escherichia coli* chromosome. *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 7370–7374. [[CrossRef](#)]
77. Kogoma, T.; Lark, K.G. DNA replication in *Escherichia coli*: Replication in absence of protein synthesis after replication inhibition. *J. Mol. Biol.* **1970**, *52*, 143–164. [[CrossRef](#)]

78. Guzman, E.C.; Jimenez-Sanchez, A.; Orr, E.; Pritchard, R.H. Heat stress in the presence of low RNA polymerase activity increases chromosome copy number of *Escherichia coli*. *Mol. Genet. Genom.* **1988**, *212*, 203–206. [[CrossRef](#)]
79. Kogoma, T.; Skarstad, K.; Boye, E.; von Meyenburg, K.; Steen, H.B. RecA protein acts at the initiation of stable DNA replication in *rnh* mutants of *Escherichia coli* K-12. *J. Bacteriol.* **1985**, *163*, 439–444. [[CrossRef](#)] [[PubMed](#)]
80. Louarn, J.; Bouché, J.P.; Patte, J.; Louarn, J.M. Genetic inactivation of topoisomerase I suppresses a defect in initiation of chromosome replication in *Escherichia coli*. *Mol. Genet. Genom.* **1984**, *195*, 170–174. [[CrossRef](#)]
81. Atlung, T. Allele-specific suppression of *dnaA*(Ts) mutations by *rpoB* mutations in *Escherichia coli*. *Mol. Genet. Genom.* **1984**, *197*, 125–128. [[CrossRef](#)]
82. Torrey, T.A.; Atlung, T.; Kogoma, T. *dnaA* suppressor (*dasF*) mutants of *Escherichia coli* are stable DNA replication (*sdrA/rnh*) mutants. *Mol. Genet. Genom.* **1984**, *196*, 350–355. [[CrossRef](#)]
83. Lobner-Olesen, A.; Atlung, T.; Rasmussen, K.V. Stability and replication control of *Escherichia coli* minichromosomes. *J. Bacteriol.* **1987**, *169*, 2835–2842. [[CrossRef](#)] [[PubMed](#)]
84. Ogawa, T.; Okazaki, T. Concurrent transcription from the *gid* and *mioC* promoters activates replication of an *Escherichia coli* minichromosome. *Mol. Genet. Genom.* **1991**, *230*, 193–200. [[CrossRef](#)] [[PubMed](#)]
85. Bates, D.B.; Boye, E.; Asai, T.; Kogoma, T. The absence of effect of *gid* or *mioC* transcription on the initiation of chromosomal replication in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 12497–12502. [[CrossRef](#)] [[PubMed](#)]
86. Itoh, T.; Tomizawa, J. Formation of an RNA primer for initiation of replication of ColE1 DNA by ribonuclease H. *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 2450–2454. [[CrossRef](#)]
87. Naito, S.; Kitani, T.; Ogawa, T.; Okazaki, T.; Uchida, H. *Escherichia coli* mutants suppressing replication-defective mutations of the ColE1 plasmid. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 550–554. [[CrossRef](#)] [[PubMed](#)]
88. Dimude, J.U.; Midgley-Smith, S.L.; Stein, M.; Rudolph, C.J. Replication Termination: Containing Fork Fusion-Mediated Pathologies in *Escherichia coli*. *Genes* **2016**, *7*, 40. [[CrossRef](#)]
89. Konrad, E.B.; Lehman, I.R. A conditional lethal mutant of *Escherichia coli* K12 defective in the 5' leads to 3' exonuclease associated with DNA polymerase I. *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 2048–2051. [[CrossRef](#)]