

RESEARCH ARTICLE

Topo IV is required to allow replisomes to converge and complete replication on the chromosome

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Abstract

The ability to complete DNA replication as replisomes converge has recently been shown to be a highly regulated, multi-enzymatic process. Converging forks also are likely to generate unique supercoiled, tangled, or knotted substrates. These structures are typically resolved by one of the four topoisomerases encoded by *Escherichia coli*. However, identifying the cellular substrates and specific function for these essential enzymes which contain overlapping biochemical activities has remained challenging. Here, we show that Topo I and Topo IV are required to allow converging forks to complete chromosome replication. Impaired Topo I function leads to amplifications where forks converge, whereas inactivation of Topo IV prevents forks from converging and produces a dramatic loss of this chromosome region. The results are consistent with previous studies suggesting Topo I suppresses illegitimate initiations in the terminus region by disrupting R- and D-loops and demonstrate a specific requirement for Topo IV acting before replication completes to allow convergent forks to reach their doubling point. We propose that the positive supercoils arising between convergent forks are converted to precatenanes and resolved by Topo IV, when diminishing space may preclude gyrase from binding and functioning.

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Author summary

DNA topoisomerases resolve twists, knots, and other topological problems arising from the need to unwind the double-helical DNA molecule in order to replicate or transcribe the genome. *E. coli*, like most organisms, including humans, encode several topoisomerase enzymes. Many of these are essential to maintain viability and are useful targets in several chemotherapeutic and antimicrobial treatments. However, identifying the specific role of each enzyme in the cell can prove challenging since their fundamental nature can lead to multiple phenotypes and they often have partially overlapping biochemical activities. Here we show that both

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Topo I and Topo IV of *E. coli* play a role in the critical process of completing DNA replication on the chromosome when two replication forks converge. The function of Topo IV appears specific, acting prior to the completion of replication, and is required to allow the converging forks to reach their doubling point.

Introduction

Cells tightly regulate the processes of DNA replication initiation, elongation and completion to ensure that each cell receives an identical copy of the genetic information. Whereas initiation and elongation have been well characterized (reviewed in [1,2]), less is known about how cells complete replication. To complete replication accurately, cells must encode an enzymatic system that is capable of recognizing pairs and joining the nascent strands of converging replication forks faithfully, without gain or loss of genetic information. The failure to complete even a single replication event would be expected to result in a loss of genomic stability, or cell lethality. Yet, this reaction must be remarkably efficient, occurring thousands of times per generation along the chromosomes of human cells. Given this critical role, it is not surprising that this final step is tightly regulated and controlled by a complex multi-enzymatic pathway [3–7].

E. coli represents a useful model to dissect how completion occurs as its genome contains a single 400-kb region where replication forks converge opposite to its bidirectional origin of replication (reviewed in [8]). Completion events are contained within this region by Tus, which binds to termination (*ter*) sequences and prevents replication forks from progressing beyond this region [9,10]. Although Tus confines completion to this region, it does not appear to directly participate in the reaction since mutants lacking Tus or *ter* sites complete replication normally [11,12].

Whereas Tus does not appear to directly participate, several gene products have been identified that impair the completion reaction when mutated and result in abnormalities specifically at loci where replication forks converge [3,5,13,14]. Current models suggest that the converging replisomes transiently bypass each other, creating a partially over-replicated region that contains a third copy of the genetic information. These over-replicated intermediates can be observed in the absence of exonucleases, which allow these intermediates to persist, both in vitro and in vivo [3,5,7,15,16]. RecG is a helicase with branch migration activity that limits the amount of over-replication and appears to prevent these DNA end intermediates from re-initiating replication [3,17–19]. It is thought that the over-replicated intermediate is first incised by SbcCD and ExoI, a cohesin-like structure-specific nuclease and 3' to 5' exonuclease [5,7,20], before the heterotrimeric RecBCD helicase-nuclease complex further processes and resects this region [5,6,20]. RecBCD function is also thought to be needed before DNA Ligase and Polymerase I can join the nascent strand ends of convergent forks, as in its absence, extensive degradation in this chromosome region occurs, severely compromising viability [5,13]. Importantly, and perhaps surprisingly, the completion reaction does not require recombination, as *recA* mutants complete replication normally [5]. However, RecA is required for many of the

aberrant amplifications and duplications that arise when completion is impaired, suggesting that recombination occurring under these conditions is directly associated with genomic instability [5–7,20].

Completion events would also be expected to generate a number of topological challenges on the DNA. During normal replication, negative and positive supercoils accumulate behind and in front of the progressing fork DNA, respectively [21,22] and reviewed in [23]. Additionally, if the fork DNA is free to swivel, positive supercoils can be relieved, generating precatenanes in the newly replicated sister chromatids behind the fork as these become twisted around each other [24–26]. As replisomes converge, the space available for enzymes to access and resolve these twists and tangles diminishes. Resolution of such structures may require unique enzymes to access these regions or structures. Additionally, over-replicated intermediates at loci where the forks converge would potentially compound these issues should swiveling of the DNA ends be prevented by tethering to proteins or membranes. Cells typically process these topological constraints using DNA topoisomerases, making it likely that some of these enzymes will play a role in the completion of replication. However, at present, it remains unclear, how topological complexities generated during completion are processed or resolved.

E. coli encodes two type I and two type II DNA topoisomerases. These enzymes form transient protein-DNA complexes that break either one (type I) or both (type II) strands, before swiveling and rejoining the DNA to relieve or introduce twists in the double-stranded helix (reviewed in [23]).

Topo I and III, encoded by *topA* and *topB* respectively, are type I topoisomerases [27,28]. Topo I is an essential enzyme that relaxes negatively supercoiled plasmids *in vitro* and *in vivo* [29–34]. It is thought to act during both transcription and replication, by reducing negative supercoiling behind the replisome and transcription machinery [35]. Suppressor mutations that restore viability appear to either alleviate transcriptionally generated R-loops, *rpoB**35, *rmh*, or decrease negative supercoiling during replication via reduced gyrase activity or upregulated Topo III or Topo IV activity [14,34,36–41]. Consistent with a general role in these essential processes, hypomorphic or suppressed *topA* mutants have altered transcription profiles and reduced replication rates [38,39,42,43].

Topo III is the only nonessential topoisomerase in *E. coli* and mutants exhibit normal growth [28]. *In vitro*, its activity is stimulated by RecQ and relaxes negative supercoils and resolves precatenane plasmid substrates [14,44–46].

Gyrase, encoded by *gyrA* and *gyrB*, and Topo IV, encoded by *parE* and *parC*, are Type II topoisomerases ([36,47,48] and reviewed in [23]). *In vitro*, gyrase catalyzes negative supercoils in an ATP-dependent manner and is thought to operate ahead of the replication fork [22,49,50]. The counteracting activities of gyrase and Topo I are thought to balance and maintain supercoiling levels in the overall genome. Inactivation of DNA gyrase slows the rate of DNA replication, consistent with the idea that the protein travels with the replication machinery to relieve the positive supercoils generated ahead of the replication fork [51–54].

Topo IV has been proposed to have many roles that partially overlap with gyrase and the enzymes share ~40% homology [36]. However, Topo IV is significantly more efficient in decatenating plasmid molecules than DNA gyrase and this appears to be related to its function *in vivo* [21,22,49,55]. Temperature-sensitive Topo IV mutants accumulate catenated plasmids following Topo IV inactivation [21,56]. Some evidence based on single-molecule, fluorescence microscopy has suggested Topo IV travels behind the replisome during replication, although a genomic analysis of Topo IV cleavage sites indicate that its activity increases in the terminus region on the chromosome [57–59].

Here, we focused on which of these topoisomerases participate in completing DNA replication on the chromosome. Characterizing how topoisomerases participate in specific cellular events can be challenging because they are often essential, and can affect a range of metabolic activities that elicit pleiotropic phenotypes *in vivo*. Plasmid minichromosomes, which contain a bi-directional origin of replication, have been used effectively to characterize aspects of how converging replication forks complete replication *in vivo* [9,15,20,60–62]. We characterized topoisomerase mutants for their ability to propagate these substrates and correlated it with events observed on the *E. coli* chromosome. We show that both Topo I and Topo IV are required to maintain substrates containing convergent forks and that Topo IV plays a specific and critical role in allowing replisomes to reach their meeting point on the chromosome.

Results

Topo I, but not Topo III, enhances the ability of replication forks to converge on plasmids

To begin to assess the role of the type I DNA topoisomerases in completing DNA replication, we compared the ability of *topA2103* and Δ *topB* mutants to maintain minichromosome plasmid, pCL01, which contains a bi-directional origin and replicates using two replisomes, to that of plasmid pBR322, which replicates using a single replisome from a uni-directional origin and therefore does not require that replisomes converge [63,64]. The ability to maintain the two-replisome plasmid has been shown previously to correlate with the cell's ability to maintain the chromosome region where replication forks converge [20,61]. We reasoned that if a topoisomerase contributes to the ability of replication forks to converge, then propagation of the 2-replisome plasmid would be more severely impaired than that of the 1-replisome plasmid.

To determine transformation efficiency, a single solution containing both plasmids was transformed into all strains (Fig 1A). Transformation of both the 1-replisome and 2-replisome plasmid was similar in the parental strain (Fig 1B). By contrast, when the plasmid mixture was transformed into Δ *recBCD* mutants, the transformation frequency of the 2-replisome plasmid was severely reduced. As noted above, RecBCD is required to maintain the chromosomal region where replication forks converge, demonstrating that the 2-replisome plasmid can be used to identify gene products participating in the completion reaction [5,20]. Most reports suggest Topo I is essential [34,38,41,65]. Therefore, we initially examined a *topA2103* mutant along with a Δ *topB* mutant. *topA2103* was isolated as a spontaneous frameshift mutation that removes the protein's C-terminal domain, but retains partial function. As shown in Fig 1B, transformation of the 2-replisome plasmid was significantly reduced in *topA2103* but not Δ *topB* mutant strains, although the 1-replisome plasmid could still propagate in both strains.

Since the *topA2103* mutant retains partial function, we could not eliminate the possibility its presence has a dominant effect that poisoned or impaired some aspect of the completion reaction. Therefore, despite reports that *topA* is essential, we attempted to construct deletion mutants of Topo I in our parental background, eventually obtaining two separate isolates. However, upon sequencing, both Δ *topA* mutants were found to have secondary, presumable suppressor, mutations in other topoisomerases. Isolate 1 contained an arginine to glycine mutation at amino acid 630 in *gyrB*, whereas isolate 2 contained an ~900 kb genomic duplication encompassing *parE* and *parC* region. However, since the *topA* deletion was present and the strains remained viable, we continued characterization of these strains. As shown in Fig 1C, transformation of the 2-replisome plasmid was impaired in both Δ *topA* strains, even in the presence of these suppressor mutations. Taken together, the observations argue against *topA2103* having a dominant effect and suggest that Topo I, but not Topo III, activity promotes the propagation of substrates containing two replisomes.

Topo IV, but not DNA gyrase, enhances the ability of replication forks to converge on plasmids

Both type II DNA topoisomerases are essential for viability. However, temperature-sensitive *gyrB* and *parE* mutants exist which are viable at 30°C but lose viability at 42°C [36,66]. We first confirmed that these mutant alleles remained temperature sensitive when present in our parental background. Both mutants retained viability and grew similar to wild-type cells at 30°C (Fig 2). However at 39°C, *parE*^{ts} mutants began to lose viability, grew at a slower rate, and Topo IV function became impaired as evidenced by elevated levels of negative topoisomers on plasmids purified from these cells. Similarly, *gyrB*^{ts} mutants also grew more slowly at 39°C and gyrase function was impaired as evidenced by the appearance of positively supercoiled plasmids purified from these cultures. In contrast to *parE*^{ts}, *gyrB*^{ts} mutants retained viability at this temperature. At 43°C, both mutants lost viability and failed to grow. *gyrB*^{ts} mutants remained viable up to 42°C, and we were unable to identify a temperature where these mutants lost partial viability as seen with *parE*^{ts}. For the purposes of comparison and as a control, *recBCD* mutants were also examined and shown not to be temperature sensitive, but did grow

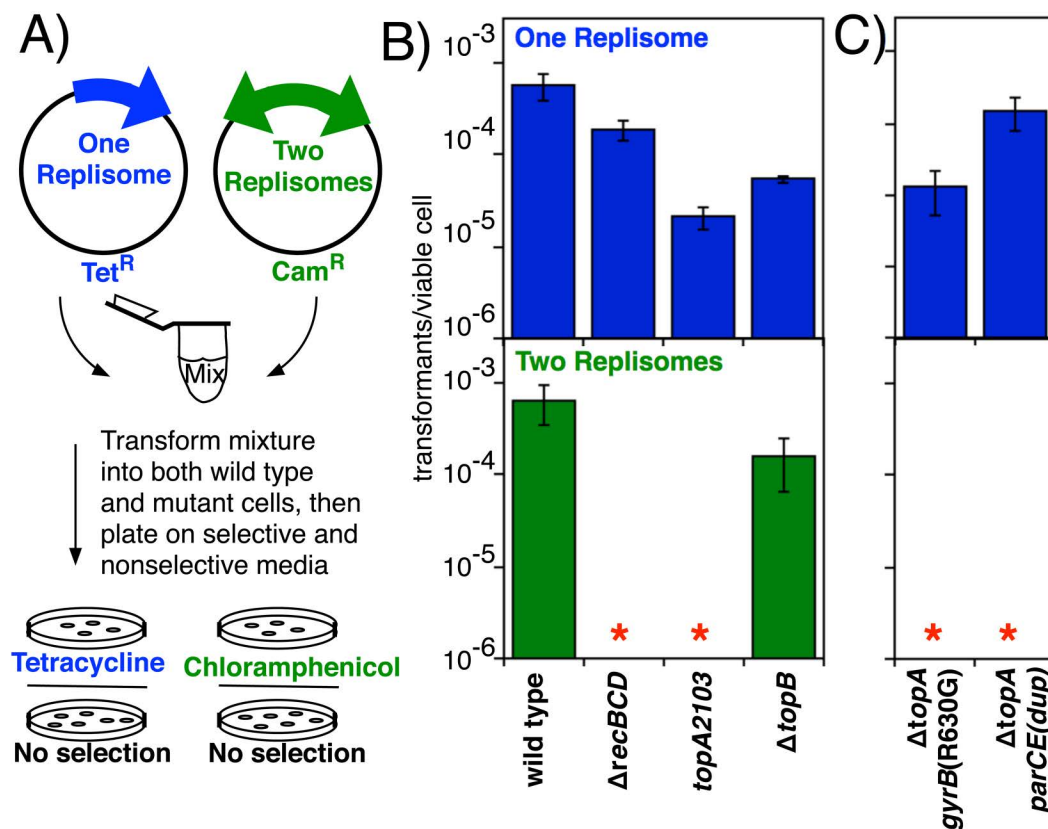


Fig 1. Mutations in Topo I but not Topo III impair the ability to maintain plasmids containing convergent replication forks. A) Illustration of how transformation frequencies were measured. A mixture containing the 1- and 2-replisome plasmids was transformed into both parental and mutant strains that were then plated on selective and nonselective media. The ratio of cells containing each plasmid was then determined by counting colonies following overnight incubation. B) Loss of Topo I activity impairs the ability to maintain plasmids containing convergent replication forks. The number of transformants per viable cell for the 1-replisome (pBR322) and 2-replisome (pCL01) plasmid is plotted for wild-type, $\Delta recBCD$, $topA2103$, and $\Delta topB$. C) Other Topo I mutants similarly compromise the ability to maintain plasmids containing convergent replication forks. The number of transformants per viable cell for the 1-replisome (pBR322) and 2-replisome (pCL01) plasmid is plotted for $\Delta topA gyrB(R630G)$ and $\Delta topA parCE^{dup}$. $\Delta topA parCE^{dup}$ contains a chromosome duplication encompassing the *parC parE* region. Plots represent the average of between three and nine independent experiments. Error bars represent the standard error of the mean. Asterisks indicate transformants were below our limit of detection.

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more slowly, an observation which has been previously reported and shown to relate to the impaired ability to complete replication [5,20]. Thus, although both enzymes are partially inactivated at the semipermissive temperature, they produce phenotypically distinct outcomes for the cell. However, we cannot rule out that *gyrB^{ts}* and *parE^{ts}* viability differences reflect different degrees of protein inactivation. Since viability was lost at 43°C, we elected to use 39°C as a semi-permissive temperature to examine if these enzymes compromise the ability to maintain substrates containing convergent forks when their activity is partially impaired.

Following transformation of the plasmid mixture into these strains at the 30°C permissive temperature, the parental, *gyrB^{ts}* and *parE^{ts}* strains were all able to propagate both the 1- and 2-replisome plasmids. Similarly, $\Delta recBCD$ mutants remained impaired for the 2-replisome plasmid as before (Fig 3). However, differences emerged at the 39°C semi-permissive temperature. *gyrB^{ts}* remained capable of propagating the 1- and 2-replisome plasmids; while transformation of the 2-replisome plasmid into *parE^{ts}* was compromised relative to the 1-replisome plasmid (Fig 3), indicating that impaired Topo IV function affects the cell's ability to maintain substrates containing convergent replisomes.

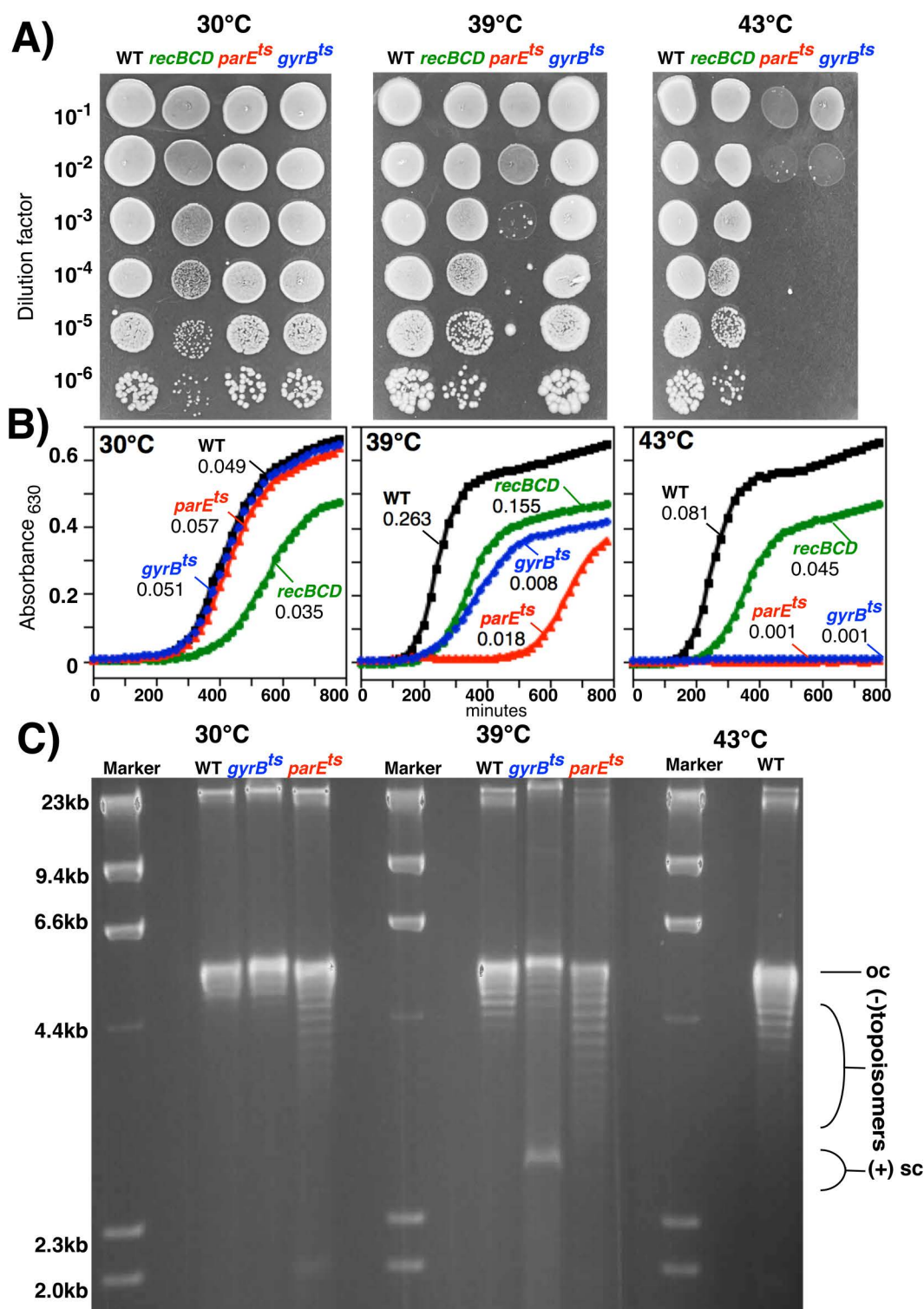


Fig 2. Properties of *gyrB^{ts}* and *parE^{ts}* strains at the permissive, semi-permissive, and restrictive temperatures used in this study. A) Viability of *gyrB^{ts}* and *parE^{ts}* strains at 30°C, 39°C, and 43°C. Ten- μ l drops of 10-fold serial dilutions were spotted onto nonselective plates for each strain and grown at the indicated temperature. B) Growth of *gyrB^{ts}* and *parE^{ts}* strains at 30°C, 39°C, and 43°C. The Absorbance at 630nm is plotted for each strain at the indicated temperature over time. C) Strains containing plasmid pBR322 were grown at the indicated temperature before plasmids were purified and examined following electrophoresis in a 1.1% agarose gel containing 8 μ g/ml chloroquine. Oc- open circle, sc – supercoils, M- lambda Hind III molecular size markers.

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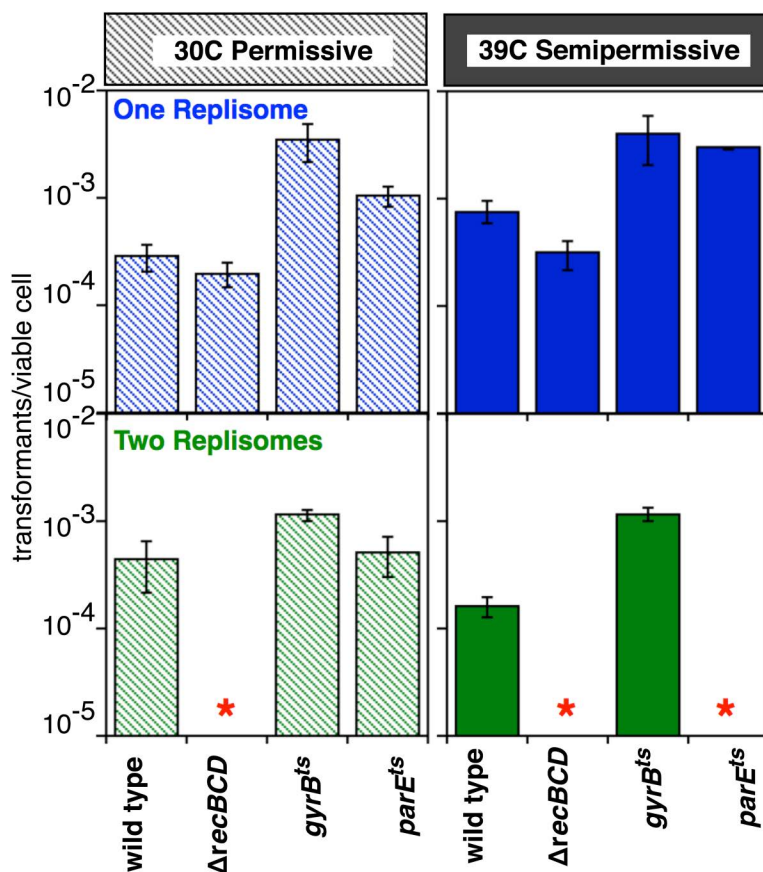


Fig 3. Loss of Topo IV activity impairs the ability to maintain plasmids containing converging replication forks. The number of transformants per viable cell for the 1-replisome and 2-replisome plasmids is plotted at the permissive (30°C) and semi-permissive (39°C) temperatures for wild-type, $\Delta recBCD$, $gyrB^{ts}$ and $parE^{ts}$ mutants. Transformants per viable cell were determined as in Fig 1. Plots represent the average of three independent experiments. Error bars represent the standard error of the mean. Asterisks indicate transformants were below our limit of detection.

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Impaired Topo IV activity generates abnormal replication intermediates when replisomes converge

The results above demonstrate that impaired Topo I or Topo IV function compromises the cell's ability to maintain plasmids containing convergent replisomes. Although we were unable to identify conditions where *topA* mutants could propagate the 2-replisome plasmid, the ability to transform $gyrB^{ts}$ and $parE^{ts}$ mutants with the 2-replisome plasmid at the permissive temperature provided an opportunity to examine the replication intermediates associated with this mutation. To this end, we used 2-dimensional (2D) agarose gel electrophoresis, a technique that can be used to visualize the structure of replicating DNA fragments in vivo [67,68]. Total genomic DNA was purified from growing cultures that contained either the 1-replisome or 2-replisome plasmid. The DNA was then digested with a restriction enzyme that cleaved the plasmids at their origin of replication, and the plasmid DNA was analyzed by Southern analysis following 2D agarose gel electrophoresis. In this technique, nonreplicating plasmids migrate as a linear fragment, forming the prominent spot observed on the 2D gel. Replicating fragments migrate more slowly because of their larger size and nonlinear shape. On plasmids containing a unidirectional origin, these replicating fragments form a simple Y-arc that extends out from the linear fragment. Plasmids with a bidirectional origin of replication produce fragments with double Y-shapes, migrating in an inverted V-shape that extends up from the linear monomer fragment and down to the linear dimer fragment (Fig 4A & 4B). In theory, the two-replisome plasmid should contain only double-Y structures. In practice however, when the two replisomes

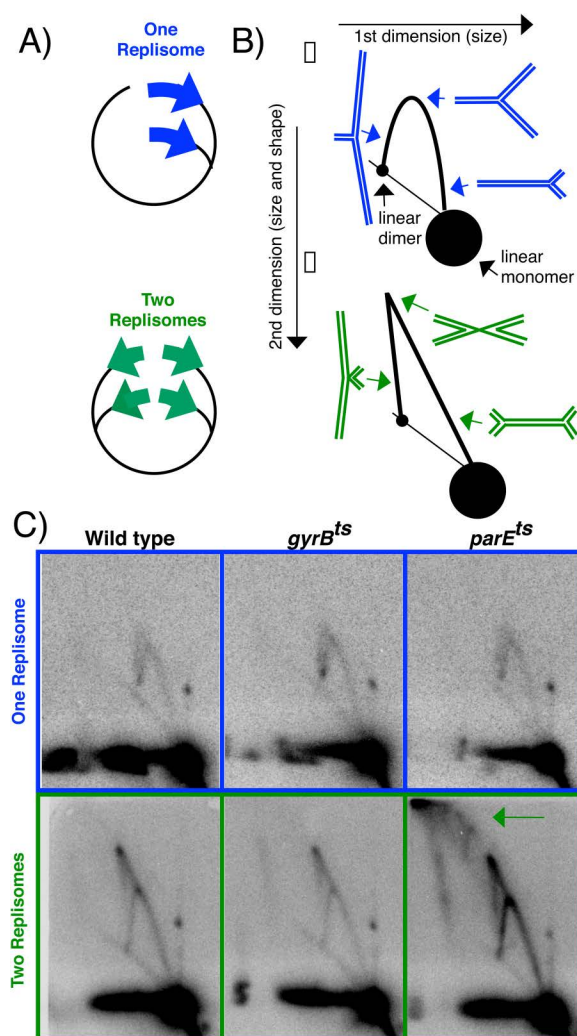


Fig 4. Topo IV mutants accumulate abnormal replication intermediates on substrates with convergent replication forks. A & B) Illustration depicting the expected migration pattern of 1- and 2-replisome plasmids when linearized at the origin of replication. Following restriction enzyme digestion, nonreplicating plasmids run as a linear monomer fragment, forming the prominent spot observed in gels. Plasmids with unidirectional origins have replicating fragments that form Y-shaped molecules, which form an arch that extends out from the linear monomer fragment and returns to the linear dimer fragment. Plasmids with bidirectional origins have replicating fragments that form double Y- or X-shaped molecules. Double Y-shaped molecules form a line that extends out from the linear monomer fragment, while X-shaped molecules appear as a line extending up from the linear dimer fragment to a point where the two lines meet. C) When Topo IV activity is compromised, large abnormal replication intermediates accumulate on substrates containing convergent replication forks. DNA was purified from wild-type, *gyrB^{ts}* and *parE^{ts}* cells containing the 1- or 2-replisome plasmids and analyzed by 2D-agarose gel electrophoresis. (Top panels) Normal replication intermediates are observed in wild-type, *gyrB^{ts}* and *parE^{ts}* cells containing plasmids with one replisome. (Bottom panels) Normal replication intermediates are observed in wild-type and *gyrB^{ts}* cells containing plasmids with two replisomes. However, abnormal intermediates accumulate in *parE^{ts}* cells that correspond to large branched fragments containing single-stranded regions (green arrow). Gels shown represent one of three independent experiments for each strain.

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initiate at slightly different times, molecules will initially appear as a single-Y structure, rather than a double-Y, which can be seen in the Southern analysis of the molecules. To demonstrate that both replisomes are active on the plasmid, two *ter* sequences, which block replication progression in an orientation-specific manner [9], were cloned into plasmid pCL01 and then re-analyzed by 2D agarose gel analysis. S1 Fig shows that replication arrest events are observed at both *ter* sequences, demonstrating that both replisomes are active on the 2-replisome plasmid.

In the case of the 1-replisome plasmid, both *gyrB*^{ts} and *parE*^{ts} strains formed normal Y-shaped intermediates, similar to the parental strain. Similarly, the replication intermediates of 2-replisome plasmid formed the expected inverted-V shaped in the parental and *gyrB*^{ts} strains. However, in *parE*^{ts} mutants, replication of the 2-replisome plasmid led to the formation of large, multimeric intermediates containing either one or two branch points (Figs 4C and S2). The abnormal intermediates likely comprise molecules having single-strand regions or gaps, since these multimers remain resistant to digestion by the restriction endonuclease. The observation that abnormal intermediates only accumulated on plasmids with two replisomes, but not one replisome, argues that suboptimal Topo IV activity impairs the ability to complete replication specifically on substrates containing convergent replication forks.

Loss of Topo IV activity impairs the ability of convergent replisomes to complete chromosome replication

The results above demonstrate that Topo I and Topo IV activity are required to maintain plasmid substrates that contain convergent replication forks. To determine if these enzymes function similarly on the chromosome, we profiled replication across the genome in growing parental and mutant cultures. To profile replication, genomic DNA was extracted from growing cultures, fragmented, and then sequenced using high-throughput sequencing. The frequency each sequence occurs is then plotted relative to its position on the chromosome (Fig 5A). In parental cultures, the frequency of sequences surrounding the origin is highest because it replicates first. The frequency of sequences then decreases inversely with their distance from the origin, until reaching the region where forks converge and replication completes (Fig 5B). Consistent with previous work, in $\Delta recBCD$ mutants which fail to join the strands of convergent forks, a dramatic loss of sequences is observed specifically at the locus where replication completes (Fig 5B and [6,20]).

Using this technique, we first examined the profiles of the type I topoisomerase mutants. In *topA2103* or $\Delta topB$ mutants, the profile of replication looked similar to that of the parental strain. However, in the $\Delta topA$ strains which contained suppressors in DNA gyrase or Topo IV, a modest increase in copy number, or over-replication, of the region where replication forks converge was observed (Fig 5C). The results are consistent with those obtained with the 2-replisome plasmid and suggest that the presence of Topo I but not Topo III is required to allow replication to complete normally.

To examine the role of essential type II topoisomerases, temperature-sensitive *gyrB* and *parE* mutants were grown at 30°C then shifted to 43°C for 90 or 180 minutes to follow the progression of the ongoing replication in the cultures. At these time points, the genomic DNA was then purified and sequenced as described above. Under these conditions (i.e., three hours of replication at 43°C), the cultures were reaching maximum density and cell growth and DNA replication had ceased. In the replication profiles of wild-type cultures, this is observed as a damping in the difference between the frequency of origin and terminus sequences, as new initiations become less frequent by 90 minutes. By 180 minutes, new initiations have ceased and ongoing rounds of replication have completed, leaving equal frequencies at all regions around the chromosome (Fig 6). Following a shift to the semi-permissive temperature, the profile of the *gyrB*^{ts} mutants resembles and resolves in a manner similar to wild-type cultures with the exception of a modest over-replicated region that persists at the 180 minutes time point. Interestingly, in the *parE*^{ts} replication profile, 90 minutes after shifting to the nonpermissive temperature, a similar modest over-replicated region is present at the loci where replication completes. Additionally however, the regions in the latter half of the chromosome are under-represented, suggesting that replication is not progressing through this portion of the chromosome. By 180 minutes when replication has ceased, this unreplicated region becomes clear and pronounced, leaving the terminus region unreplicated. The results demonstrate a specific defect in completing replication when Topo IV activity is inactivated. Taken together, the plasmid and chromosomal observations imply that the activities of Topo I and Topo IV promote the efficient completion of replication when replication forks converge. When these activities are absent or impaired, genetic instabilities arise specifically at these loci.

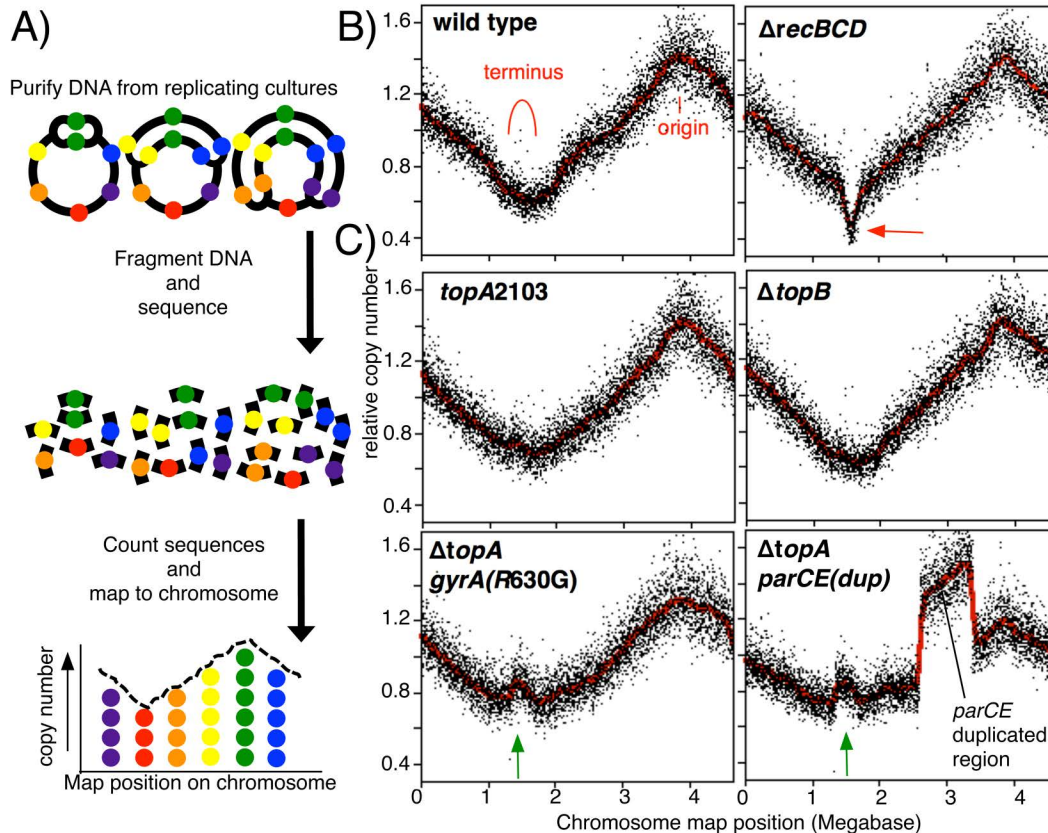


Fig 5. Impaired Topo I function leads to amplifications at loci where replication forks converge on the chromosome. A) An illustration depicting how replication profiles are determined. Genomic DNA is purified from replicating cultures, fragmented, and sequenced using high throughput sequencing. The relative number of sequence reads within each kilobase of the genome is then plotted. B) The profiles of wild-type cells and *recBCD* mutants are plotted. In replicating cultures of wild-type cells, replication proceeds bidirectionally from the origin and completes in the terminus region, as indicated. In the absence of *recBCD*, the nascent DNA ends fail to join and remain susceptible to exonucleolytic degradation, leading to a loss of sequence reads in this region of the genome (red arrow). C) *topA* mutants, but not *topB* mutants, exhibit amplification in the terminus region. Profiles of *topA2103*, *ΔtopB*, *ΔtopA gyrA(R630G)* and *ΔtopA parCE^{dup}* are plotted. *ΔtopB* does not lead to alterations within the terminus region of the chromosome. Amplified terminus region with increased copy number in *ΔtopA gyrA(R630G)* and *ΔtopA parCE^{dup}* is observed (green arrow). Profiles shown represent one of two independent experiments for each strain.

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Discussion

The results demonstrate that impaired Topo I or Topo IV function can lead to an inability to maintain substrates containing convergent replication forks and that this correlates with abnormalities on the chromosome at sites where replication forks converge. In the case of Topo I, extensive work from the Drolet lab and others suggests the effect is largely related to the stabilization of R-loops, three-stranded nucleic acid structures, consisting of an RNA-DNA hybrid and a displaced single-stranded DNA [14,41,51,69–77]. These three-stranded structures form when transcribed RNA remains hybridized with the DNA template, and are stabilized under conditions when negative supercoiling increases. D-loops, three-stranded DNA structures, are similarly stabilized under these conditions. The hybridized strands of R-loops and D-loops can serve as primers that lead to RecA-dependent illegitimate initiations of replication [14,41,75,78,79]. Early studies often characterized R- and D-loop replication events as novel, or cryptic origins of replication based on copy number analysis of these regions in replicating cultures, and found they either localized to the origin or the terminus regions, where replication forks meet [80–85]. Loss of Topo I function was found to lead to an increase in these initiation events and could bypass the

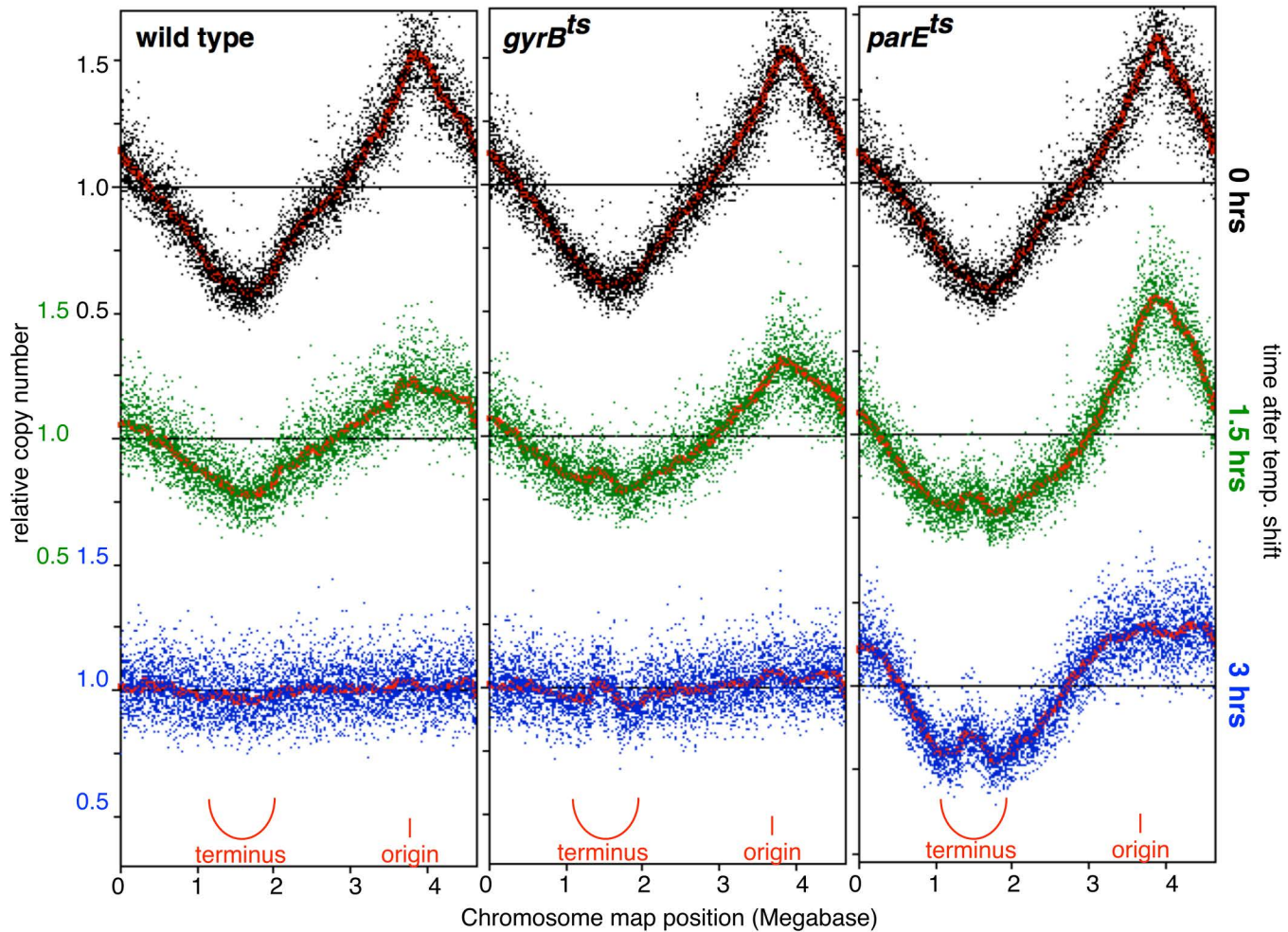


Fig 6. Inactivation of Topo IV leads to a specific inability to replicate the terminus region of the chromosome. Replication profiles of growing wild-type, *gyrB^{ts}*, and *parE^{ts}* cells are plotted at 0, 1.5, and 3 hrs after temperature shift from 30°C to 43°C. In actively growing wild-type cultures, the copy number of sequences decreases inversely with distance from the origin until reaching the terminus region. After three additional hours, replication in the culture has largely ceased, and the copy number of all sequences across the chromosome is equal. Following inactivation of gyrase (in *gyrB^{ts}* mutants) the ongoing rounds of replication are able to complete, similar to wild-type cells. However, inactivation of Topo IV (in *parE^{ts}* mutants) prevents the completion of ongoing replication events, leading to a loss of sequence reads in the terminus region and an inability to maintain the region of the chromosome where forks converge. Profiles shown represent one of two independent experiments for each strain.

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requirement for DnaA, encoding the essential replication initiation factor at *oriC* [14,41,73–75,78,84,86]. Consistent with these studies, we observe an over-replication of the terminus in suppressed strains lacking Topo I activity (Fig 5).

Although the over-replication of the terminus region in *topA* mutants is likely associated with elevated chromosomal instabilities, it seems unlikely to account for the essential role of Topo I in viability. While mutants which fail to maintain (under-replicate) the terminus region often compromise cell growth and viability, *E. coli* are generally tolerant of mutations that lead to over-replication in the terminus region [3,5,7,87]. Mutants lacking exonucleases, or the RecG helicase severely over-replicate the terminus region, yet remain viable and grow well. Thus, while the specific nature of these over-replicated intermediates remains unknown, it is clear that their presence does not necessarily compromise cell growth or viability. One possible reason for the essentiality of Topo I that has been proposed is that the supercoiling

alters and detrimentally affects cellular transcription in an essential way. This could occur directly by altering promoter recognition and efficiency, or impairing elongation [35,42,88]. It could also be an indirect effect of the stabilized R-loops, which may block essential processes of transcription or even replication progression, or a combination of both [14,35,41,54,69,89]. Excessive R-loops are associated with cellular toxicity. Growth defects in *topA* mutants can be partially suppressed by RNase HI overexpression [39,73]. However, another study, which relied on a plasmid maintenance assay, did not observe this RNase HI suppression, although *mhaA* mutants did exacerbate the *topA* effect [65]. Our observation that *topA* mutants can have issues maintaining plasmids in some contexts (Fig 1), could suggest why this latter study did not observe such an effect. The pleiotropic effects Topo I has on both transcription and replication make deciphering the essential function targeted by this enzyme difficult to identify and *topA* lethality may result from a combination of these possibilities. Nevertheless, we observed that Topo I activity was required to maintain plasmids containing convergent replication forks. Considering that it was not required on substrates containing a single replicosome, our results suggest some aspect of Topo I activity is contributing to the success or efficiency of events occurring during the completion of replication. Of interest is the modest over-replicated intermediate or transient amplifications observed between proximal *ter* sites in *topA* deletion mutants that contain suppressor mutations in gyrase or Topo IV. These amplifications are also observed in the *gyrB*^{ts} and *parE*^{ts} mutants alone but is reduced in the nonsuppressed, *topA2103* point mutation, raising the possibility that the amplification relates to alterations in type II activities of gyrase or Topo IV, rather than Topo I. Several other genes associated with the normal completion of replication exhibit similar over-replicated intermediates when mutated, although the structural form of these intermediates remains unclear [3,7,13,16,20,87]. Curiously, while the over-replication is likely associated with increased genomic instability and mutation, it does not affect viability or growth. Additionally, these mutants, including *recG* and *sbcCDxonA*, remain capable of replicating plasmids containing convergent replication forks [7,20]. In contrast, mutants which under-replicate, or fail to maintain this region, such as *recBCD* or *sbcCDxonA* lacking RecA, compromises growth and viability, similar to that seen in *parE*^{ts} mutants. Notably, these mutants also fail to propagate plasmid substrates containing convergent replication forks.

An essential function for Topo IV in allowing replication to reach the site where completion occurs was also observed. Topo IV inactivation leads to a specific loss of plasmids containing convergent replication forks which correlates with an accumulation of large unresolved plasmid intermediates containing single-strand regions (Figs 3–4). On the chromosome, inactivation of Topo IV manifests as an inability to replicate the terminus region of the chromosome, a phenotype which is likely to account for its essential role in viability (Fig 6). A number of genetic and biochemical studies from the Coz-zarelli lab distinguished the substrate preferences for gyrase and Topo IV resolving positive supercoils and catenanes, respectively [21,47,49,56]. In some elegant biochemical studies using *oriC*-plasmid substrates, Hiasa and Mariani took this further and showed that Topo IV was preferentially acting on the precatenanes behind the fork [22]. Interestingly and consistent with the observations on the chromosome reported here, they show that although either gyrase or Topo IV is sufficient to allow replication fork progression (Fig 7A), precatenane removal by Topo IV was essential to support the final stages of DNA replication. Notably, the under-replicated region spans several megabases, encompassing almost half of the chromosome, which may suggest that precatenane accumulation in the absence of Topo IV may reach a critical point which eventually inhibits replication elongation more generally.

Several studies have since supported the conclusion that Topo IV acts behind the fork throughout the replication cycle. Using fluorescently tagged version of a replisome protein (SSB), a replisome-trailing protein (SeqA), and Topo IV to localize these proteins in living cells, the Skarstad lab inferred that Topo IV followed the replication machinery during the cell cycle [59]. High throughput sequencing studies have also mapped Topo IV binding and cleavage sites across the genome, while the Sherratt lab found that Topo IV expression levels correlated with the time sister chromatids remained associated [57,58,90]. A mutation in *dnaX* that prevents Topo IV association with the travelling replisome was also shown to result in filamentation and segregation defects [91].

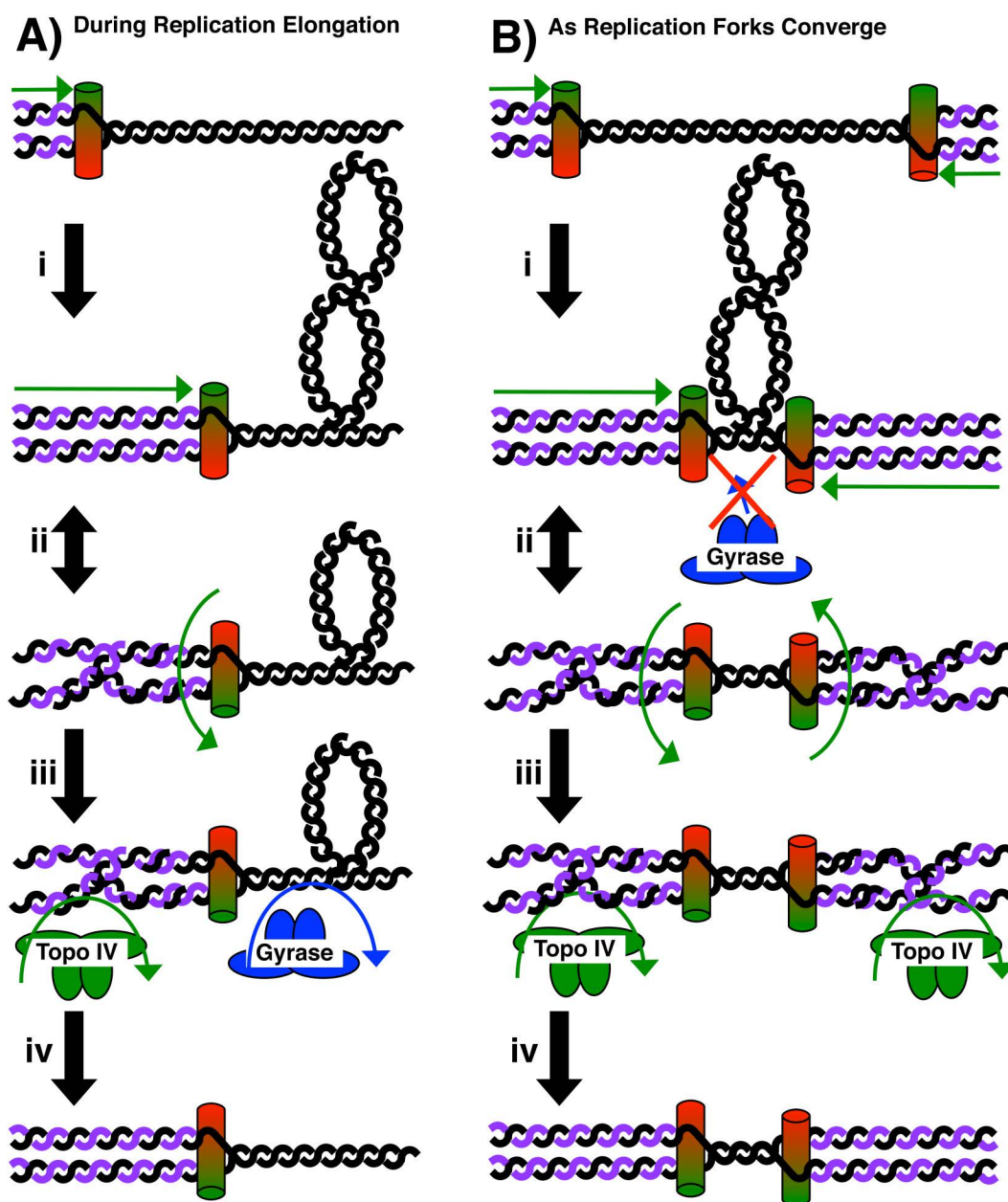


Fig 7. Model for the role of Topo IV in completing replication on the chromosome. A) During the normal progression of replication (i), unwinding of the DNA helix generates positive supercoiling, or overtwisting ahead of the DNA fork (ii). Rotating of the replisome and fork DNA can relieve the positive supercoiling, but generates twists, or precatenanes behind the fork (iii). Most data suggest that gyrase relieves positive supercoiling ahead of the fork, while Topo IV relieves precatenanes behind the fork (iv). B) When replisomes converge (i), gyrase is unable to relieve positive supercoiling between completing replisomes due to limited binding space (ii) This makes relieving this tension entirely dependent on the resolution of the precatenanes behind the fork (iii) by Topo IV (iv).

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Whereas, the above studies indicate Topo IV travels throughout the genome during replication, other observations suggest it has a unique activity at the end of replication. The genomic mapping of Topo IV binding and cleavage sites shows an enrichment of its activity in the terminus region proximal to the *dif* site [57–59]. Consistent with this, Topo IV

interacts directly with FtsK and cleaves proximal to the *dif* site in a XerCD resolvase-dependent manner [92,93]. The activities promoted by *dif* and FtsK, chromosome dimer resolution and cell septation, respectively, are events that occur after chromosome replication has completed. This would seem to indicate that either the Topo IV interactions are involved in signaling or regulating these later events, or that Topo IV also has resolving functions which operate after replication has completed [94]. Irrespectively, the lack of replication of the terminus region on the chromosome following Topo IV inactivation establishes an essential role for the enzyme in allowing convergent replication forks to progress to their meeting point. During the normal progression of DNA replication, gyrase is thought to act ahead of the fork to relieve the positive supercoils. However, the ability of Topo IV to resolve precatenanes can also relieve this tension if the replication forks swivel, which converts positive supercoiling ahead of the fork to precatenanes located behind the fork (Fig 7A). As demonstrated by Hiasa and Marians, either activity is sufficient to allow replication fork progression [22]. However, as the replication forks converge, the space remaining ahead of the fork diminishes, and at some point is likely to preclude gyrase from binding and acting. At this point, we would speculate that further progression of the converging replisomes may then depend entirely on the activity of Topo IV relieving this tension through the precatenane resolution which occurs behind the convergent replication forks (Fig 7B). In the absence of Topo IV, this superhelical tension would remain, preventing the replication forks from converging and replication of this region on the chromosome.

Several studies have also suggested that Topo III may act at convergent replication forks. In an elegant biochemical study by Suski and Marians, Topo III, encoded by *topB*, was shown to be capable of resolving convergent replication forks on *oriC* plasmids in the presence of RecQ [95]. Additionally, Topo III localizes at the replication fork and is active on precatenanes, similar to Topo IV [95]. It also acts as a multicopy suppressor of Topo IV mutants [62]. However, similar to others, we were unable to discern a phenotype in *topB* mutants associated with completing DNA replication, either on plasmids containing convergent forks, or on the chromosome in vivo [14]. While the observations indicate the enzyme is not required for this reaction to occur, its association with the RecQ family of helicases, and the replication fork suggests an important cellular function that our current assays or approaches have been unable to reveal.

Whereas completing replication occurs only once on the bacterial chromosome, it occurs hundreds to thousands of times per generation along the multi-origin, linear chromosomes of eukaryotic organisms as has been previously characterized [96–101]. Many of these bacterial proteins have structural or functional homologs in eukaryotes, suggesting that this fundamental aspect of cellular metabolism will be conserved throughout evolutionarily divergent organisms.

Materials and methods

Strains and plasmids

The parental strain used for this study is BW25113, which has the genotype *rrnB3 ΔlacZ4787 hsdR514 Δ(araBAD)567 Δ(rhaBAD)568 rph-1*. Strains used in this study are listed in Table 1. BW25113 *ymjA::FRT*-minikan (JW1288), BW25113 *yleL::FRT*-minikan (JW5612), and BW25113 *topB::FRT*-mini-kan (JW1752) are part of the Keio single gene knockout mutant collection [102]. CL4079 (BW25113 *sbcD::FRT*-mini-kan *topA2103*) containing a single base pair deletion in *topA* at nucleotide 2103 was isolated as a spontaneous secondary mutation that resulted in plasmid instability in laboratory stocks of BW25113 *sbcD::FRT*-mini-kan. To construct a BW25113 *topA2103* mutant, the kanamycin-resistance cassette from the *sbcD* deletion mutant was first removed using FLP recombinase expression from the pCP20 plasmid as described previously [103,104]. *topA2103* was then linked to a kanamycin-resistance cassette approximately 25 kb away by P1 transduction of *ymjA::Kan* from JW1288 and selecting for resistance to kanamycin and plasmid instability to generate CL5110 (BW25113 *sbcD::FRT topA2103 ymjA::FRT*-mini-kan). CL5111 was made by P1 transduction of *topA2103 ymjA::FRT*-mini-kan from CL5110 into BW25113. A second *topA* mutation, *Δ(topA-cysB)*, was similarly linked to a kanamycin-resistance cassette by P1 transduction of *ymjA::kan* from JW1288 into RFM475 (31) and selecting for kanamycin resistance and cysteine auxotrophy to generate CL5427. CL5379 (large colony morphology) and CL5380 (small colony morphology) were made by P1 transduction of *Δ(topA-cysB) ymjA::kan* from CL5427 into BW25113. All strains were

Table 1. Bacterial strains used in this study.

Bacterial Strain	Genotype	Source
BW25113 Parent	(<i>araD-araB</i>)567, Δ <i>lacZ</i> 4787(:: <i>rrnB</i> -3), λ -, <i>rph</i> -1, Δ (<i>rhaD-rhaB</i>)568, <i>hsdR</i> 514	[104]
CL4079	BW25113 <i>sbcD</i> ::FRT-mini- kan <i>topA</i> 2103	This study
CL5110	BW25113 <i>sbcD</i> ::FRT <i>topA</i> 2103 <i>ymjA</i> ::FRT-mini- kan	This study
CL5111	BW25113 <i>topA</i> 2103 <i>ymjA</i> ::FRTminikan	This study
CL5250	BW25113 <i>recBCD</i> ::kan	[20]
CL5328	N99 <i>gyrBts yieL</i> ::kan	This study
CL5379	BW25113 Δ (<i>topA-cysB</i>) <i>ymjA</i> ::kan <i>gyrA</i> (R630G)	This study
CL5380	BW25113 Δ (<i>topA-cysB</i>) <i>ymjA</i> ::kan <i>parEC</i> (duplication)	This study
CL5427	N99 Δ (<i>topA-cysB</i>) <i>ymjA</i> ::kan	This study
CL5428	BW25113 <i>parE</i> ^{ts} ::Tn10	This study
CL5429	BW25113 <i>parE</i> ^{ts} <i>tet</i> ^R	This study
CL5431	BW25113 <i>gyrB</i> ^{ts} <i>yieL</i> ::FRT- minikan	This study
JW1752	BW25113 <i>topB</i> ::FRTminikan	[102]
JW1288	BW25113 <i>ymjA</i> ::FRTminikan	[102]
JW5612	BW25113 <i>yieL</i> ::FRTminikan	[102]
RFM445	<i>rpsL galk2 gyrB</i> 221(<i>cou</i> ^R) <i>gyrB</i> 203(Ts) Δ <i>trpE</i> Δ <i>lac</i> 74	[39]
RFM475	<i>rpsL galk2 gyrB</i> 221(<i>cou</i> ^R) <i>gyrB</i> 203(Ts) Δ <i>trpE</i> Δ (<i>topA- cysB</i>)204 <i>trp</i> ⁺ Δ <i>lac</i> 74	[39]
<i>parE</i> (Ts)	MG1655 <i>parEts</i> ::Tc	[105]

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further verified by genome sequencing. CL5428 was made by P1 transduction of *parEts*::Tn10 from MG1655 *parEts*::Tc [105] into BW25113 then selected for loss of tetracycline resistance using chlortetracycline hydrochloride as previously described [106] to generate CL5429. A temperature-sensitive mutation in *gyrB* was linked to a kanamycin-resistance cassette by P1 transduction of *yieL*::kan, located approximately 25 kb away from *gyrB*, from JW5612 into RFM445 [39] and selected for kanamycin resistance and temperature sensitivity to generate CL5328. CL5431 was made by P1 transduction of *gyrBts yieL*::kan from CL5328 into BW25113. CL5429 (BW25113 *parEts*) and CL5431 (BW25113 *gyrBts yieL*::kan) were verified for temperature sensitivity.

Plasmid pBR322 used in this study contains a unidirectional origin of replication, which has been described previously [63] and confers ampicillin and tetracycline resistance. pCL01 plasmid used in this study contains a bidirectional origin of replication from bacteriophage λ [64] and ampicillin- and chloramphenicol-resistance cassettes [20]. Plasmids used in this study are listed in Table 2.

Plasmid assay

Electro-competent cells were prepared by growing a 100-fold dilution of a fresh overnight culture in 50-mL Luria-Bertani broth without salt (LB, no NaCl) to an OD₆₀₀ of 0.4. Strains were grown at 37°C or at 30°C for the temperature-sensitive strains. Cells were pelleted, washed with 50-mL of ice-cold 10% glycerol then resuspended in 200–300 μ L of ice-cold 10% glycerol, and immediately frozen at -80°C. pBR322 and pCL01 were combined in a single mixture such that equivalent transformants per viable cells were obtained for each plasmid in wild-type cultures. The same plasmid mixture was then

Table 2. Plasmids used in this study.

Plasmids	Genotype	Source
pBR322	<i>amp</i> ^R <i>tet</i> ^R ColE1 origin	[63]
pCL01	<i>amp</i> ^R <i>cam</i> ^R λ origin	[20]

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used for all strains analyzed. 50- μ L of competent cells were mixed with the plasmid mixture, electroporated at 2.5 kV, capacitance 25 μ F, resistance 200 Ohms, and allowed to recover in 1-mL SOC media for 60 minutes at 37°C. Transformation reactions were then 10-fold serially diluted and spotted in triplicate 10- μ L aliquots on sets of LB plates with no antibiotic, 15 μ g/mL tetracycline, or 20 μ g/mL chloramphenicol supplementation to determine the number of viable cells, pBR322, and pCL01 transformants, respectively. Plates were incubated overnight at 37°C and colonies were counted the next day. The relative transformation efficiency was calculated as the ratio of transformants per viable cells in the mutants to the transformants per viable cells in wild-type cultures.

For temperature-sensitive strains, transformation efficiencies were compared between the permissive (30°C) and semi-permissive (39°C) temperatures. 50- μ L of competent cells were mixed with plasmid mixture and electroporated using the conditions described above, except 2-mL of SOC was added to the cells post-electroporation, then each transformation reaction was divided equally into two 1-mL aliquots that were incubated for 1 hour at 30°C or 39°C. Following the 1-hour recovery period, cells were serially diluted and plated as described above. Plates were incubated overnight at each respective temperature and the next day, viability and transformation efficiency was determined as described above.

DNA purification for 2D gel electrophoresis

Overnight cultures of cells containing pBR322 or pCL01 grown in LB supplemented with 15 μ g/mL tetracycline or 20 μ g/mL chloramphenicol, respectively, were diluted 100-fold into 10-mL of LB and grown to an OD_{600} of 0.5 at 37°C or 30°C for temperature-sensitive strains. A 0.75-mL aliquot of cells was then collected into an equal volume of ice-cold NET buffer (100 mM NaCl; 10 mM Tris, pH 8; 10 mM EDTA, pH 8), pelleted then flash frozen, and stored at -80°C.

Cell pellets were resuspended in 120- μ L of lysis buffer (1 mg/mL lysozyme and 0.2 mg/mL RNaseA in 10 mM Tris, pH 8.0; 1 mM EDTA, pH 8.0) and incubated at 37°C for 30 minutes. Then, 10- μ L of 10 mg/mL Proteinase K and 10- μ L of 20% Sarkosyl were added to each sample, and incubation was continued at 37°C for 1 hour. Samples were extracted once using four volumes of phenol-chloroform, followed by two volumes of chloroform, then dialyzed on a 47-mm Millipore 0.05- μ m pore disk floating on 200-mL of 1 mM Tris, pH 8; 1 mM EDTA, pH 8.0 for 30 minutes.

2D gel electrophoresis and Southern analysis

Purified DNA from cells containing the pBR322 plasmid were digested with PvuII restriction enzyme, while purified DNA from cells containing the pCL01 plasmid were digested with BstEII restriction enzyme. Following a 24-hour incubation at 37°C, digested DNA was extracted with one volume of chloroform then loaded onto a 0.4% agarose gel in 1x TBE and electrophoresed at 1 V/cm for 14–16 hours. For the second dimension, lanes were excised, rotated 90°, recast in a 1% agarose gel in 1x TBE, and electrophoresed at 6.5 V/cm for 6–8 hours.

DNA from the gels was transferred to Hybond N+ nylon membrane. Plasmid DNA was detected using either 32 P-labeled pBR322 or pCL01 plasmid DNA prepared by random-primer labeling (Agilent Technologies) using alpha- 32 P-labeled-dCTP (3000 Ci/mmol) (Perkin-Elmer), and visualized using a STORM PhosphorImager (Molecular Dynamics) and analyzed using ImageJ software.

Sample preparation for replication profiles

Overnight cultures of each strain were diluted 100-fold into 10-mL of LB and grown to an OD_{600} of 0.5 at 37°C or 30°C for temperature-sensitive strains. Then, 0.75-mL aliquots of each culture were collected in an equal volume of ice-cold NET buffer (100 mM NaCl; 10 mM Tris, pH 8; 10 mM EDTA, pH 8). Cells were pelleted, flash frozen, and stored at -80°C.

For replication profiles following temperature shift in wild-type and temperature-sensitive strains, overnight cultures were diluted as described above and grown to an OD_{600} of 0.3 at the permissive temperature of 30°C. Half of the cells were then collected by filtration on 0.45- μ m membranes (Fisher Scientific) and resuspended in media pre-warmed to the

non-permissive (43°C) temperature, while the remaining culture was allowed to continue growing at 30°C. 0.75-mL aliquots of each culture were collected in an equal volume of ice-cold NET buffer immediately following resuspension in pre-warmed media and after a further 90- and 180-minutes incubation at 30°C, or 43°C, as described above. Stationary-phase wild-type cultures were grown in LB for 36 hours at 37°C for use as a normalization control.

Replication profiles

Genomic DNA was prepared as described for 2D gel electrophoresis above. Genomic DNA samples were sequenced using paired-end, 51-bp, bar-coded reads prepared and run using seqWell library prep kits (seqWell, Beverly MA, USA) and Illumina Next Seq 2000 (Illumina, San Diego, CA, USA). Breseq software is a command line tool that identifies mutations in DNA, relative to a reference genome [107]. This software was used to identify mutations between each strain and the wild-type BW25113 reference genome. Sequencing reads were aligned to the reference genome using Bowtie 1.0.0 software. Aligned reads were characterized to determine the nucleotide frequency at each position. Sequences were divided into 1-kb bins and plotted using a custom Python script developed by our lab. Copy numbers for each strain were normalized against the wild-type stationary culture. Plots represent the relative copy numbers at each genomic location in 1-kb bins and depict the replication profile for each strain.

Supporting information

S1 Fig. Both replisomes are active on the two-replisome plasmid. *ter* sequences were cloned into the plasmid, pCL01 in a manner that mimics their position on the chromosome, and the plasmids were analyzed by 2D agarose gel analysis as before. Two prominent sites where replisomes arrest, corresponding with the location of the *ter* sequences, are observed on the plasmid containing *ter* sequences (right panel) but not in their absence (left panel). Arrest sites are indicated by arrows. DNA preparation and 2D agarose gel analysis was performed as in Fig 4. (EPS)

S2 Fig. High-order intermediates accumulate on 2-replisome plasmids in *parE*^{ts} mutants. The percent of each replication intermediate in wild type, *gyrB*^{ts}, and *parE*^{ts} mutants is shown. The color-coded areas quantified from Fig 4 are indicated. Nonreplicating plasmids, red; Y-structure and Early double-Y- structures, green; Late double-Y structures, blue; Complex, high order intermediates, purple. (EPS)

S1 Data. Data files for reproducing graphs contained in the figures. (XLSX)

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References

1. Bleichert F, Botchan MR, Berger JM. Mechanisms for initiating cellular DNA replication. *Science*. 2017;355.
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(7):a010108. <https://doi.org/10.1101/cshperspect.a010108> PMID: 23818497
3. Rudolph CJ, Upton AL, Stockum A, Nieduszynski CA, Lloyd RG. Avoiding chromosome pathology when replication forks collide. *Nature*. 2013;500(7464):608–11. <https://doi.org/10.1038/nature12312> PMID: 23892781
4. Dimude JU, Midgley-Smith SL, Stein M, Rudolph CJ. Replication termination: containing fork fusion-mediated pathologies in *Escherichia coli*. *Genes (Basel)*. 2016;7.
5. Wendel BM, Courcelle CT, Courcelle J. Completion of DNA replication in *Escherichia coli*. *Proc Natl Acad Sci U S A*. 2014;111(46):16454–9. <https://doi.org/10.1073/pnas.1415025111> PMID: 25368150
6. Courcelle J, Wendel BM, Livingstone DD, Courcelle CT. RecBCD is required to complete chromosomal replication: implications for double-strand break frequencies and repair mechanisms. *DNA Repair (Amst)*. 2015;32:86–95.
7. Wendel BM, Cole JM, Courcelle CT, Courcelle J. SbcC-SbcD and ExoI process convergent forks to complete chromosome replication. *Proc Natl Acad Sci U S A*. 2018;115(2):349–54. <https://doi.org/10.1073/pnas.1715960114> PMID: 29208713
8. Hill TM. Arrest of bacterial DNA replication. *Annu Rev Microbiol*. 1992;46:603–33. <https://doi.org/10.1146/annurev.mi.46.100192.003131> PMID: 1444268
9. Hill TM, Mariani KJ. *Escherichia coli* Tus protein acts to arrest the progression of DNA replication forks in vitro. *Proc Natl Acad Sci U S A*. 1990;87(7):2481–5. <https://doi.org/10.1073/pnas.87.7.2481> PMID: 2181438
10. Hill TM, Tecklenburg ML, Pelletier AJ, Kuempel PL. Tus, the trans-acting gene required for termination of DNA replication in *Escherichia coli*, encodes a DNA-binding protein. *Proc Natl Acad Sci U S A*. 1989;86:1593–7.
11. Kuempel PL, Duerr SA, Seeley NR. Terminus region of the chromosome in *Escherichia coli* inhibits replication forks. *Proc Natl Acad Sci U S A*. 1977;74(9):3927–31. <https://doi.org/10.1073/pnas.74.9.3927> PMID: 333449
12. Duggin IG, Wake RG, Bell SD, Hill TM. The replication fork trap and termination of chromosome replication. *Mol Microbiol*. 2008;70(6):1323–33. <https://doi.org/10.1111/j.1365-2958.2008.06500.x> PMID: 19019156
13. Wendel BM, Hernandez AJ, Courcelle CT, Courcelle J. Ligase A and RNase HI Participate in Completing Replication on the Chromosome in *Escherichia coli*. *DNA*. 2021;1:13–25.
14. 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(9):e1007668. <https://doi.org/10.1371/journal.pgen.1007668> PMID: 30222737
15. Hiasa H, Mariani KJ. Tus prevents overreplication of oriC plasmid DNA. *J Biol Chem*. 1994;269:26959–68.
16. Midgley-Smith SL, Dimude JU, Rudolph CJ. A role for 3' exonucleases at the final stages of chromosome duplication in *Escherichia coli*. *Nucleic Acids Res*. 2019;47(4):1847–60. <https://doi.org/10.1093/nar/gky1253> PMID: 30544222
17. Whitby MC, Lloyd RG. Branch migration of three-strand recombination intermediates by RecG, a possible pathway for securing exchanges initiated by 3'-tailed duplex DNA. *EMBO J*. 1995;14(14):3302–10. <https://doi.org/10.1002/j.1460-2075.1995.tb07337.x> PMID: 7628432
18. Fukuoka A, Iwasaki H, Ishioka K, Shinagawa H. ATP-dependent resolution of R-loops at the ColE1 replication origin by *Escherichia coli* RecG protein, a Holliday junction-specific helicase. *EMBO J*. 1997;16(1):203–9. <https://doi.org/10.1093/emboj/16.1.203> PMID: 9009281
19. Rudolph CJ, Upton AL, Harris L, Lloyd RG. Pathological replication in cells lacking RecG DNA translocase. *Mol Microbiol*. 2009;73(3):352–66. <https://doi.org/10.1111/j.1365-2958.2009.06773.x> PMID: 19538444
20. Hamilton NA, Wendel BM, Weber EA, Courcelle CT, Courcelle J. RecBCD, SbcCD and ExoI process a substrate created by convergent replisomes to complete DNA replication. *Mol Microbiol*. 2019;111(6):1638–51. <https://doi.org/10.1111/mmi.14242> PMID: 30883946
21. Zechiedrich EL, Cozzarelli NR. Roles of topoisomerase IV and DNA gyrase in DNA unlinking during replication in *Escherichia coli*. *Genes Dev*. 1995;9(22):2859–69. <https://doi.org/10.1101/gad.9.22.2859> PMID: 7590259
22. Hiasa H, Mariani KJ. Two distinct modes of strand unlinking during theta-type DNA replication. *J Biol Chem*. 1996;271(35):21529–35. <https://doi.org/10.1074/jbc.271.35.21529> PMID: 8702938

23. Wang JC. Cellular roles of DNA topoisomerases: a molecular perspective. *Nat Rev Mol Cell Biol.* 2002;3:430–40.
24. Peter BJ, Ullsperger C, Hiasa H, Mariani KJ, Cozzarelli NR. The structure of supercoiled intermediates in DNA replication. *Cell.* 1998;94(6):819–27. [https://doi.org/10.1016/s0092-8674\(00\)81740-7](https://doi.org/10.1016/s0092-8674(00)81740-7) PMID: 9753328
25. Cebrián J, Castán A, Martínez V, Kadomatsu-Hermosa MJ, Parra C, Fernández-Nestosa MJ, et al. Direct Evidence for the Formation of Precatenanes during DNA Replication. *J Biol Chem.* 2015;290(22):13725–35. <https://doi.org/10.1074/jbc.M115.642272> PMID: 25829493
26. Postow L, Crisona NJ, Peter BJ, Hardy CD, Cozzarelli NR. Topological challenges to DNA replication: conformations at the fork. *Proc Natl Acad Sci U S A.* 2001;98(15):8219–26. <https://doi.org/10.1073/pnas.111006998> PMID: 11459956
27. Trucksis M, Depew RE. Identification and localization of a gene that specifies production of *Escherichia coli* DNA topoisomerase I. *Proc Natl Acad Sci U S A.* 1981;78:2164–8.
28. DiGate RJ, Mariani KJ. Molecular cloning and DNA sequence analysis of *Escherichia coli* topB, the gene encoding topoisomerase III. *J Biol Chem.* 1989;264(30):17924–30. PMID: 2553698
29. Zhu Q, Pongpech P, DiGate RJ. Type I topoisomerase activity is required for proper chromosomal segregation in *Escherichia coli*. *Proc Natl Acad Sci U S A.* 2001;98(17):9766–71. <https://doi.org/10.1073/pnas.171579898> PMID: 11493711
30. Stupina VA, Wang JC. Viability of *Escherichia coli* topA mutants lacking DNA topoisomerase I. *J Biol Chem.* 2005;280:355–60.
31. Kirkegaard K, Wang JC. Bacterial DNA topoisomerase I can relax positively supercoiled DNA containing a single-stranded loop. *J Mol Biol.* 1985;185:625–37.
32. Pruss GJ, Manes SH, Drlica K. *Escherichia coli* DNA topoisomerase I mutants: increased supercoiling is corrected by mutations near gyrase genes. *Cell.* 1982;31(1):35–42. [https://doi.org/10.1016/0092-8674\(82\)90402-0](https://doi.org/10.1016/0092-8674(82)90402-0) PMID: 6297751
33. Brown PO, Cozzarelli NR. Catenation and knotting of duplex DNA by type 1 topoisomerases: a mechanistic parallel with type 2 topoisomerases. *Proc Natl Acad Sci U S A.* 1981;78(2):843–7. <https://doi.org/10.1073/pnas.78.2.843> PMID: 6262776
34. Heddle JG, Lu T, Zhao X, Drlica K, Maxwell A. gyrB-225, a mutation of DNA gyrase that compensates for topoisomerase I deficiency: investigation of its low activity and quinolone hypersensitivity. *J Mol Biol.* 2001;309:1219–31.
35. Massé E, Drolet M. Relaxation of transcription-induced negative supercoiling is an essential function of *Escherichia coli* DNA topoisomerase I. *J Biol Chem.* 1999;274(23):16654–8. <https://doi.org/10.1074/jbc.274.23.16654> PMID: 10347233
36. Kato J, Nishimura Y, Imamura R, Niki H, Hiraga S, Suzuki H. New topoisomerase essential for chromosome segregation in *E. coli*. *Cell.* 1990;63:393–404.
37. Hammond GG, Cassidy PJ, Overbye KM. Novobiocin-dependent topA deletion mutants of *Escherichia coli*. *J Bacteriol.* 1991;173(17):5564–7. <https://doi.org/10.1128/jb.173.17.5564-5567.1991> PMID: 1653212
38. DiNardo S, Voelkel KA, Sternglanz R, Reynolds AE, Wright A. *Escherichia coli* DNA topoisomerase I mutants have compensatory mutations in DNA gyrase genes. *Cell.* 1982;31(1):43–51. [https://doi.org/10.1016/0092-8674\(82\)90403-2](https://doi.org/10.1016/0092-8674(82)90403-2) PMID: 6297752
39. Drolet M, Phoenix P, Menzel R, Massé E, Liu LF, Crouch RJ. Overexpression of RNase H partially complements the growth defect of an *Escherichia coli* delta topA mutant: R-loop formation is a major problem in the absence of DNA topoisomerase I. *Proc Natl Acad Sci U S A.* 1995;92(8):3526–30. <https://doi.org/10.1073/pnas.92.8.3526> PMID: 7536935
40. Broccoli S, Phoenix P, Drolet M. Isolation of the topB gene encoding DNA topoisomerase III as a multicopy suppressor of topA null mutations in *Escherichia coli*. *Mol Microbiol.* 2000;35(1):58–68. <https://doi.org/10.1046/j.1365-2958.2000.01671.x> PMID: 10632877
41. Leela JK, Raghunathan N, Gowrishankar J. Topoisomerase I Essentiality, DnaA-Independent Chromosomal Replication, and Transcription-Replication Conflict in *Escherichia coli*. *J Bacteriol.* 2021;203(17):e0019521. <https://doi.org/10.1128/JB.00195-21> PMID: 34124945
42. Collins I, Weber A, Levens D. Transcriptional consequences of topoisomerase inhibition. *Mol Cell Biol.* 2001;21(24):8437–51. <https://doi.org/10.1128/MCB.21.24.8437-8451.2001> PMID: 11713279
43. Sternglanz R, DiNardo S, Voelkel KA, Nishimura Y, Hirota Y, Becherer K, et al. Mutations in the gene coding for *Escherichia coli* DNA topoisomerase I affect transcription and transposition. *Proc Natl Acad Sci U S A.* 1981;78(5):2747–51. <https://doi.org/10.1073/pnas.78.5.2747> PMID: 6265907
44. Harmon FG, DiGate RJ, Kowalczykowski SC. RecQ helicase and topoisomerase III comprise a novel DNA strand passage function: a conserved mechanism for control of DNA recombination. *Mol Cell.* 1999;3(5):611–20. [https://doi.org/10.1016/s1097-2765\(00\)80354-8](https://doi.org/10.1016/s1097-2765(00)80354-8) PMID: 10360177
45. Lee CM, Wang G, Pertsinidis A, Mariani KJ. Topoisomerase III Acts at the Replication Fork To Remove Precatenanes. *J Bacteriol.* 2019;201(7):e00563-18. <https://doi.org/10.1128/JB.00563-18> PMID: 30617245
46. DiGate RJ, Mariani KJ. Identification of a potent decatenating enzyme from *Escherichia coli*. *J Biol Chem.* 1988;263(26):13366–73. PMID: 2843517
47. Brown PO, Peebles CL, Cozzarelli NR. A topoisomerase from *Escherichia coli* related to DNA gyrase. *Proc Natl Acad Sci U S A.* 1979;76(12):6110–4. <https://doi.org/10.1073/pnas.76.12.6110> PMID: 230498
48. Kato J, Nishimura Y, Yamada M, Suzuki H, Hirota Y. Gene organization in the region containing a new gene involved in chromosome partition in *Escherichia coli*. *J Bacteriol.* 1988;170(9):3967–77. <https://doi.org/10.1128/jb.170.9.3967-3977.1988> PMID: 2842295
49. Ullsperger C, Cozzarelli NR. Contrasting enzymatic activities of topoisomerase IV and DNA gyrase from *Escherichia coli*. *J Biol Chem.* 1996;271(49):31549–55. <https://doi.org/10.1074/jbc.271.49.31549> PMID: 8940171

50. Gellert M, O'Dea MH, Itoh T, Tomizawa J. Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. *Proc Natl Acad Sci U S A*. 1976;73(12):4474–8. <https://doi.org/10.1073/pnas.73.12.4474> PMID: 794878
51. Phoenix P, Raymond MA, Massé E, Drolet M. Roles of DNA topoisomerases in the regulation of R-loop formation in vitro. *J Biol Chem*. 1997;272(3):1473–9. <https://doi.org/10.1074/jbc.272.3.1473> PMID: 8999816
52. Oram M, Fisher LM. An *Escherichia coli* DNA topoisomerase I mutant has a compensatory mutation that alters two residues between functional domains of the DNA gyrase A protein. *J Bacteriol*. 1992;174(12):4175–8. <https://doi.org/10.1128/jb.174.12.4175-4178.1992> PMID: 1317847
53. Mirkin SM, Shmerling ZG. DNA replication and transcription in a temperature-sensitive mutant of *E. coli* with a defective DNA gyrase B subunit. *Mol Gen Genet*. 1982;188(1):91–5. <https://doi.org/10.1007/BF00332999> PMID: 6294483
54. Khodursky AB, Peter BJ, Schmid MB, DeRisi J, Botstein D, Brown PO, et al. Analysis of topoisomerase function in bacterial replication fork movement: use of DNA microarrays. *Proc Natl Acad Sci U S A*. 2000;97(17):9419–24. <https://doi.org/10.1073/pnas.97.17.9419> PMID: 10944214
55. Kato J, Suzuki H, Ikeda H. Purification and characterization of DNA topoisomerase IV in *Escherichia coli*. *J Biol Chem*. 1992;267(36):25676–84. PMID: 1334483
56. Adams DE, Shekhtman EM, Zechiedrich EL, Schmid MB, Cozzarelli NR. The role of topoisomerase IV in partitioning bacterial replicons and the structure of catenated intermediates in DNA replication. *Cell*. 1992;71:277–88.
57. El Sayyed H, Le Chat L, Lebailly E, Vickridge E, Pages C, Cornet F, et al. Mapping Topoisomerase IV Binding and Activity Sites on the *E. coli* Genome. *PLoS Genet*. 2016;12(5):e1006025. <https://doi.org/10.1371/journal.pgen.1006025> PMID: 27171414
58. Sutormin D, Galivondzhyan A, Gafurov A, Severinov K. Single-nucleotide resolution detection of Topo IV cleavage activity in the *Escherichia coli* genome with Topo-Seq. *Front Microbiol*. 2023;14:1160736. <https://doi.org/10.3389/fmicb.2023.1160736> PMID: 37089538
59. Helgesen E, Sætre F, Skarstad K. Topoisomerase IV tracks behind the replication fork and the SeqA complex during DNA replication in *Escherichia coli*. *Sci Rep*. 2021;11(1):474. <https://doi.org/10.1038/s41598-020-80043-4> PMID: 33436807
60. Evans J, Maccabee M, Hatahet Z, Courcelle J, Bockrath R, Ide H, et al. Thymine ring saturation and fragmentation products: lesion bypass, misinsertion and implications for mutagenesis. *Mutat Res*. 1993;299(3–4):147–56. [https://doi.org/10.1016/0165-1218\(93\)90092-r](https://doi.org/10.1016/0165-1218(93)90092-r) PMID: 7683083
61. Hamilton NA, Jehru AE, Samples WN, Wendel BM, Mokhtari PD, Courcelle CT. Chi sequences switch the RecBCD helicase-nuclease complex from degradative to replicative modes during the completion of DNA replication. *J Biol Chem*. 2023;:103013. <https://doi.org/10.1074/jbc.RA123456789>
62. Nurse P, Levine C, Hassing H, Mariani KJ. Topoisomerase III Can Serve as the Cellular Decatenase in *Escherichia coli*. *Journal of Biological Chemistry*. 2003;278:8653–60.
63. Bolivar F, Rodriguez RL, Greene PJ, Betlach MC, Heyneker HL, Boyer HW, et al. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. *Gene*. 1977;2(2):95–113. PMID: 344137
64. Boyd AC, Sherratt DJ. The pCLIP plasmids: versatile cloning vectors based on the bacteriophage lambda origin of replication. *Gene*. 1995;153(1):57–62. [https://doi.org/10.1016/0378-1119\(94\)00788-t](https://doi.org/10.1016/0378-1119(94)00788-t) PMID: 7883185
65. Stockum A, Lloyd RG, Rudolph CJ. On the viability of *Escherichia coli* cells lacking DNA topoisomerase I. *BMC Microbiol*. 2012;12:26. <https://doi.org/10.1186/1471-2180-12-26> PMID: 22373098
66. Orr E, Staudenbauer WL. An *Escherichia coli* mutant thermosensitive in the B subunit of DNA gyrase: effect on the structure and replication of the colicin E1 plasmid in vitro. *Mol Gen Genet*. 1981;181(1):52–6. <https://doi.org/10.1007/BF00339004> PMID: 6261085
67. Friedman KL, Brewer BJ. Analysis of replication intermediates by two-dimensional agarose gel electrophoresis. *Methods Enzymol*. 1995;262:613–27.
68. Courcelle J, Donaldson JR, Chow KH, Courcelle CT. DNA Damage-Induced Replication Fork Regression and Processing in *Escherichia coli*. *Science*. 2003;299:1064–7.
69. Drolet M, Bi X, Liu LF. Hypernegative supercoiling of the DNA template during transcription elongation in vitro. *J Biol Chem*. 1994;269(3):2068–74. PMID: 8294458
70. Usongo V, Nolent F, Sanscartier P, Tanguay C, Broccoli S, Baaklini I, et al. Depletion of RNase HI activity in *Escherichia coli* lacking DNA topoisomerase I leads to defects in DNA supercoiling and segregation. *Mol Microbiol*. 2008;69(4):968–81. <https://doi.org/10.1111/j.1365-2958.2008.06334.x> PMID: 18554330
71. Usongo V, Drolet M. Roles of type 1A topoisomerases in genome maintenance in *Escherichia coli*. *PLoS Genet*. 2014;10(8):e1004543. <https://doi.org/10.1371/journal.pgen.1004543> PMID: 25102178
72. Martel M, Balleydier A, Sauriol A, Drolet M. Constitutive stable DNA replication in *Escherichia coli* cells lacking type 1A topoisomerase activity. *DNA Repair (Amst)*. 2015;35:37–47.
73. Usongo V, Martel M, Balleydier A, Drolet M. Mutations reducing replication from R-loops suppress the defects of growth, chromosome segregation and DNA supercoiling in cells lacking topoisomerase I and RNase HI activity. *DNA Repair (Amst)*. 2016;40:1–17. <https://doi.org/10.1016/j.dnarep.2016.02.001> PMID: 26947024
74. Brochu J, Breton É-V, Drolet M. Supercoiling, R-loops, Replication and the Functions of Bacterial Type 1A Topoisomerases. *Genes (Basel)*. 2020;11(3):249. <https://doi.org/10.3390/genes11030249> PMID: 32120891

75. Brochu J, Vlachos-Breton É, Irsenco D, Drolet M. Characterization of a pathway of genomic instability induced by R-loops and its regulation by topoisomerases in *E. coli*. *PLoS Genet*. 2023;19(5):e1010754. <https://doi.org/10.1371/journal.pgen.1010754> PMID: 37141391
76. Sutormin D, Galivondzhyan A, Musharova O, Travin D, Rusanova A, Obratsova K, et al. Interaction between transcribing RNA polymerase and topoisomerase I prevents R-loop formation in *E. coli*. *Nat Commun*. 2022;13(1):4524. <https://doi.org/10.1038/s41467-022-32106-5> PMID: 35927234
77. Lang KS, Merrikh H. Topological stress is responsible for the detrimental outcomes of head-on replication-transcription conflicts. *Cell Rep*. 2021;34(9):108797. <https://doi.org/10.1016/j.celrep.2021.108797> PMID: 33657379
78. Asai T, Kogoma T. D-loops and R-loops: alternative mechanisms for the initiation of chromosome replication in *Escherichia coli*. *J Bacteriol*. 1994;176(7):1807–12. <https://doi.org/10.1128/jb.176.7.1807-1812.1994> PMID: 8144445
79. Hong X, Cadwell GW, Kogoma T. *Escherichia coli* RecG and RecA proteins in R-loop formation. *Embo J*. 1995;14:2385–92.
80. Magee TR, Asai T, Malka D, Kogoma T. DNA damage-inducible origins of DNA replication in *Escherichia coli*. *EMBO J*. 1992;11(11):4219–25. <https://doi.org/10.1002/j.1460-2075.1992.tb05516.x> PMID: 1396602
81. Hong X, Kogoma T. Absence of a direct role for RNase HI in initiation of DNA replication at the *oriC* site on the *Escherichia coli* chromosome. *J Bacteriol*. 1993;175:6731–4.
82. Cao Y, Rowland RR, Kogoma T. DNA polymerase I and the bypassing of RecA dependence of constitutive stable DNA replication in *Escherichia coli* *rnhA* mutants. *J Bacteriol*. 1993;175(22):7247–53. <https://doi.org/10.1128/jb.175.22.7247-7253.1993> PMID: 8226671
83. Cao Y, Kogoma T. Requirement for the polymerization and 5'→3' exonuclease activities of DNA polymerase I in initiation of DNA replication at *oriK* sites in the absence of RecA in *Escherichia coli* *rnhA* mutants. *J Bacteriol*. 1993;175(22):7254–9. <https://doi.org/10.1128/jb.175.22.7254-7259.1993> PMID: 8226672
84. Maduike NZ, Tehranchi AK, Wang JD, Kreuzer KN. Replication of the *Escherichia coli* chromosome in RNase HI-deficient cells: multiple initiation regions and fork dynamics. *Mol Microbiol*. 2014;91(1):39–56. <https://doi.org/10.1111/mmi.12440> PMID: 24164596
85. de Massy B, Patte J, Louarn JM, Bouche JP. *oriX*: a new replication origin in *E. coli*. *Cell*. 1984;36:221–7.
86. Louarn J, Bouché JP, Patte J, Louarn JM. Genetic inactivation of topoisomerase I suppresses a defect in initiation of chromosome replication in *Escherichia coli*. *Mol Gen Genet*. 1984;195(1–2):170–4. <https://doi.org/10.1007/BF00332741> PMID: 6092846
87. Midgley-Smith SL, Dimude JU, Taylor T, Forrester NM, Upton AL, Lloyd RG, et al. 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(15):7701–15. <https://doi.org/10.1093/nar/gky566> PMID: 29982635
88. Peter BJ, Arsuaga J, Breier AM, Khodursky AB, Brown PO, Cozzarelli NR. Genomic transcriptional response to loss of chromosomal supercoiling in *Escherichia coli*. *Genome Biol*. 2004;5(11):R87. <https://doi.org/10.1186/gb-2004-5-11-r87> PMID: 15535863
89. Gan W, Guan Z, Liu J, Gui T, Shen K, Manley JL, et al. R-loop-mediated genomic instability is caused by impairment of replication fork progression. *Genes Dev*. 2011;25(19):2041–56. <https://doi.org/10.1101/gad.17010011> PMID: 21979917
90. Wang X, Reyes-Lamothe R, Sherratt DJ. Modulation of *Escherichia coli* sister chromosome cohesion by topoisomerase IV. *Genes Dev*. 2008;22(17):2426–33. <https://doi.org/10.1101/gad.487508> PMID: 18765793
91. Espeli O, Levine C, Hassing H, Mariani KJ. Temporal regulation of topoisomerase IV activity in *E. coli*. *Mol Cell*. 2003;11(1):189–201. [https://doi.org/10.1016/s1097-2765\(03\)00013-3](https://doi.org/10.1016/s1097-2765(03)00013-3) PMID: 12535532
92. Espeli O, Lee C, Mariani KJ. A physical and functional interaction between *Escherichia coli* FtsK and topoisomerase IV. *J Biol Chem*. 2003;278:44639–44.
93. Hojgaard A, Szerlong H, Tabor C, Kuempel P. Norfloxacin-induced DNA cleavage occurs at the *dif* resolvase locus in *Escherichia coli* and is the result of interaction with topoisomerase IV. *Mol Microbiol*. 1999;33(5):1027–36. <https://doi.org/10.1046/j.1365-2958.1999.01545.x> PMID: 10476036
94. Shimokawa K, Ishihara K, Grainge I, Sherratt DJ, Vazquez M. FtsK-dependent XerCD-*dif* recombination unlinks replication catenanes in a step-wise manner. *Proc Natl Acad Sci U S A*. 2013;110(52):20906–11. <https://doi.org/10.1073/pnas.1308450110> PMID: 24218579
95. Suski C, Mariani KJ. Resolution of converging replication forks by RecQ and topoisomerase III. *Mol Cell*. 2008;30(6):779–89. <https://doi.org/10.1016/j.molcel.2008.04.020> PMID: 18570879
96. McGuffee SR, Smith DJ, Whitehouse I. Quantitative, genome-wide analysis of eukaryotic replication initiation and termination. *Mol Cell*. 2013;50(1):123–35. <https://doi.org/10.1016/j.molcel.2013.03.004> PMID: 23562327
97. Heintzman DR, Campos LV, Byl JAW, Osheroff N, Dewar JM. Topoisomerase II Is Crucial for Fork Convergence during Vertebrate Replication Termination. *Cell Reports*. 2019;29:422–436.e5.
98. Deegan TD, Baxter J, Ortiz Bazán MÁ, Yeeles JTP, Labib KPM. Pif1-Family Helicases Support Fork Convergence during DNA Replication Termination in Eukaryotes. *Mol Cell*. 2019;74(2):231–244.e9. <https://doi.org/10.1016/j.molcel.2019.01.040> PMID: 30850330
99. Claussin C, Vazquez J, Whitehouse I. Single-molecule mapping of replisome progression. *Mol Cell*. 2022;82(7):1372–1382.e4. <https://doi.org/10.1016/j.molcel.2022.02.010> PMID: 35240057
100. Campos LV, Van Ravenstein SX, Vontalge EJ, Greer BH, Heintzman DR, Kavlashvili T, et al. RTEL1 and MCM10 overcome topological stress during vertebrate replication termination. *Cell Rep*. 2023;42:112109.

101. Polo Rivera C, Deegan TD, Labib KPM. CMG helicase disassembly is essential and driven by two pathways in budding yeast. *EMBO J*. 2024;43(18):3818–45. <https://doi.org/10.1038/s44318-024-00161-x> PMID: [39039287](https://pubmed.ncbi.nlm.nih.gov/39039287/)
102. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, et al. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol Syst Biol*. 2006;2:2006.0008. <https://doi.org/10.1038/msb4100050> PMID: [16738554](https://pubmed.ncbi.nlm.nih.gov/16738554/)
103. Cherepanov PP, Wackernagel W. Gene disruption in *Escherichia coli*: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. *Gene*. 1995;158(1):9–14. [https://doi.org/10.1016/0378-1119\(95\)00193-a](https://doi.org/10.1016/0378-1119(95)00193-a) PMID: [7789817](https://pubmed.ncbi.nlm.nih.gov/7789817/)
104. Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci U S A*. 2000;97(12):6640–5. <https://doi.org/10.1073/pnas.120163297> PMID: [10829079](https://pubmed.ncbi.nlm.nih.gov/10829079/)
105. Conin B, Billault-Chaumartin I, El Sayyed H, Quenech'Du N, Cockram C, Koszul R, et al. Extended sister-chromosome catenation leads to massive reorganization of the *E. coli* genome. *Nucleic Acids Res*. 2022;50(5):2635–50. <https://doi.org/10.1093/nar/gkac105> PMID: [35212387](https://pubmed.ncbi.nlm.nih.gov/35212387/)
106. Maloy SR, Nunn WD. Selection for loss of tetracycline resistance by *Escherichia coli*. *J Bacteriol*. 1981;145(2):1110–1. <https://doi.org/10.1128/jb.145.2.1110-1111.1981> PMID: [7007341](https://pubmed.ncbi.nlm.nih.gov/7007341/)
107. Barrick JE, Colburn G, Deatherage DE, Traverse CC, Strand MD, Borges JJ, et al. Identifying structural variation in haploid microbial genomes from short-read resequencing data using breseq. *BMC Genomics*. 2014;15(1):1039. <https://doi.org/10.1186/1471-2164-15-1039> PMID: [25432719](https://pubmed.ncbi.nlm.nih.gov/25432719/)