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Replication-transcription conflicts in bacteria

Houra Merrikh^{1,2}, Yan Zhang³, Alan D. Grossman^{1*} and Jue D. Wang^{3*}

¹ Department of Biology; Building 68-530; Massachusetts Institute of Technology;
Cambridge, MA 02139

² Department of Microbiology; University of Washington; Seattle, WA, 98195

³ Department of Molecular and Human Genetics; Baylor College of Medicine; Houston,
TX 77030

* Corresponding authors

Running title: Replication-transcription conflicts

Summary: Replication-transcription conflicts in bacteria

- DNA replication and transcription share the same DNA template. Encounters between the replication and transcription machineries can lead to conflicts that result in disruption of replication, genome instability and reduced fitness.
- Replication-transcription conflicts can occur at DNA lesions, both during normal growth and upon stresses.
- Replication-transcription conflicts can occur when replication and transcription are co-directional (when genes are encoded on the leading strand), and are more severe when transcription is oriented head-on to replication (genes on the lagging strand).
- Cells utilize various mechanisms to prevent replication-transcription conflicts from occurring and to resolve conflicts once they have occurred.
- Factors involved in avoiding and resolving replication-transcription conflicts include evolutionary pressures on genome organization that favor genes on the leading strand, and accessory helicases, and modulators of transcription and translation.
- When replication-transcription conflicts do occur, cells use a variety of mechanisms to repair and restart stalled replication forks.

Preface

DNA replication and transcription use the same template and occur concurrently in bacteria. The lack of temporal and spatial separations of these two processes leads to their conflict. Failure to deal with these conflicts can result in genome alterations and reduced fitness. In recent years, significant advances have been made in understanding how cells avoid conflicts between replication and transcription, and how conflicts are resolved when they do occur. In this review, we summarize these findings, which shed light on the significance of the problem and on how cells deal with unwanted encounters between the replication and transcription machineries.

Introduction

Many bacteria have a circular chromosome with a single origin of replication, *oriC*. The bacterial replication initiation protein DnaA binds to sites in *oriC* and causes local unwinding of a region in the origin. This region serves as a platform for the assembly of the replication machinery (the replisome). The replisome includes the catalytic subunit of DNA polymerase, the beta-clamp (processivity clamp) that enables replication to proceed processively around the chromosome, the clamp loader complex, the replicative helicase that unwinds the duplex DNA during replication, and the primase,^{1,2}. Replication forks proceed bi-directionally in a clockwise and counterclockwise manner around the chromosome, duplicating the genome at a rate of approximately 500 - 1,000 base pairs (bp) per second. Replication finishes in a region known as the terminus (*ter*) located approximately 180° from *oriC* (Fig. 1A).

When the forks are moving on the chromosome, they can face various obstacles, including the transcription apparatus, other DNA binding proteins, and DNA/RNA secondary structures. Encounters between replication and transcription machineries

lead to conflicts, and failure to deal with these conflicts can lead to genome instability, including chromosomal deletions and rearrangements^{3,4}. Recent work shows that genome-wide conflicts are more prevalent than previously appreciated. Not only do conflicts occur at DNA lesions⁵, they also occur independently of preexisting DNA damage⁶ and replication is disrupted not only upon head-on encounters with the transcription machinery^{7,8}, but also during co-directional encounters on the chromosome⁹ and on a plasmid¹⁰. Relatively little is known about what happens to the replication machinery at sites of conflict, and different types of conflicts likely result in different repair mechanisms. Some conditions that cause arrest of replication forks appear to cause dissociation of some components of the replisome (DnaN) while other components remain in place^{11,12}. Even though little is known about what exactly happens at sites of conflict and replication arrest, it is clear that cells have a variety of strategies to avoid and resolve these conflicts (Figs. 2, 3) and to preserve genomic integrity.

Two types of replication-transcription conflicts

Depending on the orientation of a given gene, the replication machinery can face RNA polymerases (RNAPs) in either a head-on or a co-directional manner (Fig. 1B and 1C). Genes that are encoded on the leading strand are co-oriented with replication, and genes encoded on the lagging strand are in the opposite orientation. Early work in the field suggested that head-on conflicts (lagging strand encounters) in bacteria cause DNA replication to stall, but co-directional conflicts were generally not detected *in vivo* and were therefore thought to be benign^{8,13-18}. However, recent findings indicate that both types of conflicts (head-on and co-directional) can disrupt replication *in vivo* and that auxiliary factors are involved in resolution of these conflicts^{9,10}.

Head-on conflicts

Evidence over the years from several different studies in *E. coli*, *B. subtilis* and eukaryotic cells has shown that head-on conflicts negatively affect DNA replication more than co-directional conflicts^{8,13-23}. In an early elegant study, a replication origin was inserted either upstream or downstream of an rRNA operon in *E. coli*¹³. When the rRNA operon was head-on to replication, but not when it was co-directional, significant slowing of replication fork progression was observed. Recent studies revealed that when rRNA genes were inverted such that transcription and replication were head-on in *E. coli*, replication stalling was observed only when factors that help reduce conflicts are mutated¹⁷. Similar manipulation has stronger consequences in *B. subtilis*, as inverting rRNA genes such that replication faces these genes head-on is sufficient to stall and disrupt replication^{8,16}.

It is not clear what aspect of head-on encounters makes them more deleterious than co-directional encounters. One previously articulated aspect is the asymmetric distribution of components of the replication machinery on the two replicating strands; for example, the primase and replicative helicase move on the lagging strand template^{1,2}. These factors are ahead of the DNA polymerases and would be the first to encounter RNAPs on the lagging strand in a head-on conflict. If encountering RNAP head-on inactivates these components, the replication fork will stall. Another potential contributing factor to the difference between head-on and co-directional conflict is the difference in DNA supercoiling. As the replisome synthesizes new DNA, it generates positive supercoils in front of the replication fork. The same is true for RNAP; as a new transcript is being generated, positive supercoils build up in front of the transcription machinery. It has been proposed that the over-wound DNA template caught between

the two enzymes approaching each other causes replication forks to arrest during a head-on encounter^{7, 13, 21, 24}. The negative supercoiling behind RNAPs in a co-directional encounter would cancel the positive supercoils generated by the fork, potentially explaining the difference between the two types of conflicts. However, when the replication pause sites were mapped they were found to be strictly within the transcribed region, implicating direct replication-transcription collision, and largely ruling out topological constraints as being the primary cause of replication pausing during head-on conflicts^{15, 17}. There is also asymmetry and directionality to RNAP. It is possible that in co-directional conflicts, RNAP is easier to displace than in head-on conflicts.

Co-directional conflicts

Bacteria, and even some phages, have evolved in such a way that the majority of their genes are typically encoded on the leading strand and are transcribed co-directionally with replication. Although this co-orientation bias plays a significant role in reduction of head-on conflicts, it increases the likelihood of co-directional conflicts. In addition, the eventual meeting of replication and co-directional transcription seems inevitable because in bacteria the replisome moves 10-20 times faster than the transcription complex^{2, 7}. Therefore, potential co-directional encounters between RNA and DNA polymerases are likely to be more frequent than head-on encounters.

The potential for co-directional conflicts has been widely recognized. Two separate studies early on suggested that co-directional conflicts could disrupt replication. First, a study of phage phi29 replication proteins showed some disruption of replication upon encountering a transcription unit *in vitro*²⁵. Second, transcription terminators were shown to disrupt replication of *E. coli* plasmids when they were co-oriented with

replication²². However, co-directional conflicts with initiating or elongating RNAPs were generally thought to be benign to replication due to a lack of evidence from the *in vivo* studies.

Recent work indicates that co-directional replication-transcription conflicts are not benign. Co-directional conflicts between replication and transcription can occur at highly transcribed rRNA genes *in vivo* in *B. subtilis*, causing disruption of replication⁹. *B. subtilis* helicase loader proteins (DnaD and DnaB), which are required for restart of replication forks, were found to preferentially associate with rRNA operons in a transcription-dependent manner. By contrast, *in vitro* work found co-directional collisions between the *E. coli* replication machinery and a single RNAP were benign^{26,27}. In these studies, the replication machinery was designed to encounter a single RNAP, and this was not disruptive to replication. Under the fast growth conditions *in vivo* there are probably 50-100 RNAPs on each rRNA operon (e.g., see⁹), perhaps partly accounting for the different findings between *in vivo* and *in vitro* situations. In addition, co-directional replication and transcription appear to cause double-stranded breaks on a plasmid template *in vivo*, when transcription is induced from the very strong lambda pL promoter, and this is likely exacerbated by RNAP backtracking¹⁰. Together, these findings indicate that co-directional transcription of genes can be disruptive to replication *in vivo*, most likely when more than one or an array of RNAPs is encountered by the replication apparatus. In agreement with this phenomenon, co-directional conflicts at rRNA genes were detected under fast growth conditions where there is a high level of transcription of rRNA genes, but were not detected under slow growth conditions when there is decreased transcription⁹.

Dealing with conflicts

In order to preserve genome integrity, cells use various mechanisms to 1) prevent conflicts from occurring (Fig. 2), and 2) resolve conflicts once they have occurred (Figs. 2, 3). Genes involved in both types of mechanisms are crucial for bacterial survival, highlighting the fact that conflicts take place in cells routinely. Most avoidance mechanisms identified to date involve factors that destabilize or remove RNAP from the template, allowing a clearer path for the replisome (Fig. 2). This occurs during normal growth and during periods of stress, and at DNA lesions where RNAP gets stuck as well as undamaged DNA regions where RNAP pauses. Below, we discuss the key factors involved and their function in reducing replication-transcription conflicts. We start with mechanisms of avoidance (genome organization, modulators of RNAP, and accessory helicases) and continue with mechanisms of conflict resolution (replication fork intermediates, replication restart, and the role of recombination proteins).

Selection for Genome Organization

Virtually all bacteria have their highly transcribed rRNA/tRNA genes co-oriented with replication (Table 1). The majority of other genes also show a co-directional bias, although this bias is not 100%. In *B. subtilis*, ~75% of all genes are co-oriented with replication²⁸. In *E. coli*, there is little bias when looking at the entire genome; only ~55% of *E. coli* genes are co-oriented with replication²⁹. However, ~70% and ~90% of the essential genes (i.e. rRNA, tRNA, and other essential genes) in *E. coli* and *B. subtilis*, respectively, are transcribed co-directionally to replication, with 100% of the rRNA/tRNA genes co-directionally oriented³⁰⁻³⁴.

The quantitative difference of bias between different classes of genes in different organisms might be explained by difference of fitness cost due to their inversion. The consequences of inverting a gene from co-directional to head-on range from mild to severe. Inverting an extended genomic region with different classes of genes on average mildly (~30%) slows replication fork progression¹⁶. By contrast, inversion of rRNA genes not only stalls replication¹⁷, it also disrupts replication forks⁸. Here, disruption is defined by observation of DNA repair and recombination events, including the induction of the SOS response (the prominent DNA damage response mechanism). Disruption of replication is much more deleterious than fork stalling, because the former can trigger cell cycle checkpoint and delay cell division. Failure to fix replication disruption leads to the complete failure of cell growth¹⁷ and cell death⁸. Other than rRNA/tRNA genes, it is not clear what provides the selective pressure for maintaining co-orientation of transcription and replication of other highly expressed and essential genes. To further complicate this matter, essential genes are also often highly expressed; however, comparative genomic studies indicate that selection is specific for the essentiality of the genes, irrespective of their expression levels^{31,32}. Potential consequences due to head-on conflicts at essential genes include: 1) the formation of truncated mRNAs and/or proteins due to disruption of transcription, 2) the formation of aberrant un-translated RNAs (rRNAs and tRNAs) due to disruption of transcription and, 3) genome instability due to disruption of replication resulting in loss of function or synonymous mutations lowering the expression of a gene. The potential effects of the formation of truncated mRNAs and proteins do not appear to be a strong selective constraint, as the average 1 kb gene will be replicated in 1-2 seconds, a very small fraction of the cell cycle. Even if truncated mRNAs are generated from replication-transcription conflicts during this short time, cells have efficient mechanisms to release

ribosomes from any truncated mRNAs and degrade the resulting protein fragments³⁵. On the other hand, loss of function mutations of any essential gene are detrimental and will be removed by selection, hence are more likely to be the major factor influencing the co-orientation bias of essential genes. This issue will only be resolved by systematic investigation of the relationship between replication-transcription directionality and mutagenesis, as has been done in yeast³⁶. Most recently, new evidence has been obtained showing that replication-transcription conflict causes genome rearrangement near the location of the conflict in *E. coli* and in HeLa cells, providing a good system that can be used to investigate the effects of transcription directionality on genome stability⁴.

In summary, the conservation of the co-orientation bias strongly suggests that head-on conflicts are more detrimental than co-directional ones, and experimental data agree with this notion. The evolutionary pressure to maintain co-orientation of genes with replication, particularly for highly transcribed and/or essential genes, is at least partly to prevent head-on conflicts with transcription, to minimize disruption of replication by transcription, to promote the speed of genome duplication, and possibility also to maintain genome stability.

RNAP modulators

Factors that modulate transcription and RNAP can have profound effects on replication fork progression. Multiple factors have been identified that act on elongating, stalled, or terminating RNAPs, reducing the quantity or stability of the transcription barriers to replication. Genetic evidence indicates that the transcription-repair coupling factor Mfd, the small nucleotide ppGpp, and the RNAP secondary channel-interacting proteins DksA and GreA/B are involved in avoiding or resolving conflicts between replication and transcription^{5,6,10}. These factors also interact genetically with several DNA repair

factors, in particular the Holliday junction resolvase RuvABC, to enable cells to survive DNA damage²³. Recently, the detailed functions of these RNAP modulators in preventing replication-transcription conflict have been elucidated, and additional factors have been identified, pointing to a new model for the basis of, and environmental influence on, replication-transcription conflict.

Transcription-Repair Coupling Factor Mfd

DNA template lesions, created by exogenous (e.g., UV and gamma irradiation) and endogenous (e.g., reactive oxidative species generated by respiration) damage, can block the elongation of RNAP³⁷. In *E. coli*, the transcription-repair coupling factor Mfd facilitates the repair of these DNA lesions by carrying out two functions: 1) displacing the inactive RNAP that is stalled on the lesion, and 2) binding to UvrA to recruit DNA excision repair factors to the lesion site³⁸ (Fig. 2A). Mfd displaces the inactive RNAP by pushing arrested backtracked RNAP along the direction of transcription to dissociate it from DNA or to resume transcription in the presence of substrate NTPs³⁹. Mfd is required to ensure cell viability in the presence of DNA damage by UV irradiation *in vivo*. *In vitro*, Mfd promotes replication fork restart after head-on conflict between the replisome and stalled RNAP by causing dissociation of RNAP from the template²⁷.

ppGpp

Another RNAP modulator that helps prevent RNAP from blocking replication at DNA lesions is the small nucleotide guanosine tetraphosphate, ppGpp⁵. Production of ppGpp is induced by multiple stress conditions in bacteria and triggers an array of cellular responses to enhance survival⁴⁰. *In vitro* studies have shown that ppGpp can reduce the accumulation of RNAP arrays by decreasing the stability of the transcription elongation complex⁵. *In vivo*, high levels of ppGpp and a subclass of stringent RNAP mutations (*rpo**) that mimic the presence of ppGpp both increase the viability of DNA

repair-deficient strains when exposed to UV irradiation⁵. These studies indicate that ppGpp functions to remove replication barriers by dislodging stalled RNAP complexes at pre-existing DNA lesions.

RNAP secondary channel-interacting proteins: DksA and GreA/B

GreA, GreB (Gre proteins) and DksA are transcription factors with coiled coil domains that insert into the secondary channel of RNAP⁴¹⁻⁴³. *greA* and *dksA* mutants are sensitive to UV irradiation, indicating that DksA and Gre also help to promote DNA replication across DNA lesions by acting on transcription⁵. Recent work has shown that DksA and Gre have roles in removing transcription barriers to replication independently of pre-existing DNA damage⁶ (Fig. 2B).

DksA is a transcription initiation factor that acts with ppGpp to modulate transcription of rRNA and many other genes in response to starvation⁴⁴. In addition, DksA also prevents stalling of transcription elongation complexes at RNAP pause sites on a linear phage DNA *in vitro*⁴³, suggesting it also acts on transcription elongation complexes. Recent *in vivo* studies showed that DksA plays crucial roles in reducing replication-transcription conflict⁶. Deletion of DksA results in a chronic DNA damage response, independently of DNA damaging agents. This indicates that DNA damage itself originates from replication-transcription conflicts in the absence of DksA. DksA most likely prevents this conflict by acting during transcription elongation, as the replication block in the absence of DksA can be rescued by more processive RNAP mutants, by overexpressing the factor GreA that acts on the transcription elongation complex, and by overexpressing an apparent separation-of-function mutant of DksA that retains its activity in transcription elongation but has greatly reduced activity in transcription initiation^{6,43}.

Amino acid starvation dramatically elevates replication-transcription conflicts in *dksA* mutants, fully arresting replication forks⁶. One hypothesis to explain this observation is that DksA prevents formation of backtracked RNAP complexes that act as replication barriers. RNAPs can be stalled spontaneously or at regulatory pause sites, and undergo backtracking to displace the 3' end of the transcript from the active site, forming stable complexes^{45, 46, 47}. Amino acid starvation causes translational blockage, potentially abolishing the coupling between transcription and translation that normally prevents RNAP backtracking¹⁰. Under starvation conditions, in the absence of *dksA*, backtracked RNAP likely creates a barrier to replication that results in replication fork stalling and DNA damage. The requirement of DksA during common environmental fluctuations such as nutrient limitation indicates that the intrinsic conflict between replication and transcription is far wider than previously assumed.

In addition to DksA, GreA and GreB also prevent replication-transcription conflict. GreA and GreB are transcription cleavage factors that can insert into the secondary channel of RNAP and stimulate the intrinsic cleavage activity of RNAP^{48,42, 49}, and are important for reviving stalled transcription complexes^{50,51}. *In vitro*, GreB promotes the displacement of arrested transcription elongation complexes when they are encountered with head-on replisomes²⁷. *In vivo*, GreA compensates for loss of DksA in promoting replication elongation upon amino acid starvation⁶. Gre factors prevent loss of genome integrity caused by RNAP backtracking especially during translational blockage, at least on a plasmid¹⁰. This study shows that by interacting with the secondary channel of RNAP, Gre factors remove backtracked RNAP complexes to clear the template for replication forks.

Finally, this conflict-prevention strategy appears to be conserved in other bacteria. CarD is a recently identified transcription factor that protects *Mycobacterium tuberculosis*

cells against DNA damage⁵². The N-terminal region of CarD is similar to the region of Mfd that interacts with RNAP, suggesting that CarD functions similarly to Mfd in facilitating removal of RNAP from DNA. CarD partially compensates for the loss of DksA in *E. coli*⁵², indicating that the roles of Mfd-like and DksA-like modulators in promoting genome integrity can overlap.

R-loops: RNase H, DinG

The formation of R-loops is a major cause of replication-transcription conflicts in many organisms^{17,53}. An R-loop is a nucleic acid structure in which RNA is annealed with one strand of otherwise double-stranded DNA causing looping out of the region of complementary ssDNA. R-loops often form during transcription and were originally characterized for their ability to initiate DNA replication independently of the chromosomal origin of replication^{54,55}. Excess R-loops can induce the SOS response in *E. coli*⁵⁶, and affect genome stability by inducing chromosome rearrangement and recombination in eukaryotic cells⁵⁷. Recent evidence supports the idea that transcription-induced R-loop stalls replication forks in *E. coli*¹⁷, and R-loops cause genome rearrangement in *E. coli* and eukaryotic cells by stalling replication⁴.

Several well-characterized factors that remove R-loops reduce replication-transcription conflicts. RNase H1 is a major factor that removes R-loops by degrading RNA from an RNA:DNA duplex⁵⁸. Over-expression of RNase H1 alleviates the growth defect of *dinG* mutant in the presence of inverted rRNA operons, supporting the hypothesis that R-loops cause replication-transcription conflicts at rRNA operons and that RNase H1 prevents these conflicts¹⁷. This finding also agrees with the reported function of the helicase DinG in unwinding R-loops *in vitro*⁵⁹, indicating that DinG might prevent the growth defect caused by transcription of rRNA head-on with replication¹⁷. Overproduction of RNase H1 also can suppress transcription-replication

conflicts caused by loss of *greA*, at least on in a plasmid system ¹⁰, indicating that R-loops are likely contributing to these problems.

It is possible that other factors that reduce R-loop formation might also reduce replication-transcription conflicts. The negative supercoiling generated during transcription behind an RNAP favors formation of RNA-DNA hybrids ⁶⁰. In *E. coli* the topoisomerase TopA removes negative DNA supercoiling behind RNAP, reducing R-loop formation ⁶¹. It is therefore anticipated that TopA also decreases conflicts between transcription and replication via its inhibitory effects on R-loop formation. In addition, RNAP mutations isolated for their ability to suppress replication-transcription conflicts caused by inversion of an rRNA operon both lower the stability of RNAP binding to DNA and reduce R-loop formation ^{17,62}. However, it is not yet clear whether an R-loop itself is a replication barrier, or if the RNAPs slowed or stalled by R-loops form the replication barrier.

Transcription termination factors: NusG, Rho

Transcription termination factors also play a role in reducing replication-transcription conflicts. Removal of inactive transcription elongation complexes via transcription termination can prevent them from disrupting replication. Bacteria employ two distinct types of transcription termination: intrinsic termination mediated by a GC-rich RNA hairpin structure followed by a run of Us, and Rho-dependent termination (reviewed in ⁶³). Rho is a homohexameric helicase that translocates on RNA. Its ability to cause transcription termination requires NusG ⁶⁴. Loss of function mutations in NusG and RNase H1 are synthetically lethal ⁶⁵, indicating that Rho-dependent premature transcription termination prevents the formation of R-loops on the chromosome. Recently, Rho, NusG and another Rho-accessory factor, NusA, were found to be important for preventing disruption of replication by transcription elongation

complexes and for maintaining genome integrity^{10,66}. Inhibiting Rho activity by the drug bicyclomycin has strong synthetic effects with loss of function mutations in *nusG*, *nusA* and in DNA repair genes, which then can be alleviated by a destabilizing mutation in RNAP⁶⁶. These findings indicate that Rho-dependent termination facilitates replisome progression by releasing stable obstructing transcription elongation complexes.

Transcription-translation coupling

In bacteria, transcription and translation are coupled both in time and space. Newly synthesized transcripts are utilized as templates for translation while transcription elongation is still occurring. Structural studies indicate that the transcription and translation machineries are physically associated via NusG⁶⁷. The amino-terminal of NusG contacts RNAP and the carboxyl-terminal of NusG can bind to the ribosome-associated NusE, enabling NusG to link transcription with translation⁶⁷. *In vivo*, the rate of transcription elongation is controlled by the rate of translation⁶⁸. This suggests that the coupling between transcription and translation in bacteria is important for preventing RNAP backtracking, which recently was shown to cause DNA double strand breaks by affecting DNA replication, at least on a plasmid¹⁰. If transcription is uncoupled from translation, Rho/NusG and GreA prevent the formation of and reactivate the backtracked RNAP complexes, respectively¹⁰. The importance of Rho, NusG, Gre and DksA for maintaining genome integrity highlights the potential of RNAP backtracking and conflicts with replication due to the absence of translation-transcription coupling.

Accessory helicases

In order to ensure that replication progresses without disruption, cells possess a variety of factors that clear the path for the replication fork. One mechanism by which the replisome can successfully translocate across high-traffic, RNAP-coated regions is via the activity of specialized accessory helicases that remove these roadblocks. In addition, accessory helicases are involved in resolving conflicts after they occur. The first accessory helicase shown to move RNAPs out of the path of the replication fork was the Dda helicase of bacteriophage T4⁶⁹. It was later shown that a group of helicases perform similar functions in yeast (reviewed in⁷⁰). These studies established that accessory helicases can remove RNAPs that block replication. Inversion of rRNA operons, such that they are head-on to replication, does not significantly affect viability in *E. coli*^{17,71}. To date, three helicases have been identified in *E. coli* that are important for progression of replication forks through inverted rRNA operons: Rep, UvrD, and DinG¹⁷ (Fig. 2C). Rep and UvrD are 3' to 5' helicases and DinG is a 5' to 3' helicase^{17,72,73}. A homologue of Rep and UvrD, PcrA, has been identified as an accessory helicase in *B. subtilis*. Below, we review the functions of these helicases and their potential roles in conflict avoidance.

E. coli accessory helicases

It has been hypothesized that the *E. coli* accessory helicases cooperate to promote cell survival during harmful conflicts between replication and transcription¹⁷. Support for this comes from the fact that, in the absence of DinG, both Rep and UvrD are important for the fitness of cells harboring inverted rRNA loci¹⁷. Additionally, the roles of Rep and UvrD appear to be redundant to some degree, since loss of either helicase is tolerated, but the loss of both is lethal^{74,75}. This indicates that UvrD and Rep cooperate to help cells deal with replication-transcription conflicts.

In vitro, both UvrD and Rep can remove DNA-bound proteins from the path of the replication machinery⁷⁶. Additionally, *in vivo*, *uvrD rep* double mutants are sensitive to presence of *lac* operators bound by Lac repressors, implying that UvrD and/or Rep can remove DNA-bound proteins⁷⁶. UvrD (and to a lesser degree, Rep) is also required for the viability of cells in which ectopic replication termination (*ter*) sites are inserted and bound by the replication termination protein Tus⁷⁷. Rep seems to be the main motor that helps replication get through DNA-protein complexes. Replication fork speed is reduced in cells lacking Rep and not in cells lacking UvrD^{73,78,79}. Rep, but not UvrD, interacts directly with the main replicative helicase, indicating that Rep is associated with the replisome⁷⁶.

UvrD seems to play an important role in turnover of recombination intermediates formed at stalled replication forks. Deletion of any of the RecFOR gap repair pathway proteins rescues the lethality of a *uvrD rep* double deletion, implying that UvrD (and/or Rep) likely plays an essential role in removing RecA filaments, which are recruited to gaps in the DNA by the RecFOR pathway^{75,78,80}. *In vitro* studies have shown that UvrD can remove RecA filaments from DNA. This function has been hypothesized to be important for replication fork reactivation *in vivo*^{75,78,81-83}. Additionally, *in vivo*, the number and intensity of RecA-GFP foci increase in the absence of *uvrD*⁸³. RecA filaments are postulated to cause formation of toxic recombination intermediates when replication forks are stalled and need to be reactivated. These studies together suggest that UvrD helps the replisome get through dense transcription units by clearing RecA filaments that form when replication forks are stalled and need to be reactivated.

In summary, Rep and UvrD seem to have complementary, partly redundant, and also distinct functions in ensuring that the replisome gets through genomic regions. Both helicases have the ability to remove proteins from DNA, but likely different

proteins (RNAP vs. RecA) at different stages of replication conflicts. The activities of these accessory helicases contribute to the removal of RNAP and other proteins bound to DNA and the restart of stalled replication forks.

B. subtilis accessory helicase PcrA

PcrA of *B. subtilis* and other Gram-positive bacteria is a 3' to 5' accessory helicase that is essential in *B. subtilis* and is required for rolling circle replication of plasmids⁷⁴. PcrA is approximately 40% similar to both Rep and UvrD from *E. coli* and is a functional homolog of UvrD which potentially has some aspects of Rep function. The lethality caused by *pcrA* null mutations is suppressed by deletion in any of the genes in the RecFOR recombination pathway⁸⁰. Additionally, PcrA can remove RecA filaments from DNA *in vitro* similar to UvrD^{84,85}. Expression of *B. subtilis* PcrA in *E. coli* complements the UV sensitivity defect of a *uvrD* null mutant⁷⁴. In addition, PcrA can restore growth of a *uvrD* null mutant in which replication is blocked due to ectopic insertions of extra copies of replication termination sites⁷⁷. Expression of *pcrA* also rescues the lethality of a *rep uvrD* double mutant, indicating that PcrA might perform some functions of both proteins⁸⁰. It is not known if PcrA moves with the replication fork or whether it has any direct involvement in clearing RNAPs in front of the replication machinery. PcrA does interact with the *B. subtilis* RNA polymerase^{86,87}, making this possibility seem plausible.

Conflict resolution

Although there are several mechanisms in place that prevent conflicts from occurring, encounters between replication and transcription do occur *in vivo*. Depending on the extent of the damage to the template and disruption of the replisome components, stalled replication forks and the replisome may have to be reactivated. *In vitro*, the *E. coli* replication machinery can go past a single RNAP on a DNA template without

disruption of the replisome components²⁷. However, *in vivo* the situation is much different (Fig. 3). Recent work suggests that replication forks can be disrupted during encounters with RNAP^{5,6,66}, and that replication-transcription conflicts occur at rRNA loci⁹. It is not yet clear what the fate of the replisome components are in these conflicts. Replication restart proteins (PriA, and helicase loaders DnaB and DnaD of *B. subtilis*) are probably required for reactivation of forks following replication-transcription conflicts at rRNA loci (Fig. 3A)⁹. These findings imply that at least the replicative helicase is either inactivated or released and is then reassembled. PriA is a 3' to 5' helicase that is essential for origin-independent activation of replication forks⁸⁸. PriA can recognize both branched structures that form at stalled forks and D-loops that may form if there is strand invasion by RecA. It is not yet clear whether one or both of these structures are present at sites of replication-transcription conflict. It is possible that RecA is needed for reactivation of forks and generation of the PriA substrate for restart. If so, upon generation of the PriA substrate, RecA is probably removed by the accessory helicases (UvrD and PcrA) in order to prevent unnecessary recombination events.

Recent work with *E. coli* found that RecBC is the only recombination enzyme needed for resolving head-on transcription-replication conflicts between RNA polymerase and the replisome at inverted rRNA loci⁸⁹. Based on analyses of conflict-generated products and the genetic requirements, it appears that after these conflicts, there are two potential outcomes for the replication fork. First, prolonged replication stalling can block the next round of replication, resulting in replication fork collapse and the generation of double strand ends. This is repaired by homologous recombination followed by replication restart (Fig. 3B). Second, there can be replication fork reversal. The reversed fork is processed by either homologous recombination or by direct degradation via the RecBCD nuclease. Both pathways produce a replication fork that is

further away from the conflict (Fig. 3C)⁸⁹. This replication fork can then provide a place to load a new replisome, and perhaps accessory helicases (Rep and DinG or UvrD) that are involved in removing the obstacle and enabling replication across what had been a barrier.

It is not known if primase is used to generate the initial primer during replication fork reactivation. Interesting work recently indicated that the transcript being synthesized by RNAP could be used as a primer to reinitiate the replication fork *in vitro*²⁶. In co-directional conflicts, upon encounter with RNAP, leading strand synthesis by the replisome was terminated, but then reinitiated using the transcript as a primer²⁶. This is an appealing model for reactivation of replication forks after a conflict, but it is not yet clear whether this occurs *in vivo*.

Conclusions and future directions

Conflicts between the transcription and replication machineries occur on the DNA template. Head-on conflicts between transcription and replication have adverse effects on fitness and genome stability. Additionally, we now know that co-directional conflicts can also stall replication and cause breaks in the DNA. Furthermore, several auxiliary factors have been characterized that play important roles in dealing with replication-transcription conflicts *in vivo*.

The current understanding of transcription-replication conflicts and the factors involved in avoiding and resolving them provides the potential to answer many of the remaining questions. For example, we still do not understand the mechanisms that cause replication fork stalling during encounters with RNAP. Data from *in vitro* experiments indicate that RNAPs dissociate from the DNA template when they encounter the replisome. The simplest expectation from these findings is that the

replisome should get through transcription by simply pushing RNAPs off of DNA. However, most findings, especially *in vivo*, indicate that the replisome does not easily get through chromosomal regions that are coated with RNAP and there is replication fork stalling. Further experiments are needed to understand how replication stalls during encounters with the transcription machinery.

Until recently, the significance and occurrence of co-directional conflicts was not appreciated. Clearly, head-on conflicts are more severe than co-directional conflicts, and genomes are organized to reduce the head-on conflicts. We can now begin to ask questions about the mechanistic differences between head-on and co-directional conflicts. Understanding these differences could elucidate the basis of the evolutionary selection for a genome that is co-oriented with replication.

Whereas it is clear that transcription-replication conflicts happen, it is not yet clear precisely what happens to the replisome during these conflicts. The involvement of auxiliary replication restart factors indicates that replication may need to be reactivated in some cases, by reloading of the replicative helicase. This could be due to a break in the DNA and a need to reestablish the replication fork. Alternatively, replication restart proteins may be involved because some components of the replisome are inactivated or dissociated from the fork and need to be reassembled. These possibilities are not mutually exclusive and further work is needed to understand what happens to the replisome when there is a conflict with the transcription machinery, and how this compares to what happens when replication is arrested by other mechanisms.

Much remains to be understood about the nature and mechanistic properties of replication-transcription conflicts. The expansion of the field and the advances in the technological front should facilitate further progress in understanding transcription-replication conflicts and conflict resolution.

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Table 1. Distribution of different types of genes on the leading or lagging strands^a.

organism	Essential		Nonessential		rRNA operons		r-protein genes		All genes	
	leading	lagging	leading	lagging	leading	lagging	leading	lagging	leading	lagging
<i>E. coli</i>	63-76%	24-37%	53-54%	46-47%	100%	none	93%	7%	55%	45%
<i>B. subtilis</i>	93-94%	6-7%	72-73%	27-28%	100%	none	94%	6%	74%	26%

^a Percentages of different classes of genes (essential, nonessential, rRNA operons, all genes) in the chromosome of *E. coli* and *B. subtilis* that are on the leading or lagging strands are shown. Percentages are taken from ^{28-31, 90}. In cases where the estimates differ between references, the ranges are indicated.

Figure legends

Figure 1. Cartoon of bidirectional replication and co-directional and head-on transcription and replication.

A. Cartoon depicting a partly duplicated bacterial chromosome. Bacterial

chromosomes are generally circular with a single origin of replication (*oriC*). The replication machinery assembles at *oriC* and replication occurs bi-directionally, with replication forks moving clockwise and counter-clockwise away from the origin. Partly duplicated chromosomes have two copies of *oriC* and other regions that have been duplicated. Replication ends in the terminus region, denoted *terC*. The *ter* region extends from $\sim 152^\circ$ to $\sim 187^\circ$ on the 360° circular map, with most termination events occurring at $\sim 172^\circ$ ⁹¹. Replication forks and their directionality are indicated by the black arrows.

B. Co-directional conflicts. A co-directional conflict between replication and transcription is depicted. Co-directional conflicts occur when a gene is coded for on the leading strand. In these conflicts, transcription occurs in the same direction as leading strand replication. Small black arrows represent the direction of movement of the replication machinery on each of the two strands. The longer black arrow represents the overall movement of the replication fork and the direction of unwinding by the replicative helicase. Leading and lagging strands, and directionality of the parent strands are indicated. RNAPs are represented by pentagons pointing in the direction of transcription. A thin gray arrow marks the beginning of an open reading frame (ORF).

C. Head-on conflicts. A head-on conflict between replication and transcription is depicted. Head-on conflicts occur when a gene is coded for on the lagging strand. In these conflicts, transcription occurs in the opposite direction as leading strand replication. Shapes are as described for panel B.

Figure 2. Representative mechanisms of avoiding/resolving replication-transcription conflicts.

A. Conflict at DNA lesions. RNAP modulators (GreA/B, DksA, ppGpp, Mfd) are proposed to inhibit formation of arrays of stalled RNAPs at lesions on the DNA template. Mfd (orange), for instance, can dislodge the inactive RNAPs, recruit UvrA₂B complex (gold) to repair the lesion, and prevent replication forks from encountering an array of stalled RNAPs.

B. Conflict due to backtracked RNAPs. Coupling transcription with translation prevents RNAP backtracking, promoting transcription processivity. The RNAP secondary channel factors, DksA and GreA/B (blue) are also proposed to reduce the replication-transcription conflict by preventing and resolving RNAP backtracking, respectively. For both panels A, and B, the replication forks are shown, but the forks likely do not need to be near RNAP for the indicated factors to function.

C. Conflict at rRNA (*rrn*) operons. DNA accessory helicases DinG, Rep and UvrD (green) cooperate to reduce replication-transcription conflict at highly transcribed *rrn* operons.

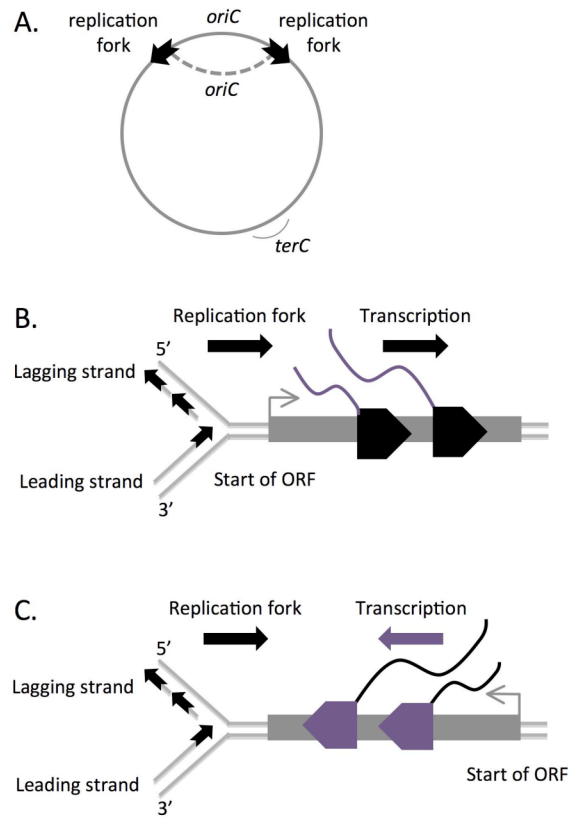
Figure 3. Possible fates of a stalled replication fork due to conflicts with transcription.

Three possible fates of stalled replication forks following conflict with RNAP.

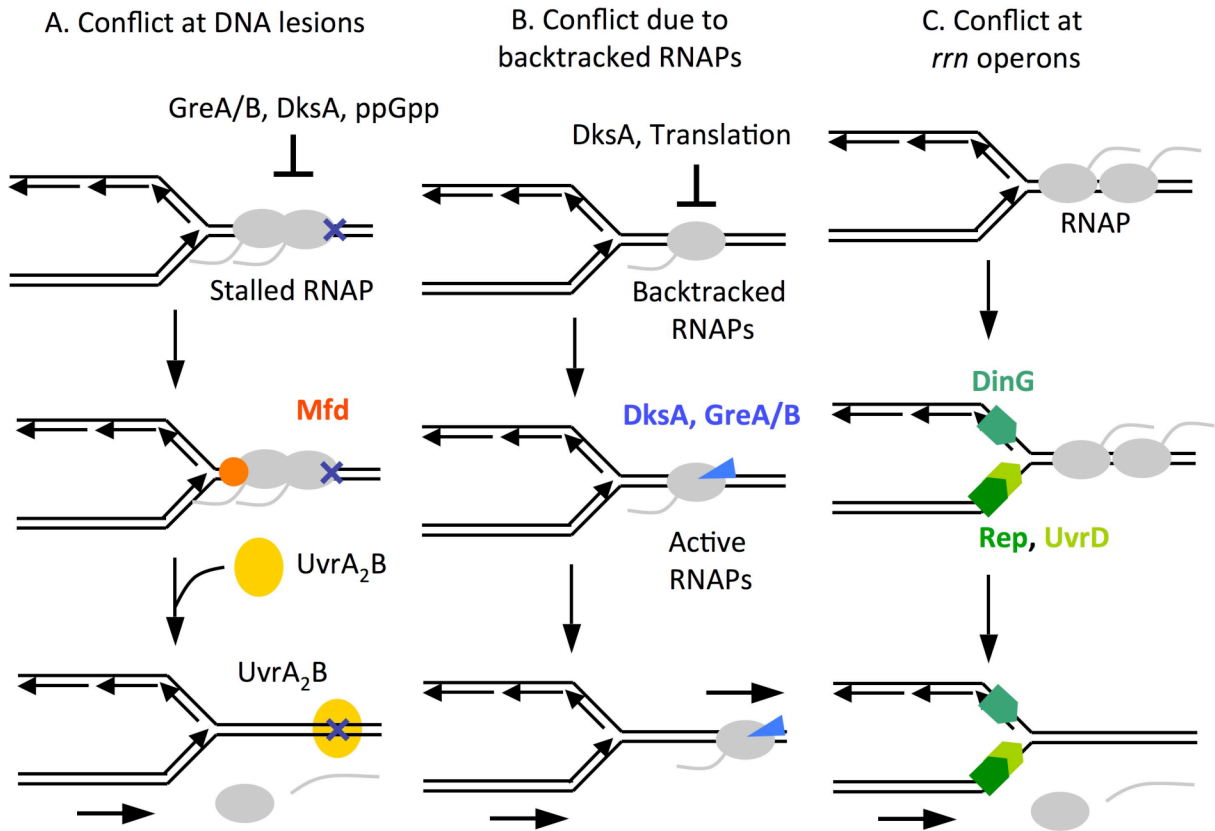
A. Direct replication restart. Replication restart proteins (e.g. PriA, and helicase loaders DnaB and DnaD in *B. subtilis*) are recruited to the stalled replication fork and directly reactivate replication without DNA recombination.

B. Double strand DNA ends. Prolonged replication stalling can result in the next round of replication reaching the stalled fork and generating double strand ends, which are repaired by homologous recombination.

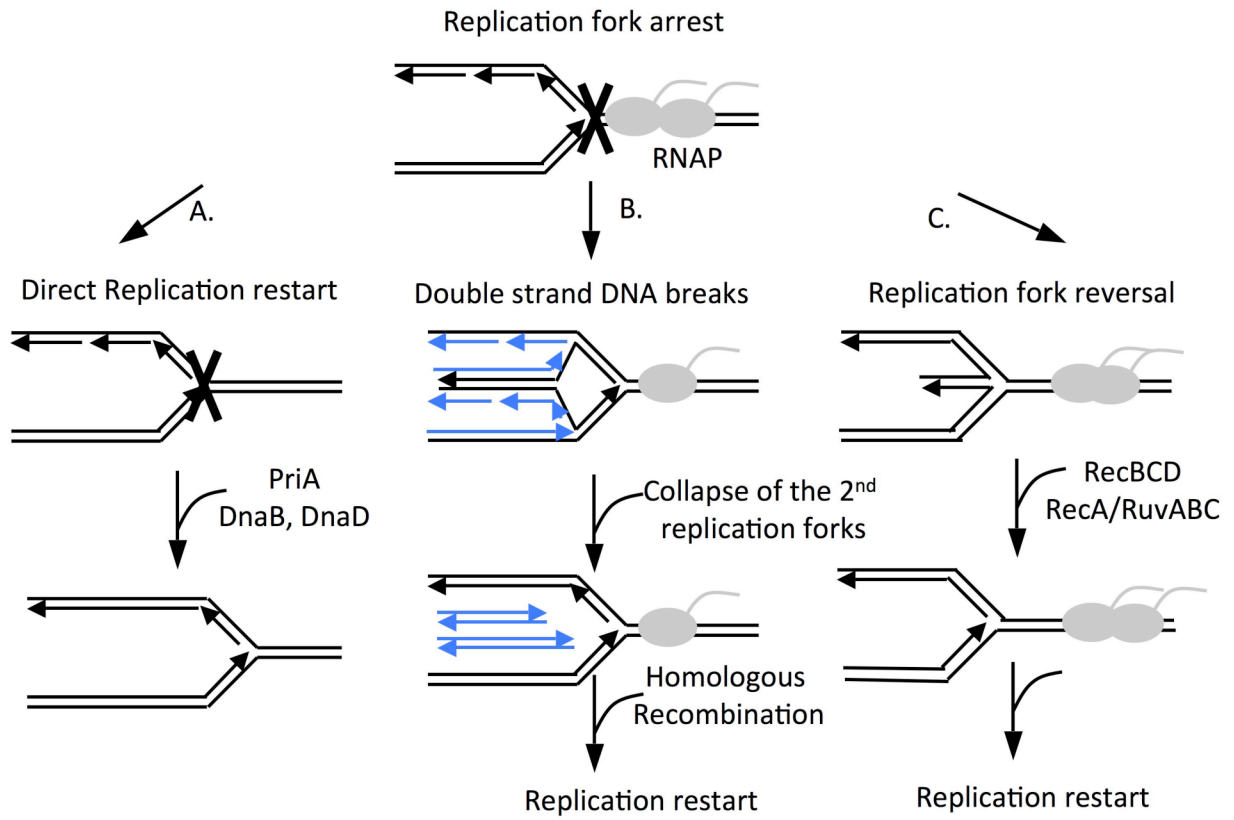
C. Replication fork reversal. A replication fork encountering conflict with RNAP undergoes replication fork reversal. RecBC(D), RecA and RuvABC are recruited to the reversed fork for DNA degradation and homologous recombination, resulting in a replication fork at a new position further away from the conflict. Replication can then restart with the help of other auxiliary proteins.



Merrikh et al., Fig. 1



Merrickh et al., Fig. 2



Merrickh et al., Fig. 3