Polymerase trafficking
A role for transcription factors in preventing replication fork arrest

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Replication forks and transcription complexes often collide, which can result in mutagenesis and chromosomal rearrangements. Recent studies of *E. coli* demonstrate a role for transcription factors in reducing conflicts between replication and transcription. These findings suggest that transcription regulators preserve genome integrity by preventing replication fork arrest.

**Introduction**

The arrest of replication forks leads to genome instability in all domains of life. This is due to the fact that DNA is often broken or extensively unwound following replication fork arrest, which leaves the genome susceptible to mutation, such as during DNA recombination and repair. Transcription complexes represent the main obstacle to replication forks due to their high stability and abundance on DNA. Mechanisms that prevent replication fork arrest following encounters with transcription complexes, however, remain poorly understood.

Mounting evidence indicates that collisions between replication and transcription compromise genome integrity in bacteria and eukaryotes. For example, studies in bacteria and eukaryotes demonstrate that replisome-RNAP collisions cause DNA damage response, mutagenesis and chromosomal rearrangements, which indicates that replication forks collapse within certain transcription units. Several reports demonstrate that the orientation and expression level of genes determine the severity of conflict between replication and transcription. For instance, in vivo and in vitro studies indicate that bacterial and eukaryotic replication forks become arrested upon collision with head-on transcription complexes, but are mostly unaffected by co-directional transcription complexes. In bacteria, highly expressed head-on genes have the greatest inhibitory effect on fork progression, which has been shown to result in DNA damage response and cell death in *B. subtillus*. Collisions within less active head-on transcription units result in mutagenesis. In yeast, conflict between replication and transcription also causes mutations, especially within highly expressed genes. Interestingly, mutations observed due to collisions in yeast require error-prone DNA polymerase activity and possibly stem from mixing of putative compartmentalized nucleotide pools.

Conflict between replication and transcription is highest in bacteria since the rate of replication (~630 nt/s) is 12–30 times greater than the rate of transcription (~20–50 nt/s) and there is no temporal separation of these processes. Furthermore, the pressure for heavily active transcription during chromosomal duplication can be quite high. For example, ribosomal gene expression may account for up to 50% of all transcription in the cell. Yet, the progression of the replication fork is apparently unaffected within these highly transcribed co-directional genes. This indicates that highly efficient mechanisms exist to resolve replication-transcription encounters.

Recent reports indicate that auxiliary helicases Rep, UvrD and DinG in *E. coli* and Rrm3 in yeast are necessary for promoting replication past protein...
roadblocks. Rep and Rrm3 interact with replisome components and Rrm3 has been shown to move with the replication fork. Helicases that do not interact with the replisome, such as UvrD, may be recruited to assist the replication fork after it has become arrested. Importantly, omitting certain auxiliary helicases results in replication fork arrest, double-strand breaks and in some cases cell death. Thus, DNA helicases clearly play an important role in preventing replication fork arrest due to encounters with protein roadblocks such as transcription.

Recent studies of E. coli demonstrate that transcription factors such as Mfd, DksA and GreA/B, which promote elongation or dissociation of RNAP, play an important role in reducing conflicts between replication and transcription. These findings reveal an unexpected link between DNA replication and transcription regulation and suggest the possibility that proper transcription elongation is necessary for reducing backed-up arrays of transcription complexes that may block the replication fork. Regardless of the detailed mechanisms, these findings suggest that transcription factors contribute to genome integrity by preventing conflicts between replication and transcription. Below we highlight recent biochemical and cell-based studies in E. coli that demonstrate roles for RNAP modulators in preventing replication fork arrest due to encounters with transcription.

The Transcription-Repair Coupling Factor Mfd Resolves Conflicts Between Replication and Transcription In Vitro

The idea that modulation of RNAP activity could prevent conflicts between replication and transcription is not entirely new. Philip Hanawalt postulated over fifteen years ago that the transcription-coupled repair (TCR) pathway, which promotes elongation or dissociation of a halted RNAP, might promote replication by removing frozen RNAP roadblocks from DNA. Since RNAP is arrested by DNA damage in the transcribed strand and remains stably bound to the DNA thereafter, a mechanism to displace the stalled RNAP is necessary if the lesion is to be repaired. In bacteria, TCR specifically displaces a halted RNAP from DNA and recruits the nucleotide excision repair (NER) pathway to the site, which results in preferential repair of the transcribed strand. TCR is performed by a single polypeptide in E. coli called Mfd (reviewed in ref. 22). Mfd is an ATP-dependent DNA translocase that binds to the rear of a halted elongation complex and rewind the transcription bubble behind the RNAP. Depending on whether conditions for forward translocation along the DNA are unfavorable or favorable, Mfd binding results in displacement or elongation of the polymerase, respectively. Mfd also binds to the NER factor UvrA and therefore recruits the DNA repair machinery to the stall site. Mfd displacement of a halted RNAP, however, is not exclusive to sites of DNA damage. For example, Mfd displaces transcription complexes halted by other means including nucleotide starvation, protein blocks and secondary structure of DNA. This suggests that TCR may act as a general mechanism to prevent halted RNAPs from accumulating along chromosomal DNA, which may block the replication fork.

Recent in vitro studies support a direct role for Mfd in resolving replisome-RNAP collisions. These studies reconstituted collisions of the E. coli replisome with a head-on RNAP halted elongation complex in vitro. Consistent with previous in vivo studies, the replication fork was impeded by a head-on collision with a transcription complex (Fig. 1). Yet, the replication fork was surprisingly stable and remained intact following the collision and eventually resumed elongation after slowly displacing the RNAP (left). These data are consistent with in vivo studies indicating that paused replication forks remain active for extended periods without the need for reassembling the replication apparatus. However, since replication is repeatedly initiated from a single replication origin in bacteria, prolonged replication fork stalling leads to double-strand breaks due to replication run-off of a replisome approaching from upstream. Thus, factors must exist to promote fork progression following the collision. Indeed, the in vitro study showed that the addition of Mfd following the collision resulted in direct restart of the stalled replication fork, which was due to the ability of Mfd to facilitate displacement of the RNAP ahead of the fork (right). Although these data demonstrate a convincing role for TCR in resolving collisions, it is important to note that mfd cells grow normally. This suggests that additional factors exist that can also resolve or prevent conflicts between replication and transcription. Indeed, additional factors have recently been identified which include auxiliary helicases discussed above and other transcription factors (discussed below).

Transcription Elongation Factors Prevent Conflicts Between Replication and Transcription In Vivo

Recent in vivo data indicate a role for transcription factors such as DksA in preventing conflicts between replication and transcription following nutritional starvation. DksA is mostly known for its ability to act with the stringent response regulator (p)ppGpp in modulating transcription initiation at certain promoters, such as those within ribosomal operons. For example, following amino acid starvation, cellular levels of (p)ppGpp are rapidly increased, which inhibit transcription initiation at ribosomal promoters in conjunction with DksA. DksA, however, also acts as a transcription elongation factor independently of (p)ppGpp and in this capacity DksA has been shown to reduce RNAP pausing.

Using genome-wide DNA arrays to monitor the progression of replication forks in synchronized E. coli cells, Tehranchi et al. showed that the function of DksA in promoting transcription elongation was necessary for preventing replication fork arrest following amino acid starvation in vivo. Nutrient starvation potentially leads to a higher distribution of RNAP to genes other than ribosomal operons. Since a large fraction (~45%) of transcription units opposes the direction of replication, this would raise the energetic barrier to fork progression along most of the chromosome. Strikingly, addition of the transcription initiation inhibitor rifampicin restored replication elongation in dksA cells under...
Transcription

Figure 1. The transcription repair factor Mfd resolves a head-on replisome-RNAP collision. The replication fork pauses after encountering a head-on RNAP (top). The replication fork slowly displaces the RNAP then resumes elongation (left). Mfd binds to the rear of the halted transcription complex then uses the energy of ATP to displace the RNAP from DNA, which results in direct restart of the replication fork (right). Figure adapted from ref. 12.

nutrient starvation, which indicated that transcription was indeed the main cause of fork arrest. Tehranchi et al. further showed that dksA cells induced the SOS response and recruited RecA to replication forks following amino acid starvation, which indicated replication fork collapse.

Interestingly, GreA/B transcription proofreading factors, which prevent prolonged RNAP pausing by reducing backtracking activity, demonstrated a similar effect as DksA in preventing replication fork arrest due to collisions with transcription under the same conditions. Likewise, cells harboring RNAP mutants that exhibit either reduced pausing or a higher elongation rate also prevented replication fork arrest under nutritional starvation. Finally, Tehranchi et al. showed that omitting multiple transcription elongation factors greA/B and dksA significantly inhibited replication fork progression even in the absence of nutrient starvation. Notably, GreB has previously been shown to prevent replication fork arrest due to an encounter with a transcription termination complex, which further supports a role for transcription factors in reducing conflicts between replication and transcription.

These studies indicate that proper transcription elongation is necessary for preventing replication forks from becoming arrested within transcription units. Although the mechanistic details of how transcription factors DksA and GreA/B reduce replisome-RNAP conflicts remains to be determined, the role of transcription factors in preventing prolonged RNAP pausing is probably an important factor. For example, cells deficient in transcription elongation factors, such as DksA and GreA/B, exhibit a higher frequency of transcriptional pausing. Furthermore, a single halted RNAP has been shown to lead to a backed-up array of halted RNAPs, which presumably arrests replication. Since replication is mostly unaffected by co-directional transcription complexes, head-on RNAP arrays probably have the greatest inhibitory effect on replication. Yet, it is important to note that under certain conditions co-directional transcription complexes also arrest the fork; R-loops and RNAP backtracking contribute to this effect. We favor a model whereby deficient transcription elongation leads to the accumulation of replication fork blocking RNAP arrays (Fig. 2, right). In contrast, when proper regulation of transcription elongation is in place, such as in wild-type cells, replication forks probably have little difficulty passing individually spaced elongating transcription complexes (Fig. 2, left). A similar model of RNAP modulators preventing conflicts between replication and transcription by limiting RNAP arrays has been proposed previously. The prior model, however, was based on the ability of RNAP modulators Mfd, GreA, DksA and (p)ppGpp to confer UV resistance to cells deficient in DNA repair. Thus, although these innovative studies were among the first to suggest a role for RNAP modulators in reducing replication-transcription conflicts, direct evidence for transcription factors affecting fork progression was still lacking. The more recent studies described herein have, for the first time, directly demonstrated that transcription factors are necessary for replication to proceed in the face of transcription.

Conclusion

In conclusion, recent in vitro and in vivo studies of E. coli directly demonstrate a role for transcription factors in preventing replication fork arrest due to transcription. These data suggest that proper transcription regulation and repair prevents transcriptional pausing that leads to replication barriers. The current findings provide direct insight into a complex interplay of replication, transcription and
transcription regulation and repair that is necessary for ensuring complete DNA replication in the face of highly stable transcription complexes. Since obstructing replication leads to mutagenesis and chromosomal rearrangements, transcription factors that prevent replication fork arrest likely play an important role in maintaining genome integrity.

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References