Molecular Microbiology (2012) 85(1), 12–20
doi:10.1111/j.1365-2958.2012.08102.x
First published online 31 May 2012

MicroReview

The conflict between DNA replication and transcription

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Summary

There is mounting evidence that there are frequent conflicts between complexes that replicate DNA and those that transcribe the same template, and that these conflicts lead to blockage of replication and genome instability. Such problems are perhaps best understood in bacteria, but it is becoming apparent that replicative barriers associated with transcription are a universal feature of life. This review summarizes what is currently known about how collisions between replisomes and transcription complexes are minimized and the mechanisms that help to resolve such collisions when they do occur. Although our understanding of these processes is still far from complete, a picture is emerging of a wide variety of different types of transcriptional blocks to replication that have resulted in a complex, overlapping system of mechanisms to avoid or tolerate such collisions.

The problem with transcription

Several recent reports have highlighted the problems that occur when bacterial replication forks encounter transcription complexes, linking conflicts between genome duplication and gene expression to replication fork breakdown and genetic instability. How does gene expression create problems for genome duplication? As DNA replication complexes move faster than transcription complexes, the two must inevitably collide given that they occupy the same template (Fig. 1). A simple solution to this problem would be provided if the replisome induced dissociation of RNA polymerases (RNAPs) from the template but the evidence linking transcription with replication blockage suggests this is not an efficient solution *in vivo* (Brewer, 1988; Mirkin and Mirkin, 2007; Rudolph *et al*., 2007). Thus either collisions between replisomes and RNAPs are avoided or specific mechanisms exist to resolve such collisions.

It is not only actively transcribing RNAPs that could pose a problem for replisomes (Fig. 1). In many genes RNAP pauses at regulatory sequences, and RNAP can also pause or stall at sites of DNA damage (Selby and Sancar, 1990; Selby *et al*., 1997; Landick, 2006; Tornaletti *et al*., 2006). Some paused or stalled transcription complexes translocate backwards along the template, and these backtracked complexes cannot resume transcription since the 3′ end of the transcript is no longer at the active site (Komissarova and Kashlev, 1997; Nudler *et al*., 1997; Cheung and Cramer, 2011). Halted transcription complexes represent stable barriers not only to replisomes, but also to other RNAPs transcribing the same template. Consequently arrays of immobile transcription complexes can form upon collision of upstream RNAPs with a stalled RNAP, especially if the gene is highly transcribed (Trautinger *et al*., 2005). One potential replicative barrier can therefore give rise to a tandem array of barriers.

The orientation of collisions between replisomes and RNAPs has an important impact on the outcome of such events (Fig. 1). Head-on collisions inhibit fork movement to a greater extent than co-directional collisions *in vivo* in both bacteria and lower eukaryotes (Vilette *et al*., 1995; Deshpande and Newlon, 1996; Mirkin and Mirkin, 2005; Prado and Aguilera, 2005; Wang *et al*., 2007a; Srivatsan *et al*., 2010), a pattern that may also hold in humans (Jorgensen and Schierup, 2009). One potential explanation for this difference is that transcription generates positive supercoiling ahead of the transcription bubble, potentially providing a topological rather than a direct physical block to continued fork movement (Wu *et al*., 1988; Olavarrieta *et al*., 2002). However, mapping of head-on collisions between bacterial forks and transcription complexes suggests that blockage occurs by direct contact between replisomes and RNAPs rather than indirectly via positive supercoiling (Mirkin and Mirkin, 2005). Indeed, on topologically unconstrained DNA the *Escherichia coli* replisome

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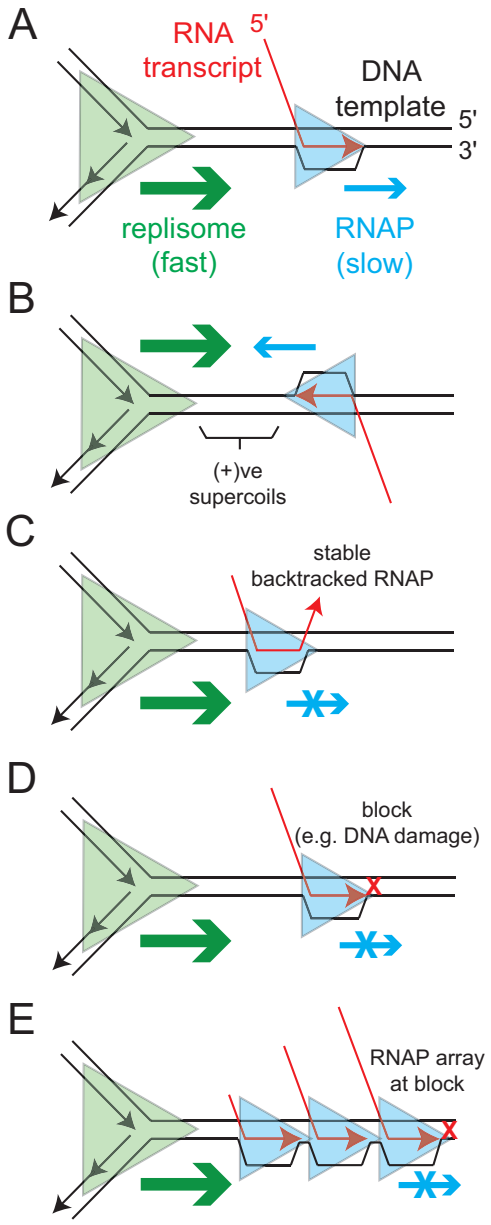

Fig. 1. The problem with transcription. The cartoon depicts different scenarios in which RNA polymerase may cause problems for replication. The replisome and RNAP are shown as green and blue triangles, respectively, with arrows indicating the direction of movement along the DNA template. The DNA and RNA are shown as black and red lines, respectively, with arrows indicating the 3′ ends of recently synthesized nucleic acid strands.
A. Co-directional collision of the replisome and transcribing RNAP is inevitable because the complexes move at different speeds.
B. Head-on collision between the replisome and transcribing RNAP causes accumulation of positive supercoils between the moving complexes.
C. Stable backtracked RNAP complexes form at specific sequences, at sites containing roadblocks or under conditions of nucleotide starvation.
D. RNAP is unable to progress through roadblocks such as DNA damage or tightly bound proteins.
E. A single blocked RNAP leads to the formation of RNAP arrays behind the stalled complex in highly transcribed genes.
Note that, although (C)–(E) depict stalled RNAPs as co-directional with replication, these same problems will also occur in a head-on context.

can displace a stalled RNAP rapidly *in vitro* when co-directional but not when head-on, providing a simple explanation for *in vivo* patterns of fork pausing (Pomerantz and O'Donnell, 2008; 2010). However, it remains unclear why direct head-on collisions are more inhibitory to fork movement. It should be noted that topology may play a greater role in eukaryotes than in prokaryotes, since tethering of highly transcribed genes to the nuclear envelope may result in generation of topological stress as a fork approaches (Bermejo *et al*., 2011).

Although most research has focused on collisions between replisomes and transcription elongation complexes, other aspects of transcription may also affect replication. Transcription initiation complexes, in which the promoter duplex DNA has melted, are remarkably stable and are known to undergo repeated rounds of abortive initiation before vacating the promoter (Murakami *et al*., 2002). Pausing of transcribing RNAP just downstream of promoters may also be commonplace (Core *et al*., 2008; Hatoum and Roberts, 2008; Nechaev *et al*., 2010; Perdue and Roberts, 2011). Artificially stabilized transcription initiation complexes can impede replication fork progression (Mirkin *et al*., 2006) but the impact of promoter proximal complexes, and most promoter-bound complexes, is unknown. There is also a major indirect problem caused by transcription since strand exchange can occur between exposed RNA and duplex DNA to form R-loop structures, formation of which in bacteria may be at least partially dependent on the strand exchange protein RecA (Hong *et al*., 1995; Kasahara *et al*., 2000; Zaitsev and Kowalczykowski, 2000). In bacteria such R-loops promote the unscheduled initiation of replication via PriA (Masai *et al*., 1994). RNAP backtracking may also promote the formation of RNA:DNA hybrids (Dutta *et al*., 2011) although the mechanisms behind this promotion are unclear. Genome instability associated with R-loop formation in eukaryotes indicates similar problems are faced by all organisms but it is unclear exactly how R-loops generate replicative stress in eukaryotes (Huertas and Aguilera, 2003; Prado and Aguilera, 2005; Helmrich *et al*., 2011; Wahba *et al*., 2011; Aguilera and Garcia-Muse, 2012).

Recent studies have begun to reveal the multiple strategies that have evolved to deal with these conflicts between gene expression and genome duplication. That there are so many different strategies indicates the vital importance of ensuring that transcription does not prevent completion of genome duplication.

Minimizing collisions between replisomes and transcription complexes

Avoiding co-occupancy of the DNA template

Collisions could potentially be avoided by spatially or temporally separating DNA replication and transcription. Spatial separation could be achieved by separating replication and transcription into different 'factories', allowing concurrent gene expression and genome duplication but ensuring that replisomes and transcription complexes rarely occupy the same section of the DNA template. While this might occur to some extent in eukaryotes (Sutherland and Bickmore, 2009), DNA replication factories are not a universal feature of bacterial cells (Reyes-Lamothe *et al*., 2008). A complete temporal separation of transcription and DNA replication is clearly difficult in rapidly dividing bacteria where there is no window during the cell cycle in which DNA replication is not occurring. Furthermore, the need to produce proteins such as histones during genome duplication (Heintz *et al*., 1983), as well as the existence of very large genes whose transcription takes longer than a cell cycle (Tennyson *et al*., 1995), indicates that complete temporal separation of transcription and replication is not a viable solution in eukaryotes. Thus organisms must have developed alternative strategies that minimize the more deleterious types of collision and/or resolve collisions when they do occur.

Genome organization

If collisions cannot be avoided then minimizing the frequency of the more deleterious head-on collisions could reduce potential replicative problems. Indeed, many bacterial and viral genomes have evolved so that the majority of highly transcribed genes are oriented co-directionally with replication fork movement (Brewer, 1988). Mechanisms to avoid head-on collisions also exist in eukaryotes. Each highly transcribed ribosomal DNA transcription unit in *Saccharomyces cerevisiae* contains a potential origin of replication, risking multiple head-on collisions between forks and RNAPs. However, these are prevented by a specific replication fork barrier downstream of the transcribed region (Brewer and Fangman, 1988). Similar mechanisms to prevent head-on collisions within rDNA exist in other eukaryotes, indicating that the more deleterious nature of such collisions shapes the organization of eukaryotic as well as bacterial genomes (Mirkin and Mirkin, 2007). However, co-directional collisions are not without hazards. In both *Bacillus subtilis* and *E. coli* replisome blockage is most frequent within highly transcribed *rrn* operons, all of which are oriented co-directionally with genome duplication (Ton-Hoang *et al*., 2010; Merrikh *et al*., 2011). Other highly transcribed genes with this orientation also present barriers to replication in *B. subtilis* (Soultanas, 2011). Thus high RNAP density creates problems for replisomes regardless of orientation.

Reducing the number of immobile RNAPs on the genome

Reducing the accumulation of stalled RNAPs, and the consequent risk of formation of RNAP arrays, can be achieved by reducing the probability of stalling occurring, reactivating stalled RNAPs to allow transcription to resume or dissociation of stalled RNAPs (Fig. 2). The presence of multiple RNAPs translocating on a single gene can reduce pausing because backtracking of any one RNAP is inhibited by the motion of those behind it. Furthermore, trailing RNAPs can promote forward movement of a stalled RNAP if backtracking has already occurred (Epshtein and Nudler, 2003; Epshtein *et al*., 2003). Such a mechanism may help to reduce the formation of arrays of stalled RNAPs in, for example, ribosomal RNA operons. Ribosomes translocating along the emerging transcript also suppress backtracking and associated genome instability in *E. coli*, implying that the coupling of translation and transcription facilitates RNAP movement (Proshkin *et al*., 2010; Dutta *et al*., 2011). However, upstream RNAPs and translation are not sufficient safeguards against RNAP stalling since multiple additional factors that reduce accumulation of stalled RNAPs are needed to minimize the need for replication repair in *E. coli* (Trautinger *et al*., 2005; Mahdi *et al*., 2006).

The RNA translocase Rho also reduces the conflict between gene expression and genome duplication (Fassler *et al*., 1985; Washburn and Gottesman, 2011). Rho catalyses programmed termination of transcription and abortion of transcripts that are not being utilized (Roberts *et al*., 2008) but may also translocate 5′–3′ along emerging transcripts to displace stalled RNAPs (Dutta *et al*., 2011). This Rho function may be particularly important when transcription is not coupled to translation, such as in *rrn* operons (Klumpp and Hwa, 2008). NusG plays a key role in co-ordinating Rho activity with transcription–translation coupling. The N-terminus of NusG interacts with RNAP while the C-terminus interacts with both the ribosomal protein S10 (also called NusE) and Rho in a mutually exclusive manner (Burmann *et al*., 2010). Absence of translating ribosomes might therefore facilitate NusG-promoted displacement of paused RNAP by Rho (Cardinale *et al*., 2008). NusG also forms an anti-termination system with NusA, NusB and NusE together with specific anti-terminator sequences within highly transcribed *rrn* operons (Roberts *et al*., 2008). This anti-termination complex both inhibits access to the untranslated transcripts by Rho and minimizes RNAP pausing, thus reducing RNAP 'traffic jams' (Klumpp and Hwa, 2008). RfaH, a protein with an N-terminal domain

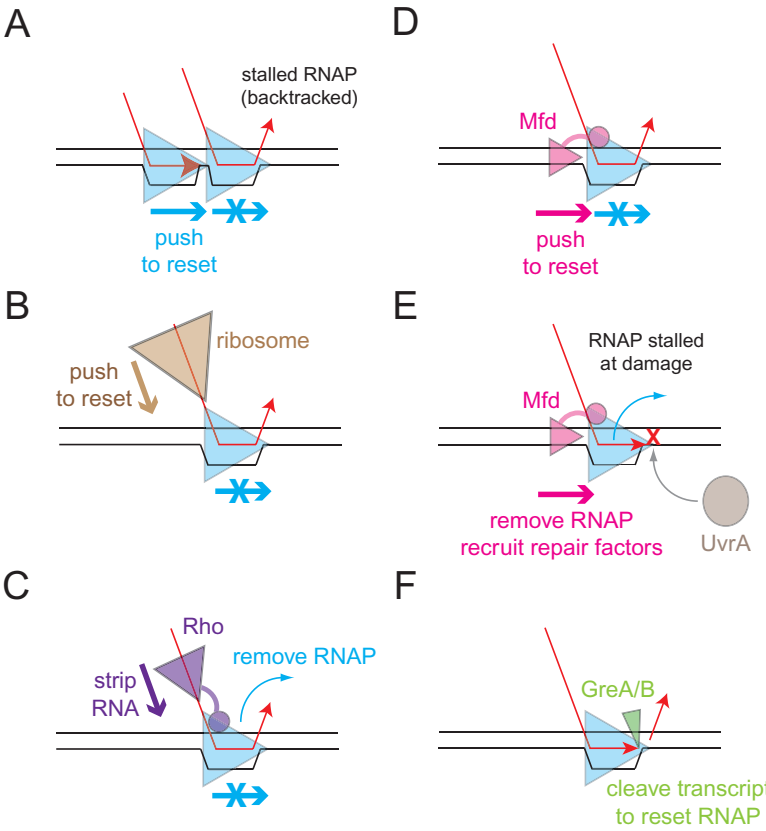

Fig. 2. Reducing immobile RNAPs on the genome. The cartoon depicts different strategies for removing stalled RNAPs from DNA, thereby clearing the path for the replisome.
A. Trailing RNAPs may help to re-activate backtracked RNAP complexes.
B. Translation of the RNA transcript by a moving ribosome may help to re-activate a backtracked RNAP complex. This process may be assisted by bridging interactions between the ribosome and RNAP formed by NusG (see main text for details).
C. The RNA helicase Rho can interact directly with RNAP to strip out the nascent RNA strand and terminate transcription.
D. The DNA motor protein Mfd directly engages and re-activates a backtracked RNAP.
E. The DNA motor protein Mfd acts as a transcription coupled repair factor to engage and displace RNAP stalled at a site of DNA damage, and recruits the nucleotide excision repair protein UvrA.
F. GreA/B binds backtracked RNAP in the secondary channel causing phosphodiester hydrolysis at the active site for RNA polymerization to generate a new 3′-OH transcript end.

structurally homologous to NusG (Belogurov *et al.*, 2007), also inhibits RNAP pausing in operons containing specific promoter-proximal DNA sequences (Artsimovitch and Landick, 2002) probably via conformational alterations within RNAP (Svetlov *et al.*, 2007; Sevostyanova *et al.*, 2011).

Other anti-backtracking factors include a double-stranded DNA translocase, Mfd, that binds to stalled transcription complexes and the DNA upstream of the complex (Park *et al.*, 2002). Translocation by Mfd along the DNA results in forward translocation of backtracked RNAP and resumption of transcription (Selby and Sancar, 1993; Park *et al.*, 2002). Mfd also acts to dissociate RNAP that is stalled by template damage, and in this situation facilitates lesion repair (Park *et al.*, 2002; Manelyte *et al.*, 2010).

GreA and GreB reactivate backtracked RNAP using a distinctive mechanism. Both proteins bind within the *E. coli* RNAP secondary channel to stimulate hydrolysis of backtracked transcripts by the RNAP active site, creating new 3′ transcript ends to allow transcription to resume (Orlova *et al.*, 1995). Like Mfd, both GreA and B reduce the need for fork repair (Trautinger *et al.*, 2005) supporting the idea that RNAP backtracking is a major problem for genome duplication. A similar function in eukaryotes is also critical for efficient transcription and viability (Sigurdsson *et al.*, 2010).

Escherichia coli DksA may also bind to the secondary channel within RNAP (Perederina *et al.*, 2004) and is known to reduce the need for replication repair (Trautinger *et al.*, 2005; Mahdi *et al.*, 2006). DksA acts in conjunction with ppGpp, a signalling molecule generated by amino acid starvation, to inhibit many promoters, especially those controlling *rrn* expression (Paul *et al.*, 2004; Perederina *et al.*, 2004; Potrykus and Cashel, 2008). Like DksA, ppGpp also reduces the requirement for replication fork repair (McGlynn and Lloyd, 2000). However, there is no direct correlation between suppression of ppGpp-related transcription initiation defects and replication repair defects (McGlynn and Lloyd, 2000; Baharoglu *et al.*, 2010). Thus altered levels of transcription initiation cannot be the sole mechanism by which ppGpp and DksA reduce conflicts between replication and transcription. There is some evidence that ppGpp can decrease blocked transcription complex stability (Trautinger *et al.*, 2005) while DksA inhibits transcriptional arrest *in vitro* (Perederina *et al.*, 2004). DksA also prevents transcription-dependent inhibition of replication upon amino acid starvation *in vivo*, an effect that is unrelated to the inhibition of transcription initiation by DksA (Tehranchi *et al.*, 2010). Although the mechanism underlying this DksA function is unknown, one possibility is that absence of this function results in uncoupling of transcription and translation (Tehranchi *et al.*, 2010; Dutta *et al.*, 2011). However, this starvation

phenotype is independent of ppGpp (Tehranchi *et al.*, 2010). ppGpp can also reduce replication–transcription conflicts by a very different mechanism in *B. subtilis* where amino acid starvation results in a ppGpp-dependent shutdown of replication by inhibiting primase (Wang *et al.*, 2007b). Bacteria can therefore minimize replication–transcription collisions under starvation conditions by minimizing transcriptional barriers to replication or by inhibiting DNA replication itself.

Resolving collisions after they occur

Displacement of RNAP by the replisome

There are multiple ways in which, if collisions do occur between forks and RNAPs, replication might be able to proceed to completion. The *E. coli* replisome itself can displace an RNAP stalled by nucleotide deprivation in the co-directional orientation *in vitro* (Pomerantz and O'Donnell, 2008), although it remains unknown whether displacement of tandem stalled RNAPs with this orientation occurs efficiently. In contrast, replication readthrough in the head-on orientation occurs very slowly (Pomerantz and O'Donnell, 2010). These data correlate with multiple *in vivo* studies indicating the greater challenge posed by head-on collisions (see above). Surprisingly, although co-directional collisions do cause inhibition of leading strand DNA synthesis, the replisome reinitiates synthesis using the 3′ end of the transcript annealed to the leading strand template (Pomerantz and O'Donnell, 2008). Replication of the leading strand can continue therefore, leaving a discontinuity in the leading strand together with a stretch of RNA incorporated downstream of the discontinuity. However, the relative importance of such transcript-dependent priming of leading strand synthesis *in vivo* is unknown. In particular, given the known replisome blockage in highly transcribed genes oriented co-directionally (Merrikh *et al.*, 2011), such a re-priming mechanism is unlikely to operate efficiently within the context of arrays of stalled RNAPs.

Displacement of RNAP by accessory replicative motors

Collisions with RNAPs clearly do cause fork pausing (Merrikh *et al.*, 2011), implying that the replicative helicase frequently fails to dislodge RNAPs *in vivo*. The dsDNA translocase Mfd (see above) can displace an RNAP after a head-on collision has occurred *in vitro,* providing a potential back-up mechanism if the replisome fails to displace a transcription complex (Pomerantz and O'Donnell, 2010). However, whether such a mechanism is operative *in vivo* has been questioned (Dutta *et al.*, 2011). An alternative means of underpinning replication would be to employ additional helicases at the fork to aid the main

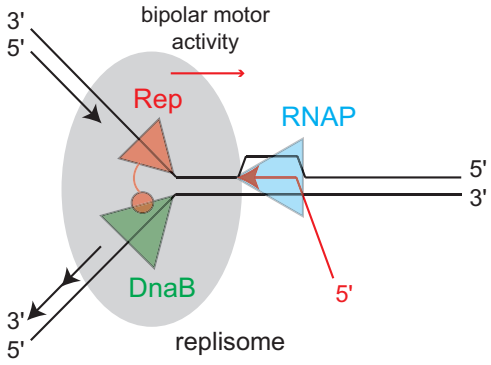

Fig. 3. Resolving collisions after they occur: the role of accessory replicative helicases. The replisome is shown as a grey transparent oval, containing the canonical replicative helicase DnaB (green), moving in the 5′–3′ direction on the lagging strand template. The accessory replicative helicase Rep (red), interacts with DnaB and is thought to engage the leading strand template allowing its 3′–5′ ssDNA translocation activity to complement that of DnaB. Together, this bipolar motor activity can assist the replisome through blocks including transcription complexes. Note that, although a head-on collision with a transcribing RNAP is depicted here, the precise nature of the replication:transcription conflicts dealt with by the activity of Rep are poorly defined.

replicative helicase in dissociation of protein–DNA complexes ahead of the fork (Matson *et al.*, 1994) (Fig. 3). Evidence is accumulating that such accessory helicases are a ubiquitous feature of genome duplication. Two homologous 3′–5′ helicases in *E. coli*, Rep and UvrD, can each promote movement of replisomes along protein-bound DNA *in vitro* and *in vivo* (Guy *et al.*, 2009). Both helicases also maintain viability of cells carrying *rrn* operons oriented head-on rather than co-directionally with replication (Boubakri *et al.*, 2010). Moreover, the inviability of cells lacking both Rep and UvrD can be suppressed by mutations in RNAP subunit genes that may inhibit RNAP backtracking (Guy *et al.*, 2009; Baharoglu *et al.*, 2010; Boubakri *et al.*, 2010; Dutta *et al.*, 2011). Thus both Rep and UvrD can act as accessory replicative helicases to promote fork movement through transcription complexes, although UvrD likely does so only in the absence of Rep (Atkinson *et al.*, 2011a,b). However, direct displacement of transcription complexes by Rep or UvrD has not yet been demonstrated. It is also possible that, even if displacement does occur, only a subset of transcription complexes can be displaced by such helicases.

A third *E. coli* helicase, DinG, has also been implicated in promoting fork movement through transcription complexes (Boubakri *et al.*, 2010). However, DinG translocates 5′–3′ along ssDNA (Voloshin *et al.*, 2003), the opposite polarity to Rep and UvrD, and so promotion of fork movement by DinG is unlikely to occur via the same mechanism as Rep and UvrD. Indeed, DinG might inhibit the formation and/or promote the removal of RNA:DNA hybrids (Boubakri *et al.*, 2010).

In *S. cerevisiae*, the Rrm3 helicase promotes fork movement through non-histone protein–DNA complexes, including many RNAP III-transcribed genes (Ivessa *et al.*, 2003). Although fork pausing within genes highly transcribed by RNAP II is not affected by Rrm3 (Azvolinsky *et al.*, 2009) it is possible that other accessory helicases in *S. cerevisiae* could perform this function (Sabouri *et al.*, 2012). Furthermore, a homologue of Rrm3 in *S. pombe*, Pfh1, promotes fork movement through a range of protein–DNA complexes including highly transcribed RNAP II genes, supporting a general role for accessory helicases in displacement of transcription complexes in eukaryotes (Sabouri *et al.*, 2012; Steinacher *et al.*, 2012).

Rebooting genome duplication if all else fails

If a bacterial replisome does become blocked then replicative function is lost within a few minutes (Marians *et al.*, 1998; McGlynn and Guy, 2008), although this relative instability has been questioned recently (Pomerantz and O'Donnell, 2010). Loss of function of the replisome necessitates reloading of the replication machinery via PriA or PriC in *E. coli* (Heller and Marians, 2006). Cells lacking PriA are viable but extremely sick in rich media (Lee and Kornberg, 1991; Nurse *et al.*, 1991), a phenotype that can be suppressed by a mutation in RNAP that reduces backtracking (Mahdi *et al.*, 2006; Dutta *et al.*, 2011). Therefore fork breakdown due to transcription complexes creates a significant need for replisome reloading under rapid growth conditions (Merrikh *et al.*, 2011) even in the presence of the many other mechanisms that reduce or resolve such conflicts.

Mutations in RNAP also suppress viability and DNA repair defects caused by the absence of recombination enzymes, suggesting that blocked fork processing by recombination enzymes is needed to tolerate conflicts between replication and transcription (McGlynn and Lloyd, 2000; Trautinger *et al.*, 2005; Mahdi *et al.*, 2006; Baharoglu *et al.*, 2010). Consistent with this, *B. subtilis* cells in which head on collisions are provoked by the inversion of *rrn* operons have a greatly increased frequency of RecA foci (Srivatsan *et al.*, 2010). To directly bypass replicative blocks, recombination must occur with downstream homologous sequences, resulting in deletion of a part of the chromosome. Alternatively, blocked fork processing by recombination enzymes might simply facilitate replication reinitiation upstream of the block, providing a second chance for a replisome to proceed successfully through the block (Payne *et al.*, 2006). Recombination may also be critical in overcoming replication/transcription conflicts in eukaryotes (Huertas and Aguilera, 2003; Aguilera and Gomez-Gonzalez, 2008) although mechanistic details are again lacking. However, the involvement of recombination in overcoming transcriptional blocks in eukaryotes implies a requirement for replisome reloading, given the intimate links between recombination and replication initiation (Aguilera and Gomez-Gonzalez, 2008). Mechanisms that stabilize stalled forks in eukaryotes do not therefore obviate the need to reinitiate replication.

Conclusions

Analysis of conflicts between genome duplication and gene expression in bacteria are providing important mechanistic insights into how such conflicts are resolved. However, the interplay between different mechanisms that help to reduce the impact of transcription on replication is still very unclear. This is partly due to the extensive functional overlap between the many different systems that act to ensure efficient replication and transcription elongation. We also have no clear idea about the outcomes of replisome collisions with different types of transcription barrier. Dissection of these complex processes will require a combination of genetic and biochemical analysis of collisions between replisomes and a range of defined types of transcription barrier. Only then can we hope to unravel the complex relationship between genome duplication and expression in living cells.

Acknowledgements

Work in the authors' laboratories on the interplay between DNA replication and transcription is funded by the Biotechnology and Biological Sciences Research Council (BB/I001859/1).

References

Aguilera, A., and Garcia-Muse, T. (2012) R loops: from transcription byproducts to threats to genome stability. *Mol Cell* **46:** 115–124.

Aguilera, A., and Gomez-Gonzalez, B. (2008) Genome instability: a mechanistic view of its causes and consequences. *Nat Rev Genet* **9:** 204–217.

Artsimovitch, I., and Landick, R. (2002) The transcriptional regulator RfaH stimulates RNA chain synthesis after recruitment to elongation complexes by the exposed nontemplate DNA strand. *Cell* **109:** 193–203.

Atkinson, J., Gupta, M.K., and McGlynn, P. (2011a) Interaction of Rep and DnaB on DNA. *Nucleic Acids Res* **39:** 1351–1359.

Atkinson, J., Gupta, M.K., Rudolph, C.J., Bell, H., Lloyd, R.G., and McGlynn, P. (2011b) Localization of an accessory helicase at the replisome is critical in sustaining efficient genome duplication. *Nucleic Acids Res* **39:** 949–957.

Azvolinsky, A., Giresi, P.G., Lieb, J.D., and Zakian, V.A. (2009) Highly transcribed RNA polymerase II genes are impediments to replication fork progression in *Saccharomyces cerevisiae*. *Mol Cell* **34:** 722–734.

Baharoglu, Z., Lestini, R., Duigou, S., and Michel, B. (2010)

RNA polymerase mutations that facilitate replication progression in the *rep uvrD recF* mutant lacking two accessory replicative helicases. *Mol Microbiol* **77:** 324–336.

Belogurov, G.A., Vassylyeva, M.N., Svetlov, V., Klyuyev, S., Grishin, N.V., Vassylyev, D.G., and Artsimovitch, I. (2007) Structural basis for converting a general transcription factor into an operon-specific virulence regulator. *Mol Cell* **26:** 117–129.

Bermejo, R., Capra, T., Jossen, R., Colosio, A., Frattini, C., Carotenuto, W., *et al.* (2011) The replication checkpoint protects fork stability by releasing transcribed genes from nuclear pores. *Cell* **146:** 233–246.

Boubakri, H., de Septenville, A.L., Viguera, E., and Michel, B. (2010) The helicases DinG, Rep and UvrD cooperate to promote replication across transcription units *in vivo*. *EMBO J* **29:** 145–157.

Brewer, B.J. (1988) When polymerases collide: replication and the transcriptional organization of the *E. coli* chromosome. *Cell* **53:** 679–686.

Brewer, B.J., and Fangman, W.L. (1988) A replication fork barrier at the 3' end of yeast ribosomal RNA genes. *Cell* **55:** 637–643.

Burmann, B.M., Schweimer, K., Luo, X., Wahl, M.C., Stitt, B.L., Gottesman, M.E., and Rosch, P. (2010) A NusE:NusG complex links transcription and translation. *Science* **328:** 501–504.

Cardinale, C.J., Washburn, R.S., Tadigotla, V.R., Brown, L.M., Gottesman, M.E., and Nudler, E. (2008) Termination factor Rho and its cofactors NusA and NusG silence foreign DNA in *E. coli*. *Science* **320:** 935–938.

Cheung, A.C., and Cramer, P. (2011) Structural basis of RNA polymerase II backtracking, arrest and reactivation. *Nature* **471:** 249–253.

Core, L.J., Waterfall, J.J., and Lis, J.T. (2008) Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. *Science* **322:** 1845–1848.

Deshpande, A.M., and Newlon, C.S. (1996) DNA replication fork pause sites dependent on transcription. *Science* **272:** 1030–1033.

Dutta, D., Shatalin, K., Epshtein, V., Gottesman, M.E., and Nudler, E. (2011) Linking RNA polymerase backtracking to genome instability in *E. coli*. *Cell* **146:** 533–543.

Epshtein, V., and Nudler, E. (2003) Cooperation between RNA polymerase molecules in transcription elongation. *Science* **300:** 801–805.

Epshtein, V., Toulme, F., Rahmouni, A.R., Borukhov, S., and Nudler, E. (2003) Transcription through the roadblocks: the role of RNA polymerase cooperation. *EMBO J* **22:** 4719–4727.

Fassler, J.S., Tessman, I., and Tessman, E.S. (1985) Lethality of the double mutations *rho rep* and *rho ssb* in *Escherichia coli*. *J Bacteriol* **161:** 609–614.

Guy, C.P., Atkinson, J., Gupta, M.K., Mahdi, A.A., Gwynn, E.J., Rudolph, C.J., *et al.* (2009) Rep provides a second motor at the replisome to promote duplication of protein-bound DNA. *Mol Cell* **36:** 654–666.

Hatoum, A., and Roberts, J. (2008) Prevalence of RNA polymerase stalling at *Escherichia coli* promoters after open complex formation. *Mol Microbiol* **68:** 17–28.

Heintz, N., Sive, H.L., and Roeder, R.G. (1983) Regulation of human histone gene expression: kinetics of accumulation and changes in the rate of synthesis and in the half-lives of individual histone mRNAs during the HeLa cell cycle. *Mol Cell Biol* **3:** 539–550.

Heller, R.C., and Marians, K.J. (2006) Replisome assembly and the direct restart of stalled replication forks. *Nat Rev Mol Cell Biol* **7:** 932–943.

Helmrich, A., Ballarino, M., and Tora, L. (2011) Collisions between replication and transcription complexes cause common fragile site instability at the longest human genes. *Mol Cell* **44:** 966–977.

Hong, X., Cadwell, G.W., and Kogoma, T. (1995) *Escherichia coli* RecG and RecA proteins in R-loop formation. *EMBO J* **14:** 2385–2392.

Huertas, P., and Aguilera, A. (2003) Cotranscriptionally formed DNA:RNA hybrids mediate transcription elongation impairment and transcription-associated recombination. *Mol Cell* **12:** 711–721.

Ivessa, A.S., Lenzmeier, B.A., Bessler, J.B., Goudsouzian, L.K., Schnakenberg, S.L., and Zakian, V.A. (2003) The *Saccharomyces cerevisiae* helicase Rrm3p facilitates replication past nonhistone protein–DNA complexes. *Mol Cell* **12:** 1525–1536.

Jorgensen, F.G., and Schierup, M.H. (2009) Increased rate of human mutations where DNA and RNA polymerases collide. *Trends Genet* **25:** 523–527.

Kasahara, M., Clikeman, J.A., Bates, D.B., and Kogoma, T. (2000) RecA protein-dependent R-loop formation *in vitro*. *Genes Dev* **14:** 360–365.

Klumpp, S., and Hwa, T. (2008) Stochasticity and traffic jams in the transcription of ribosomal RNA: intriguing role of termination and antitermination. *Proc Natl Acad Sci USA* **105:** 18159–18164.

Komissarova, N., and Kashlev, M. (1997) RNA polymerase switches between inactivated and activated states by translocating back and forth along the DNA and the RNA. *J Biol Chem* **272:** 15329–15338.

Landick, R. (2006) The regulatory roles and mechanism of transcriptional pausing. *Biochem Soc Trans* **34:** 1062–1066.

Lee, E.H., and Kornberg, A. (1991) Replication deficiencies in *priA* mutants of *Escherichia coli* lacking the primosomal replication n' protein. *Proc Natl Acad Sci USA* **88:** 3029–3032.

McGlynn, P., and Guy, C. (2008) Replication forks blocked by protein–DNA complexes have limited stability *in vitro*. *J Mol Biol* **381:** 249–255.

McGlynn, P., and Lloyd, R.G. (2000) Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression. *Cell* **101:** 35–45.

Mahdi, A.A., Buckman, C., Harris, L., and Lloyd, R.G. (2006) Rep and PriA helicase activities prevent RecA from provoking unnecessary recombination during replication fork repair. *Genes Dev* **20:** 2135–2147.

Manelyte, L., Kim, Y.I., Smith, A.J., Smith, R.M., and Savery, N.J. (2010) Regulation and rate enhancement during transcription-coupled DNA repair. *Mol Cell* **40:** 714–724.

Marians, K.J., Hiasa, H., Kim, D.R., and McHenry, C.S. (1998) Role of the core DNA polymerase III subunits at the replication fork. α is the only subunit required for processive replication. *J Biol Chem* **273:** 2452–2457.

Masai, H., Asai, T., Kubota, Y., Arai, K., and Kogoma, T. (1994) *Escherichia coli* PriA protein is essential for inducible and constitutive stable DNA replication. *EMBO J* **13:** 5338–5345.

Matson, S.W., Bean, D.W., and George, J.W. (1994) DNA helicases: enzymes with essential roles in all aspects of DNA metabolism. *Bioessays* **16:** 13–22.

Merrikh, H., Machon, C., Grainger, W.H., Grossman, A.D., and Soultanas, P. (2011) Co-directional replication–transcription conflicts lead to replication restart. *Nature* **470:** 554–557.

Mirkin, E.V., and Mirkin, S.M. (2005) Mechanisms of transcription–replication collisions in bacteria. *Mol Cell Biol* **25:** 888–895.

Mirkin, E.V., and Mirkin, S.M. (2007) Replication fork stalling at natural impediments. *Microbiol Mol Biol Rev* **71:** 13–35.

Mirkin, E.V., Castro Roa, D., Nudler, E., and Mirkin, S.M. (2006) Transcription regulatory elements are punctuation marks for DNA replication. *Proc Natl Acad Sci USA* **103:** 7276–7281.

Murakami, K.S., Masuda, S., and Darst, S.A. (2002) Structural basis of transcription initiation: RNA polymerase holoenzyme at 4 A resolution. *Science* **296:** 1280–1284.

Nechaev, S., Fargo, D.C., dos Santos, G., Liu, L., Gao, Y., and Adelman, K. (2010) Global analysis of short RNAs reveals widespread promoter-proximal stalling and arrest of Pol II in *Drosophila*. *Science* **327:** 335–338.

Nudler, E., Mustaev, A., Lukhtanov, E., and Goldfarb, A. (1997) The RNA–DNA hybrid maintains the register of transcription by preventing backtracking of RNA polymerase. *Cell* **89:** 33–41.

Nurse, P., Zavitz, K.H., and Marians, K.J. (1991) Inactivation of the *Escherichia coli priA* DNA replication protein induces the SOS response. *J Bacteriol* **173:** 6686–6693.

Olavarrieta, L., Hernandez, P., Krimer, D.B., and Schvartzman, J.B. (2002) DNA knotting caused by head-on collision of transcription and replication. *J Mol Biol* **322:** 1–6.

Orlova, M., Newlands, J., Das, A., Goldfarb, A., and Borukhov, S. (1995) Intrinsic transcript cleavage activity of RNA polymerase. *Proc Natl Acad Sci USA* **92:** 4596–4600.

Park, J.S., Marr, M.T., and Roberts, J.W. (2002) *E. coli* transcription repair coupling factor (mfd protein) rescues arrested complexes by promoting forward translocation. *Cell* **109:** 757–767.

Paul, B.J., Barker, M.M., Ross, W., Schneider, D.A., Webb, C., Foster, J.W., and Gourse, R.L. (2004) DksA: a critical component of the transcription initiation machinery that potentiates the regulation of rRNA promoters by ppGpp and the initiating NTP. *Cell* **118:** 311–322.

Payne, B.T., van Knippenberg, I.C., Bell, H., Filipe, S.R., Sherratt, D.J., and McGlynn, P. (2006) Replication fork blockage by transcription factor–DNA complexes in *Escherichia coli*. *Nucleic Acids Res* **34:** 5194–5202.

Perdue, S.A., and Roberts, J.W. (2011) Sigma(70)-dependent transcription pausing in *Escherichia coli*. *J Mol Biol* **412:** 782–792.

Perederina, A., Svetlov, V., Vassylyeva, M.N., Tahirov, T.H., Yokoyama, S., Artsimovitch, I., and Vassylyev, D.G. (2004) Regulation through the secondary channel – structural framework for ppGpp-DksA synergism during transcription. *Cell* **118:** 297–309.

Pomerantz, R.T., and O'Donnell, M. (2008) The replisome uses mRNA as a primer after colliding with RNA polymerase. *Nature* **456:** 762–766.

Pomerantz, R.T., and O'Donnell, M. (2010) Direct restart of a replication fork stalled by a head-on RNA polymerase. *Science* **327:** 590–592.

Potrykus, K., and Cashel, M. (2008) p)ppGpp: still magical? *Annu Rev Microbiol* **62:** 35–51.

Prado, F., and Aguilera, A. (2005) Impairment of replication fork progression mediates RNA polII transcription-associated recombination. *EMBO J* **24:** 1267–1276.

Proshkin, S., Rahmouni, A.R., Mironov, A., and Nudler, E. (2010) Cooperation between translating ribosomes and RNA polymerase in transcription elongation. *Science* **328:** 504–508.

Reyes-Lamothe, R., Possoz, C., Danilova, O., and Sherratt, D.J. (2008) Independent positioning and action of *Escherichia coli* replisomes in live cells. *Cell* **133:** 90–102.

Roberts, J.W., Shankar, S., and Filter, J.J. (2008) RNA polymerase elongation factors. *Annu Rev Microbiol* **62:** 211–233.

Rudolph, C.J., Dhillon, P., Moore, T., and Lloyd, R.G. (2007) Avoiding and resolving conflicts between DNA replication and transcription. *DNA Repair (Amst)* **6:** 981–993.

Sabouri, N., McDonald, K.R., Webb, C.J., Cristea, I.M., and Zakian, V.A. (2012) DNA replication through hard-to-replicate sites, including both highly transcribed RNA Pol II and Pol III genes, requires the *S. pombe* Pfh1 helicase. *Genes Dev* **26:** 581–593.

Selby, C.P., and Sancar, A. (1990) Transcription preferentially inhibits nucleotide excision repair of the template DNA strand *in vitro*. *J Biol Chem* **265:** 21330–21336.

Selby, C.P., and Sancar, A. (1993) Molecular mechanism of transcription–repair coupling. *Science* **260:** 53–58.

Selby, C.P., Drapkin, R., Reinberg, D., and Sancar, A. (1997) RNA polymerase II stalled at a thymine dimer: footprint and effect on excision repair. *Nucleic Acids Res* **25:** 787–793.

Sevostyanova, A., Belogurov, G.A., Mooney, R.A., Landick, R., and Artsimovitch, I. (2011) The beta subunit gate loop is required for RNA polymerase modification by RfaH and NusG. *Mol Cell* **43:** 253–262.

Sigurdsson, S., Dirac-Svejstrup, A.B., and Svejstrup, J.Q. (2010) Evidence that transcript cleavage is essential for RNA polymerase II transcription and cell viability. *Mol Cell* **38:** 202–210.

Soultanas, P. (2011) The replication–transcription conflict. *Transcription* **2:** 140–144.

Srivatsan, A., Tehranchi, A., MacAlpine, D.M., and Wang, J.D. (2010) Co-orientation of replication and transcription preserves genome integrity. *PLoS Genet* **6:** e1000810.

Steinacher, R., Osman, F., Dalgaard, J.Z., Lorenz, A., and Whitby, M.C. (2012) The DNA helicase Pfh1 promotes fork merging at replication termination sites to ensure genome stability. *Genes Dev* **26:** 594–602.

Sutherland, H., and Bickmore, W.A. (2009) Transcription factories: gene expression in unions? *Nat Rev Genet* **10:** 457–466.

Svetlov, V., Belogurov, G.A., Shabrova, E., Vassylyev, D.G., and Artsimovitch, I. (2007) Allosteric control of the RNA polymerase by the elongation factor RfaH. *Nucleic Acids Res* **35:** 5694–5705.

Tehranchi, A.K., Blankschien, M.D., Zhang, Y., Halliday, J.A., Srivatsan, A., Peng, J., *et al*. (2010) The transcription factor DksA prevents conflicts between DNA replication and transcription machinery. *Cell* **141:** 595–605.

Tennyson, C.N., Klamut, H.J., and Worton, R.G. (1995) The human dystrophin gene requires 16 hours to be transcribed and is cotranscriptionally spliced. *Nat Genet* **9:** 184–190.

Ton-Hoang, B., Pasternak, C., Siguier, P., Guynet, C., Hickman, A.B., Dyda, F., *et al*. (2010) Single-stranded DNA transposition is coupled to host replication. *Cell* **142:** 398–408.

Tornaletti, S., Maeda, L.S., and Hanawalt, P.C. (2006) Transcription arrest at an abasic site in the transcribed strand of template DNA. *Chem Res Toxicol* **19:** 1215–1220.

Trautinger, B.W., Jaktaji, R., Rusakova, E., and Lloyd, R.G. (2005) RNA polymerase modulators and DNA repair activities resolve conflicts between DNA replication and transcription. *Mol Cell* **19:** 247–258.

Vilette, D., Ehrlich, S.D., and Michel, B. (1995) Transcription-induced deletions in *Escherichia coli* plasmids. *Mol Microbiol* **17:** 493–504.

Voloshin, O.N., Vanevski, F., Khil, P., and Camerini-Otero, R.D. (2003) Characterization of the DNA damage-inducible helicase DinG from *Escherichia coli*. *J Biol Chem* **278:** 28284–28293.

Wahba, L., Amon, J.D., Koshland, D., and Vuica-Ross, M. (2011) RNase H and multiple RNA biogenesis factors cooperate to prevent RNA:DNA hybrids from generating genome instability. *Mol Cell* **44:** 978–988.

Wang, J.D., Berkmen, M.B., and Grossman, A.D. (2007a) Genome-wide coorientation of replication and transcription reduces adverse effects on replication in *Bacillus subtilis*. *Proc Natl Acad Sci USA* **104:** 5608–5613.

Wang, J.D., Sanders, G.M., and Grossman, A.D. (2007b) Nutritional control of elongation of DNA replication by (p)ppGpp. *Cell* **128:** 865–875.

Washburn, R.S., and Gottesman, M.E. (2011) Transcription termination maintains chromosome integrity. *Proc Natl Acad Sci USA* **108:** 792–797.

Wu, H.Y., Shyy, S.H., Wang, J.C., and Liu, L.F. (1988) Transcription generates positively and negatively supercoiled domains in the template. *Cell* **53:** 433–440.

Zaitsev, E.N., and Kowalczykowski, S.C. (2000) A novel pairing process promoted by *Escherichia coli* RecA protein: inverse DNA and RNA strand exchange. *Genes Dev* **14:** 740–749.