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Transcription and replication

Breaking the rules of the road causes genomic instability

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Replication and transcription machineries progress at high speed on the same DNA template, which inevitably causes traffic accidents. Problems are not only caused by frontal collisions between polymerases, but also by cotranscriptional R-loops. These RNA-DNA hybrids induce genomic instability by blocking fork progression and could be implicated in the development of cancer.

Key words: transcription-associated recombination, R-loops, DNA replication, genomic instability

Abbreviations: RNAP, RNA polymerase; TAR, transcription-associated recombination; DSB, DNA double-strand break; rDNA, ribosomal DNA; RFB, replication fork barrier; Top1, topoisomerase I

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Introduction

DNA replication and transcription are fundamental genetic processes that are essential for cell growth and division. They are carried out by large protein complexes progressing at high speed and for long distances along the chromosomes. Head-on collisions inevitably arise when replisomes and RNA polymerases (RNAP) face each other on the same DNA template. A large body of evidence from both prokaryotes and eukaryotes indicates that frontal collisions induce replication fork stalling and genomic instability.[1-7] In *E. coli*, the replisome moves 15 to 30 times faster than transcription complexes and the replication machinery can also rear-end RNA polymerases.[8,9] In general, the replisome is given priority and RNAP are displaced. However, cells lacking both RNAP modulators and fork recovery factors have much reduced viability, indicating that collisions are frequent and have deleterious consequences.[10] To limit frontal collisions, bacterial genomes show a bias for genes to be encoded on the leading strand, resulting in coorientation of transcription and replication.[11] Reversing this collinear organization in *Bacillus subtilis* reduces the rate of DNA synthesis by ~50% specifically in regions with reversed bias.[12] Altogether, these data indicate that bacteria have evolved multiple means to minimize interference between replication and transcription.[10] Whether the same mechanisms operate in eukaryotic cells is currently unclear. In this review, we focus on the nature of replication/transcription interference in eukaryotes and on its consequences on genome integrity.

Functional Links between Transcription, Replication and Recombination

Although it is now well established that transcription increases genomic instability in eukaryotes,[13-15] the molecular mechanisms involved remain poorly understood. Evidence from budding yeast indicates that, like in bacteria, gene expression induces replication fork pausing in eukaryotic cells.[7,16-19] Since arrested forks are prone to recombination,[20] it has been proposed that transcription induces genomic instability by blocking fork progression.[6] This view is supported by yeast studies showing that transcription, replication and recombination are functionally linked.[15] Similarly, transcription-associated recombination (TAR) is only detected during S phase in mammalian cells.[21] Moreover, TAR is associated with signatures of one-ended double-strand break (DSB) recombination, and not with classical two-ended DSB repair, suggesting that recombination occurs at broken replication forks.[21,22] Non-exclusive models have been proposed to explain how transcription interferes with DNA

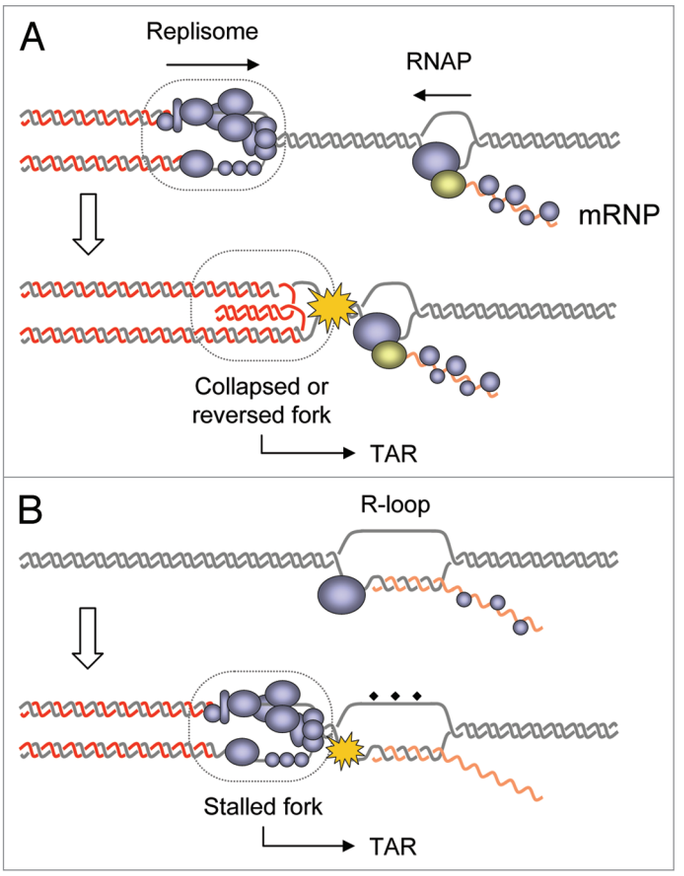

Figure 1. Models for interference between DNA replication and transcription. (A) The head-on collision model stipulates that a direct clash between the replisome and the RNA polymerase causes fork stalling, dissociation of the replisome and/or formation of recombinogenic reversed fork. This model implies that DNA replication and transcription occur simultaneously on the same DNA template. (B) In the cotranscriptional R-loops model, RNA-DNA hybrids formed during transcription interfere with replication fork progression and induce TAR. It is not clear whether fork arrest and recombination are caused by the DNA-RNA hybrid itself or by DNA lesions accumulating on the exposed ssDNA strand (diamonds).

replication in eukaryotes. These models are discussed in the following sections.

Collision between Replication and Transcription Machineries

Several lines of evidence suggest that TAR is promoted by frontal collisions between replication and transcription complexes in eukaryotes, as it is the case in bacteria. These collisions would in turn provoke fork collapse or reversion, which would be resolved by recombinational repair (**Fig. 1A**). In agreement with this view, transcription by RNA polymerase II and III induces fork arrest in *S. cerevisiae* only when it opposes the direction of replication.[7,17] Like bacteria, eukaryotes have also evolved polar replication fork barriers (RFBs) to prevent head-on collisions between polymerases.[20] The best-characterized RFB is located downstream of 35S rRNA genes in budding yeast (**Fig. 2**). This barrier blocks replication forks progressing opposite to the direction of transcription[23,24] and inactivation of RFBs increases collisions between forks and highly-expressed rRNA genes.[18] Mammalian genomes also contain rDNA RFBs[25] and their genes are potentially organized collinearly with replication.[26] Altogether, these data indicate that eukaryotes have evolved systems to prevent collisions between transcription and replication complexes.

However, other observations also argue against the collision model and indicate that additional mechanisms contribute to replication/transcription interference in eukaryotic cells. Indeed, unlike in bacteria, replication and transcription occur within spatially and temporally separated domains in the eukaryotes.[27,28] Moreover, it is generally believed that DNA and RNA polymerases do not travel along the chromosomes but rather form large replication and transcription factories through which DNA is pulled.[29,30] Inactive or blocked RNAPs could remain attached to DNA and interfere with replication. Alternatively, replication-impeding structures could form behind RNAPs and persist on DNA after transcription has ceased. These include RNA-DNA hybrids called R-loops, which were shown to affect genomic integrity in eukaryotes and prokaryotes.[31-34] Recent data suggesting that R-loops interfere with replication forks are discussed in the following section.

R-Loops Impede Replication Fork Progression

Cotranscriptional R-loops form during transcription when the nascent RNA reanneals with the template DNA strand, leaving the other strand unpaired (**Fig. 1B**). R-loop formation occurs preferentially at GC-rich regions and is favored by negative DNA supercoiling accumulating behind the RNAP.[31] Eukaryotic transcription is coupled with multiple processes such as pre-mRNA maturation, surveillance and export. Defects in any of these processes have been implicated in the formation of R-loops.[2,6,35] This is best illustrated with the yeast THO/TREX complex, mutation in which leads to defects in transcription elongation and induces TAR.[15,34] Importantly, fork progression is impaired at actively transcribed genes in THO mutants and induces a constitutive activation of the replication stress response.[36] Overexpression of RNase H, an enzyme that degrades R-loops, suppresses fork arrest and TAR in THO mutants.[15] Moreover, one particular THO/TREX mutant showing transcription and RNA export defects but no increased replication fork pausing does not have increased TAR.[37] Altogether, these data indicate that replication fork stalling induced by R-loops promote recombinational

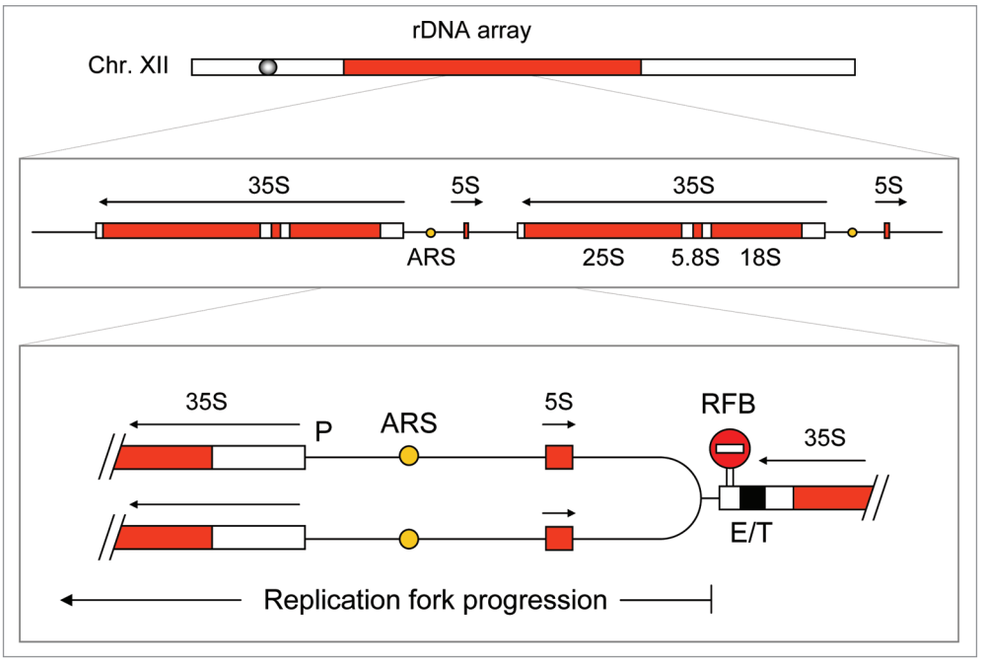

Figure 2. Schematic representation of the replication fork barrier at the *S. cerevisiae* rDNA array. This array is composed of ~200 identical repeats (9.1 kb) containing a large 35S rRNA gene, a small 5S rRNA gene and a replication origin (ARS). Replication forks progressing opposite to the direction of 35S transcription are arrested at the replication fork barrier (RFB). P: Promoter of the 35S gene. E/T, enhancer/terminator.

repair and increase TAR in yeast THO mutants.[36]

In vertebrates, R-loops also form in cells deficient in the splicing factor ASF/SF2 and induce a type of genomic instability that is suppressed by RNase H.[32,35] Other studies indicate that TAR depends on DNA replication in human cells,[21,22] suggesting that similar mechanisms operate in yeast and in higher eukaryotes. This view is supported by a recent report showing that depletion of Topoisomerase I (Top1) in mammalian cells induces replication fork stalling and DNA damage at highly-expressed genes.[38] Besides its well-characterized relaxation function, Top1 displays a kinase activity that is implicated in the regulation of splicing factors of the SR family such as ASF/SF2.[39] It has been shown that Top1 prevents interference between replication and transcription not only by relaxing topological constraints ahead of RNA and DNA polymerases but also by promoting ASF/SF2 function in order to prevent the formation of R-loops.[38] Interestingly, other mRNA-processing factors have been recently identified in a genome-wide siRNA screen for new factors preventing genomic instability.[40] As for Top1-deficient cells, spontaneous DNA damage was partially suppressed by RNase H in cells depleted for these factors, indicating that it is at least partly caused by R-loops.[38,40] How R-loops interfere with DNA replication is currently unclear. RNA-DNA hybrids could directly impede the progression of the replisome. Alternatively, these structures could also promote the accumulation of DNA lesions on the non-coding strand, which would in turn interfere with DNA replication (**Fig. 1B**). In both cases, it is tempting to speculate that these structures are able to interfere with DNA replication long after transcription has ceased. However, the stability of R-loop structures in the context of chromatin is currently unknown. Moreover, R-loop formation requires the presence of clusters of G on the non-transcribed strand, which may restrict their formation to specific regions of the genome.[41]

Conclusion and Perspectives

Besides frontal collisions between replication and transcription machineries, a growing body of evidence indicates that cotranscriptional R-loops affect the integrity of eukaryotic genomes. Although these RNA-DNA hybrids play a positive role in developmentally-regulated processes such as class switch recombination at immunoglobulin genes,[2,33] they also represent a major source of genomic instability.[15] Since patterns of gene expression and DNA replication are altered in oncogene-activated cells, it is tempting to speculate that transcription contributes to the replication stress observed in precancerous lesions[42] and that cotranscriptional R-loops are involved in cancer development. Future experiments taking advantage of new ChIP-seq and RNA-seq technologies are required to test this hypothesis.

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