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AUTHOR'S VIEWS

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Topoisomerase I prevents transcription-replication conflicts at transcription termination sites

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ABSTRACT

R-loops have both positive and negative impacts on chromosome functions. To identify toxic R-loops, we mapped RNA:DNA hybrids, markers of replication fork stalling and DNA double-strand breaks along the human genome. This analysis indicates that transient replication fork pausing occurs at the transcription termination sites of highly expressed genes enriched in R-loops and prevents head-on conflicts with transcription, in a topoisomerase I-dependent manner.

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Author's view

During the S phase of the cell cycle, tens of thousands of replication origins are coordinately activated to ensure the complete duplication of the human genome. The execution of the DNA replication program is challenged by endogenous and exogenous stresses, which contributes to the onset of various pathologies. This replication stress (RS) represents an important cause of genomic instability, and strong evidence in recent years indicates that oncogene-induced RS is a major driver of carcinogenesis and tumor progression.[1]

The transcription and replication machineries share the same DNA template, which renders head-on (HO) or co-directional (CD) collisions inevitable. HO collisions are considered as more deleterious to the genomic stability.[2] Moreover, transcription-replication conflicts (TRCs) can also be caused by the three-stranded nucleic acid structures called R-loops, containing a RNA:DNA hybrid and a displaced DNA strand (Figure 1). R-loops are formed co-transcriptionally when the nascent RNA reanneals with the template DNA strand, leaving the non-coding strand unpaired.[3] R-loops have been proposed to play both positive and negative roles in gene expression and other chromosome functions.[1,3] Many factors have been identified to prevent the formation of R-loops or remove them. These include RNA splicing factors, DNA topoisomerases, RNase H and specialized RNA:DNA helicases.[1,4] The analysis of the genome-wide distribution of R-loops with DNA:RNA hybrid immunoprecipitation and next-generation sequencing (DRIP-seq) revealed that R-loops are abundant structures, covering up to 5% of mammalian genomes.[5] Importantly, R-loops also form preferentially at regions of HO collision between replication and transcription.[2] However, the mechanism by which R-loops interfere with fork progression and promote genomic instability in human cells remains poorly understood.

We have recently developed a new method to directly measure the genome-wide replication fork directionality (RFD) along human genome by sequencing of Okazaki fragments (OK-seq),[6] which are only present on the lagging replicating strand of the replication fork. These novel data obtained by OK-seq revealed a significant CD bias of replication and transcription within active genes.[6] Such a CD bias may help to minimize the accumulation of the HO collisions and deleterious R-loops. By combining OK-seq with the mapping of RNA:DNA hybrids (DRIP-seq), phosphorylated replication protein A32 subunit on S33 (called thereafter *p*-RPA), phosphorylation of histone variant H2AX on S139 (γ-H2AX) and DNA double-strand breaks (DSBs, by i-BLESS; double-strand Breaks Labeling, Enrichment on Streptavidin and next-generation Sequencing), we found that although R-loops are enriched at both transcription start site (TSS) and transcription termination site (TTS) of highly expressed genes, *p*-RPA was only detected at TTS, where forks mostly progress in a HO orientation relative to the direction of transcription. In topoisomerase I (TOP1)-deficient cells, we also observed a broad γ-H2AX signal at active genes and the presence of DSBs at TTS enriched in R-loops and *p*-RPA (Figure 1).[7] Since *p*-RPA is a mark of ATM-Rad3-related (ATR) pathway activation at paused forks and γ-H2AX is a mark of collapsed forks and DSBs, these data indicate that forks transiently pause at TTS but do not break, whereas prolonged fork pausing and DSBs occur in TOP1-deficient cells, presumably because of unresolved torsional stress. The impact of R-loops in this process was further confirmed by the overexpression of RNase H1, which at

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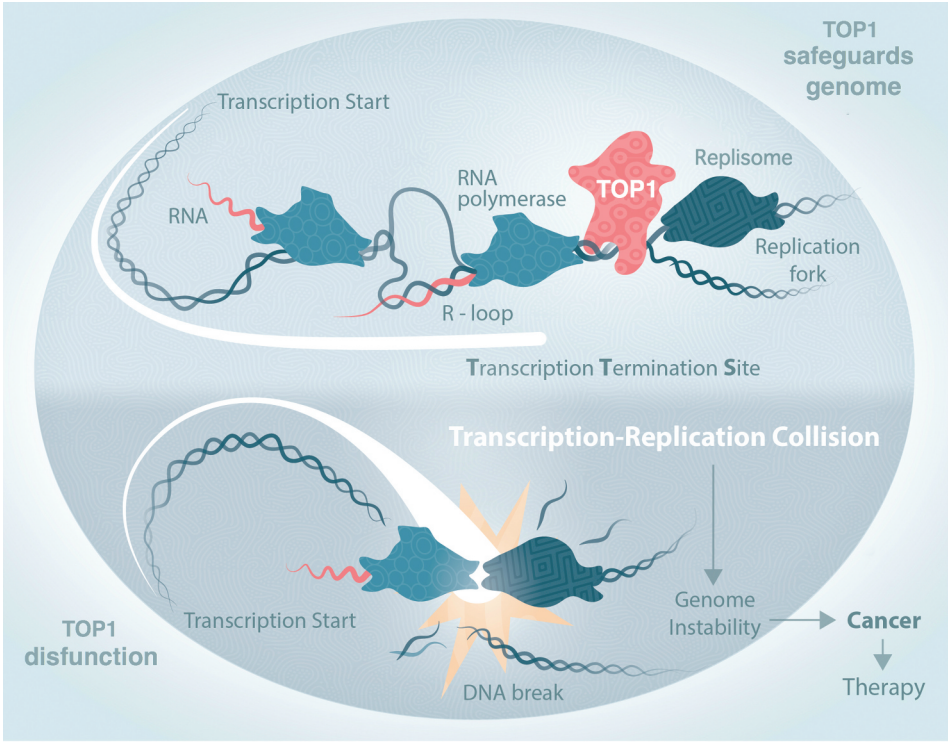

Figure 1. Topoisomerase I prevents transcription-replication conflicts and safeguards genome stability. During DNA replication, in normal condition (top panel), replisome preferentially encounters RNA polymerases at transcription termination sites (TTS) due to the presence of replication origins in front of of active genes. Replication fork pausing occurs at TTS enriched in R-loops, presumably because of the accumulation of positive DNA supercoiling. Since transcription is a discontinuous process at the level of individual cells, replication could resume locally when transcription stops and torsional stress is relieved. This would prevent conflicts between RNA and DNA polymerases, in a topoisomerase I- (TOP1)-dependent manner. However, in the case of TOP1 dysfunction (bottom panel), DNA supercoiling may increase the persistence of R-loops. They would promote fork collapse, increase DNA double-strand breaks (DSBs) and genome instability, which provides a way for the cancer therapy by using TOP1 inhibitors.

least partially alleviated replication stress in TOP1-deficient cells.[4,7]

Altogether, these results provide a global picture of how the functional organization of the human genome limits the deleterious consequences of fork collisions with transcription and R-loops. In this model, the preferential co-directional orientation of replication and transcription at highly expressed genes and the controlled pausing of replication forks at TTS are both important to prevent HO collisions (Figure 1). The molecular mechanisms ensuring stable fork pausing and restart at TTS are currently unclear, but it may require a tight control of DNA torsional stress as it is perturbed in TOP1-deficient cells. In addition, transient replication fork stalling and local ATR activation might help displace RNA polymerases ahead of the replisome, as reported earlier in budding yeast.[8] In TOP1-deficient cells, the chronic activation of the ATR pathway may also actively slow down fork progression to prevent further head-on collisions and maintain genome integrity.[4,7]

Recent evidence indicates that deregulated oncogenic pathways increase TRCs in precancerous lesions.[1] Interestingly, these oncogene-induced origins are located within gene bodies and generate forks that are not protected from collisions with RNA polymerases. Moreover, deregulated transcriptional programs may also increase RS in cancer cells. For example, in estrogen receptor (ER)-positive breast cancer cells (MCF7 cells), the R-loops resulting from the estrogen transcriptional response colocalize with DNA damage sites in a replication-dependent manner.[9] These data provide a possible mechanistic link between R-loops and estrogen-dependent carcinogenesis and indicate that R-loops might play an important role in breast cancer development. Since TOP1 plays a central role in the regulation of TRCs, these results may also explain why TOP1 inhibitors such as Topotecan and Irinotecan are effective against tumor cells, for instance, during breast cancer treatment, presumably because they increase RS to unbearable levels for tumor cells.[10] Further studies of TRCs in normal and cancer cells exposed or not to topoisomerase inhibitors will shed light on this important process as well as get a better understanding on drug resistance, and may also lead to the development of new therapeutic strategies to fight cancer.

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Disclosure of potential conflicts of interest

We declare no competing interests and no potential conflicts of interest.

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