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Review

The Top1 paradox: Friend and foe of the eukaryotic genome

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ABSTRACT

Topoisomerases manage the torsional stress associated with the separation of DNA strands during transcription and DNA replication. Eukaryotic Topoisomerase I (Top1) is a Type IB enzyme that nicks and rejoins only one strand of duplex DNA, and it is especially important during transcription. By resolving transcription-associated torsional stress, Top1 reduces the accumulation of genome-destabilizing R-loops and non-B DNA structures. The DNA nicking activity of Top1, however, can also initiate genome instability in the form of illegitimate recombination, homologous recombination and mutagenesis. In this review, we focus on the diverse, and often opposing, roles of Top1 in regulating eukaryotic genome stability.

1. Introduction

Topoisomerases transform the topological state of helical DNA by first creating a break in the DNA backbone and, following the swiveling of DNA strands, catalyzing re-ligation of the broken strand(s). From bacteria to humans, these enzymes are critical for maintaining topological homeostasis and ensuring proper function and stability of a dynamic genome. Topoisomerases are classified as either Type I or Type II enzymes depending on whether they cleave one or both strands of DNA, respectively (reviewed in [1]). The Type I enzymes are further subdivided into Type IA and IB enzymes, which differ in their preference for double- *versus* single-stranded DNA as substrate and in the type of covalent linkage made with a nicked DNA strand. Type IA enzymes prefer single-stranded DNA and hence are specialized to deal with an underwound duplex, which has single-strand character and accumulates negative supercoils. Type IB enzymes only cleave double-stranded DNA [2] and can resolve both positive (overwound DNA) and negative supercoils. Like Type II enzymes, Type IA enzymes form a covalent link between an active-site tyrosine and the 5′-phosphate of a nicked DNA backbone, leaving a 3′-OH on the other side of nick. By contrast, Type IB enzymes form a 3′-phosphotyrosyl link when they nick DNA and leave a 5′-OH on the other side of the nick. In this perspective, we focus on the role of the highly conserved eukaryotic Topoisomerase I (Top1), which is a Type 1B enzyme. The myriad functions of Top1 related to genome stability can be divided into two opposing categories. Top1 is critically important for maintaining genome integrity, especially in areas facing the unique topological challenges associated with transcription. Even very transient breaking of the DNA backbone can be hazardous, however, turning Top1 from a helpful friend into a destabilizing foe that can initiate both small- and large-scale genetic changes. Here, we discuss these opposing roles of eukaryotic Top1.

2. Top1 as a regulator of genome stability

2.1. Top1 and transcription

The movement of the transcription machinery and the obligatory separation of DNA strands create twin domains of positive and negative supercoils ahead of and behind the transcription complex, respectively (Fig. 1; [3]). This necessitates topoisomerase action in order to avoid levels of helical tension that interfere with DNA metabolic processes. In bacteria, for example, activation of a single strong promoter in a plasmid results in negative supercoiling detectable by cruciform-structure formation at AT repeats embedded upstream of the transcribed gene [4]. In yeast, *TOP1* deletion results in excessively negative-supercoiled plasmid DNA [5,6], which highlights the key role of Top1 in managing transcription-induced negative supercoiling.

A recent yeast study used two closely-spaced promoters to examine

Abbreviations: Top1, topoisomerase I; DSB, double-strand break; RNAP, RNA polymerase; GCR, gross chromosomal rearrangement; CPT, camptothecin; G4, guanine quadruplex; TNR, trinucleotide repeat; CSR, class-switch recombination; AID, activation-induced cytosine deaminase; NHEJ, non homologous end joining; IR, illegitimate recombination; ribo, ribonucleotide; Top1cc, Top1 cleavage complex

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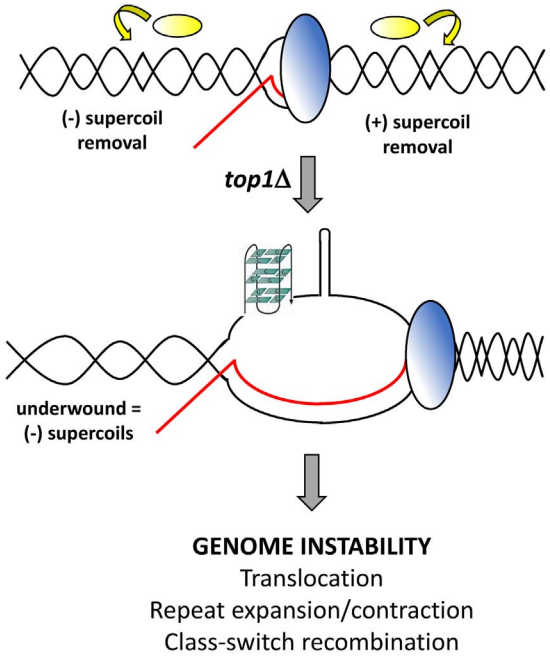

Fig. 1. Genome stabilization by Top1 during transcription. During normal transcription by RNAP (blue oval), topological homeostasis is maintained by the activity of Top1 (yellow oval). In the absence of Top1, underwound and negatively supercoiled DNA that accumulates behind RNAP supports the formation of co-transcriptional R-loops in which the RNA transcript (red) pairs extensively with the DNA (black) template strand, and the non-template DNA strand is single-stranded. Single-stranded DNA folds into non-B secondary structures such as G4 DNA and hairpins. R-loops and non-B structures initiate genome instability.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

how eukaryotic topoisomerases deal with transcription-driven topological changes that have the potential to affect genome stability [7]. Activation of promoters arranged in a divergent configuration led to loss of a terminal segment of the corresponding chromosome arm, which reflects double-strand break (DSB) formation. Activation of two convergently arranged promoters, however, did not have any appreciable effect on such gross chromosomal rearrangements (GCRs). The DSBs initiating the GRC events associated with divergent promoters were attributed to excessive negative supercoils produced when two RNA polymerase (RNAP) complexes move away from each other, supporting the argument that negative torsional stress is the main transcription-associated source of genome instability. Neither loss of Top1 nor reduction of Top2 activity (Top2 is the sole Type II enzyme in yeast and is essential for chromosome segregation; [8]) affected the GCR rate when the promoters diverged. Reduced Top2 activity, however, sharply elevated GCRs when the promoters converged; loss of Top1 had no effect. These results suggest that Top2 can largely complement Top1 function in removing negative supercoils, but that Top1 cannot complement Top2 removal of positive supercoils that are potentially pathological. Top3, a Type IA topoisomerase that mainly functions during the resolution of Holliday junctions formed during homologous recombination [9], does not appear to be involved in regulating transcription-associated topological dynamics.

For transcribed genic regions, the regulation of transcription-associated topological stress by Top1 involves its physical association with the RNAPII complex. In yeast, the Top1 occupancy of a gene correlates with its level of transcription [10], and Top1 specifically interacts with the phosphorylated C-terminal repeat domain of the RNAPII catalytic subunit [11]. In human cell lines, Top1 occupancy also is enriched at highly transcribed genes and the N-terminal domain of Top1 mediates its physical interaction with the C-terminal domain of RNAPII [12]. Importantly, the DNA relaxation activity of Top1 in human cell lines is stimulated by interaction with the phosphorylated C-terminal domain of RNAPII and that this association facilitates promoter escape as well as elongation past natural pause sites. In addition to a direct interaction with RNAPII, human Top1 is recruited to transcriptionally active chromatin *via* interaction with chromatin remodeling factors [13].

In human cells, Top1 (and Top2) are required for the transcription of extremely long genes that are several hundred kb in length [14], and this could reflect the accumulation of inhibitory positive or negative supercoils. Negative supercoiling promotes the formation of R-loops (see below) whereas excessive positive supercoiling prevents the continued unwinding of DNA. Intriguingly, transcription-associated Top1 effects have been linked to several human neurological diseases, suggesting that the use of topoisomerase inhibitors could have therapeutic value [15]. In neurons, for example, genes linked to autism spectrum disorder are extremely long, and their expression is reduced by Top1 inhibitors [14]. Furthermore, a reduction in Top1 activity reduces expression of an anti-sense transcript that silences the paternal *Ube3a* allele in Angelman syndrome [16].

2.2. Top1 and RNA-DNA hybrids

The transcription bubble within elongating RNAP is ~15 nt, with pairing between the nascent transcript and template DNA strand extending 9 bp [17]. More extensive and stable hybridization between the template DNA strand and the nascent RNA can occur through a threadback mechanism after the transcript and duplex DNA exit the RNAP through separate channels [18]. The structure thus generated (a stable RNA:DNA hybrid and the displaced, non-template single strand) is referred to as an R-loop and occurs co-transcriptionally when RNA processing is disrupted. Reduction of the THO/TREX complex involved in mRNA packaging/export or the ASF1/SF2 splicing factor, for example, leads to transcription-dependent accumulation of extensive R-loops [19,20]. R-loops can be removed by the RNA:DNA hybrid-specific *ribo*-endonucleases RNase H1 and H2, or by the RNA:DNA helicases senataxin (SETX) and aquarius (AQR); disruption of any of these factors leads to accumulation of RNA:DNA hybrids [21–23]. Although R-loops have physiological functions (*e.g.*, in transcription termination), they have emerged as an important pathological structure that instigates genome instability through disruption of transcription and replication (reviewed in [22,24,25]).

Negative torsional stress favors R-loop formation, and preventing the accumulation of R-loops is an important role of Type I enzymes that is conserved from prokaryotes to mammalian systems (Fig. 1). In *E. coli*, disruption of the Type IA topoisomerase topA leads to growth defects that are rescued by overexpression of RNase H, indicating RNA:DNA hybrid accumulation as the major consequence of excessive negative supercoils [26–28]. In yeast, loss of Top1 is associated with transient melting of the DNA duplex and R-loop accumulation in the very highly transcribed ribosomal RNA genes [29,30]. The accumulation of R-loops and the disruption of transcription worsens in *top1*Δ cells when the degradation of RNA:DNA hybrids is blocked by additional depletion of RNase H enzymes. In yeast cells lacking both RNase H1 and H2, the loss of Top1 is lethal [29]. Genome-wide mapping of R-loops using the RNA:DNA hybrid-specific monoclonal antibody S9.6 showed that conditional depletion of Top1 in yeast cells deficient in both RNase H1 and H2 leads to further accumulation of R-loops throughout the genome, especially at ribosomal RNA and transfer RNA genes [31]. A more recent study identified two additional features that predispose genomic regions to R-loop formation in the absence of RNase H1/H2: either high expression or presence of polyA tracts [32]. Whereas yeast cells tolerate the loss of Top1, RNase H1 or RNase H2 individually and in some combinations, each is an essential enzyme in mammals.

The function of Top1 in preventing R-loop accumulation is conserved in mammalian systems. Top1-deficient mouse lymphocytes accumulate stalled replication forks and DNA breaks at actively transcribed regions [33]. Both replication-fork stalling and DNA breaks are reduced by overexpression of RNase H1, implicating transcription-

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