

Topoisomerases, chromatin and transcription termination

Mickaël Durand-Dubief,¹ J. Peter Svensson,² Jenna Persson² and Karl Ekwall^{2,*}

¹Chromatin and Gene Expression; Babraham Institute; Babraham, Cambridge, UK; ²Department of Biosciences and Medical Nutrition; Center for Biosciences; Karolinska Institutet; Novum, Sweden

In eukaryotes transcription is complicated by the DNA being packed in nucleosomes and by supercoils induced by opening of the DNA double helix during elongation. Here we discuss our recent genome-wide work regarding topoisomerases and their role in chromatin remodeling during the transcription cycle and we report a novel function for topoisomerases in transcription termination.

Introduction

DNA topoisomerases solve the topological problems associated with DNA replication, transcription and recombination by introducing transient nicks in DNA and thereby relieving the helical torsion.^{1,2} Topoisomerases relax the structure of DNA by generating single-strand (type I subfamily) or double-strand (type II subfamily) breaks.¹ The fundamental need for topoisomerases originates from the DNA double helix, as (1) during DNA replication, the two strands of the DNA must be untangled and as (2) during transcription, the opening of DNA structure to allow RNA elongation, generates DNA supercoils that must be relaxed in order for the polymerase to move rapidly along the template.^{1,3-6} In eukaryotic cells, even though the bulk of the genomic DNA is relaxed,⁷ supercoiled microdomains may appear at sites of active transcription.⁸⁻¹⁰ This has been demonstrated by studies of transcription at divergent promoters, shown to induce local negative supercoiling in human cells.¹¹

In multicellular organisms, Top1 is essential for early development. However, the yeast deletion mutant is viable, likely

through compensation from Top2.^{1,2,12} Inactivation of either Top1 or Top2 does not significantly affect transcription, whereas inactivation of both enzymes clearly reduces RNA levels and, to a lesser extent, mRNA synthesis.¹³⁻¹⁵ Although both type I and II topoisomerases have been implicated in transcription, Top1 seems to be the main actor in releasing transcription-generated helical stress.^{1,2,16} Recently, systematic mapping of 53 different chromosomal proteins combined with principal component analysis in *Drosophila* showed that Top1 is found in two of five principal chromatin types, and these two types are also characterized by having the active chromatin mark H3K4me and high levels of transcription.¹⁷

DNA dependent processes including transcription, replication, recombination and repair are complicated by the fact that chromosomes are compacted by DNA being wrapped around histone octamers, forming nucleosomes. Nucleosomes can form on both negatively and positively supercoiled circular DNA templates *in vitro* and, in the latter case, this increases the superhelical stress of the template molecule.¹⁸⁻²⁰ In contrast, when nucleosomes are formed on negatively coiled circular templates this relaxes the superhelical tension by one turn per nucleosome.²¹ Negative supercoiling of DNA templates facilitates nucleosome formation *in vitro*.¹⁸⁻²⁰ According to the 'twin supercoiling model' a transcribing RNA polymerase generates positive supercoils ahead of the enzyme and negative coils behind assuming that the DNA ends are not free to rotate.² As a consequence nucleosome assembly behind the polymerase and disassembly ahead of the polymerase should be favored.

Key words: topoisomerases, gene expression, nucleosome, transcription termination, nucleosome free region

Abbreviations: ChIP, chromatin immunoprecipitation; Top1, topoisomerase I; Top2, topoisomerase II; Pol II, RNA polymerase II; CTD, carboxy terminal domain; TSS, transcription start site; TTS, transcription termination site; NFR, nucleosome free region

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*Correspondence to: Karl Ekwall;
Email: karl.ekwall@ki.se

However, the relationships between topoisomerases and chromatin assembly and disassembly mechanisms are poorly understood and the existing data are mostly based on *in vitro* or single gene studies. In our recent genome-wide study,²² we provided a holistic view of the changes in transcription and nucleosome occupancy that occur in topoisomerase mutants. The results support a role for Top1 in facilitating the eviction of nucleosomes to create 5'NFR regions at gene promoters and stimulate transcription initiation. The data also suggest that Top1 and Top2 are required for efficient elongation of transcription, presumably by removing positive supercoiling ahead of the polymerase. Here, we review these findings and bring novel insights into topoisomerase functions linked chromatin dynamics at 3'NFR regions and control of transcription termination.

Topoisomerases Broadly Contribute to the Control of Gene Expression and Chromatin Structures

We have carried out a genome-wide analysis of topoisomerases of type I and II in the fission yeast *Schizosaccharomyces pombe*.²² We found that both topoisomerases occupy intergenic regions and Top1, but not Top2, is linked to actively transcribed gene promoters. In contrast, both topoisomerase enzymes were found in 3' coding regions and 3' intergenic regions. *In vitro* studies have suggested that nucleosome assembly is tightly coupled to DNA topology.^{23,24} To determine the influence of topoisomerase activity on chromatin organization as suggested in earlier studies,²⁵ we used the double mutant *top1Δ top2-191^{ts}* carrying a gene deletion for *top1* and a temperature sensitive allele of *top2*. This strain displays reduced topoisomerase II activity when grown at the semi-permissive temperature.¹² We showed that topoisomerase activity influences histone density and detected higher histone H3 occupancy in intergenic regions in *top1Δ top2^{ts}*, especially in genes that are highly transcribed in wild-type cells. The same set of genes also showed reduced expression and Pol II occupancy in the *top1Δ top2^{ts}* mutant. These results suggested that topoisomerase

activity maintains a low histone density in promoters to allow for efficient Pol II recruitment and high transcription levels. Additionally, we found that Pol II accumulates on longer genes in the absence of topoisomerase activity. In longer genes, Top2 occupancy is markedly increased in 3' coding regions. These findings support an essential role for topoisomerases *in vivo* to facilitate transcription elongation (and especially Top2 at longer genes) through the removal of positive DNA supercoils generated ahead of Pol II.^{1,2}

Topoisomerases and the Chd1 Chromatin Remodeler Hrp1 are Both Needed for Nucleosome Disassembly

In vitro studies have shown that the activity of the ATP-dependent chromatin remodeler Snf2 generates superhelical torsion during nucleosome assembly and requires DNA topoisomerases to relieve the DNA tension.²⁶ In *S. pombe*, the Snf2 remodeler and Chd1 homolog Hrp1 interacts with the histone chaperone Nap1 and stimulates nucleosome disassembly *in vivo*.²⁷ Our analysis showed a significant overlap between Hrp1, Nap1 and Top1/Top2 occupancy where histone H3 density is changed in the respective mutants.²² It is conceivable that the remaining intergenic regions (unaffected by *hrp1* and where H3 density is increased in *top1Δ top2-191^{ts}*) require other Snf2 chromatin remodelers that evict nucleosomes together with Top1 and Top2.

Topoisomerases are Required for Efficient Transcription Termination

Genome-wide mapping of yeast nucleosomes show that promoters (5'NFR) and sites of transcript termination (3'NFR) are depleted in nucleosomes.^{28,29} In budding yeast the 3' NFR was shown to be dependent on transcription by Pol II and it was hypothesized to be linked to some aspect of RNA 3' end formation.²⁸ An interesting aspect of topoisomerases is their occupancy at the 3' end of genes.²² By revisiting our genome-wide data, we set out to test whether topoisomerase activity could play a role in modifying the histone density at the 3' end of genes.

We determined the average H3 density in the region surrounding the 3' TTS (using TTS annotations obtained from ref. 29). We filtered the data to include only genes transcribed above average and partitioned the data by the distance from a gene's stop codon to the start/stop codon of the next 3' gene. With this step, we avoid interference by adjacent transcription (next gene within 500–1,000 bp are orange and next gene >1,000 bp away are green in Fig. 1). In wild-type cells, longer intergenic regions between transcribed genes show the most pronounced depletion of histone H3 (Fig. 1A). By comparing the high-resolution H3 maps for *top1Δ top2-191^{ts}* and wild-type cells, we observed that there is a significant increase of histone occupancy regardless of the distance to adjacent genes (Fig. 1B). This indicates that topoisomerase activity participates in histone disassembly in the 3' end of the gene.

Levels of histone H3 is generally linked to regulation of transcription, therefore we asked whether transcription termination defects could be observed at the 3' end of genes. Using our transcriptome data,²² we performed the same analysis for wild-type cells and the *top1Δ top2-191^{ts}* mutant at regions encompassing the 3' TTS. As expected, we found increased transcription levels in the 3' TTS regions of the *top1Δ top2-191^{ts}* mutant compared to wild-type cells (Fig. 1C). Also the elevated RNA levels were accompanied by an increase of Pol II occupancy in the *top1Δ top2-191^{ts}* mutant (Fig. 1D). Together, the data presented here suggest that topoisomerase activity is involved in controlling the supercoil structures at the 3' end of genes and thereby contributing to nucleosome disassembly and efficient transcription termination.

We illustrate our findings with two pairs of convergent genes. By comparing *top1Δ top2-191^{ts}* and wild-type cells, we observed an increased H3 occupancy at intergenic regions (Fig. 1E). In addition, the antisense transcription in *top1Δ top2-191^{ts}* is increased whereas the sense transcription seems unaffected. The Pol II occupancy in wild type is slightly increased at the end of the transcript and, consistent with run-on transcription, Pol II occupancy in *top1Δ top2-191^{ts}*

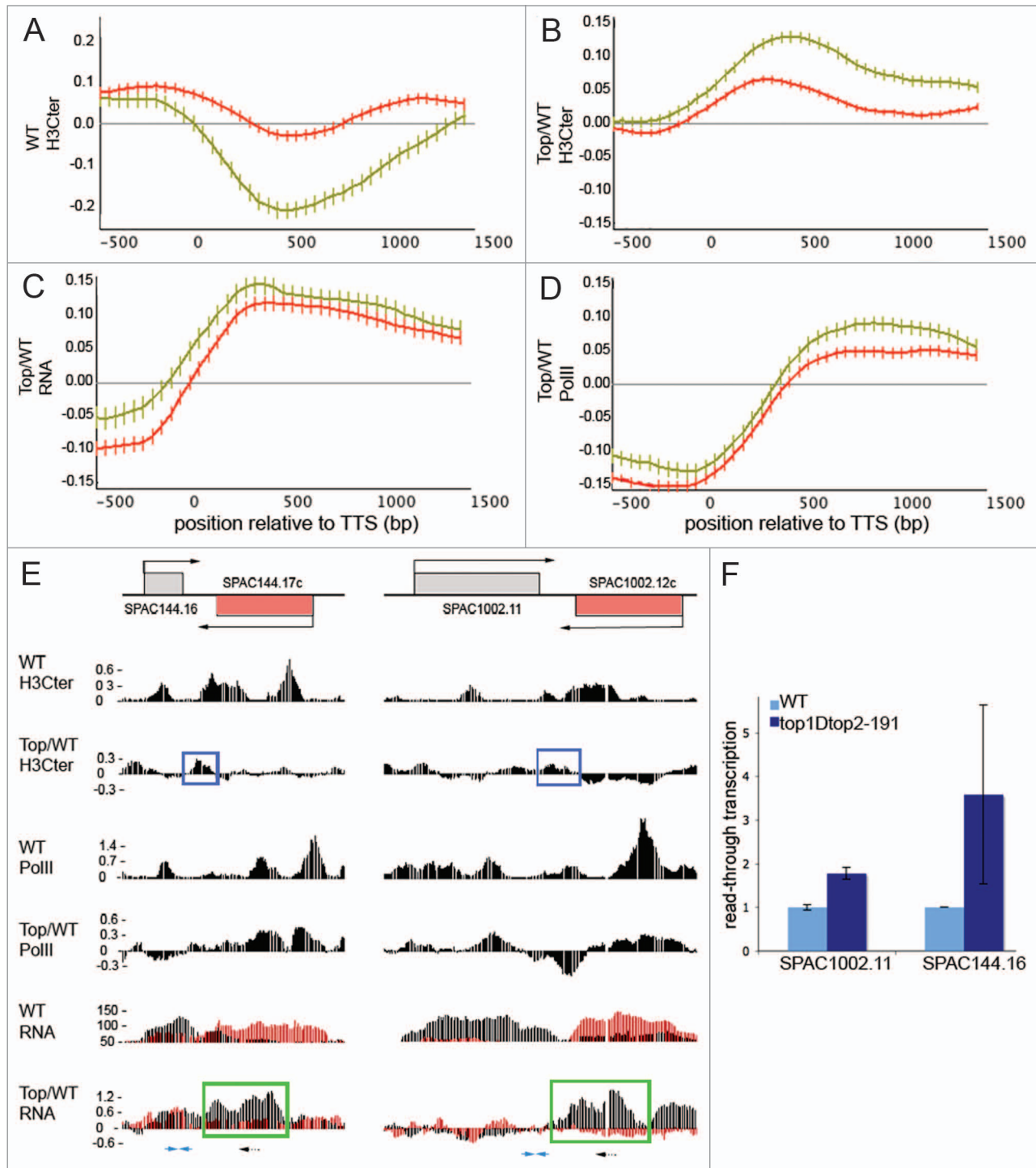


Figure 1. Topoisomerases are required for the global formation of 3'NFR and transcription termination. (A) Histone H3 occupancy in wild-type cells is dependent on the proximity to the adjacent gene, (B) H3 occupancy in *top1Δ top2-191^{ts}* mutant relative to levels in wild-type cells, (C) RNA levels in *top1Δ top2-191^{ts}* mutant relative to levels in wild-type cells, (D) Pol II occupancy in *top1Δ top2-191^{ts}* mutant relative to levels in wild-type cells. Genes making up the green lines have no other transcript <1 kb downstream, genes making up the orange line have another transcript between 500 and 1,000 bp away. The genomic data are aligned at the transcript termination site (TTS). Error bars represent the 99% confidence intervals. Graphs were generated in Podbat (www.podbat.org). (E) Transcription from the forward strand is shown in black and transcription from the reverse strand is shown in red. The green boxes highlight the aberrant transcript in the *top1Δ top2-191^{ts}* mutant. Blue boxes indicate the increased level of H3 in the intergenic regions. Dotted arrows indicate cDNA primer position and blue arrows indicate the qRT-PCR primer positions used in (F). Browser views are generated in Podbat. (F) qRT-PCR reveals aberrant transcript elongation in *top1Δ top2-191^{ts}* cells. The primers specifically detect read-through transcripts from the indicated gene. Data are normalized to endogenous control.

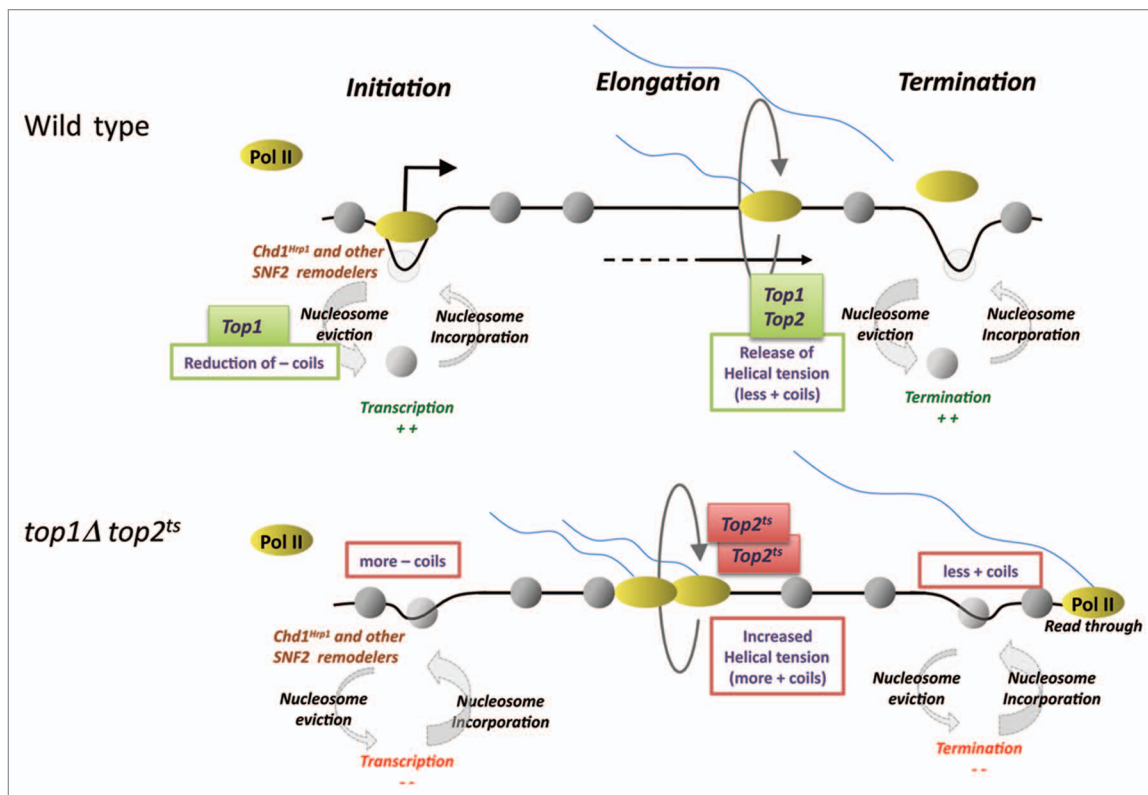


Figure 2. A model of transcriptional regulation by Top1, Top2 and chromatin remodelers. All stages of the transcription cycle are affected by Top1 and Top2.

cells is increased and shifted 3', within the coding region of the neighboring gene. Detection by quantitative 5' nested RT-PCR shows an increase of SPAC144.17c antisense transcripts initiated from SPAC144.16 in the *top1Δ top2-191^{ts}* mutant (Fig. 1F), as well as a SPAC1002.12c antisense transcription from SPAC1002.11. This suggests that the defects in transcription termination in the neighboring converging gene causes Pol II read-through.

Normally, transcription termination is linked to a shift in phosphorylation levels of the C-terminal domain (CTD) of Pol II largest subunit. As the polymerase approaches the end of the open reading frame, serine 2 phosphorylation increases and serine 5 gets dephosphorylated. Factors such as Pcf11, needed for mRNA polyadenylation, bind to the serine 2 phosphorylated CTD, and the nascent RNA is cleaved at the polyA site. Pol II continues to transcribe the DNA but the resulting RNA is rapidly degraded by the Rat1/Xrn2 exonuclease.³⁰ Both transcription initiation and termination are highly

regulated processes and are associated with a checkpoint pause. This pause is controlled by the chromatin remodeler Isw1 in *Saccharomyces cerevisiae*.³¹ Mutation of Isw1 results in a denser nucleosome positioning around the 5'NFR as well as transcription termination defects. It has also been shown that Isw1 interacts physically with Top1.³² However, *S. pombe* has no Isw1 homolog and these functions could have been taken over by other Snf2 remodelers acting together with Top1/Top2. In the case of both Isw1 and the topoisomerases, the effect of decreased activity leads to a less accessible chromatin structure. We speculate that a possible consequence of the altered chromatin structure we observe at the 3'NFR obstructs the signals for transcript termination. For example this could happen through reduced availability for interactions with the CTD. The increase of read-through RNA that we observe could be from parts of the RNA molecules that normally are degraded by the Rat1/Xrn2 exonuclease.

We propose a model for the function of Top1 and Top2 in controlling DNA

supercoiling states and chromatin structure during all stages the transcription cycle: initiation, elongation and termination (Fig. 2, top part). First, in the initiation phase and pre-initiation complex formation, Top1 has a key role in removing negative coils to facilitate nucleosome disassembly by remodelers at the 5'NFR. Then, during transcription elongation Top1 and Top2 cooperate to decrease helical tension introduced by Pol II activity, as predicted by the 'twin supercoiling' model. Finally, in the process of transcription termination, topoisomerases are required to maintain the 3'NFR to allow for proper termination. It is possible that the positive supercoils that presumably accumulate in the 3' region of the gene destabilize nucleosomes in the 3' NFR region. Chromatin remodelers could potentially also stimulate 3'NFR formation. Interestingly, the Chd1 remodeler Hrp1 has also been implicated in facilitating transcription termination of individual genes in *S. pombe*.³³ In the *top1Δtop2^{ts}* mutant we assume that negative coils accumulate in the 5' region leading to a

reduced 5'NFR (Fig. 2: bottom part). Furthermore, the partially inactive Top2^{ss} enzyme is not sufficient to allow for proper elongation by Pol II leading to a 'piling up' of Pol II 500–1,000 bp into the coding region, and the accumulation of extra positive supercoils in the same region. As a consequence of this elongation defect the positive supercoiling is reduced in the 3' region thus inhibiting the nucleosome eviction process and the 3'NFR disappears and thereby obstructing the signals for transcript termination.

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