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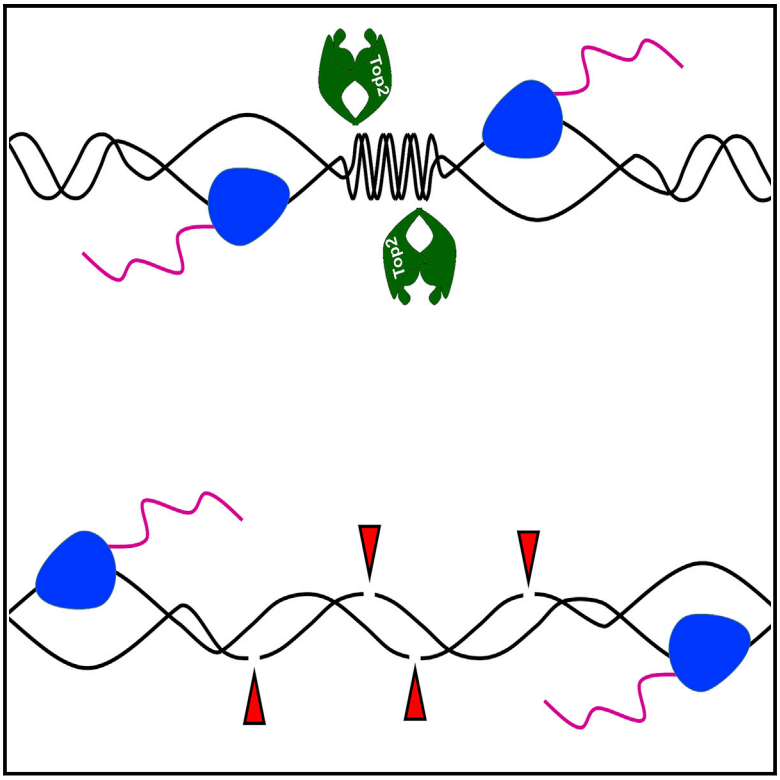
Report

Dissecting the Roles of Divergent and Convergent Transcription in Chromosome Instability

Graphical Abstract



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In Brief

Pannunzio and Lieber demonstrate that, in wild-type cells, divergent, but not convergent, transcription increases genome instability measured by gross chromosomal rearrangements. For convergent promoters, the function of topoisomerase II is critical for preventing instability at convergent promoters.

Highlights

- Convergent transcription does not increase genome instability in wild-type cells
- Divergent transcription leads to a significant increase in genome instability
- Top2 is critical for relieving topological tension at closely spaced promoters
- Direction of transcription affects chromosome instability via topological tension

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Dissecting the Roles of Divergent and Convergent Transcription in Chromosome Instability

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SUMMARY

The interplay of transcription, topological tension, and chromosome breakage is a subject of intense interest, but, with so many facets to the problem, it is difficult to test. Here, we vary the orientation of promoters relative to one another in a yeast system that permits sensitive detection of chromosome breaks. Interestingly, convergent transcription that would direct RNA polymerases into one another does not increase chromosome breakage. In contrast, divergent transcription that would create underwound and potentially single-stranded DNA does cause a marked increase in chromosome breakage. Furthermore, we examine the role that topoisomerases are playing in preventing genome instability at these promoters and find that Top2 is required to prevent instability at converging promoters.

INTRODUCTION

The events surrounding collision of RNA polymerases during transcription are complex and of universal relevance for all living organisms (García-Rubio and Aguilera, 2012; Hobson et al., 2012; Liu and Alberts, 1995; Prescott and Proudfoot, 2002). Much of the early analysis of this phenomenon was from work performed in bacteria where it became clear that not only must the steric nature of two transcription complexes be considered, but also the effect that the complexes have on the DNA they are transcribing. The twin domain model proposed by Liu and Wang (1987) explains how the movement of an RNA polymerase can generate both positive and negative supercoiling in the DNA. In eukaryotes, where linear chromosomes are packaged into nucleosomes, the dynamics of transcription and torsional stress, and the effect of the positioning of transcription units on DNA structure has been an active area of study (Naughton et al., 2013; Teves and Henikoff, 2014). Our interest is in how these dynamics affect an organism physiologically, particularly as it relates to DNA breakage.

In both yeast and mammals, promoter regions represent a nucleosome-depleted region (NDR) where the pre-initiation complex (PIC) assembles between a −1 and a +1 nucleosome before traveling to a downstream transcription start site (TSS). Divergent promoters can occur in a single NDR where two separate PICs form and direct the transcription units away from each other, creating an area of active chromatin and an expansive NDR determined by the distance between TSSs (Rhee and Pugh, 2012; Scruggs et al., 2015). As the RNA polymerase II (RNAP2) machinery departs the TSS and moves into the gene body, further repositioning of nucleosomes would be necessary, but a key question concerns what happens in the underwound region behind the two RNAP2 complexes? One facet of this question is that this promoter orientation is somehow used to regulate gene expression (Wei et al., 2011). Another facet is that this interplay of transcription, torsional stress, and active chromatin can cause genome instability. Determining the latter is especially important, given that it is now clear that expression of small RNAs is tissue and cell stage specific; that is, a region that does not have divergent protein-coding promoters in one tissue may have divergent promoters in another cellular context (Lu et al., 2005, 2015; Schotte et al., 2011).

Importantly, closely spaced convergent promoters may also be a source of potential instability. While convergently expressed genes would have their promoter regions separated by the two gene bodies, some convergent promoters may be close enough to occupy the same NDR. Work has shown that as two RNAP2 complexes approach each other from convergent promoters in a head-on collision; the two complexes stall and prevent each other from proceeding (García-Rubio and Aguilera, 2012; Hobson et al., 2012; Saeki and Svejstrup, 2009). However, the consequences of very close convergent promoters in vivo has only recently begun to be studied, as it now appears that antisense transcription can lead to convergent transcription, especially at proposed super-enhancer regions (Lu et al., 2015; Meng et al., 2014).

Recently, two studies have reported that closely positioned transcription units appear to be correlated with chromosomal translocations (Meng et al., 2014; Pefanis et al., 2014). The conclusions of these large-scale, genome-wide studies make it unclear whether it is divergent or convergent transcription that more greatly potentiates the risk of a DSB. Here, we sought to develop a simple genetic assay as a starting point to determine whether, indeed, closely spaced convergent or divergent promoters result in genome instability. We compare divergent and convergent transcription chromosomal regions in a yeast system that permits detection of gross chromosomal rearrangements (GCRs) (Chen and Kolodner, 1999). We find that convergent transcription that would direct RNA polymerases into one another