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A New Twist on Transcriptional Bursting

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Abstract

Transcriptional bursting has been observed across species and is one of the primary causes of variable gene expression in cells and tissue. In this issue, Chong et al. describe how DNA topology results in transcriptional bursting in *E. coli*.

Gene expression heterogeneity, or “noise,” in gene expression has now been observed in bacteria, yeast, slime mold, flies, and mammals (Sanchez and Golding, 2013). Within the last several years, it has become clear that this variability is not static but is dynamic: expression of a gene of interest can fluctuate on timescales of minutes to hours to days. Most of this heterogeneity is thought to originate during transcription: genes are infrequently transcribed in stochastic “bursts” of RNA synthesis interspersed with long periods of inactivity (Larson, 2011). However, the causes and consequences of transcriptional bursting are still largely unknown. Because this phenomenon has only been directly observed in vivo, usually by advanced live-cell microscopy techniques, it has been difficult to probe the underlying biochemical mechanism. Chong and colleagues now describe a general mechanism of transcriptional bursting in *E. coli* that is based on DNA topology (Chong et al., 2014). For the first time, they are able to visualize transcriptional bursting in vitro using a single-molecule assay. They demonstrate that the torque introduced by the very act of transcriptional elongation is responsible for the bursts of RNA synthesis. In fact, this same mechanism seems to operate in vivo, suggesting that DNA mechanics may play a fundamental role in gene expression heterogeneity observed in clonal populations.

When RNA polymerase transcribes DNA into RNA, the DNA double helix experiences a torque. In front of the polymerase, the DNA becomes more tightly wound (positive supercoiling), and behind the polymerase, the DNA becomes more loosely wound (negative supercoiling). In prokaryotes, there are two topoisomerases that relieve this tension: topoisomerase 1A passes one strand of an unwound segment through a transient break in the other to relieve negative supercoiling, whereas DNA gyrase relieves positive supercoils by passing a double-helical segment through a transient double-stranded break (Figure 1). Because the activity of gyrase is limiting, the authors hypothesized that positive supercoiling might accumulate in front of the transcribing polymerase, leading to an effective stall force that eventually brings transcription to a halt. The key insight is that this unsynchronized push and pull between transcription and torsion might be responsible for bursting.

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The authors first test this idea in vitro by observing transcription from torsionally constrained 12 kb templates. With their single-molecule assay, they were able to observe bursts of RNA synthesis from the template (Figure 2D in Chong et al., 2014). As transcription proceeds, positive supercoiling continues to accumulate, eventually resulting in a reduced rate of initiation from the template. Addition of DNA gyrase relieves this stall force, and transcription restarts. This constrained-template assay is reminiscent of the actual organization of the bacterial chromosome into topologically constrained loops, suggesting that this same principle might be operating in living cells. Indeed, the rate of DNA gyrase catalysis is similar to the length of transcriptional bursts in *E. coli*, suggesting that the in vitro observation might be recapitulated in vivo.

This jump from an in vitro system to an in vivo one, interrogated with the same single-molecule resolution, is one of the primary advances in this paper. To visualize transcriptional bursting in cells, one can either directly image the production of nascent pre-mRNA in real time or infer the underlying behavior by measuring steady-state distribution of mRNA in the cell (Larson, 2011). The idea behind the latter approach is that transcription dynamics have a “signature” that can be observed in the population of cells: as the gene turns “on” and “off” with a certain duty cycle, the steady-state distribution will change. Chong and coworkers find that, by overexpressing DNA gyrase, they increase this duty cycle. Essentially, by relieving positive supercoiling, the gene is maintained in the active “on” state. However, one could argue that, if bursting is the rule, then any disruption of a kinetically contributory transcriptional regulatory step will, by definition, result in some change to the property of bursts, for example, the duty cycle. The critical experiment then is to specifically recruit the DNA gyrase downstream of the actively transcribing polymerase, which the authors achieve by introducing a strong gyrase site into the reporter plasmid. In this case, in which the gyrase is specifically bound and presumably active, the reporter has the highest duty cycle, indicating greatly reduced bursting (Figure 7 in Chong et al., 2014).

Though the role of supercoiling in transcription elongation is well known and has recently been elucidated at the single-molecule level (Ma et al., 2013), the key finding in the current work is that positive supercoiling is abruptly relieved by gyrase, allowing the polymerase to surge and resulting in a burst of transcription (Figure 1). Thus, this work draws a direct line between DNA mechanics and stochastic gene expression. The implication of this observation is that gyrase itself may be the limiting factor in determining the duration of the “off” time, meaning that this fundamental kinetic rate is a general property of *all* transcribed genes in *E. coli* rather than a gene-specific one. This idea that DNA topology might be a general factor in determining expression variation in *E. coli* has been raised before, based on the observation that single-molecule mRNA distributions from a number of expressed genes follow similar steady-state behavior (So et al., 2011). The work by Chong et al. provides the first mechanistic support for this observation. Conversely, Paulsson and colleagues have also argued that expression heterogeneity is modulated in a manner that is gene nonspecific, but they attribute this phenomenon to transcription-independent mechanisms such as cell division (Huh and Paulsson, 2011). The question of whether noise in bacteria is gene specific or general and what the underlying cause might be is still an area of active research.

Will this same model extend to eukaryotes? The same topological constraints are present, and DNA supercoiling has been shown to be ubiquitous, for example, in the human genome (Kouzine et al., 2013), but the timescales of bursting are much longer than bacteria (Suter et al., 2011). Moreover, when single gene bursting is directly visualized in eukaryotes, it can be seen that gene-specific mechanisms such as the concentration of active transcription factor determine transcription kinetics (Larson et al., 2013; Larson et al., 2011), though not necessarily independent from topology. Nevertheless, the role of DNA topology has been perhaps underappreciated in gene regulation, and these recent results in bacteria point toward a prominent role of DNA mechanics in expression variability.

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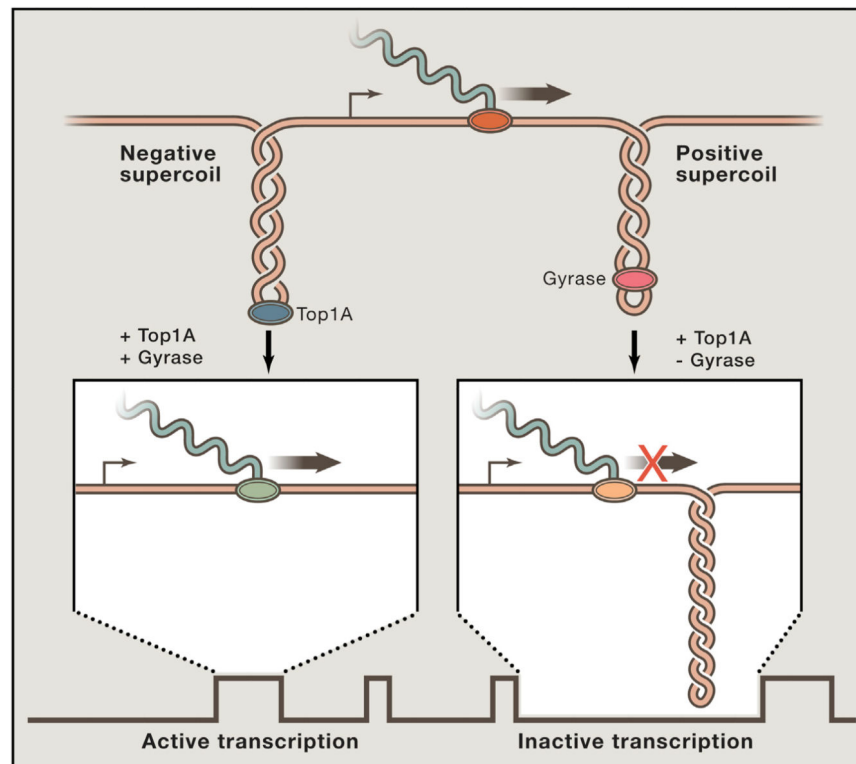


Figure 1. The Connection between DNA Topology and Transcriptional Bursting in *E. coli*
 Transcription of DNA into RNA by RNA polymerase results in positive supercoiling in front of the polymerase and negative supercoiling behind the polymerase, indicated here as supercoiled DNA plectonemes. Topoisomerase IA relieves negative supercoiling; gyrase relieves positive supercoiling. In the absence of a positive supercoiling stall force, RNA synthesis can proceed (lower-left). As positive supercoiling accumulates or in the absence of DNA gyrase, transcription comes to a halt (lower-right). This alternation between active and inactive transcription accounts for the “on” and “off” behavior that characterizes transcriptional bursting (lower schematic).