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RNA polymerase is a powerful torsional motor

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DNA supercoiling has been recognized as an important regulator of gene expression in vivo. As RNA polymerase (RNAP) tracks the helical groove of DNA, it generates (+) DNA supercoiling ahead and (–) DNA supercoiling behind, as described by the "twin supercoiled domain model" proposed by James Wang.[1] Initial studies suggested that transcription generated DNA supercoiling when RNAP and DNA are topologically confined in systems lacking topoisomerases.[1] More recent studies have convincingly demonstrated that DNA supercoiling can be dynamically built up and is broadly present during transcription.[2] Bulk studies, along with single molecule experiments, have provided important insights into how DNA supercoiling affects transcription initiation,[2,3] and they have also estimated the lower bound of the torque that an *E. coli* RNAP can generate.[4] Despite the functional significance of transcription-generated supercoiling, the interplay between DNA supercoiling and RNAP was not well understood. In particular, there was a lack of quantitative studies, stemming from the technical challenges of measuring minute biological torques.

How much torque can an RNAP generate before stalling? How does torque regulate transcription speed? How does an RNAP respond to a changing DNA topology that is constantly modified by other proteins? Answering these questions required an approach that allowed accurate control of DNA supercoiling, measurement of torque on RNAP, and simultaneous detection of RNAP movement along DNA. Utilizing an angular optical trap (AOT)[5] and a novel supercoiling assay, we have now monitored RNAP translocation in real-time, as the RNAP worked under a defined torque.[6] Our assays incorporated features from the elegant earlier approaches[3,4] while substantially extending them to allow for torque measurements.

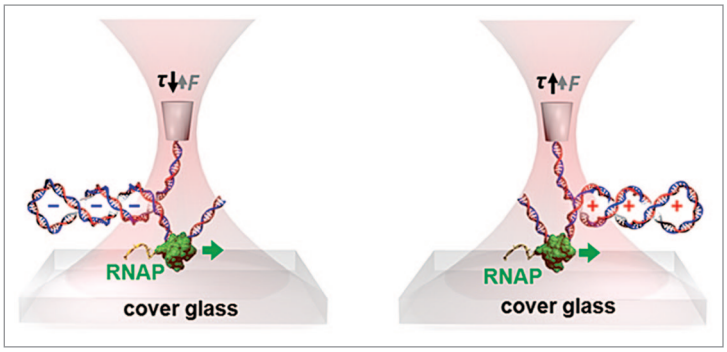

Figure 1. Experimental configurations of transcription under torsion using an angular optical trap. RNA polymerase transcribes against either an upstream (–) DNA supercoiling (left) or downstream (+) DNA supercoiling (right). Artwork by Robert A Forties.

Our experimental results have revealed new insights into the behavior of RNAP under DNA supercoiling and suggest ways by which DNA supercoiling may regulate transcription in vivo. We found that both (+) DNA supercoiling downstream or (–) DNA supercoiling upstream (**Fig. 1**) can slow RNAP, induce its pausing, and eventually lead to its stalling. In both cases, the stall torque was measured to be ~11 pN×nm, significantly greater than a previously estimated lower bound.

These results thus demonstrate that *E. coli* RNAP is a powerful torsional motor. Interestingly, the stall torque value coincides with what is required to melt DNA of arbitrary sequence.[7] This torque is more than sufficient to induce structural transitions of AT-rich sequences that are prone to melting, as well as GC repeats that are known to form right-handed Z-DNA.[8] We speculate that RNAP has evolved with the capacity to reliably melt DNA. Such melting could serve an important regulatory role, for example, to facilitate initiation at an adjacent promoter, to allow binding of regulatory factors, or even to promote the initiation of replication. Transcription-generated torque could also potentially alter or dissociate bound proteins. Since supercoiling can transmit through DNA, the polymerase can effectively act at a distance. Thus torque may serve as a global factor to remotely control gene regulation and affect DNA stability genome-wide.

We also found that RNAP is resilient to torque changes. In our studies, RNAP could often recover from longer periods of torque-induced stalling upon torque relaxation. Recovery is likely a mechanistic adaptation to prevent the accumulation of stalled RNAPs and reduce the number of unfinished transcripts. We found that RNAP was immune to brief torque fluctuations, and transcription elongation was unabated by short exposures to strong torques. In vivo torque changes can occur due to spontaneous loss of torsional constraints in DNA, action of a topoisomerase, or the action of motor proteins such as polymerases and helicases. In particular, the replication

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fork is known to move at a speed much faster than transcription, creating a source of torque accumulation that could potentially impact transcription without direct contact or collision between a replisome and transcription machinery.

Our work provides a quantitative framework for understanding how DNA supercoiling can dynamically regulate transcription and how transcription can greatly influence DNA supercoiling and structure. We show that RNAP can generate torque, which, in turn, regulates transcription rate and pausing, and that excessive torque accumulation leads to transcription stalling and DNA structural alterations. It has long been suggested that the positive torque generated by Pol II during transcription could destabilize histone–DNA interactions and thus assist Pol II transcription through a nucleosome. Utilizing the experimental platform developed here, it may now be possible to determine how Pol II transcribes under torsion and whether it can generate sufficient torque to alter nucleosome structure. This platform may also be expanded to examine other DNA-based torsional motors, such as DNA polymerases and chromatin remodelers. The resulting information could significantly expand our understanding of the complex, transcriptional in vivo topography.

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