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Transcription Under Torsion

Jie Ma,1,2 Lu Bai,3,4 Michelle D. Wang1,2*

In cells, RNA polymerase (RNAP) must transcribe supercoiled DNA, whose torsional state is constantly changing, but how RNAP deals with DNA supercoiling remains elusive. We report direct measurements allowing for the determination of the RNAP position on the DNA template as it transcribed under torque (11).

DNA supercoiling is a regulator of gene expression (1–5). RNA polymerase (RNAP) must transcribe supercoiled DNA, and transcription elongation, in turn, generates DNA supercoiling. As RNAP moves along the helical groove of DNA, it generates (+) DNA supercoiling ahead and (−) DNA supercoiling behind (the “twin supercoiled domain model”) (1, 3–6). DNA supercoiling is broadly present during transcription (3–5). Active transcription can accumulate dynamic DNA supercoiling on DNA templates that are not bound by topological constraints (3), as well as in the presence of a normal complement of topoisomerases in vivo (4). However, little is known about some basic properties of the interplay between transcription and DNA supercoiling. We have developed an assay to directly monitor RNAP translocation in real time as it worked under a defined torque. An RNAP was torsionally anchored to the surface of a coverslip, and either the downstream or upstream end of the DNA template was torsionally anchored to the bottom of a nanofabricated quartz cylinder held in an angular optical trap (AOT) (Fig. 1A and fig. S1 (7–11)). An AOT allows simultaneous control and measurement of rotation, torque, displacement, and force of the trapped cylinder (8–11). Analysis of these measurements allowed for the determination of the RNAP position on the DNA template as it transcribed under torque (11).

We investigated how RNAP stalled as it worked against (+) supercoiling downstream or (−) supercoiling upstream. Before the cylinder was trapped, RNAP translocation could be directly visualized by rotation of a tethered cylinder (movie S1). Once trapped, the cylinder’s orientation was controlled by the AOT. RNAP translocation rotated the DNA, forming a (+) plectoneme in downstream stalling experiments (Fig. 1B and fig. S5A) or a (−) plectoneme in upstream stalling experiments (figs. S4 and SSB). Resisting torque build-up eventually led to transcription stalling. Our method was inspired by previous magnetic tweezers–based studies to monitor transcription and amplify its detection (12–14) but is distinct from those studies in its real-time transcription elongation detection and/or flexible torque control and readout.

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The measured downstream stall torque distribution is well fit by a Gaussian function, yielding a mean torque of $11.0 \pm 3.7$ pN·nm (mean ± SD), with the largest measured value being $\sim 18$ pN·nm (Fig. 2A and fig. S6A). This mean torque is sufficient to create (+) plectonemic DNA under the low forces used in our experiments. In contrast, the upstream stall torque distribution shows an asymmetry (Fig. 2B and fig. S6B). Unlike (+) supercoiled DNA, which can sustain a much higher torque before structural changes, (−) supercoiled DNA undergoes a transition at $10.5$ pN·nm consistent with melting (fig. S3) (I). The upstream stall torque distribution shows a singular peak immediately before a sharp cutoff near the DNA melting torque, and $\sim 60\%$ of RNAPs were stalled between 10 to 12 pN·nm. These data indicate that RNAP is able to generate an upstream torque sufficient to alter DNA structure.

**Fig. 2. Transcription stalling and resumption.**

(A) Distribution of the measured downstream stall torques. The smooth blue curve is a fit with a Gaussian function, yielding a mean of $11.0 \pm 3.7$ pN·nm (mean ± SD). (B) Distribution of measured upstream stall torques. The smooth curve is a fit with a Gaussian function assuming that the peaked fraction generated torques of at least 10 pN·nm, yielding a mean of $10.6 \pm 4.1$ pN·nm (mean ± SD). (C) Example traces showing RNAP reverse translocation upon stalling. Both axes are shifted for clarity. For each trace, the arrow indicates the entry into a stall. (D) Fraction of RNAPs that resumed transcription after torque release versus time. After stalling, torque on RNAP was relaxed, and transcription was detected by an experiment similar to that shown in step 1 of Fig. 1B. Error bars indicate SEM.

**Fig. 3. Transcription response to a transient torque pulse.** (A) (Top) Cartoon illustrating steps of the torque pulse experiments and (bottom) representative traces of data. RNAP initially transcribed under a low downstream torque of $\sim +7$ pN·nm and then was subjected to a higher torque pulse for either 5 or 0.5 s before restoration of the initial low torque. Traces 1 and 4 are controls. The extension and time axes are shifted for clarity. (B) Probability of maintaining active transcription during the 5-s torque pulse. The blue solid line is a fit to a Boltzmann function: $f = 1 / [1 + e^{(t - t_c)/t_0}]$, where $t_c$ is the characteristic cutoff torque, and $t_0$ is the characteristic width of the transition torque. Error bars indicate SEM. (C) Probability of resuming transcription immediately (within 5 s) after the torque pulse. Error bars indicate SEM.
variations and single-molecule stochasticity, according to a thermal-ratchet kinetic model for transcription elongation that we previously developed (15–17).

Thus, RNAP is fully capable of generating torque sufficient to melt DNA of arbitrary sequence (11), not just AT-rich sequences that are prone to melting (3, 4, 11). The strong \((\sim)\) supercoiling generated by RNAP may facilitate initiation of transcription from adjacent promoters (18), binding of regulatory proteins (3, 4), and initiation of replication (19).

We found that, in some traces, RNAP reverse translocated upon stalling (Fig. 2C). This reverse motion suggests that torque may induce stalling via backtracking, during which RNAP translocates back along the template DNA and displaces the \(3’\) transcript from the active site, preventing RNA synthesis (20–22).

In vivo, torsional stress accumulated by RNAP may be relaxed by the arrival of a topoisomerase at the DNA template or by DNA rotation. We found that stalled RNAPs gradually resumed transcription following torque release (Fig. 2D). At 90 s after torque release, \(~50\%\) of stalled RNAPs had resumed transcription. Thus, in vivo torque relaxation should allow a large fraction of stalled RNAPs to resume transcription, preventing them from becoming obstacles or inducing DNA damage that disrupts genome stability (23).

In vivo, torsional stress in local DNA segments may be present transiently due to actions of motor proteins and dynamic reconfiguration of topological domains. However, it is not known how these sudden changes in torsional stress might influence a transcribing RNAP. We thus carried out transient torque pulse experiments to determine how RNAP responded to a brief exposure of a resisting torque on a time scale comparable to those of topoisomerases (24–26) (0.5 or 5 s) (Fig. 3A). We found that the fraction of active RNAPs during the 5-s pulse decreased as the torque was jumped to an increasingly higher value (Fig. 3B). The characteristic cutoff torque was 10.6 \(\pm\) 4.0 pN·nm, a value similar to the mean stall torque. A substantially larger fraction of RNAPs was able to transcribe immediately (within 5 s) after the 0.5-s pulse, as opposed to after the 5-s pulse (Fig. 3C), indicating that a 0.5-s torque pulse does not give sufficient time for RNAP to backtrack substantially. Thus, RNAP can effectively resist transient torque fluctuations (<0.5 s) but is unable to withstand prolonged exposure to a large torque without stalling or arresting.

We investigated the torque-velocity relationship, which characterizes how the transcription speed is regulated by torque (Fig. 4A). To maintain a constant torque, we monitored transcription in the presence of a DNA plectoneme under a small and constant force. The measured transcription traces showed that continuous elongation was interrupted by frequent pausing (Fig. 4B and fig. S7). Because of the sensitivity of the assay, it was possible to resolve pauses as short as 0.2 s. By analyzing the velocity between pauses, we obtained the torque-velocity relation of RNAP. Figure 4C shows how the transcription rate increased with an assisting torque and decreased with a resisting torque. In addition, both pause density and duration decreased with an assisting torque and increased with a resisting torque (Fig. 4D).

We show that RNAP can generate torque; torque, in turn, regulates transcription rate and pausing; and excessive torque accumulation leads to transcription stalling and DNA structural alteration. A transcription-generated supercoiling wave can propagate through DNA to provide action at a distance, not only to alter DNA structure (3, 4) but also to potentially alter or dissociate bound proteins (3, 4, 27). Torsion generated by eukaryotic RNAP may alter chromatin fiber and evict histones (4, 27, 28), and torsional relaxation by chromatin may, in turn, facilitate transcription (28).

References and Notes
Fe-S Cluster Biosynthesis Controls Uptake of Aminoglycosides in a ROS-Less Death Pathway

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All bactericidal antibiotics were recently proposed to kill by inducing reactive oxygen species (ROS) production, causing destabilization of iron-sulfur (Fe-S) clusters and generating Fenton chemistry. We find that the ROS response is dispensable upon treatment with bactericidal antibiotics. Furthermore, we demonstrate that Fe-S clusters are required for killing only by aminoglycosides. In contrast to cells, using the major Fe-S cluster biosynthesis machinery, ISC, cells using the alternative machinery, SUF, cannot efficiently mature respiratory complexes I and II, resulting in impedance of the proton motive force (PMF), which is required for bactericidal aminoglycoside uptake. Similarly, during iron limitation, cells become intrinsically resistant to aminoglycosides. Furthermore, we demonstrate that Fe-S clusters are required for killing only by aminoglycosides.

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The lack of evidence for a link between oxidative stress and bactericidal antibiotics also held true when testing the same strains for minimal inhibitory concentrations (MIC) and growth rates in subinhibitory antibiotic amounts (table S1 and fig. S4). Taken together, these results revealed no association between ROS and bactericidal antibiotic sensitivity, in agreement with two recent reports using complementary approaches (6, 7).

Kohanski et al. (1) proposed that protein-bound Fe-S clusters are required for killing by bactericidal antibiotics because they release Fe²⁺ ions that fuel ROS production by Fenton chemistry. This assumption was based on the fact that mutants lacking the major Fe-S cluster biogenesis system ISC were resistant to both Gm and Amp. The iscS gene codes for the ISC cysteine desulphurase that, in addition to Fe-S protein maturation, is involved in all sulfur trafficking pathways (8, 9). We found that the iscS mutant, as previously reported (1), was fully resistant to Gm killing and showed partial resistance to Amp in a time-dependent killing experiment using 5 μg/ml for both drugs (Fig. 2A and B).

However, the enhanced resistance of the iscS mutant was only recapitulated for Gm, but not for Amp at lower antibiotic concentrations (fig. S5A and B) or when measuring MICs and growth rates in subinhibitory antibiotic concentrations (table S1 and fig. S5CA).

We then tested an iscUA mutant, because in contrast to the pleiotropic iscS mutant, it is specifically compromised in Fe-S cluster biogenesis, as it lacks both the scaffold for assembling the Fe-S cluster and the transport machinery that inserts the Fe-S cluster into apo-proteins (9, 10). Interestingly, the iscUA mutant was resistant to Gm and sensitive to Amp in all tests used (fig. 2A and B, table S1, and fig. S5C).

Fe-S clusters are required for the bactericidal effect of aminoglycosides but not for that of β-lactams. If killing by aminoglycosides is not caused by ROS, why does eliminating the ISC system render E. coli resistant to these antibiotics? Fe-S clusters are essential for growth, and E. coli has a second assembly system, called SUF (10). To