Transcription Under Torsion

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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 of individual Escherichia coli RNAPs as they transcribed supercoiled DNA. We found that a resisting torque slowed RNAP and increased its pause frequency and duration. RNAP was able to generate 11 ± 4 piconewton-nanometers (mean ± standard deviation) of torque before stalling, an amount sufficient to melt DNA of arbitrary sequence and establish RNAP as a more potent torsional motor than previously known. A stalled RNAP was able to resume transcription upon torque relaxation, and transcribing RNAP was resilient to transient torque fluctuations. These results provide a quantitative framework for understanding how dynamic modification of DNA supercoiling regulates transcription.

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 cover slip, 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).

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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 S5B). 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.

Fig. 1. Stall torque experiments. (A) (Top) Cartoon depicting the twin supercoiled domain model (1). (Bottom) Experimental configuration that mimics the twin supercoiled domain model for transcription against (−) supercoiling upstream or (+) supercoiling downstream. τ, torque; F, force; HA, hemagglutinin. (B) Representative set of data for downstream stall torque measurements. After the introduction of nucleoside triphosphates (NTPs), the force on the DNA was clamped at a low value while DNA was mechanically unwound to form a (−) plectoneme. Subsequent translocation of RNAP neutralized the (−) plectoneme (steps 1 and 2) and resulted in (+) plectoneme formation (step 3). The force clamp was then turned off (step 4). RNAP translocation increased the force (directly measured) and the corresponding torque (derived (11)) until reaching a stall ([<1 base pair (bp)/s for 20 to 50 s] for 20 to 50 s). Data were filtered: extension to 200 Hz (black) and 1 Hz (red) and force to 40 Hz (black) and 1 Hz (red). The RNAP template position is defined as the distance of RNAP from the transcription start site (in base pairs).
The measured downstream stall torque distribution is well fit by a Gaussian function, yielding a mean torque of 11.0 ± 3.7 pN·nm (mean ± SD), with the largest measured value being ~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) (11). The upstream stall torque distribution shows a singular peak immediately before a sharp cutoff near the DNA melting torque, and ~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. The upstream data were fit with a Gaussian function, yielding a Gaussian centered at 10.6 ± 4.1 pN·nm, comparable to the downstream stall torque (Fig. 2B). The spreads in the measured stall torque distributions are attributed to DNA sequence asymmetry (Fig. 2B and fig. S6B).

**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 ± 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 ± 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 ~+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 (−) 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 ± 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

Fig. 4. Determination of transcription torque-velocity relationship. (A) Representative set of data for transcription measurement under a constant torque. Transcribing RNAP, under a small and constant tension of 0.15 pN, was subjected to multiple cycles of resisting and assisting torque. For each cycle, the downstream DNA was mechanically unwound to remove any (+) plectoneme (step 1) and create a (−) plectoneme (step 2). Subsequent RNAP transcription was assisted by the (−) DNA supercoiling (step 3), until the generation of (+) supercoiling, which hindered transcription (step 4). In the absence of a plectoneme, the torque on the DNA was constant for a given force (9) (fig. S3), and RNAP velocity was derived from the slope of the extension-versus-time curve (21). Also, we define a resisting torque to be (+) and an assisting torque to be (−). Data were filtered to 200 Hz (blue and red) and 1 Hz (gray). (B) Representative transcription traces under a torque of +7.5 pN·nm. Continuous transcription (green smoothed data) was interrupted by pauses (red smoothed data). (C) Transcription torque-velocity relationship. Transcription velocity was obtained by weighting each transcript position equally, and the resulting velocity reflected primarily transcription rates between pauses (11, 29). Vertical error bars indicate SEM; horizontal error bars denote SD. (D) Pause density (top) and duration (bottom) as a function of torque. A pause is defined as having a duration of ≥0.2 s at a given nucleotide position (21). Zero-torque data (fig. S8) had lower sensitivity to transcription due to lack of plectonemes in DNA, precluding detection of pauses of 0.2 to 2 s in duration, and were thus excluded from analysis. Vertical error bars indicate SEM; horizontal error bars denote SD.
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. 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.

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 desulfurase 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. S5). We conclude that Fe-S proteins promote aminoglycoside uptake by enabling their uptake.

E. coli mutants, hypersensitive to O2− (lacking both cytoplasmic superoxide dismutases, ΔsodA and ΔsodB) or to H2O2 (lacking the H2O2-sensing master activator, ΔoxyR), exhibited similar sensitivities to Gm and Amp as the wild type (WT) in a time-dependent killing experiment, with ΔoxyR being more resistant to Amp at the last time point, 4.5 hours after drug addition (Fig. 1A and B). When tested in a concentration-dependent killing experiment, the two mutants were more sensitive as WT to Gm (Fig. 1C) but exhibited small differences to the WT at intermediate Amp concentrations, at levels that provided no support for a prominent role for ROS defense mechanisms during treatment with bactericidal antibiotics (Fig. 1D). Interestingly, the iscU4 mutant was resistant to Gm and sensitive to Amp in all tests used (Fig. 2, A and B, table S1, and fig. S5). We conclude that 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. To...