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Multiple modes of *Escherichia coli* DNA gyrase activity revealed by force and torque

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E. coli **DNA gyrase uses the energy of ATP hydrolysis to introduce essential negative supercoils into the genome, thereby working against the mechanical stresses that accumulate in supercoiled DNA. Using a magnetic-tweezers assay, we demonstrate that small changes in force and torque can switch gyrase among three distinct modes of activity. Under low mechanical stress, gyrase introduces negative supercoils by a mechanism that depends on DNA wrapping. Elevated tension or positive torque suppresses DNA wrapping, revealing a second mode of activity that resembles the activity of topoisomerase IV. This 'distal T-segment capture' mode results in active relaxation of left-handed braids and positive supercoils. A third mode is responsible for the ATP-independent relaxation of negative supercoils. We present a branched kinetic model that quantitatively accounts for all of our single-molecule results and agrees with existing biochemical data.**

The level of negative (denoted as (−)) DNA supercoiling of the *E. coli* chromosome is tightly regulated in the cell and influences processes such as replication, transcription, repair and recombination. DNA topoisomerases are enzymes that interconvert different topological forms of DNA: they can add or remove supercoils, catenations and knots by catalyzing the passage of one DNA segment through a transient break in another. This reaction changes the linking number (Lk) of DNA, defined for topologically closed DNA circles as one-half the number of signed crossings of the two DNA strands in any projection of the molecule. Topoisomerases are divided into two classes depending on whether one (type-1) or two (type-2) DNA strands are broken during the topoisomerization reaction[1]. Prokaryotic topoisomerases are the primary targets of many important antibacterial drugs[2].

E. coli has two type-2 topoisomerases, DNA gyrase and topoisomerase IV (Topo IV). Topo IV ensures proper unlinking of sister chromosomes after replication. DNA gyrase is unique among topoisomerases in its ability to introduce negative supercoils into DNA. Gyrase also shows low levels of decatenation and unknotting activities and is able to relax (−) supercoiled DNA in an ATP-independent manner[3–9]. The (−) supercoiling activity by gyrase has been explained by a 'sign inversion' mechanism, in which one duplex DNA segment (transport or T segment) passes through a transient double-strand break in a second DNA segment (gate or G segment), producing a net change in Lk of –2 (ref. 10).

Gyrase is a heterotetramer composed of two A and two B subunits ($GyrA_2GyrB_2$). DNase I footprinting and exonuclease III digestion experiments have shown that gyrase binds a DNA fragment of ~140 base pairs (bp) and cleaves it nearly at its center in an ATP-independent manner[11–13]. The binding of gyrase to DNA has been shown to stabilize positive (denoted as (+)) supercoils[14,15] generated by the chiral wrapping of DNA around the C-terminal domains of the GyrA subunits (GyrA-CTD)[14,16–18] (**Fig. 1a**). The C-terminal domains of GyrB are responsible for interacting with DNA and GyrA[15,19]. The N-terminal domains of GyrB are responsible for binding and hydrolyzing ATP[20,21]. These domains form the entrance gate (N gate) for the T segment and dimerize upon nucleotide binding (**Fig. 1a**).

A simplified catalytic cycle for the introduction of (−) supercoils by gyrase[22] is shown in **Figure 1b**. First, the G segment is bound and can be cleaved in an ATP-independent fashion (1). A proximal T-segment DNA then forms a chiral wrap around the enzyme (2). This process brings the T segment into the N gate. The N gate is closed upon T-segment capture and ATP binding (3), triggering a conformational change in the enzyme structure that transports the T segment through the gap in the cleaved G segment (4). The transported T segment and the products of hydrolysis are released (5) and the enzyme is reset to start a new cycle of catalysis. We define this mode of catalysis as the α mode, to reflect the path of DNA wrapping around GyrA-CTD.

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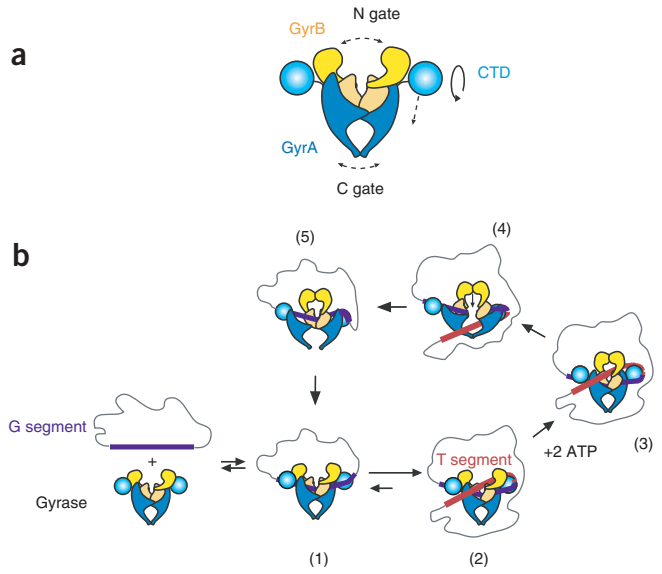

Figure 1 Gyrase subunit composition and mechanism of action. (**a**) Gyrase is a heterotetramer composed of two monomers of GyrA (blue) and two of GyrB (yellow). The N-terminal domains of GyrA (dark blue) interact with DNA and GyrB and form the DNA exit, or C gate. The C-terminal domains of GyrA (GyrA-CTDs, blue spheres) fold independently and are linked to the N-terminal domain of GyrA by a flexible linker, which gives them rotational and translational flexibility[47] (arrows). GyrA-CTDs are responsible for DNA binding and wrapping. GyrB interacts with DNA and GyrA, is responsible for ATP binding and hydrolysis, and forms the DNA entrance, or N gate. (**b**) Model for introduction of (−) supercoiling by gyrase. A gyrase complex binds a DNA fragment, called the G segment (1). From this state, there is a force-dependent kinetic competition between DNA wrapping and dissociation. After wrapping (2) and ATP binding (3), the enzyme can proceed with the strand-passage reaction (4), which leads it to a state (5) that can be reset to commence a new catalytic cycle.

We recently studied the α mode by tracking the rotation of a submicrometer bead (rotor bead) attached to the side of a stretched linear DNA molecule[23]. Changes in DNA tension over a range of a few tenths of piconewtons (pN) did not appreciably change the rate of supercoiling but greatly affected the processivity of the reaction. We explained this behavior by a mechanochemical model in which the processivity of the reaction is determined by a kinetic competition between tension-sensitive DNA wrapping and dissociation of gyrase from the DNA.

The α mode of gyrase activity describes the (−) supercoil–introduction reaction but cannot account for the other activities of gyrase. Here, we use magnetic tweezers to investigate (−) supercoil introduction and relaxation, (+) supercoil relaxation, and decatenation activities of *E. coli* gyrase. We found that small changes in force and torque on the DNA can cause gyrase to switch among these different modes of activity. Under low mechanical stress, gyrase uses a wrapping-mediated proximal T-segment capture mode to introduce (−) supercoils into DNA. However, at forces and torques that inhibit DNA wrapping, gyrase favors a wrapping-independent distal T-segment capture mode that provides an explanation for the low level of decatenation activity of gyrase *in vivo*[9].

RESULTS

Single-molecule assay

Precise amounts of force and supercoiling density can be applied to single topologically constrained DNA substrates by using a magnetic-tweezers setup. We tethered a single constrained DNA molecule to a glass slide on one end and to a paramagnetic bead on the other end[24] (**Fig. 2**). Tension and twist were applied to the DNA molecule by translating and rotating a pair of permanent magnets. We define the linking number of a topologically constrained linear DNA molecule as the linking number of the idealized DNA molecule that is obtained by circularizing the duplex DNA ends attached to the bead and to the glass slide surface. ΔLk is the difference between the Lk of a molecule and that of the same molecule in an unconstrained, relaxed state (Lk_0). We define supercoiling density (σ), a length-independent measure of the degree of DNA supercoiling, as the number of turns (n) introduced into the DNA molecule from its relaxed state divided by the total number of DNA helical repeats (number of base pairs/10.5). By convention, σ is positive for (+) supercoiled DNA and negative for (−) supercoiled DNA.

The changes in DNA twist and writhe produced by tension and torque are well understood[25]. The properties of the extension-supercoiling curves were used to calculate ΔLk from given values of DNA extension and force. The torque on the DNA in the plectonemic region was approximated by $\tau = (2BF)^{1/2}$, where $B = \sim 230$ pN · nm^2 is the bending rigidity of DNA and F is the tension on the DNA.

It is sometimes assumed that a single mechanism is responsible for the relaxation of (+) supercoils and the introduction of (−) supercoils by gyrase. Here we show that, under mechanical stress, gyrase can also relax (+) supercoils by a DNA interaction mode that is different from the one it uses to introduce (−) supercoils. Therefore, we define gyrase activities on the basis of the starting substrate to avoid making assumptions about the underlying mechanism responsible for each activity: relaxation of (+) supercoils converts a (+) supercoiled substrate to a relaxed DNA product, whereas introduction of (−) supercoils converts a relaxed substrate to a (−) supercoiled product.

Gyrase activities at low force

We first investigated whether gyrase was able to relax (+) supercoils and introduce (−) supercoils into a constrained DNA molecule at low forces, as expected for the canonical wrap-mediated pathway. At

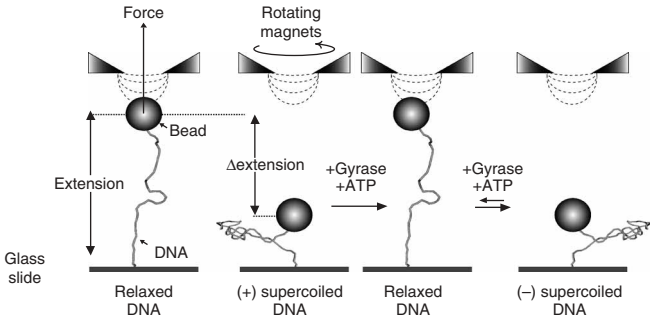

Figure 2 Magnetic tweezers experimental setup. A magnetic trap consists of a magnetic bead (sphere) tethered to a glass surface by one DNA molecule (string). Magnets above the sample (triangles) can be rotated and translated to exert tension and torque on the DNA molecule. (+) plectonemic DNA structures are formed by the application of torque. The relaxation of (+) supercoils by gyrase can be measured by monitoring the extension of the DNA tether in real time. Introduction of (−) supercoils into a relaxed DNA substrate by gyrase forms (−) plectonemic DNA regions that make the DNA extension decrease.

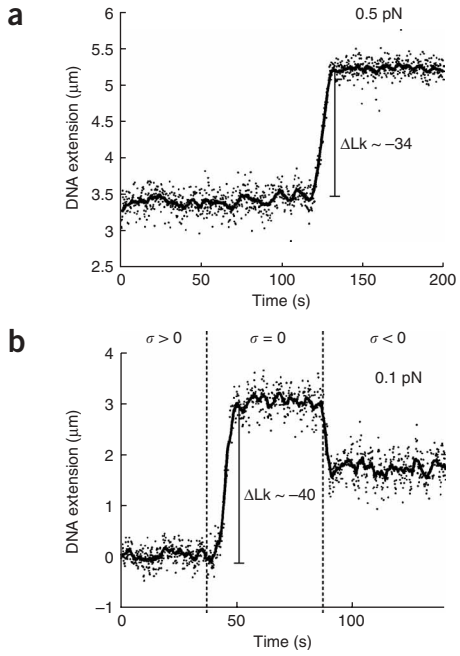

Figure 3 Activities of gyrase at low forces. (**a**) Processive relaxation of (+) supercoiled DNA. Shown is a representative single-gyrase-molecule relaxation of a (+) supercoiled DNA molecule ($\sigma = +0.025$) at 0.5 pN. In this case, ~34 (+) supercoils were relaxed in a single burst of activity. Raw data are plotted as dots and a 1-s moving average is shown as a solid curve. (**b**) Processive relaxation of (+) and introduction of (−) supercoils by a single gyrase enzyme at 0.1 pN of tension. In the first burst of activity (at ~40 s), gyrase relaxed ~40 (+) supercoils, changing σ from +0.03 to ~0. Later, in a second burst of activity (at ~90 s), gyrase processively introduced ~20 (−) supercoils, changing σ to its final value of ~ –0.015. Raw data are plotted as dots and a 1-s moving average is shown as a solid curve.

saturating ATP concentrations (1 mM), gyrase relaxed (+) supercoiled DNA processively (**Fig. 3a**), causing the extension of a supercoiled molecule to increase as plectonemes were removed. Under the same conditions, gyrase was also able to introduce (−) supercoils into relaxed DNA molecules (causing decreases in extension), but only against low forces and torques (**Fig. 3b**). The wrapping-mediated activity that leads to (−) supercoiling introduction is also likely to be responsible for the processive relaxation of (+) supercoils at these low forces. Ensemble measurements taken in standard gyrase reaction conditions (1.8 mM spermidine, 37 °C) yielded similar results to experiments done under conditions optimized for single-molecule analysis (0.2 mM spermidine, 25 °C; **Supplementary Fig. 1** online).

Gyrase relaxes (+) supercoils at high force

The experiments described above showed that gyrase was able to relax (+) supercoils at low forces in the presence of ATP (**Fig. 3a**). In our previous work, the processivity and initiation rate of the α mode decreased rapidly as the force increased over ~1 pN, owing to the inhibition of DNA wrapping[23]. Wrapping was strongly inhibited by tension but only mildly affected by torque. Accordingly, we did not expect gyrase to be able to relax (+) supercoils at high forces (>1–2 pN). To test this hypothesis, we mechanically introduced (+) supercoils into a topologically constrained DNA molecule at high force (>2 pN) and monitored the activity of gyrase (1 nM) in the presence of 1 mM ATP. To our surprise, gyrase was able to relax (+) supercoils processively at tensions up to 4 pN (**Fig. 4a** and **Supplementary Fig. 2** online). The coexistence of B-DNA and P-DNA in (+) supercoiled substrates at high torques[26,27] prevented the straightforward interpretation of relaxation experiments at forces higher than 4 pN. The velocity of relaxation (the number of cycles per unit time during a processive burst of activity) was found to be independent of force between 1.5 and 4 pN (see below). This gyrase activity could be explained by a wrapping-independent mode of catalysis that, in contrast to the α mode mechanism, involves the capture of a distal as opposed to a proximal T segment. We define this activity as the χ mode. Such an activity would not have been observed in our previous single-molecule assay[23] because of the absence of plectonemic crossings that juxtapose DNA segments.

Next, we investigated whether high force (+) supercoiling relaxation activity requires (+) DNA crossings. At low supercoiling densities, the extension of the DNA is not appreciably affected by the number of turns in the DNA. After the buckling transition, however, the DNA extension decreases linearly with the number of (+) turns introduced. (+) supercoiled plectonemic DNA was generated in the presence of gyrase at high tension ($F = 3$ pN). After complete removal of plectonemic crossings by gyrase, the change in DNA extension was measured by mechanically introducing new (+) supercoils. If gyrase

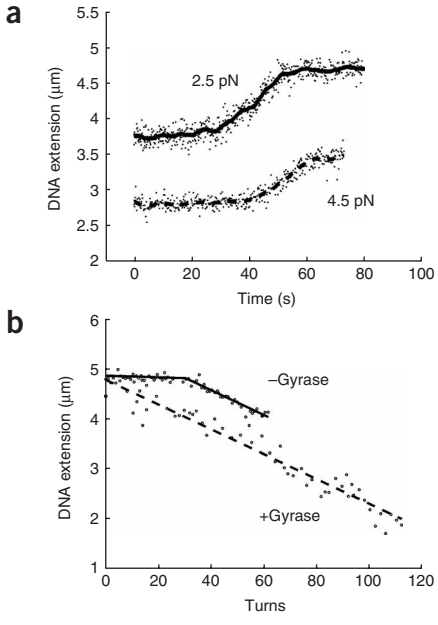

Figure 4 Activity of gyrase at high DNA tensions. (**a**) Processive relaxation of (+) supercoiled DNA at high forces. Curves show representative single-gyrase relaxation traces of (+) supercoiled DNA molecules ($\sigma = +0.022$) at 4.5 (solid) and 2.5 pN (dashed). Here, ~30 (+) supercoils were relaxed in single bursts of activity. Raw data are plotted as dots and 1-s moving averages are shown as solid curves. (**b**) Relaxation of (+) supercoils at high forces requires (+) crossings. In the absence of enzyme (solid line), the introduction of (+) supercoils from $\sigma = 0$ leaves the DNA extension unchanged up to the buckling transition and decreases extension linearly thereafter. In the presence of 1 nM gyrase and 1 mM ATP, 110 (+) supercoils were introduced in a topologically constrained DNA molecule. After plectonemic DNA relaxation ceased, 110 new (+) supercoils were mechanically introduced while the DNA extension was monitored in real time (dashed curve). Raw data are plotted as dots and piecewise linear fits are shown as solid curves.

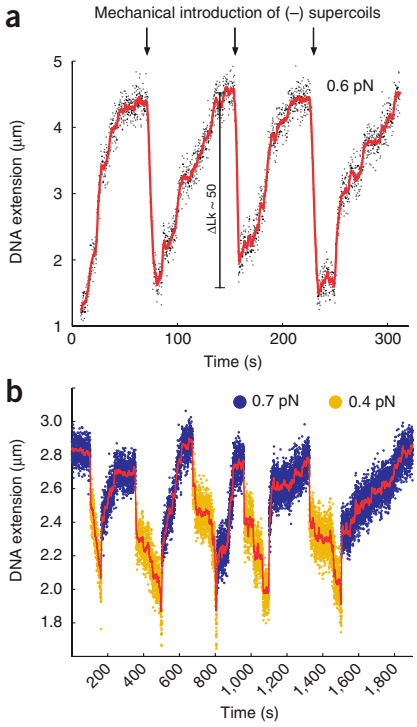

Figure 5 Passive mode of relaxation by gyrase. (**a**) Relaxation of (−) supercoils by gyrase in the absence of ATP. The DNA at time zero was (–) supercoiled to $\sigma = -0.035$, and relaxation by 20 nM gyrase was followed by monitoring the DNA extension in real time. Four cycles of (−) supercoiling relaxation are shown. Raw data are plotted as dots and a 1-s moving average is shown as a solid curve. (**b**) Modulation of gyrase activity by force. In the presence of 20 nM enzyme and 1 mM ATP, gyrase was able to relax (−) supercoils at high force ($F \sim 0.7$ pN, blue dots) and introduce (−) supercoils at low force (F ~0.4 pN, gold dots). A 1-s moving average is shown as a red curve.

had relaxed DNA twist beyond the buckling transition for (+) plectonemes, the mechanical introduction of new (+) turns would have led to a plateau followed by a linear decrease in DNA extension (**Fig. 4b**, solid line). In contrast, in the presence of gyrase (5 nM), the DNA extension decreased linearly with the number of new (+) turns introduced, demonstrating that gyrase was unable to relax the DNA beyond the critical buckling torque (**Fig. 4b**, dashed line). These results suggest that at high forces, gyrase relaxes (+) supercoiled DNA by a mode of activity that requires juxtaposed DNA segments for strand passage.

ATP-independent relaxation of (–) supercoils

In addition to the ATP-dependent introduction of (−) supercoils, gyrase has been shown to relax (−) supercoils in the absence of ATP (hereafter referred to as the passive mode)[3,4,28]. In our assay, no relaxation activity was observed when (−) supercoils were introduced into DNA in the absence of ATP, with the same gyrase concentration used previously (1 nM), under a wide force range (0.1–0.5 pN; data not shown). At higher concentrations (~20 nM), however, gyrase was able to relax (−) supercoils distributively (**Fig. 5a**), in agreement with ensemble experiments[28,29]. We observed no ATP-independent relaxation of (+) supercoils under the same conditions (data not shown).

By measuring the relaxation rates at different forces ($F < 0.65$ pN), we found that the passive mode was enhanced by force and negative torque (data not shown). Gyrase required a plectonemic DNA substrate to achieve ATP-independent relaxation of (−) supercoils (**Supplementary Fig. 3** online), consistent with a requirement for pre-existing (−) DNA crossings, as previously suggested[29].

At high protein concentrations (~20 nM) and in the presence of ATP, the contrasting mechanical sensitivities of the (−) supercoil relaxation and introduction activities can be exploited to modulate gyrase activity by force. This phenomenon was illustrated by an experiment in which the tension on a topologically constrained DNA molecule was cycled between 0.7 and 0.4 pN (**Fig. 5b**). Remarkably, this small change in force was sufficient to switch gyrase between the passive and α modes of activity, respectively.

We did not observe relaxation of (+) supercoils in the absence of ATP (data not shown). Thus, ATP is necessary for the relaxation of (+) supercoils but not of (−) supercoils. To test whether the (−) supercoil relaxation activity is stimulated by ATP, we measured the rate of relaxation in the presence and absence of ATP at forces that inhibit the α mode ($F = 0.6$ pN). The rates for relaxation of (−) supercoils by gyrase (20 nM) from 20 independent runs in the absence and presence of ATP (1 mM) were 0.06 ± 0.03 and 0.08 ± 0.03 Hz, respectively. From these measurements, we conclude that ATP does not substantially stimulate the relaxation of (−) supercoils by gyrase.

In light of these results, we propose that gyrase has three modes of action: (i) an ATP-dependent, wrapping-mediated proximal T-segment capture mode, responsible for (+) supercoil relaxation and (−) supercoil introduction (α mode); (ii) an ATP-dependent distal T-segment capture mode, responsible for the relaxation of (+) DNA crossings (χ mode); and (iii) an ATP-independent mode, responsible for the relaxation of (−) supercoils (passive mode) that requires both (−) crossings and negative torque. According to this scheme, a gyrase enzyme, once bound to DNA, enters a kinetic competition among multiple modes regulated by tension and torque.

Gyrase relaxes left-handed but not right-handed braids

Our model for multiple modes of gyrase activity makes testable predictions concerning the activity of gyrase on DNA braids. First, under conditions that do not allow gyrase to wrap DNA, the χ mode responsible for high-force relaxation of (+) supercoils should also relax the left-handed crossings (L crossings) found in left-handed braids (L braids). These crossings are geometrically equivalent to those found in plectonemic regions of (+) supercoiled DNA (**Fig. 6a**). Second, the failure of ATP to stimulate (−) supercoil relaxation implies a chirality preference for distal T-segment capture; therefore, right-handed braids (R braids) should not be relaxed. To test these predictions, we performed unbraiding experiments using nicked DNA molecules[30,31].

We generated DNA braids by attaching one end each of two nicked DNA molecules to a glass slide and the other ends to a paramagnetic bead in magnetic tweezers. The mechanics of DNA braids and their use in single-molecule enzymology have been extensively described[24,30,31]. Following ref. 31, we define braiding density (σ_{br}) as the total number of turns mechanically introduced (n) divided by the number of helical repeats in each DNA molecule. σ_{br} is positive for R braids and negative for L braids. At any constant force, the DNA-braid extension curve is symmetric with respect to σ_{br}. At low braiding densities ($|\sigma_{br}| < |\sigma_{br,buckle}|$), the DNA extension decreases with n. At $|\sigma_{br}| > |\sigma_{br,buckle}|$, the braids buckle to form plectonemic superhelices (second-order braids[32]). This transition is observed when the DNA extension is approximately two-thirds of its maximum extension and is characterized by a discontinuity in the slope of DNA extension plotted against n (**Fig. 6b**).

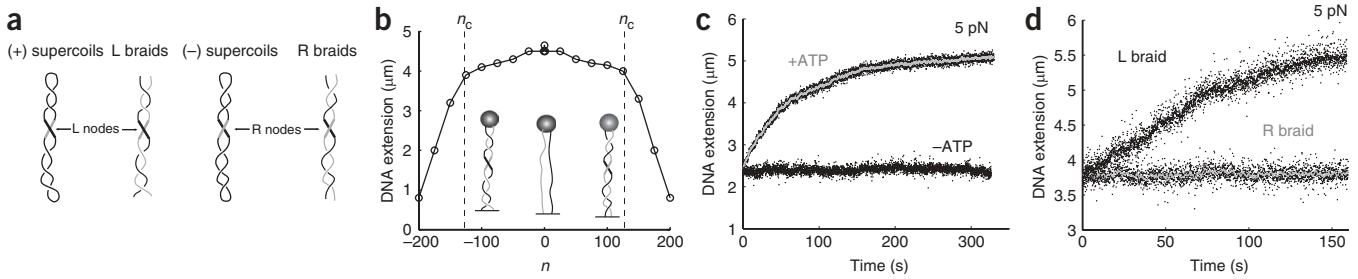

Figure 6 Gyrase unbraids DNA. (**a**) DNA crossings in plectonemic and braided DNA. In (+) supercoiled DNA, the crossings are left-handed (L crossings), as in left-handed braids (L braids). In contrast, DNA crossings are right handed (R crossings) on (–) supercoiled DNA and right-handed braids (R braids). (**b**) Variation of the extension of DNA with the number of turns n introduced for a tether with two nicked DNA molecules under constant tension. Introduction of (–) turns by rotation of the magnets leads to the formation of first-order L braids, whereas the introduction of (+) turns produces first-order R braids. As in the case of a single topologically constrained DNA molecule, beyond a critical number of turns ($n > n_c$), the DNA braids buckle to form second-order DNA braids (supercoils of braids). (**c**) Gyrase relaxes L braids only in the presence of ATP. Gyrase activity was followed by monitoring the braid extension at constant tension (5 pN). Only in the presence of 1 mM ATP was gyrase able to relax an L braid ($\sigma_{br} = -0.2$; black curve, no ATP; gray curve, 1 mM ATP). Raw data are plotted in black and 1-s moving averages are shown as solid curves. (**d**) Preferential relaxation of L braids (5 pN). In the presence of 1 mM ATP, gyrase was able to relax first-order L braids ($\sigma_{br} = -0.07$, black curve) but not first-order R braids ($\sigma_{br} = +0.07$, gray curve). The mean unbraiding rate was $\sim 10 \pm 3$ nm s^{-1}. Raw data are plotted in black and 1-s moving averages are shown as solid curves.

We tested the ability of gyrase to relax L and R braids. An L braid with $\sigma_{br} = -0.21$ was generated at 5 pN in the presence of 1 nM gyrase, and relaxation activity was followed by monitoring the DNA extension as a function of time. Gyrase was able to rapidly and completely relax L braids in an ATP-dependent manner (**Fig. 6c**). At the high gyrase concentrations used for these experiments, multiple enzymes could act on the substrate, and thus, the unbraiding relaxation activity did not result in a burst as seen for the relaxation of (+) supercoils. The mean unbraiding rate under these conditions was ~0.6 Hz. Immediately after the relaxation of a first-order L braid with $\sigma_{br} = -0.07$, an R braid of the same $|\sigma_{br}|$ was generated and the DNA extension was monitored for the same amount of time that was required for complete relaxation of the L braid. In this case, however, we detected no relaxation (**Fig. 6d**). These results were independent of the presence of ATP, enzyme concentration (from 5 to 50 nM) or force (0.2 to 5 pN) (data not shown). Unlike (–) supercoils, R braids are not a substrate for ATP-independent relaxation, consistent with a requirement for negative torque to activate the passive mode. These results clearly show that gyrase preferentially relaxes DNA crossings with a left-handed geometry by a wrapping-independent mode of activity (χ mode) that involves the intermolecular capture of a distal T segment.

Mechanochemical model for gyrase activity

We have presented evidence for three modes of gyrase activity. The mechanochemical cycle for each of these modes begins with a complex between the enzyme and the G segment. We propose that kinetic competition ensues between three pathways for T-segment capture and subsequent strand passage. On (+) supercoiled DNA, there is a competition between the α mode, which requires the capture of a proximal T segment (the mode that dominates at low mechanical stresses and leads to the ATP-dependent introduction of (–) supercoils), and the χ mode, which instead captures a distal T segment (the mode that dominates at high forces and leads to the ATP-dependent relaxation of (+) supercoils or left-handed braids). On (–) supercoiled DNA, there is a competition between the α mode, which introduces (–) supercoils, and the passive mode, which relaxes (–) supercoils and is favored at high negative torques. To test the validity of our model and determine the unknown mechanical and kinetic parameters for the different modes of activity, we quantitatively described the model as a branched kinetic pathway with mechanically sensitive rate constants (**Fig. 7**). We then compared the predictions of the model with force-velocity data obtained using the plectonemic assay (**Fig. 7a**). The basic kinetic parameters used for each of the modes are described and justified below (for detailed calculations, see **Supplementary Data** online). All mechanically sensitive rates were assumed to depend on force and torque, according to a simple transition-state model:

$$k_n'(F,\tau) = k_n e^{\frac{F\Delta x_n + \tau\Delta\theta_n}{k_b T}}$$

n stands in for the reaction pathway being modeled (here, α, χ or p, for passive), k_n is the rate at zero force and torque, Δx_n is the distance to the transition state along the extension coordinate (which determines the sensitivity to force), $\Delta\theta_n$ is the angle to the transition state along the twist coordinate (which determines the sensitivity to torque), k_b is the Boltzmann constant and T is temperature. For the plectonemic DNA template, torque was assumed to remain at the critical buckling value, which depends on the square root of the tension as described earlier: $\tau = (2BF)^{1/2}$.

The α mode was characterized in detail in our previous study[23]. Wrapping was deduced to be rapid, but the rate was measured only as a ratio of the wrapping and dissociation rates: $k_{\alpha,0}/k_{off} = 250$ (see **Supplementary Data**). Here, we assume $k_{off} = 10$ Hz (a lower-limit estimate; see **Supplementary Data**), fixing $k_{\alpha,0} = 2{,}500$ Hz. Wrapping was found to be very sensitive to tension, with a distance to the transition state $\Delta x_\alpha = -31$ nm; we retain this value as a fixed parameter in our expanded model presented here. Wrapping is chiral and therefore sensitive to torque as well as tension. We previously obtained a rough estimate of the torque sensitivity of wrapping ($\Delta\theta_\alpha \sim 1.5$ rad) from changes in processivity owing to rotor drag[23]. We allow $\Delta\theta_\alpha$ to vary in the present analysis, leading to a comparable fit value $\Delta\theta_\alpha = 0.8$ rad. An additional feature of the α mode must be introduced to explain the peaked appearance of the force-velocity curve (**Fig. 7a**): a slight torque sensitivity of the rate-limiting 'reset' step for the cycle. Our previous experiments used very low torques resulting only from rotor drag, and we were unable to detect a torque dependence for the rate-limiting step. However, the initial rising phase in the force-velocity curve on (+) supercoiled DNA may be explained if the rate-limiting (RL) step is slightly sensitive to torque. Here, we obtain a fit value

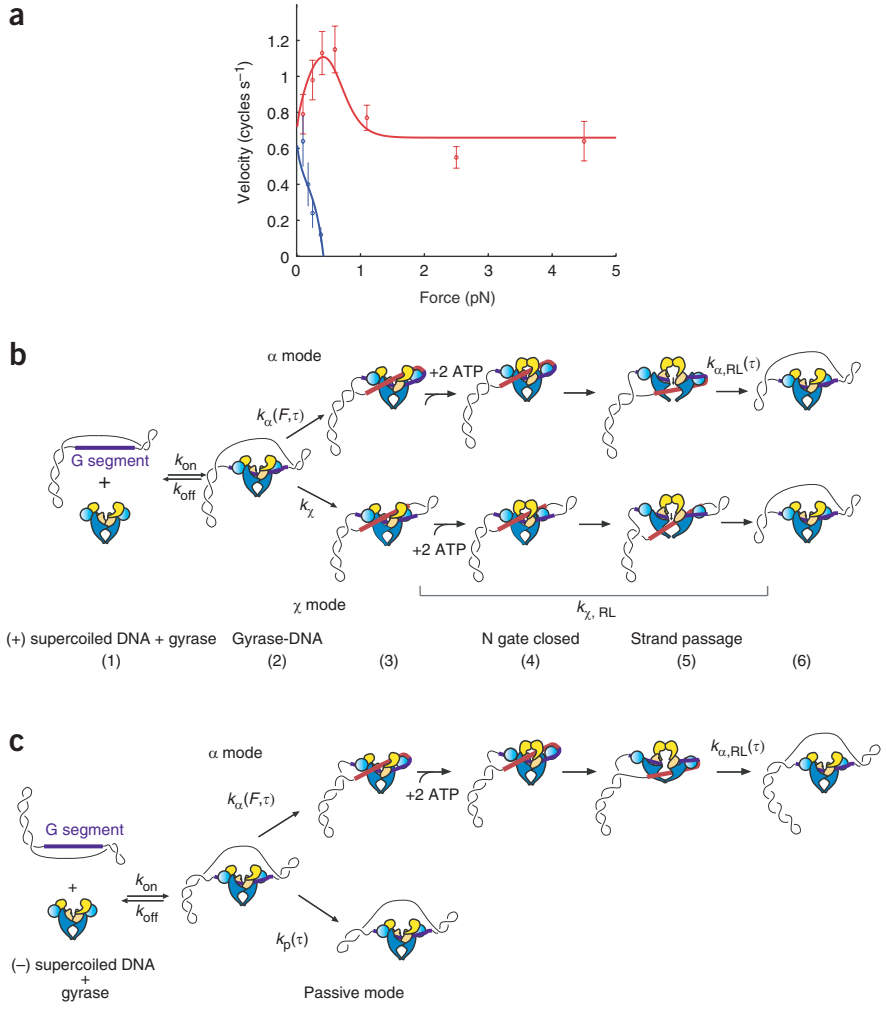

Figure 7 Force-velocity curves and proposed mechanochemical model. (**a**) The effect of force on the rate of gyrase activity. The rates of (+) supercoil relaxation (red circles) and (−) supercoil introduction (blue circles) were calculated as averages of single bursts of single-molecule activity and were fit to the kinetic equations derived from the proposed mechanochemical model (solid lines). Error bars represent s.e.m. (**b**) Branched model for gyrase activity. A gyrase molecule binds a G segment on a (+) supercoiled DNA molecule (1). From this state, there is a kinetic competition between proximal and distal T-segment capture (2 and 3). At low forces and torques, wrapping is very fast and gyrase rapidly wraps DNA around its GyrA-CTDs (2 and 3, α mode), leading it into the upper pathway. In this pathway, wrapping and ATP binding (3 and 4) can commit the enzyme to a full catalytic cycle in which two negative supercoils are introduced into DNA. A step at the end of the cycle is rate limiting and sensitive to torque (5 and 6). At forces and torques that inhibit DNA wrapping, gyrase captures instead a distal T segment (2 and 3, χ mode). In this case, ATP binding can commit the enzyme to strand passage, which results in the relaxation of two (+) supercoils. The overall rate of this pathway ($k_{\chi,RL}$) is assumed to be independent of force and torque. (**c**) Branched model for gyrase activity on (−) supercoiled DNA. A gyrase molecule binds a G segment on a (−) supercoiled DNA molecule (1). From this state, there is a kinetic competition between the α mode and the passive mode (2 and 3). At low forces and torques, gyrase rapidly wraps DNA around its GyrA-CTDs, leading it into the upper pathway. At forces and torques that inhibit DNA wrapping, gyrase relaxes (−) supercoils in an ATP-independent manner, leading it into the lower pathway. The overall rate of this pathway (k_p) is assumed to be torque-dependent.

$\Delta\theta_{\alpha,RL} = 0.2$ rad, too small to have been noticeable in the rotor bead assay. This small value of $\Delta\theta_{\alpha,RL}$ is consistent with kinetic experiments performed in bulk, which show only a small chiral preference for positive over negative supercoiling (**Supplementary Fig. 1**).

The χ mode is assumed to act only on the L crossings found in (+) supercoiled DNA (as we observed no ATP stimulation of (−) supercoil relaxation) and to be insensitive to mechanical stresses. The pathway can therefore be defined using only the rate of T-segment capture (k_χ) and the overall rate-determining parameter $k_{\chi,RL}$. The χ mode dominates at high forces and positive torques, when k_α becomes small compared with k_χ. The value of k_χ was fixed independently of the force-velocity data, by considering the processivity ($\langle n \rangle$) of the reaction at high forces (~12 cycles) and k_{off} (~110 Hz), so that k_χ = and k_{off} ($\langle n \rangle - 1$). $k_{\chi,RL}$ was fixed at the overall reaction rate measured throughout the high-force plateau (~0.7 Hz). The constant velocity in this plateau implies that G-segment rejoining is either insensitive to tension or else faster than the rate-limiting rate in the range of forces investigated.

The ATP-independent mode of supercoil relaxation was observed to operate only on (−) supercoiled DNA and not on either (+) supercoiled DNA or braided DNA (which is not under torque). In our model, we explain these observations by assuming that the rate of entry into the passive mode $k_{p,0}$ is enhanced by negative torque. We obtain the fit parameters $k_{p,0}$ ~1.1 Hz and $\Delta\theta_p$ ~ −0.5 rad.

A branched kinetic pathway with the set of parameters described above (summarized in **Fig. 7b**,**c**) quantitatively predicts the force-velocity curves for (+) supercoiled (red line in **Fig. 7a**) and (−) supercoiled (blue line in **Fig. 7a**) substrates. The model explains the shape of the curves as follows: on (+) supercoiled DNA, the velocity initially increases with force as the buckling torque rises and accelerates the rate-limiting step of the wrap-mediated pathway (the resetting of the machine). At high forces and torques, wrapping becomes inhibited and the mechanically insensitive χ mode takes over, leading to a plateau in the plot of velocity against force. This high-force plateau is a signature of a branched pathway and is roughly analogous to the processivity plateau seen with myosin V at high ADP concentrations[33]. On (−) supercoiled DNA, the observed velocity of (−) supercoil introduction decreases with force, owing to both the torque sensitivity of the rate-limiting step of the α mode and the competition between ATP-dependent introduction of supercoils and ATP-independent relaxation of supercoils. The passive mode dominates at higher forces, causing the supercoiling velocity to drop to zero and ultimately cross the origin into the regime of net relaxation.

DISCUSSION

DNA gyrase is an essential enzyme that helps maintain the equilibrium level of (−) supercoiling in the *E. coli* chromosome. The canonical mechanism for (−) supercoil introduction by gyrase is well established

and involves chiral DNA wrapping. Several additional gyrase-catalyzed DNA transactions, however, are not readily explained by the wrapping-mediated mechanism. Among these alternative reactions are ATP-dependent decatenation and ATP-independent relaxation of (−) supercoils.

Modulation of tension is fundamental for regulating many different biological processes[34,35]; the activity of enzymes involving DNA wrapping, bending or looping is strongly affected by tension on DNA[36]. In this study, we used a magnetic-tweezers apparatus to apply a physiologically relevant range of forces and torques to DNA, modulating the activity of gyrase among three distinct modes: (i) DNA wrapping-mediated ATP-dependent introduction of (−) supercoils (α mode), (ii) DNA wrapping-independent ATP-dependent (+) supercoil relaxation (χ mode), and (iii) passive ATP-independent relaxation of (−) supercoils (passive mode). In addition, inhibition of DNA wrapping led to a relaxation activity with a chirality preference for L crossings, similar to that observed for Topo IV[30,31]. A gyrase mutant lacking the GyrA-CTD decatenates DNA and relaxes both (+) and (−) DNA crossings in an ATP-dependent manner with no chiral preference[29], reminiscent of eukaryotic Topo II. Recent biochemical experiments have shown that for Topo IV, the ParC-CTDs bend DNA and dictate its chiral selectivity[37]. Our results suggest that the GyrA-CTDs can confer a chirality preference to the χ mode in the absence of DNA wrapping.

The results presented here resolve several puzzling observations from earlier studies on DNA gyrase. Previous ensemble experiments have determined that ATP hydrolysis can be largely uncoupled from the supercoiling reaction[20,38,39], a phenomenon termed mechanochemical slippage. The existing model for mechanochemical slippage by gyrase postulates that when the supercoiling reaction reaches its energetic limit, the enzyme continues futile rounds of ATP hydrolysis in the absence of strand passage. Our single-molecule experiments reveal that the continued ATP hydrolysis may alternatively derive from a combination of two modes of catalysis, one that introduces (−) supercoils using ATP and a second that removes them without the use of ATP, resulting in a net ΔLk equal to zero. At high negative torques, the activity of the α mode will be so diminished that it will compete with the passive mode, ultimately defining the equilibrium level of supercoiling by gyrase *in vitro*. However, *in vivo*, the equilibrium (–) supercoiling level is also affected by other topoisomerase activities in the cell, notably the (−) supercoil relaxation activity of Topo I[40].

DNA minicircles have been used to study the minimal closed circular DNA size that would sustain (−) supercoil introduction and (+) supercoil relaxation by gyrase[41]. Gyrase cannot introduce supercoils on a DNA circle smaller than 174 bp but can relax (+) supercoils even on 116-bp circles. The authors interpreted these results by concluding that gyrase wrapped a stretch of DNA (~100 bp) considerably shorter than previously reported (~140 bp)[11–13,42,43]. However, the distal T-segment capture mode of gyrase catalysis described herein provides a different interpretation of these results, as (+) supercoils in circles <174 bp can be relaxed without the need for DNA wrapping.

The low level of decatenation detected *in vivo*[8,9] is difficult to reconcile with the wrap-mediated mechanism, wherein the probability of capturing a distal intermolecular T segment is exceedingly small. Our characterization of a second mode of relaxation activity provides a direct mechanism for gyrase-mediated decatenation and unknotting of DNA. Notably, a recent genetic screen in *E. coli* for high-copy suppressors of conditional lethality and partitioning defects in *parC* and *parE* (Topo IV) mutants identified an integral membrane protein (SetB) required for proper segregation of daughter chromosomes at the end of replication[44]. The overexpression of SetB rescues the conditional lethality and partitioning defects of *parC* and *parE* mutants and leads to a pronounced nucleoid stretching phenotype, in which DNA is under high tension. It has been argued that this elevated tension on DNA could lead to stimulation of the decatenase activity of gyrase[44]. In this paper, we show that the application of tension (as low as ~1 pN) on DNA could act as a mechanosensory transduction mechanism that activates the decatenase activity of gyrase at high tension.

METHODS

DNA constructs and enzyme preparations. To construct torsionally constrained DNA molecules to be immobilized in the magnetic tweezers, short DNA linker fragments (~500 bp) having either biotinylated and digoxigenated dUTP were generated by PCR as described[45], digested with BamHI and XhoI, and specifically ligated to the 14.7-kilobase (kb) BamHI-SalI digestion product of the pPIA6 plasmid. The DNA molecules for DNA unbraiding experiments were constructed in a similar manner, but the 14.7-kb insert was dephosphorylated before ligation. This process guaranteed that both DNA molecules in a braid were nicked. DNA gyrase was purified by a procedure similar to that in ref. 43.

Magnetic tweezers. Single-molecule experiments were conducted on a high-power magnetic-tweezers instrument that will be described elsewhere (S.C.H., D.E. Humphries, M.D.S., M.N., C.B. and N.R.C., unpublished data). Dynamic changes in DNA extension were measured in real time at 10 Hz by comparing the bead diffraction ring pattern with a previously calibrated set of images taken at known focal displacements.

Single molecule assays. Activity assays were conducted in gyrase reaction buffer containing 35 mM Tris-HCl (pH 7.5), 24 mM potassium glutamate, 4 mM $MgCl_2$, 2 mM DTT, 0.2 mM spermidine, 1mM ATP, 6.5% (v/v) glycerol, 0.1 mg ml^{-1} BSA and the indicated amount of DNA gyrase. The distance between the magnets and the sample was held constant throughout each experiment and the indicated degree of DNA twist was introduced by rotation of the magnets, resulting in constant force during measurements. Modified DNA molecules having multiply labeled biotinylated and digoxigenated ends were oriented between an anti-digoxigenin–coated glass surface (Roche) and 1-μm-diameter streptavidin beads (Dynal). DNA gyrase activity was measured as a change in the DNA extension resulting from the removal of (+) supercoils or the introduction of (−) supercoils. Individual bursts of gyrase activity were fit with three lines using a piecewise linear fitting algorithm. To select for observations of single DNA gyrase enzymes, waiting times before the onset of activity were required to be five times longer than the burst time. Enzymatic rates were determined by linear regression over 2-s windows during the period of activity.

Negative supercoiling relaxation single-molecule assays. We quantified the activity decay rate (β) for (−) supercoiling relaxation by fitting the number (T) of (−) supercoils in the DNA molecule at time t for each relaxation event to the equation $T = T_0 e^{-\beta t}$, where T_0 is the initial number of mechanically introduced (−) supercoils. To limit the effect of concentration on the measurement of β, alternating runs with and without ATP were done using a constant gyrase concentration and the chamber was washed with a constant amount of buffer between runs.

Ensemble gyrase kinetics. Relaxed pUC18 plasmid DNA (2.7 kb) was prepared using wheat-germ topoisomerase I. (+) supercoiled pUC18 was prepared using the archaeal histone HMfB as described[46]. Kinetics reactions contained 28 nM DNA, 12 nM gyrase, 35 mM Tris-HCl (pH 7.6), 24 mM potassium glutamate, 4 mM $MgCl_2$, 2 mM DTT, 1 mM ATP, 6.5% (v/v) glycerol, 0.1 mg ml^{-1} BSA and either 0.2 mM spermidine (25 °C reactions) or 1.8 mM spermidine (37 °C reactions). Aliquots were removed from the reaction at the indicated times and terminated by the addition of EDTA. SDS and proteinase K were added to final concentrations of 0.5% (w/v) and 0.15 mg ml^{-1}, respectively, and the samples were incubated for 30 min at 37 °C. Reactions containing relaxed DNA were analyzed on 1.2% agarose gels in TAE buffer (40 mM Tris-acetate, 1 mM EDTA

(pH 8)). For (+) supercoiled DNA reactions, the gel and buffer contained 10 μM netropsin to resolve the topoisomers. Rates were determined by assigning a ΔLk to each topoisomer and calculating the total number of links in the DNA at each time point on the basis of the fraction of each topoisomer present. Products were quantified from Southern blots of the gels using a Molecular Dynamics PhosphorImager.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

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AUTHOR CONTRIBUTIONS

M.N., M.D.S, Z.B. and J.G collected and interpreted single-molecule data, supervised by C.B. and N.R.C. N.J.C. collected and analyzed ensemble data. S.-C.H. helped with the construction of single-molecule instruments. S.M. and A.M. contributed ideas and reagents. M.N., M.D.S., Z.B., J.G., N.J.C., C.B. and N.R.C. wrote the paper.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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