COMPETING INTERESTS STATEMENT
The author declares no competing financial interests.

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Under DNA stress, gyrase makes the sign of the cross

N Patrick Higgins

In this issue, Nöllmann and colleagues report single-molecule analyses of DNA gyrase action on supercoiled DNA under different levels of strain. Surprisingly, they found that gyrase changes its reaction mechanism in response to changes in DNA strain. This explains the role of ATP in a branching topoisomerase reaction pathway and revisits an old puzzle about gyrase reversibility.

Topoisomerases (Topo) are essential enzymes for moving chromosomes to specific locations during cell growth and division. They reversibly disrupt the phosphodiester backbone by forming a covalent phosphotyrosine bond with DNA. Type I Topo enzymes break one strand at a time and Type II Topo enzymes break both strands simultaneously. In fast-growing bacteria such as *Escherichia coli*, two Type II Topo enzymes are essential. DNA gyrase catalyzes ATP-dependent negative (–) supercoiling that condenses DNA into a compact and highly interwound conformation. TopoIV is structurally related to gyrase, but it rapidly simplifies DNA networks that become tangled into knots and catenates links between sister strands during chromosomal replication, transcription and recombination. Both supercoil condensation and untangling are crucial for segregating DNA into daughter cells before cell division. In eukaryotes, (–) supercoiling is accomplished by deposition of DNA around the surface of the histone octamer, and TopoIIβ carries out the untangling function during segregation.

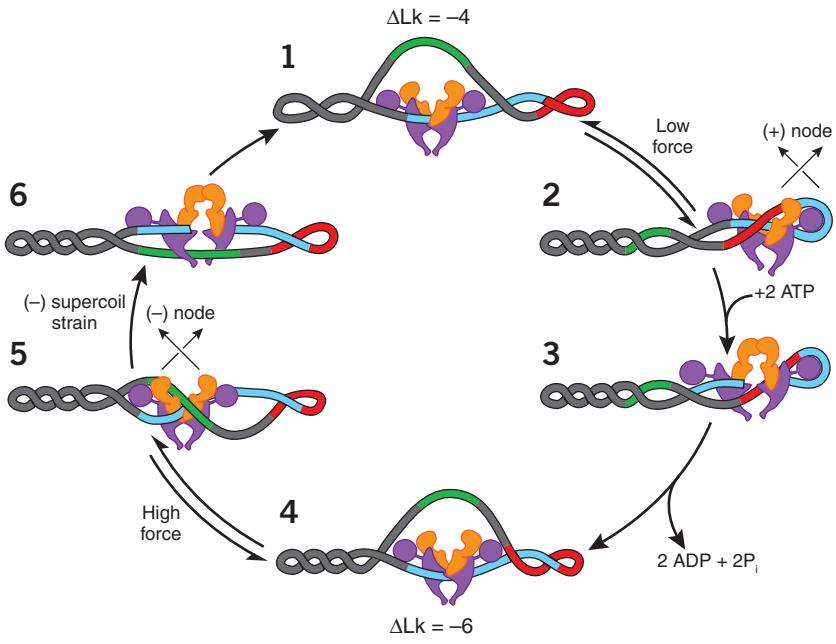

Figure 1 Gyrase reaction mechanism for producing and removing (–) supercoils. Subunits of DNA gyrase form a heterotetramer with two GyrA protomers (purple) and two GyrB protomers (orange). In the supercoiling mode, the sign of the cross or node is (+) for introducing negative supercoils and (–) for removing negative supercoils. Lk, linking number.

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Two reactions that mechanistically differentiate gyrase from eukaryotic and viral Type II Topo are (i) ATP-dependent (–) supercoiling of DNA and (ii) relaxation of (–) supercoils in the absence of ATP. The basic gyrase reaction scheme has been understood for several years. The first step is reversible binding of the gate (G) segment (shown in blue in **Fig. 1**) at the active site of the enzyme (step 1). Next, the C-terminal 'pinwheel' domain[1] of GyrA (purple circles) loops the adjacent

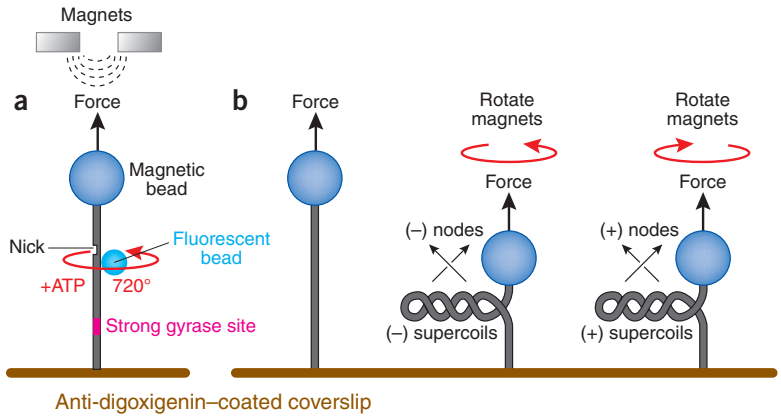

Figure 2 Magnetic tweezers and rotating magnets create single-molecule substrates for DNA gyrase. Details are explained in the text.

DNA (red), called the transfer (T) segment, so that it forms a chiral positive (+) cross above the G segment (step 2). Binding of one ATP molecule to each GyrB subunit triggers a conformational change that opens the G segment and passes the T segment through to a bottom chamber (step 3). Hydrolysis of two ATP molecules and release of the T segment yields a gain of two (–) supercoils, shown here as a linkage change from –4 to –6 (step 4).

Previous work from the Bustamante/Cozzarelli group analyzed gyrase supercoiling reactions using the experimental setup shown in **Figure 2a** (ref. 2). Double-stranded DNA linked to digoxigenin at one end and fluorescein at the opposite end was suspended between an anti-digoxigenin–coated coverslip and an anti-fluorescein–coated magnetic bead. Gyrase binds this DNA preferentially at a high-affinity site from the phage Mu genome (pink segment in **Fig. 2**) (ref. 3). A fluorescent bead covalently attached to DNA near a unique single-strand nick allowed detection of gyrase activity. ATP addition triggered two turns of the bead (a 720° rotation) around the DNA axis. Gyrase supercoiling was extremely sensitive to force applied by the magnetic tweezers. Processive supercoiling observed at 0.1 pN became more erratic and distributive as force increased to 1 pN. Thus, stressing DNA hinders the ability of gyrase to wrap DNA around the pinwheel (**Fig. 1**, step 2).

In the report on page 264 of this issue, Nöllmann *et al.*[4] have changed the single-molecule setup to study DNA in a more natural interwound conformation. They used a double-stranded, covalent (un-nicked) DNA, lacking a strong gyrase site, that could be charged with plectonemic (interwound) (+) or (–) supercoils by rotating the magnets (**Fig. 2b**). Adding supercoils reeled the bead in, whereas removing either (+) or (–) supercoils allowed the bead to move away from the coverslip. Tracking the bead to characterize gyrase activity under different forces, the authors measured rates for supercoiling and relaxation of both (+) and (–) supercoiled substrates.

Adding (–) supercoils is algebraically equivalent to removing (+) supercoils, so the first big surprise was that gyrase relaxes (+) supercoils under forces ten times stronger than the level that completely blocks (–) supercoiling. Given a +40 supercoiled substrate under a weak force of 0.1 pN, gyrase processively removed all (+) supercoils in 10 s, which represents 20 cycles of single-enzyme action. After a lag, gyrase introduced 20 (–) supercoils at the same highly processive rate. When the experiment was repeated at 0.5 pN force, gyrase removed the (+) supercoils as quickly as at low force, but it did not introduce any (–) supercoils. Indeed, unexpectedly, gyrase removed (+) supercoils from molecules at forces of 2.5 and even 4.5 pN.

The force-versus-rate relationship indicates that the (–) supercoiling and (+) supercoil relaxation pathways proceed through different mechanisms. The key is a change in the (+) supercoil pathway under force (**Fig. 3**): starting with (+) supercoiled DNA, the low-force pathway (steps 1→2A→3A→4) is the same as the (–) supercoiling pathway. However, relaxation of (+) supercoils is stimulated by forces up to 1 pN, whereas (–) supercoiling is inhibited by all levels of force tested. Under forces >0.5 pN, (+) supercoil relaxation proceeds via steps 1→2B→3B→4. A distal T segment (green) forms a (+) node over the G segment, and two molecules of ATP trigger strand passage, as in the (–) supercoiling pathway. Electrostatic repulsion of the phosphate backbone forces the interwound strands apart under low-ionic and low-stress conditions[5]. As force increases up to 1 pN, the capture of a distal T segment is more efficient, possibly due to formation of a tighter plectonemic coil.

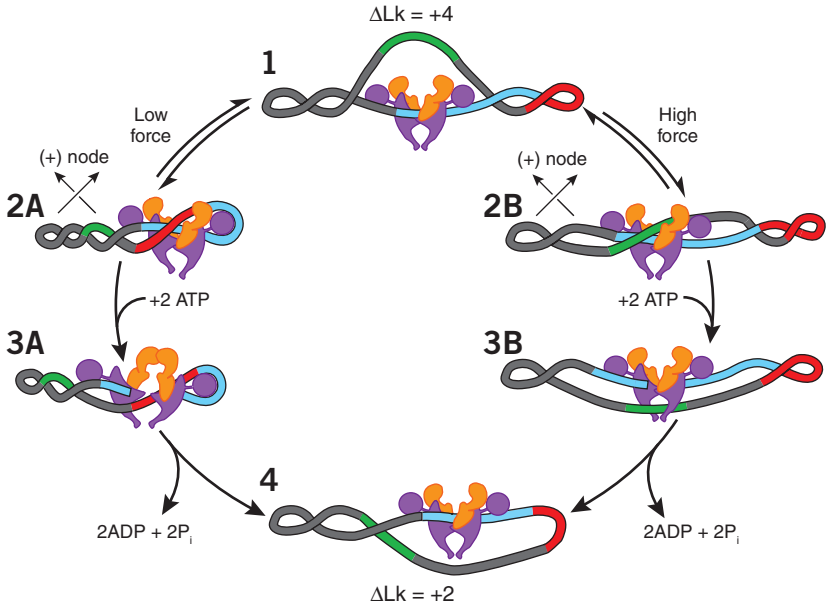

Figure 3 Pathways for relaxing (+) supercoils at low force (left) and at high force (right). In both cases, the sign of the cross is (+), but at low force, gyrase wraps the T segment (red) closest to the G segment (blue), whereas at high force a distant T segment (green) is used. Lk, linking number.

Gyrase relaxation of (–) supercoils in the absence of ATP is an old puzzle; no other type II topoisomerase performs this reaction. A second surprise in this study is that relaxation of (–) supercoils is also stimulated by force. Under a force of 0.6 pN, gyrase processively relaxed 50 (–) supercoils through four cycles of resupercoiling by 50 magnet rotations. An even more striking observation started with DNA held under 0.4 pN of force: gyrase processively supercoiled the DNA at 0.4 pN, but when the force was increased to 0.7 pN, gyrase reversed direction and removed (–) supercoils. Force changes turned gyrase into a monomolecular yoyo wizard, with one continuous trace documenting five supercoiling-relaxation cycle reversals.

How does gyrase catalyze ATP-independent (–) supercoil relaxation? One possible mechanism is that gyrase reverses the steps of the (–) supercoiling pathway (**Fig. 1**, steps 4→3→2→1). Binding of the G segment could be followed by the entrance of the T segment (red) into the bottom chamber of gyrase, forming a (–) node. Opening the gate would allow DNA to pass upward and be released when the upper chamber opens. Evidence that the reverse mechanism might work under low stress has been presented[6]. However, in a perfect reversal of the forward reaction, gyrase would make ATP from ADP and inorganic phosphate. The fact that stress stimulates both (–) and (+) supercoil relaxation suggests another possibility (steps 4→5→6→1). Like the (+) supercoil–removal reaction, introduction of a distal (green) T segment into the top chamber would form a (–) node crossing. The trigger for opening the gate is the nucleophilic attack of the reactive GyrA tyrosine on the phosphodiester backbone. The unwinding energy of (–) supercoiling could promote formation of this complex, just as it does for the *E. coli* Topo I enzyme[7]. Experiments with braided nicked DNA by Nöllmann *et al.*[4] confirm that gyrase carries out ATP-dependent unbraiding of L braids, which form chiral (+) node plectonemes, but it doesn't work, with or without ATP, on R braids. Both gyrase and the *E. coli* TopoIV enzyme, which is a classic Type II topoisomerase, prefer (+) nodes and work more slowly on (–) node substrates[8].

The work from Nöllmann and colleagues has important implications for studies on chromosome structure. Under DNA stress, gyrase reads the sign of interwound crosses and changes the reaction pathway to become a good decatenase, which may be the answer to a cell's prayer. All well-studied bacteria maintain an average supercoil density (σ) distributed around a small median value (between –0.06 and –0.07 in most studied organisms). Increases or decreases of σ by 20% cause cell-division failure. If the point of (–) supercoil introduction and reversal is controlled by the structure of gyrase (that is, by the opening and closing forces of the gate and two chambers), gyrase itself may be the major determinant of σ. DNA in *E. coli* is notably more supercoiled than that of many other enterics[9], and the hypothesis that adaptive changes have occurred in gyrase explains the surprising toxicity of *Salmonella typhimurium* GyrB protein expressed in *E. coli*[10]. It also explains how the slow-growing *Mycobacterium tuberculosis* can make do with gyrase as its sole type II Topo[11].

How do other type II Topo enzymes respond to DNA under stress, given (+) and (–) supercoiled substrates? After the human pS2 promoter is activated for transcription, a single phased nucleosome upstream of the TATA box becomes associated with TopoIIβ as well as the DNA repair proteins Ku70, Ku 86 and PARP1, and the DNA-dependent protein kinase[12]. A high transcription level is correlated with a persistent TopoIIβ-induced double-strand break in a defined internucleosomal DNA site. This break would dissipate the (–) supercoil stress associated with high transcription[13].

Finally, many stress-sensing enzymes could contribute to chromosome dynamics during normal and abnormal chromosome behavior. McClintock's chromosome breakage fusion-bridge cycle[14] is one example. When homologous recombination yields a chromosome with two centromeres (eukaryotes) or a dimer circle (prokaryotes), the machinery that moves chromosomes to sister cells causes stress-point breakage and re-formation of aberrant chromosomes, with amplification and deletion of genetic regions at the breakpoints. The proteins that pull chromosomes apart are not forceful enough (4–6 pN) to break the double helix (which requires >>100 pN), so nuclease hydrolysis or another double-strand break mechanism must make this happen. It will take more tweezers experiments to find out what proteins are involved in this and how they respond to DNA stress.

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