A Superhelical Spiral in the Escherichia coli DNA Gyrase A
C-terminal Domain Imparts Unidirectional Supercoiling Bias*

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DNA gyrase is unique among type II topoisomerases in that its DNA supercoiling activity is unidirectional. The C-terminal domain of the gyrase A subunit (GyrA-CTD) is required for this supercoiling bias. We report here the x-ray structure of the Escherichia coli GyrA-CTD (Protein Data Bank code 1ZI0). The E. coli GyrA-CTD adopts a circular-shaped β-pinwheel fold first seen in the Borrelia burgdorferi GyrA-CTD. However, whereas the B. burgdorferi GyrA-CTD is flat, the E. coli GyrA-CTD is spiral. DNA relaxation assays reveal that the E. coli GyrA-CTD wraps DNA inducing substantial (+) superhelicity, while the B. burgdorferi GyrA-CTD introduces a more modest (+) superhelicity. The observation of a superhelical spiral in the present structure and that of the Bacillus steothermophilus ParC-CTD structure suggests unexpected similarities in substrate selectivity between gyrase and Topo IV enzymes. We propose a model wherein the right-handed (+) solenoidal wrapping of DNA around the E. coli GyrA-CTD enforces unidirectional (-) DNA supercoiling.

DNA topoisomerases solve the topological problems that arise in the course of normal DNA metabolism. Among the numerous functions of these essential enzymes are resolution of DNA catenation during replication and cell division and relaxation of DNA (+) and (-) supercoiling1 resulting from transcriptional and replicative unwinding of the genome (1). In addition to relieving torsional stress, some topoisomerases are also capable of introducing supercoils (2). In particular, bacterial DNA gyrase catalyzes the ATP-dependent introduction of (-) supercoils (1). This activity, unique to gyrase among all topoisomerases, helps maintain prokaryotic genomes at a (-) superhelical density, which in turn is thought to lower the energy barrier for unwinding DNA during transcription and replication (3, 4).

Gyrase, similar to all members of the type II topoisomerase family (Topo IIs),2 catalyzes the ATP-dependent passage of one DNA duplex (T-segment) through another (G-segment), a process that requires transient introduction of a double-stranded DNA break (1). Intramolecular passage of a T-segment results in relaxation or induction of supercoils, whereas intermolecular passage results in decatenation. Most Topo IIs perform both decatenation and relaxation; yet, interestingly, gyrase exhibits an overriding preference to perform intramolecular and unidirectional (+) to (-) node transfer of a T-segment through a contiguous G-segment. Gyrase is an A,B2 tetramer comprising GyrA and GyrB subunits. ATPase activity resides in the N-terminal domain of GyrB (GyrB-NTD); the core cleavage-reunion complex is made up of the C-terminal domain of GyrB (GyrB-CTD) (5) plus the N-terminal domain of GyrA (GyrA-NTD) (2, 6).

Both of the hallmark functional features unique to gyrase, its unidirectional supercoiling activity and strong preference for compact intramolecular T-segment transfer, have been attributed to the C-terminal domain of GyrA (GyrA-CTD) (7). This domain is conserved among gyrases and also members of the Topo IV family, in which it is known as ParC-CTD. Eukaryotic Topo IIs possess a CTD, but it appears to be completely different in structure and function from the prokaryotic CTDs (1, 8–10). Selective removal of the GyrA-CTD from Escherichia coli gyrase results in loss of the ability to introduce (-) supercoils in relaxed and negatively supercoiled DNA, a gain of the ability to relax (-) supercoils, and a 30-fold increase in decatenation activity (7); addition of the GyrA-CTD in trans results in restoration of the capacity to introduce (-) supercoils (11, 12). Plasmid nicking/religation experiments indicate that the E. coli GyrA-CTD and full-length gyrase (in the absence of ATP) both bind DNA such that (+) superhelicity is introduced, whereas GyrB + GyrA-NTD does not alter global DNA topology upon binding (7, 13–15). Furthermore, E. coli gyrase preferentially binds and processes (+) supercoiled DNA (16), whereas GyrB + GyrA-NTD has no such preference (14). These data have been interpreted to suggest that DNA wraps around gyrase in a manner dependent upon the presence of the GyrA-CTD, producing (+) superhelicity, and that this wrapping plays a crucial role in controlling the directionality of supercoil in-
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**EXPERIMENTAL PROCEDURES**

**Protein Preparation**—Plasmids encoding E. coli GyrA-CTD (532–853 or 532–841) and B. burgdorferi GyrA-CTD (501–810) were cloned from E. coli D. melanogaster (American Type Culture Collection) into the pET30a (Novagen) vector containing a GigaKit (Qiagen). The E. coli GyrA-CTD was purified by E. coli overexpression in pLysS cells at 37 °C. Lysis was accomplished by sonication in 50 mM HEPES, 1.85 mM MgCl2, 5 mM spermidine, 100 μM dithiothreitol, 0.6–6.0 μM pBR322, and 0.6–6.0 μM HEPES, 1 mM EDTA (labeled protein crystals and then applied to a native data set.

The native x-ray diffraction dataset (dmax = 2.6 Å) was collected at the Macromolecular Diffraction at the Cornell High Energy Synchrotron Source (MacCHESS) A1 beamline, and a multiwavelength anomalous dispersion dataset (dmax = 3.0 Å) was obtained at the National Synchrotron Light Source X-4 station at 100 K. Reflection data were integrated and scaled with the HKL2000 program (34). Real-space locations of all twelve of the expected selenomethionines in the asymmetric unit were placed using SOLVE (35). The initial model was built using Quanta 2000 (Accelrys, San Diego, CA), into a density-modified map generated using the DM module of the Collaborative Computational Project 4 suite (36, 37). This model was subjected to rounds of simulated annealing, energy minimization, individual B-factor refinement in crystallography NMR software (version 1.1) against the native dataset interspersed with manual rebuilding to produce a model with final values of Rfree = 22.7% and Rwork = 26.7%. Noncrystallographic symmetry was not employed during the refinement. Electron density was weak or absent for a significant number of side chains: proteins A and B had 2102 and 2091 atoms built with 32 and 46 side-chains partially disordered (110 and 175 atoms not modeled), respectively. The figures were created with Ribbons 3.22 and GRASP1.2 software (the GRASP surface was created with a model in which the disordered parts of side chains with β-carbon density were modeled to most appropriately reflect surface charge distributions) (38, 39). PROCHECK and SFCHECK were used to inform model building and validate the final model (40, 41).

**Topo IB Relaxation Assays**—Negatively supercooled pBR322 was prepared using a GigaKit (Qiagen) (14, 42). The pBR322-resuspended pellet was dialyzed against 20 mM Tris, pH 8.0, 1 mM EDTA and concentrated on a 30 K polyethersulfone membrane (Vivaspin) to 0.85 μg/ml. To obtain undamaged pBR322, it is essential to never freeze the DNA. The reaction buffer included 35 mM Tris, pH 7.4, 50 mM NaCl, 1.85 mM MgCl2, 5 mM spermidine, 100 μg/ml bovine serum albumin, 9 μg/ml rRNA, 6.5% glycerol, 5.5 mM βME, 1 mM ATP, 2 mM HEPES, 0.1 mM EDTA, 300 mM NaCl, 0.6–6.0 μM GyrA-CTD (E. coli 532–841) or B. burgdorferi; the reactions are identical with or without His8 tags. T4 DNA ligase (100 units, New England Biolabs) was added to each reaction. After 10 min, DNA Topo IB from vaccinia virus (10 units, Epicentre) was added to a final volume of 10 μl, and the reactions were incubated for 4–6 h at 37 °C. Phenol:chloroform:isoamyl alcohol (25:24:1) buffered to pH 8.0 was used to extract the protein from the reactions, followed by ethanol precipitation. The DNA was resuspended in 20 mM Tris, pH 8.0, 1 mM EDTA. Gels with ~0.5 μg of DNA lane (1% agarose in 1× TPE (Tris phosphate, EDTA) or 1× TAE (Tris acetate, EDTA)) were run at 4 V/cm for 10 min and then 1.0 V/cm for 19–21 h. After ethidium bromide staining, the topoisomerase bands were quantitated in ImageQuant TL (Amersham Biosciences) and plotted in Kaleidagraph (Synergy Software), where they were fit to gaussian curves.

**RESULTS**

**Structure Analysis**—Crystals of the E. coli GyrA-CTD (residues 531–853 plus an N-terminal hexahistidine tag) produced diffraction data to a limiting resolution of 2.6 Å (supplemental Table I). Experimental phases were determined by multiwavelength anomalous dispersion phasing using selenomethionine-labeled protein crystals and then applied to a native data set. The final model includes two protomers/asymmetric unit, each comprising residues 535–841, with a large disordered loop from 555 to 576 in protomer A and 563 to 575 in protomer B (Fig. 1C). This disordered loop, hereafter referred to as the β-CA loop, contains a known trypsin cleavage site plus the “GyrA box,” an indel used to distinguish GyrA from ParC as a separate step.

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shown). The two protomers possess nearly identical overall structures and can be superimposed with a C$_{\text{r.m.s.d.}}$ of 1.2 Å. Significant structural differences between the two are localized entirely to flexible elements located at the crystal packing interface, namely the ordering of a small stretch of the $\beta$-CA loop (residues 555–562) in protomer B into a short $\beta$-helix and the minor adjustment of a nearby loop (residues 608–617) (Fig. 1D, wedges). Upon removal of these parts from the superposition, the r.m.s.d. drops to 0.7 Å. Because the short helix in protomer B appears to be a crystal packing artifact, we have used protomer A for all figures, except Fig. 1, C and D. The crystal structure of the E. coli GyrA-NTD (29) was ordered through residue 522, thus only 12 amino acids are missing between the structures of the two domains of GyrA (Fig. 2A).

The overall fold of the E. coli GyrA-CTD is similar to that recently described for the structure of the B. burgdorferi GyrA-CTD (32), with both bearing a superficial similarity to the well known $\beta$-propeller (45). Similar to $\beta$-propeller proteins, the GyrA-CTDs contain serially repeated subdomains known as “blades,” each of which is composed of a four-stranded antiparallel $\beta$-sheet (Fig. 1, A and B). Closer inspection, however, reveals that the topology of the blades in the GyrA-CTDs is completely different from that in $\beta$-propeller proteins; this distinction prompted Corbett et al. (32) to describe the GyrA-CTD fold as a $\beta$-pinwheel. Whereas the $\beta$-strands that make up the blades in a $\beta$-propeller reside on a contiguous stretch of polypeptide sequence, the $\beta$-pinwheel is a more interdigitated structure, with the polypeptide chain

**Fig. 1. Structure analysis of E. coli GyrA C-terminal domain.** A, spiraling $\beta$-pinwheel structure of the E. coli GyrA-CTD residues 535–841, with the disordered $\beta$-CA loop (555–576) represented by a dotted line. The blade subdomains (four-stranded antiparallel $\beta$-sheet and a $3_{\alpha}$ helix) are labeled 1–6 and alternate in color from red to blue. The choice of blade nomenclature used here for the spiraling $\beta$-pinwheel preserves the four-stranded sheet with strands labeled from the center proximal outward following the convention for $\beta$-propellers. Note that the topology for all three CTD structures discussed in this paper is identical, despite the different blade and strand nomenclature used by Corbett et al. (28, 32). B, topology diagram illustrating interdigitation of blades. C, asymmetric unit of the E. coli GyrA-CTD structure. Monomer A is in red, and monomer B is in blue. The perhaps spurious packing $\alpha$-helix formed from the $\beta$-CA loop is indicated by the wedge. D, Ca superposition of monomers A and B. The r.m.s.d. is 1.2 Å and falls to 0.7 Å, when the $\alpha$-helix (wedge 1) in B and the loop that abuts it (wedge 2) are excluded from the calculation. Dashed lines indicate disordered loops spanning residues 555–576 and 563–575 in protomers A and B, respectively.

**Fig. 2. Models.** A, the E. coli GyrA-NTD (ordered from 30–522) (29) and the E. coli GyrA-CTD (ordered from 535–841) showing the largest possible distance of 39 Å separating the two domains. B, model suggesting how DNA might wrap about the E. coli GyrA-CTD to impart right-handed superhelicity on the DNA. Left, DNA modeled onto the electrostatic potential surface.
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meandering back and forth between adjacent blades (Fig. 1, A and B).

Although each blade in the β-pinwheel fold of the E. coli GyrA-CTD is nearly identical to its counterpart in the B. burgdorferi GyrA-CTD (gray) (32), the spatial arrangement differs significantly; while the blades of the B. burgdorferi GyrA-CTD form a toroidal β-pinwheel, the blades of the E. coli GyrA-CTD are arranged in an ascending spiral around a screw axis (Fig. 3). If the B. burgdorferi GyrA-CTD has the appearance of a flat washer, then the E. coli GyrA-CTD has the appearance of a lock washer. Consequently, a global superposition of the B. burgdorferi and E. coli proteins yields an r.m.s.d. of 4.59 Å (5.45 Å for protomer B), in marked contrast to the excellent correspondence of blade-to-blade superpositions. The significant divergence in overall shape can be observed in a Ca superposition using the N-terminal blade of each structure (Fig. 3). To gain a more quantitative estimate of the difference in helicity of the two proteins, we computed a screw axis (Fig. 3, blue rod) and measured the displacement of the center of mass for blades 1 and 6 (Fig. 3, spheres) along the axis. Although the B. burgdorferi β-pinwheel has almost no axial displacement (0.04 Å) from blade 1 to 6, the E. coli β-pinwheel exhibits pronounced axial displacement (11.03 Å). Consonant with the axial displacement of the propeller blades, the interface between blades 1 and 6 is much more extensive in the B. burgdorferi GyrA-CTD structure than in the E. coli structure (2,150 Å² of solvent-inaccessible surface versus 466 Å², respectively). In the B. burgdorferi structure, a significant fraction of this interface is contributed by the GyrA on the β-CA loop (991 Å²), whereas the whole loop is disordered in the E. coli GyrA-CTD.

The Bacillus stearothermophilus ParC-CTD (Topo IV) structure, reported while this work was under review, also has a β-pinwheel fold with the blades arranged in an ascending spiral (28); the E. coli GyrA-CTD and the B. stearothermophilus ParC-CTD superimposed with an overall r.m.s.d. of 1.5 Å (Fig. 3). The most notable structural difference between these two domains is the presence of α-helices in the β-CA loop and at the C terminus of the ParC-CTD. The latter packs against strand A of blade 1, resulting in a much more extensive interaction between blades 1 and 6 (1086 Å²) than in the E. coli GyrA-CTD structure (466 Å²).

It is formally possible that the differences in overall fold between these three structures result from crystal packing. Two independent lines of evidence suggest this is not likely. Protomers A and B of the E. coli GyrA-CTD asymmetric unit are virtually identical in fold, despite packing differently within the unit cell (Fig. 1, C and D, and supplemental Fig. 3). Further, the B. stearothermophilus ParC-CTD, which also shares this spiral fold, packs in yet another form (space group P212121) (supplemental Fig. 3) (28).

To gain insight into the charge density and distribution on the surface of the E. coli GyrA-CTD, we calculated its surface electrostatic potential (Fig. 2B). Strikingly, a positively charged strip extends along the midriff of the protein, following the right-handed superhelical rise of the β-pinwheel. When DNA is modeled into the structure so as to maximize its electrostatic interactions with this positive strip, the right-handed superhelical rise in the positive strip imparts a corresponding superhelical rise in the bound DNA (Fig. 2B). Interestingly, although the B. burgdorferi structure also contains a positive strip (32), it lacks a spiral sense and hence is not expected to impart any superhelical rise upon its bound DNA. In either case, most of the predicted DNA contacts can be attributed to the preponderance of arginines and lysines on the long loops connecting the 3₁₀ helix of one propeller blade with the outermost β-strand in its neighbor (Figs. 1A and 4). It is conceivable and perhaps even likely that these basic loops undergo some local conformational adjustment upon DNA binding but unlikely that they change so drastically as to alter the overall course of the positive strip. The right-handed helical arrangement of blades from N to C terminus suggests a model for DNA wrapping, whereby binding along the midriff of the protein surface imparts right-handed superhelicity in the DNA (Fig. 2B).
**Topo I Readout of Wrapping—**To compare changes in DNA topology upon binding of the *E. coli* or the *B. burgdorferi* GyrA-CTDs, Topoisomerase IB was used to relax pBR322 (300 nM) in the presence of either the *E. coli* or *B. burgdorferi* GyrA-CTD at increasing molar ratios of protein, trapping the local superhelical state induced by protein binding. Nicked and linearized controls were obtained by digestion of pBR322 with N.BpuI or HindIII, respectively. A, samples run on a 1.1% agarose gel (1X TPE buffer) and stained with ethidium bromide. B, samples run on a 1.1% agarose, 0.6 μg/ml chloroquine gel (1X TAE buffer) and stained with ethidium bromide. The sign of the linking number changes were determined to be positive from the increase in mobility of the topoisomeres in the chloroquine gel (B) compared with the non-chloroquine gel (A). Further increases in mobility were observed on a 3 μg/ml chloroquine gel (supplemental Fig. 1A). The bands on both gels were quantified and fit to gaussian curves to determine the ΔLk/protein (supplemental Fig. IB).

DISCUSSION

Recent structural findings on CTDs from *B. burgdorferi* DNA gyrase and *B. stearothermophilus* Topo IV revealed β-pinwheel folds that can adopt two markedly distinct overall conformations, a planar one bearing resemblance to a flat washer and a spiral one resembling a lock washer (28, 32). That topoisomerases from different functional classes should display such a clear structural distinction led quite naturally to the suggestion that gyrase CTDs are flat and Topo IV CTDs are spiral, and that this difference gives rise to the distinctive class-specific functional characteristics of each (28). The present structural findings dispel the notion that the structure/function relationships in topoisomerase CTDs follow such a simple pattern. Specifically, here we report that the CTD from *E. coli* DNA gyrase has a spiral (not flat) arrangement of blades. Thus, the *E. coli* GyrA-CTD is more closely related in overall structure to the paralogous Topo IV CTD than to the orthologous *B. burgdorferi* GyrA-CTD.

The present structure furthermore dispels the notion that the GyrA box, which is present in GyrA-CTDs (refer to Fig. 1) but lacking in Topo IV-CTDs, is responsible for stabilizing the flat structure by lashing blades 1 and 6 together (28). It is possible that the particular GyrA box sequence in the *B. burgdorferi* GyrA-CTD does stabilize the flat structure, but this cannot be the role of the GyrA box in general, because the *E. coli* GyrA-CTD contains a GyrA box and yet adopts a spiral structure. It is worthy of note that the GyrA box of the *E. coli* protein (QRRGKG) is a perfect match to the consensus sequence (QXXGGXXG, where X is a positively charged amino acid) (44); whereas the GyrA box in *B. burgdorferi* (QGTGGKG) diverges further from the consensus than 85 of 87 GyrA orthologs (Supplementary Fig. 2). Also noteworthy is the fact that the β-CA loop, including the GyrA box, is disordered in the *E. coli* GyrA-CTD, which raises the possibility that this entire segment of the CTD becomes ordered upon docking with the remainder of gyrase or upon binding DNA.

Having established that the flat versus spiral structural divergence transcends class distinctions among topoisomerases, the question remains as to whether the two divergent shapes give rise to distinct and definable functional roles. Below, we will focus first on the implications for gyrase function and will then return to the issue of Topo IV mechanism. A predicted β-pinwheel CTD is found in all known Topo II enzymes capable of introducing (−) supercoils (9). Removal of the GyrA-CTD from *E. coli* gyrase results in loss of the bias toward introduction of (−) supercoils but retention of core Topo II activity (7). Finally, a subfragment of the *E. coli* CTD (residues 572–875) is able to complement a CTD-less gyrase variant (residues 6–571) (12), suggesting the CTD behaves as a plug-in structural module of gyrase, rather than an inextricably linked structural component. Taken together, these results lend strong support to the notion that the GyrA-CTD is responsible for the (−) supercoiling bias of DNA gyrases and bolster confidence in the extrapolation of results from the isolated CTDs to full-length gyrase.

Gyrase CTDs, both flat and spiral, possess a stripe of positive charge about their midriff that is the only obvious locus for interaction with a contiguous stretch of duplex DNA. Consistent with this notion, fluorescence resonance energy transfer experiments on the *B. burgdorferi* GyrA-CTD indicate that the...
domain has the ability to induce bends in DNA (32). Similar fluorescence resonance energy transfer and single-molecule DNA-contraction experiments on the *E. coli* GyrA-CTD reach the same conclusion (data not shown). Although the *B. burgdorferi* and the *E. coli* GyrA-CTDs both wrap DNA, the manner of wrapping is not identical, because the *B. burgdorferi* CTD does not alter the average linking number of plasmid DNA in relaxation experiments described above to the same extent as the *E. coli* CTD. Two different modes of CTD-mediated conformational changes in the bound duplex DNA could account for these changes in linking number, overtwisting, or (+) writhe induction via wrapping of the DNA around the CTD, or some combination of both. The relatively modest ΔLk/protein of +0.3 for the *B. burgdorferi* GyrA-CTD could result entirely from the twist imparted by a protein binding DNA about its radial surface. On the other hand, overtwisting alone is highly unlikely to account for the observed ΔLk/protein imparted by the *E. coli* CTD, because its large magnitude (+0.8 for *E. coli* GyrA-CTD) would require the additional linking number of nearly one complete additional turn over the <50 base-pairs of DNA (4–5 turns) estimated to be bound to the CTD (18, 21), as compared with relaxed, B-form DNA. If overtwisting alone cannot account for the observed linking number change, then writhe, most likely in the form of (+) solenoidal superhelical wrapping, must play a role. Such wrapping of DNA around a roughly cylindrical surface is well preceded in the nucleosome core particle (46). The nucleosomal DNA is wrapped in a left-handed ((−)solenoidal) superhelix (-1.67 superhelical turns) in opposition to slight overwinding (10.17 bp/turn versus 10.5 ± 0.1 for canonical B-DNA, for a total ΔLk/nucleosome of −1.2) (46). We note that, adjusting the magnitudes of writhe and twist from the nucleosome to the DNA size regime that bound by the *E. coli* GyrA-CTD and assuming the sign of twist and writhe changes are both (+), one arrives at a ΔLk/protein of +0.8 (twist = +0.2, and writhe = +0.6). Although actual magnitudes of writhe and twist cannot be determined from our present data, we favor a model with a preponderance of the *E. coli* GyrA-CTD +0.8 ΔLk/protein superhelicity in the form of writhe, because the kind of gentle superhelical writhe suggested by the structure (Fig. 2B) is less energetically costly than drastic overwinding. Importantly, the spiral sense in the *E. coli* GyrA-CTD is (+), consistent with the observed (+) linking number change in the Topo I assay. Full-length *E. coli* gyrase, which has two GyrA-CTDs, also introduces a (+) linking number change in DNA in a similar assay (14, 15). The change in linking number is +0.7 to 0.8 per enzyme, indicating the extent of DNA wrapping by each CTD must be diminished or only one CTD of the two in gyrase is engaged with DNA at a time. Thus, in summary, we propose that the spiral positively charged stripe about the midriff surface of the *E. coli* GyrA-CTD is predisposed toward binding DNA containing comple-
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Corbett et al. (32) have shown that the highly positively charged GyrA box directs DNA packing against the rest of gyrase to impose directionality of DNA supercoiling. GyrA box might play a role in the enzyme of G and T segment contiguity by gyrase. This highly charged stretch may electrostatically guide the G-segment from the cleavage/reunion core binding cleft to the CTD in the case of gyrase, whereas in Topo IV, the absence of this concentrated charge in the β-CA loop may destabilize or prevent such presentation of a continuous stretch of DNA. Unlike gyrase, Topo IV appears to bind a T-segment only transiently during processing (51). The ParC-CTD is believed to be responsible for T-segment selection (28), and the E. coli ParC-CTD is capable of inducing bends in DNA (32). Taking these earlier findings into account, we add that the right-handed spiraling nature of the ParC-CTD could be directly responsible for selection of T-segments that form (+) nodes (Fig. 7). Our model explains that the selectivity for (+) supercoiled DNA by Topo IV occurs during processing, despite the absence of any such selectivity in initial G-segment binding (26).

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GyrA-CTD Imparts Unidirectional Supercoiling Bias

Corbett et al. (32) have shown that the highly positively charged GyrA box directs DNA packing against the rest of gyrase to impose directionality of DNA supercoiling. GyrA box might play a role in the enzyme of G and T segment contiguity by gyrase. This highly charged stretch may electrostatically guide the G-segment from the cleavage/reunion core binding cleft to the CTD in the case of gyrase, whereas in Topo IV, the absence of this concentrated charge in the β-CA loop may destabilize or prevent such presentation of a continuous stretch of DNA. Unlike gyrase, Topo IV appears to bind a T-segment only transiently during processing (51). The ParC-CTD is believed to be responsible for T-segment selection (28), and the E. coli ParC-CTD is capable of inducing bends in DNA (32). Taking these earlier findings into account, we add that the right-handed spiraling nature of the ParC-CTD could be directly responsible for selection of T-segments that form (+) nodes (Fig. 7). Our model explains that the selectivity for (+) supercoiled DNA by Topo IV occurs during processing, despite the absence of any such selectivity in initial G-segment binding (26).

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