Structure of the Topoisomerase IV C-terminal Domain

A BROKEN β-PROPELLER IMPLIES A ROLE AS GEOMETRY FACILITATOR IN CATALYSIS*

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Bacteria possess two closely related yet functionally distinct essential type IIA topoisomerases (Topos). DNA gyrase supports replication and transcription with its unique supercoiling activity, whereas Topo IV preferentially relaxes (+) supercoils and is a decatenating enzyme required for chromosone segregation. Here we report the crystal structure of the C-terminal domain of Topo IV ParC subunit (ParC-CTD) from Bacillus steaothermophilus and provide a structure-based explanation for how Topo IV and DNA gyrase execute distinct activities. Although the topological connectivity of ParC-CTD is similar to the recently determined CTD structure of DNA gyrase GyrA subunit (GyrA-CTD), ParC-CTD surprisingly folds as a previously unseen broken form of a six-bladed β-propeller. Propeller breakage is due to the absence of a DNA gyrase-specific GyrA box motif, resulting in the reduction of curvature of the proposed DNA binding region, which explains why ParC-CTD is less efficient than GyrA-CTD in mediating DNA bending, a difference that leads to divergent activities of the two homologous enzymes. Moreover, we found that the topology of the propeller blades observed in ParC-CTD and GyrA-CTD can be achieved from a concerted β-hairpin invasion-induced fold change event of a canonical six-bladed β-propeller; hence, we proposed to name this new fold as “hairpin-invasion-induced β-propeller” to highlight the high degree of similarity and a potential evolutionary linkage between them. The possible role of ParC-CTD as a geometry facilitator during various catalytic events and the evolutionary relationships between prokaryotic type IIA Topos have also been discussed according to these new structural insights.

The intertwining nature of long bacterial chromosome encounters two topological problems during the process of semi-conservative replication (reviewed in Refs. 1 and 2). By pulling the interwoven complementary strands apart, the progression of replication machinery inevitably generates (+) supercoils in front of the replication fork. Failure to remove the excess (+) supercoils will cause replication elongation to stop. In addition, the newly synthesized sister chromosomes are interlinked as catenanes. Consequently, decatenation is an essential step for chromosome segregation. Although these replication-induced topological entanglements are harmful for the cell, a proper level of (−) supercoiling is considered to set the topological homeostasis, which governs certain cellular processes (2), such as transcription and replication initiation, that require DNA unwinding (3). In bacteria, the resolution of DNA entanglements and maintenance of topological homeostasis are largely handled by two essential type IIA topoisomerases (Topos),1 DNA gyrase and Topo IV (4).

All of the members of the type IIA Topo family share significant sequence similarity and essentially the same ATP-dependent catalytic mechanism (5, 6). Specifically, type IIA enzymes alter the topological state of DNA by producing a transient double-stranded break in one duplex (termed G-segment for the gate-forming segment), transporting another duplex (termed T-segment for transported segment) through the enzyme-mediated gate, and religating the cleaved G-segment. Interestingly, although DNA gyrase and Topo IV share a high degree of similarity, they possess distinct cellular functions and thus cannot complement each other in vitro (7, 8). DNA gyrase is the only type IIA enzyme capable of actively introducing (−) supercoils into DNA, an activity involved in replication initiation and transcription of (−) supercoil-dependent promoters (9, 10). Additionally, this unique supercoiling capability made DNA gyrase particularly effective for removing (+) supercoils generated during replication elongation (11, 12). In contrast, Topo IV does not possess supercoiling activity and is normally localized behind the replication forks to serve as a decatenating enzyme for disentangling the interlinked daughter chromosomes (12, 13). Recent studies further sustain its role as a decatenase by demonstrating that, through the interaction with a septal ring protein FtsK, Topo IV is only accessible to DNA at the end of replication (14). Because the decatenating activity of Topo IV is stimulated by (−) superhelical density, the two enzymes appear to work in concert to complete replication (15).

In addition to its apparent function in decatenation, in vitro analyses also revealed that Topo IV is able to catalyze relaxation of supercoiled DNA (16). However, in sharp contrast to the eukaryotic type IIA Topos, which act indistinguishably on the supercoils of both signs, recent studies show that Topo IV

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1 The abbreviations used are: Topo, topoisomerase; CTD, C-terminal domain; FRET, fluorescence resonance energy transfer; ParC, topoisomerase IV A subunit; GyrA, DNA gyrase A subunit; Se, selenium; r.m.s.d., root mean square deviation; G-segment, gate-forming segment; T-segment, transported segment.
relaxes (+) supercoils much faster than (−) supercoils (17–19). Although this distinctive substrate specificity provides an explanation of why Topo IV can promote replication elongation without counteracting the activity of DNA gyrase, it raises the question of how Topo IV preferentially recognizes (+) supercoiled DNA as preferred substrate. Substrate binding affinity does not appear to solve this question, because both (+) and (−) supercoiled DNA compete equally well with linear DNA for Topo IV binding (19). Cozzarelli's and Charvin's groups have engaged similar single-molecule relaxation assays on mechanically braided DNA molecules, and both groups hypothesized that Topo IV is able to sense the geometry of DNA cross-overs. A left-handed crossing of the T- and G-segments imposed by the (+) superhelix makes a preferred substrate for duplex passage reaction (17, 19).

How do homologous type IIA Topos diverge in catalytic activities and substrate specificities? Multiple sequence alignment revealed that, although most functional domains are conserved in all type IIA enzymes, the highly charged C-terminal domain (CTD) of the eukaryotic Topo has no detectable sequence similarity with the corresponding regions in either DNA gyrase or Topo IV (20). Instead of involving catalysis, various studies have suggested that the intracellular localization of eukaryotic Topo IIA is regulated by its CTD (21–24). In contrast, the two prokaryotic enzymes are assembled from two closely related subunits. The GyrA and GyrB subunits of DNA gyrase can be aligned over their entire lengths with the corresponding ParC and ParE subunits of Topo IV, respectively. Moreover, unlike the eukaryotic CTD, which is probably unstructured and dispensable for catalysis, the ~35-kDa CTDs of both DNA gyrase (GyrA-CTD) and Topo IV (ParC-CTD) were predicted to adopt a six-bladed β-propeller fold (25) and the negative supercoiling activity of DNA gyrase is clearly dependent on GyrA-CTD (26). Due to the absence of this domain, the involvement of GyrA-CTD during catalysis explains why the eukaryotic type IIA enzymes may differ from DNA gyrase. However, the basis for the functional differences between DNA gyrase and Topo IV remains unanswered.

Using DNA footprint assay, it was found that DNA gyrase protects ~140-bp long DNA from nuclease digestion, whereas only 35 bp of DNA is protected by Topo IV (27, 28). In addition, GyrA-CTD was found to be a nonspecific DNA-binding protein capable of bending and wrapping DNA around itself (27, 29–31). Together, these results suggested that the DNA binding properties of these two prokaryotic enzymes are very different. It has been proposed that, with the help from GyrA-CTD, a continuous stretch of DNA can be wrapped by DNA gyrase to generate a (+) supercoil-like intramolecular DNA cross-over to facilitate subsequent duplex passage reaction that produces net (−) supercoils in DNA (28, 30, 32). Consistent with its predicted catalytic function, deletion of GyrA-CTD converted DNA gyrase into a conventional type IIA Topo (26). It appears that the functional differences between DNA gyrase and Topo IV can be attributed in part to their respective CTDs.

Recently, the crystal structure of GyrA-CTD from Borrelia burgdorferi has been determined (30). Although the structure was globally reminiscent of a six-bladed β-propeller as predicted (25), GyrA-CTD folds with a novel topology and was alternatively termed to adopt a β-pinwheel fold (30). An arc-shaped positively charged patch on the outside surface was predicted as the region responsible for DNA bending. Guided by electron microscopy images of the holoenzyme (33), GyrA-CTD was positioned relative to the catalytic core of DNA gyrase and a model was constructed to explain how this domain is involved in generating (−) supercoils (30). This model was further supported by fluorescence resonance energy transfer (FRET) measurement showing that DNA can be effectively bent by GyrA-CTD. Interestingly, albeit ~10-fold less efficient, ParC-CTD was also found to possess DNA bending activity. This finding suggests that the different bending efficiency of the two CTDs may define the functional differences between DNA gyrase and Topo IV. Moreover, this previously unknown DNA bending activity raises the possibility that ParC-CTD may play significant roles during Topo IV catalysis.

In this report, we present the crystal structure of ParC-CTD from bacterium Bacillus stearothermophilus. Surprisingly, although the structure displays similar topological connectivity as observed in GyrA-CTD (30), ParC-CTD folds as a previously unseen broken form of a six-bladed β-propeller in which no close contacts are observed between the first and last propeller blades. Besides, we found that the topological connectivity of GyrA-CTD and ParC-CTD type can be achieved from a β-hairpin invasion-mediated fold change event of a canonical six-bladed β-propeller structure. Hence, we proposed to name this newly observed protein fold as “hairpin-invaded β-propeller” to highlight the high degree of structural similarity and a possible evolutionary linkage between them. Similar to GyrA-CTD, a continuous electropositive patch is present on the outside perimeter of the broken β-propeller, which may represent the DNA binding surface of ParC-CTD. Compared with GyrA-CTD, the lesser surface curvature and lower electropositive potential of this CTD region suggest why ParC-CTD bends DNA less efficiently when DNA is docked against it. Most significantly, a structural comparison revealed the reason why ParC-CTD folded into a broken β-propeller. It was due to the absence of a Lys/Arg-rich sequence motif named GyrA box, which is conserved in all of the DNA gyrases (34). This finding supports the use of GyrA box as one of the features to distinguish GyrA and ParC proteins and provides a structural basis for how this sequence motif dictates the functional differences between the two prokaryotic type IIA Topos. Our results also imply how ParC-CTD may act as a geometry facilitator during Topo IV catalysis.

EXPERIMENTAL PROCEDURES

Preparation and Crystallization of Selenomethionyl ParC-CTD—Residues constituting the CTD of prokaryotic type IIA Topo have been clearly defined by various techniques (25, 29). Based on this information and multiple sequence alignment performed using the ClustalX program (35), residues 499–810 of the B. stearothermophilus ParC were easily recognized as the region that corresponds to its CTD. The cloning, expression, purification, and crystallization of this domain have been described previously (36). Expression of selenomethionyl ParC-CTD was achieved at 20 °C after inducing for 16 h with 1 mM isopropyl-β-D-thiogalactopyranoside in Escherichia coli strain B834(DE3) in M9 medium supplemented with 40 μg/ml seleno-l-methionine (37). Purification and crystallization of labeled ParC-CTD were performed using protocols established for native protein with the exception that 1 mM dithiothreitol was used in all of the buffers to replace β-mercaptoethanol as the reducing agent (36). Unit cell parameters of selenomethionyl ParC-CTD crystals are identical to those of native protein and were summarized in Table I.

Data Collection and Structural Determination—Crystals of selenomethionyl ParC-CTD were stabilized in mother liquor containing 35% methylpentanediol plus 15% ethylene glycol before freezing in liquid nitrogen. Diffraction data sets were collected from a single Se-labeled crystal at three different wavelengths at SPring8 (Beamline SP12B2, ADSC Quantum 4 CCD detector, 100K) and processed by using HKL2000 (38). Data from each wavelength were indexed according to the same crystal orientation matrix but integrated and scaled independently. Scattered data sets from each of the three wavelengths were then scaled together from 30.0- to 1.8-Å resolution using Scaledit (39). The structure of ParC-CTD was determined to 1.8-Å resolution by the multiwavelength anomalous dispersion method. Specifically, the selenium substructure was determined with the program Shake-and-Bake (40). The refinement of selenium positions, calculation of phases, density modification, and the building of an initial model were all performed by using SHARP/autoSHARP (41). The program O (42) and
Table I
Summary of crystallographic analysis

| Space group | Unit cell dimensions (Å) |
|-------------|-------------------------|
| a = b = 83.5, c = 45.1 |
| Data collection | Se-edge | Se-peak | Se-remote |
| Resolution (Å) | 0.97984 | 0.97966 | 0.95370 |
| No. of observed reflections | 15,2385 | 152,587 | 161,160 |
| Unique reflections | 29,122 | 29,219 | 30,204 |
| Completeness (last shell) (%) | 89.4 (63.1) | 89.4 (63.1) | 92.9 (70.3) |
| Multiplicity | 5.2 | 5.2 | 5.3 |
| Rfree (last shell) (%) | 9.6 (20.1) | 9.8 (21.1) | 9.6 (22.4) |

\*Statistics for data from the resolution shell of 1.86–1.80.

\*Rfree = \(2(\sum |F_h| - |F_l|)/\sum |F_h|\), where the average intensity (I) is taken over all symmetry equivalent measurements and |F_h| is the measured intensity for any given reflection.

\*Rcryst = \(2(|F_o| - k|P|)/\sum |P|\).

REFMAC5 (39) were then used for rounds of manual model rebuilding and refinement, respectively, to a final Rcryst of 16.0% and Rfree of 21.4%. The final model includes all 312 amino acid residues of ParC-CTD and 377 water molecules. Because electron density for part of the histidine tag is well defined, the final ParC-CTD model contains an additional 11 amino acid residues (KKLAALHEHHHH from pET021b, Novagen) at the C terminus. 93.4% non-glycine residues have main-chain torsion angles in the most favored regions of the Ramachandran plot. Data collection and refinement statistics are summarized in Table I.

RESULTS AND DISCUSSION

Structure of ParC-CTD: a Broken β-Propeller with Hairpin-invaded Blade Topology—ParC-CTD is composed of six highly twisted antiparallel β-sheet modules arranged sequentially along a curved surface to give an overall arc-shaped appearance (Fig. 1A). This architecture approximates the well known β-propeller structures (45–47) and resembles the newly discovered GyrA-CTD β-pinwheel in particular (30). However, in sharp contrast to other β-propeller structures (including GyrA-CTD β-pinwheel) where the first and the last propeller blades are always tethered together to form a closed-ring structure, a gap was clearly present in the ParC-CTD between blades 1 and 6, resulting in the breakage of the six-bladed β-propeller ring (Fig. 1A). The closed ring structures seen in all of the other β-propeller proteins were achieved by one or a combination of the three following ways: 1) disulfide linkages; 2) strand exchange (the so-called Velcro topology); or 3) tight packing between the two end blades (47, 48). In ParC-CTD, no close contacts were observed between end blades 1 and 6, and the resulting \(H_2O\)-filled interblade cleft led directly to the central tunnel. Closer examination revealed that, although blades 2–6 are approximately related around a central pseudo-symmetry axis, blade 1 adopts a different orientation regarding this axis. To our knowledge, ParC-CTD revealed for the first time that a protein can fold as a broken β-propeller without ring closure. Although it has been suggested that circularization may play a critical role for structural stability and in its absence the β-propeller structures may be inherently unstable or prone for misassembly (46, 47), our structure provides an exception to this presumption. Moreover, the arc-shaped appearance of ParC-CTD indicates that the packing angle between the adjacent blades contributes significantly to the overall structural curvature.

Another prominent feature of ParC-CTD resides in the complex topology of its propeller blades (Fig. 1B). This novel folding pattern was first observed in the GyrA-CTD β-pinwheel, and an unusual type of four-stranded repeating unit that conforms to the Greek key connectivity is defined by Corbett et al. (30) to
highlight the overall pinwheel topology. However, although subdividing GyrA-CTD into Greek key modules is useful for illustrating the repeating units in the primary sequence level, it is clear that each module does not correspond to a single β-sheet as in other β-propeller structures. Such a depiction consequently emphasizes the differences and underscores the high degree of structural similarity between GyrA-CTD β-pinwheel and other β-propeller structures. To be consistent with the nomenclature used to describe the canonical β-propeller structures, we feel that a single β-sheet in GyrA-CTD and ParC-CTD should be used to define a propeller blade. We suggest that the type of β-sheets observed in GyrA-CTD and ParC-CTD can be referred to as hairpin-invaded β-propeller blades. As shown in Fig. 1B, with the exception of those involved in Velcro topology, the canonical β-propeller blade is comprised of a continuous string of residues that form a four-stranded antiparallel β-sheet with a simple up-down-up-down topology (45–47). In contrast, if the constituent β-strands of each ParC-CTD blade are named according to their spatial order regardless of the sequence connectivity using the nomenclature adapted from canonical β-propeller structures, only the middle strands (B^N and C^N) of the ParC-CTD blades are contiguous in the primary structure (Fig. 1B). The connectivity of the Nth blade of ParC-CTD starts from the middle β-hairpin B^N—C^N. The polypeptide then reaches back and forms a long guarding loop and a short β-strand (strand D^N−1) that constitutes the outer edge of the previous (N−1)th blade before traveling back to form strand A^N. Following the innermost strand A^N, the polypeptide chain extends forward into the next (N+1)th blade to form its middle β-hairpin B^N+1—C^N+1 and then comes back again to form the guarding loop and the last strand D^N to complete the folding of the Nth blade. Although the two blades shown in Fig. 1B appear very different, the interconversion between them can be straightforward if the blades of ParC-CTD are viewed as being hairpin-invaded. Specifically, instead of forming hydrogen bonds with strands A^N and D^N to complete their folding into the Nth blade with regular topology, the β-hairpin B^N+1—C^N+1, which succeeds strand A^N in the primary sequence (highlighted in blue in Fig. 1B) appeared to be displaced from the Nth blade due to the invasion of β-hairpin B^N—C^N (highlighted in green in Fig. 1B) coming from the previous (N−1)th blade. A unique structural consequence that stems from this hairpin-invaded topology is pervasive strand exchange between adjacent blades, giving ParC-CTD structure with five and six tandem Velcro systems, respectively. In addition, compared with canonical β-propeller structures where two adjacent blades are connected by a single covalent linkage between strands D^N−1 and A^N, the adjacent pair of blades of ParC-CTD are connected by three covalent linkers in between strands A^N and B^N+1, strands C^N and D^N−1, and strands D^N−1 and A^N (Fig. 1B). Both of these structural features suggest that ParC-CTD should be quite stable.

Because invasion of a β-strand(s) into existing β-sheets has been viewed as a common mechanism for triggering fold change (49), it is reasonable to hypothesize that ParC-CTD of B. stearothermophilus may have evolved from an ancestral six-bladed β-propeller structure via a concerted β-hairpin invasion-mediated fold change event. Even if this presumed structural change appears extremely drastic, the energy required for making this transition may not be too overwhelming because the interblade hydrophobic cores of a canonical β-propeller can be properly restored in the resulting hairpin-invaded β-propeller, thus warranting the possibility of such an evolutionary path. Moreover, other conserved features in a canonical β-propeller that include structurally conserved aspartate residues and the reverse turn between strands B and C (46) are also preserved in GyrA-CTD and the Topo IV-CTD. The fact that hairpin-invaded β-propellers have not been identified until very recently might be because the chance for a concerted β-hairpin invasion-induced fold change is indeed infrequent. However, it remains possible that the global similarity shared between β-propeller structures and ParC-CTD is simply due to convergent evolution, as was proposed for GyrA-CTD (30).

Surface Features of ParC-CTD—Although it has long been recognized that the unique supercoiling activity of DNA gyrase is dependent on the DNA binding/bending capability of GyrA-CTD (26, 30, 32), the functional role of ParC-CTD is not fully understood. Surprisingly, recent results obtained from FRET experiments indicated that both GyrA-CTD and ParC-CTD are capable of bending DNA, despite GyrA-CTD being more effective (30). It also was proposed based on this finding that ParC-CTD may facilitate the recognition and positioning of T-segment DNA. To understand how ParC-CTD may interact and bend DNA, its surface electrostatic features were examined. Similar to the predicted DNA binding region of GyrA-CTD (30), a continuous electropositive patch ~25 Å wide and 95 Å in length was identified on the outer surface of ParC-CTD, suggesting a potential binding region for double-stranded DNA (Fig. 2A). The dimension and curvature of this positively charged region appeared suitable for bending DNA, because 36 bp of curved duplex DNA taken directly from the nucleosome core particle (50) can fit nicely along this surface in an in silico docking analysis (Fig. 2B). The helical axis of DNA is bent smoothly by ~180° as the result of ParC-CTD binding, giving a ~65-Å end-to-end distance of the bent DNA. Both values corresponded favorably with the FRET measurement (30). In line with its potential functional significance, an analysis of conserved surface residues revealed that this prospective DNA binding region is comprised of highly conserved residues (Fig. 2C). In addition to its role in DNA bending, it appears that ParC-CTD also mediates protein-protein interactions. Results from bacterial two-hybrid assays indicated that the CTD of E. coli ParC can interact with SeqA protein, a DNA binding modulator of chromosome replication (51). As SeqA was found to preferentially bind hemimethylated DNA and thus localize primarily behind the replication fork, E. coli Topo IV was proposed to be recruited to this general area by SeqA to disentangle precatenanes caused by replication. More interestingly, it was found that SeqA not only affects Topo IV localization but also stimulates the relaxation and decatenation activity of Topo IV. Because the activator role of SeqA requires its DNA binding activity, it seems likely that SeqA-dependent stimulation is achieved by boosting the interaction between ParC-CTD and DNA. Additionally, through direct interaction between ParC and the C-terminal DNA-translocase domain of FtsK, E. coli Topo IV was shown to be concentrated in the septal ring to support segregation of newly replicated daughter chromosomes (14). Similar to the effect exerted by SeqA, the binding of FtsK also enhances Topo IV-catalyzed decatenation. Although SeqA is only found in γ-proteobacteria, FtsK is a widely conserved protein family. It remains to be determined whether ParC-CTD is required for this stimulatory effect by providing an interacting surface for FtsK binding. Since the circumstance of ParC-CTD has been predicted as the DNA binding site, the top and bottom surfaces may further represent potential regions for protein-protein interactions (Fig. 2, A and C), as had been reported for several other β-propeller proteins (52, 53).
introducing DNA bending (30). It is widely believed that, with the strong DNA bending activity of GyrA-CTD, a continuous DNA segment can be constrained by DNA gyrase to adopt a left-handed \("/H_11001\) node before subsequent strand passage converts it into a \("/H_11002\) node to produce \((/H_11002)\) supercoil (26, 28, 30, 32). In contrast, although Topo IV acts preferentially on DNA cross-over with a left-handed superhelical geometry, this enzyme has no negative supercoiling activity (16–19). This phenomenon indicates that the functional role of ParC-CTD, unlike GyrA-CTD, does not involve constraining a continuous piece of DNA into a left-handed cross-over. Instead, this domain may exert its catalytic role by interacting and positioning the T-segment in a left-handed crossing geometry relative to the G-segment to facilitate productive strand passage reaction (30) and the weaker DNA bending activity of ParC-CTD is in fact consistent with the physiological role of Topo IV as a relaxing and decatenating enzyme.

The structure of ParC-CTD provides two indications that suggest why GyrA-CTD bends DNA more efficiently. The most prominent difference between the two is that ParC-CTD does not fold as a closed-ring structure as GyrA-CTD (Fig. 3A). Therefore, the proposed DNA binding region of GyrA-CTD has a steeper surface curvature than that of ParC-CTD, which may account for their different bending efficiency. Because the FRET measurements suggested that DNA was bent by both domains to a similar extent (30), the possibility remains that ParC-CTD might undergo a conformational change upon DNA binding, resulting in ring closure. However, such a ring closure event has to be accompanied by a free energy penalty because the solvent-exposed surfaces on blades 1 and 6 to be buried upon their packing are both negatively charged (Fig. 2A). Another difference between the two structures is that the DNA binding surface of GyrA-CTD of \(B.\ burgdorferi\) is more electropositive, a property that correlates with its better DNA bending capability. Despite this fact, the net positive charge on the DNA binding surface is unlikely to be a critical determinant for DNA bending efficiency because the surface electrostatic features calculated from the homology model of \(E.\ coli\) GyrA-CTD (data not shown) closely resembles that of ParC-CTD reported here (Fig. 2A). Likewise, homology modeling suggested that ParC-CTDs from certain species, such as \(C.\ perfringens\) and \(M.\ mycoides\), have highly electropositive surfaces. Taken together, we suspect that the broken-ring structure may play a dominant role in defining the functional property of ParC-CTD.

If the structure/function relationship mentioned above does exist, the functional significance of a conserved DNA gyrase-specific short amino acid sequence termed GyrA box (34) can be readily inferred. This signature sequence motif of DNA gyrase was identified by multiple sequence alignment and was used as one of the criteria to determine whether a newly discovered bacterial type IIA enzyme should be annotated as a DNA gyrase or a Topo IV. For GyrA-CTD, ring closure was mediated mainly by the strand exchange between blades 1 and 6 in which the loop follows strand C1 wraps and packs against the strand C6 (Fig. 3B). Because GyrA box (highlighted in red in Fig. 3B) resides in the middle of this loop and makes extensive interactions with the core residues of blade 6, it is reasonable to expect
that the closed ring structure of GyrA-CTD would be significantly destabilized when the GyrA box is missing, thus resulting in a broken β-propeller as observed in ParC-CTD. Besides this evident structural role, the electropositive GyrA box located at the entrance of the proposed DNA binding region may be actively involved in shaping the local DNA geometry into a left-handed cross-over by initiating DNA wrapping. A lack of GyrA box makes DNA segments that directly flank the G-segment unable to wrap around ParC-CTD, and only 35 bp of DNA were protected in the footprinting assay (27). Our analysis supports the use of GyrA box as a key determinant in the functional annotation of newly sequenced bacterial genomes.

**Roles of CTD in Topo IV Catalysis**—Unlike eukaryotic type IIA Topos, which exhibit similar activities on (+) and (−) supercoiled DNA substrates, it is well documented that Topo IV can distinguish the spatial arrangement of the T- and G-segments imposed by the superhelix and preferentially resolves left-handed cross-overs of (+) supercoiled DNA (17–19). Furthermore, the activity of Topo IV is sensitive to the superhelical density (σ) (as defined by Bates and Maxwell (54)). The relaxation rate is proportional to higher value of (+) σ, whereas higher (−) σ has an opposite effect on relaxation rate (18). Based on these unique functional properties of Topo IV and the apparent DNA binding capability of its CTD, we proposed the following models to illustrate how ParC-CTD might affect various reactions catalyzed by Topo IV (Fig. 4). During the relaxation of (+) supercoiled substrate, the T-segment was placed at a left-handed cross-over position suitable for rapid duplex passage reaction (Fig. 4A). Although ParC-CTD interacts with the T-segment DNA in this case, this domain is probably dispensable for relaxation because the catalytic productive cross-over is already imposed by the (+) superhelix. Higher (+) σ accelerates relaxation by facilitating the formation of such a DNA-crossing geometry. In contrast, by favoring a right-handed cross-over, (−) supercoiled DNA is usually a poor substrate for relaxation (Fig. 4B-1). However, the non-productive binding mode can be converted into a catalytic proficient left-handed node with the help of ParC-CTD (Fig. 4B-2) and the slower relaxation rate is probably because of the requirement for this additional step. Negative σ values discourage relaxation by inhibiting this essential conformational change of DNA. Similarly, ParC-CTD may facilitate decatenation by positioning the T- and G-segments into a preferred orientation for duplex passage reaction (Fig. 4C). Taken together, we suspect that ParC-CTD plays different roles during Topo IV catalysis. Whereas this weak DNA binding/bending domain may be dispensable for the relaxation of (+) supercoils, it might be actively involved in the decatenation and relaxation of (−) supercoils by serving as a geometry facilitator (see “Addendum”).

**Evolution of Prokaryotic Type IIA Topoisomerases**—Although it was suggested that DNA gyrase probably evolved from Topo IV, the phylogenetic relationships between them remain to be further explored (30). The phylogenetic trees of type IIA Topo family described in Clusters of Orthologous Groups (COG0188, see Ref. 55) and by Gadelle et al. (20) are generally consistent with the widely accepted evolutionary trends (56). Three major subgroups of type IIA Topos with predictable CTD structures according to GyrA-CTD (30) and our ParC-CTD can be identified in the tree. All of the GyrA-CTDs are clustered together and appear to be with a six-bladed closed ring β-propeller, whereas Topo IV-CTDs are less conserved. In the branch of Gram-positive bacteria, Topo IV-CTDs revealed as a broken propeller with six blades, as we reported here. This structure feature is also predicted to present in the Topo IV-CTDs from bacteria in the deep branches of the phylogenetic tree (including Bacteroides, Cytophaga, and Porphyromonas), although their sequences are more divergent (data not shown). Conversely, Topo IV-CTDs in proteobacteria (in-
including α-, β-, and γ-subtypes) possibly contain only five β-sheet modules. The degree of sequence similarity in catalytic domains also corresponds to their CTDs. These analyses imply that the ancestral Topo IIA is Topo IV-like, carrying a CTD domains also corresponds to their CTDs. These analyses imply that the ancestral Topo IIA is Topo IV-like, carrying a CTD domains also corresponds to their CTDs. These analyses imply that the ancestral Topo IIA is Topo IV-like, carrying a CTD domains also corresponds to their CTDs. These analyses imply that the ancestral Topo IIA is Topo IV-like, carrying a CTD domains also corresponds to their CTDs. These analyses imply that the ancestral Topo IIA is Topo IV-like, carrying a CTD domains also corresponds to their CTDs. These analyses imply that the ancestral Topo IIA is Topo IV-like, carrying a CTD domains also corresponds to their CTDs. These analyses imply that the ancestral Topo IIA is Topo IV-like, carrying a CTD domains also corresponds to their CTDs. These analyses imply that the ancestral Topo IIA is Topo IV-like, carrying a CTD domains also corresponds to their CTDs. These analyses imply that the ancestral Topo IIA is Topo IV-like, carrying a CTD domains also corresponds to their CTDs.