Mechanisms for Defining Supercoiling Set Point of DNA Gyrase Orthologs

I. A NONCONSERVED ACIDIC C-TERMINAL TAIL MODULATES ESCHERICHIA COLI GYRASE ACTIVITY

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**Background:** The mechanisms controlling DNA supercoiling efficiency by gyrase are not understood.

**Results:** A nonconserved C-terminal tail in GyrA controls DNA binding, wrapping, and supercoiling set point.

**Conclusion:** The tail is a novel regulatory element that modulates gyrase activity.

**Significance:** Intrinsic properties of gyrase can be fine-tuned to boost enzyme output.

DNA topoisomerases manage chromosome supercoiling and organization in all cells. Gyrase, a prokaryotic type IIA topoisomerase, consumes ATP to introduce negative supercoils through a strand passage mechanism. All type IIA topoisomerases employ a similar set of catalytic domains for function; however, the activity and specificity of gyrase are augmented by a specialized DNA binding and wrapping element, termed the C-terminal domain (CTD), which is appended to its GyrA subunit. We have discovered that a nonconserved, acidic tail at the extreme C terminus of the *Escherichia coli* GyrA CTD has a dramatic and unexpected impact on gyrase function. Removal of the CTD tail enables GyrA to introduce writhe into DNA in the absence of GyrB, an activity exhibited by other GyrA orthologs, but not by wild-type *E. coli* GyrA. Strikingly, a “tail-less” gyrase holoenzyme is markedly impaired for DNA supercoiling capacity, but displays normal ATPase function. Our findings reveal that the *E. coli* GyrA tail regulates DNA wrapping by the CTD to increase the coupling efficiency between ATP turnover and supercoiling, demonstrating that CTD functions can be fine-tuned to control gyrase activity in a highly sophisticated manner.

DNA topoisomerases are essential enzymes that help counteract the topological effects of nucleic acid transactions such as transcription, replication, and repair (1). Nearly all bacteria, and some archaea, possess a unique topoisomerase, termed gyrase, which negatively supercoils DNA to maintain chromosomes in an underwound state (2, 3). Gyrase belongs to the type IIA family of topoisomerases, which are distinguished in part by their use of an ATP-dependent, duplex DNA strand-passage mechanism (1). Gyrase is an $A_B$ heterotetramer whose supercoiling activity requires a domain (the “CTD”)$^2$ that resides at the C terminus of the GyrA subunit (see Fig. 1A) (2). The GyrA CTD forms a disc- or spiral-shaped DNA binding element that constrains a positive supercoil by wrapping a duplex around its surface (5–7). This bend, in the context of the gyrase holoenzyme, allows for the introduction of two negative supercoils upon strand passage (8, 9).

The GyrA CTD constitutes a critical appendage that distinguishes gyrase from its other type IIA cousins. A paralogous bacterial enzyme, topo IV, possesses a domain that is related to the gyrase CTD (10); however, the topo IV CTD is degenerate and always lacks one or more structural features of its GyrA counterpart (11–13). Eukaryotic and phage T4 topo II appear to lack a version of the gyrase/topo IV CTD entirely. These structural differences bestow distinct activities on the different type IIA topoisomerase classes. For instance, topo IV preferentially decatenates interlocked DNA segments and removes positive DNA supercoils more efficiently than negative supercoils (14, 15). As with gyrase, this bias in activity is dependent upon its CTD (12). By contrast, topo II tends not to distinguish between the types of substrates on which it acts (16, 17), with human topo IIα being an exception (18). Thus far, it is unclear how specific evolutionary modifications to the CTD help modulate the substrate selectivity and functional output of bacterial type IIA topoisomerases. As a consequence, the extent to which the steady-state supercoiling level of the bacterial chromosome is influenced by the possession of a particular complement of type I and II topoisomerases (19, 20), *versus* specific augmentations to those topoisomerases, is poorly understood.

In comparing the supercoiling properties of *Mycobacterium tuberculosis* (Mtb) and *E. coli* gyrase, we discovered that the isolated GyrA proteins of the two species differ dramatically in their respective abilities to wrap DNA (see accompanying article (46)). Further analysis of this distinction led us to probe the function of the nonconserved stretch of amino acids that follows the CTD in *E. coli* GyrA (see Fig. 1B). We unexpectedly

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2 The abbreviations used are: CTD, C-terminal domain; topo, topoisomerase; AMP-PNP, adenosine 5’-($\beta$,γ-imino)triphosphate.
found that removal of either the entirety or an internal portion of the tail bestows *E. coli* GyrA with the capacity to wrap DNA, an activity exhibited by *Mtb* GyrA, but not by the wild-type *E. coli* protein. The isolated, full-length *E. coli* GyrA CTD is also unable to wrap, or even bind DNA, whereas ablating or trimming the tail restores these functions. Interestingly, alterations to the CTD tail have no effect on either basal or DNA-stimulated ATPase activity, but greatly reduce both the rate of negative supercoiling and the final level of superhelical density that can be introduced by gyrase. These findings indicate that species-specific appendages to the GyrA CTD can regulate its function in the context of the gyrase holoenzyme, thereby ensuring that ATP turnover is tightly coupled to supercoiling efficiency.

**EXPERIMENTAL PROCEDURES**

**Protein Purification**—A truncated *E. coli* GyrA CTD construct (residues 531–853) (5, 6), as well as full-length *E. coli* gyrA (1–875) and gyrB (1–804) genes, were cloned into pET28b. The full-length *E. coli* GyrA CTD (531–875), along with “insert-less” *E. coli* gyrA and the GyrA CTD (both missing residues 842–856), were amplified from the gyrA pET28b vector and cloned into a derivative of pET28b using an in-house ligation-independent cloning vector system (pLIC) behind an N-terminal, tobacco etch virus protease-cleavable hexahistidine tag. Proteins were expressed in *E. coli* BL21-Codon-Plus(DE3)-RIL cells (Stratagene) by inducing log-phase cells with isopropyl-β-D-thiogalactoside overnight at 18 °C. Cells were harvested by centrifugation, resuspended in 20 mM Tris-HCl, pH 7.9, 800 mM NaCl, 30 mM imidazole, 10% glycerol, and protease inhibitors (1 μM leupeptin, 1 μM pepstatin A, and 1 mM phenylmethylsulfonyl fluoride), and then frozen dropwise in liquid nitrogen for storage at −80 °C.

For purification, cells were sonicated and centrifuged, and the clarified lysate was passed over an Ni²⁺ affinity column (Amersham Biosciences). The His-tagged protein was eluted with 20 mM Tris-HCl, pH 7.9, 100 mM NaCl, 500 mM imidazole, 10% glycerol, and protease inhibitors (1 μM leupeptin, 1 μM pepstatin A, and 1 mM phenylmethylsulfonyl fluoride), and then frozen in liquid nitrogen for storage at −80 °C.

**DNA Binding Assays**—DNA binding was determined by fluorescence anisotropy using a random, 37-bp segment with 40% GC content as a substrate. Annealed oligonucleotides 5′-TAATGCTAGAGACAACCGATACTCAGGATTTA3′ and 5′-56-FAMTAATCCTCGATCTAGTGAAGTCTCTATGAACGTTTTA3′ (where 56-FAM indicates the position of a carboxyfluorescein dye for visualization) were purchased from Integrated DNA Technologies and resuspended in TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0). Varying amounts of *E. coli* GyrA full-length CTD, insert-less CTD, and tail-less CTD were incubated with 20 nM of fluorescently labeled duplex oligonucleotide at room temperature in the dark in 20 mM Tris-HCl, pH 7.5, 70 mM KCl, 10% glycerol, and 1 mM MgCl₂. Fluorescence anisotropy measurements were performed using a Victor 3V (PerkinElmer Life Sciences) multilabel plate reader. Data points represent the average of three independent measurements, where error bars represent the S.D. between measurements (see Figs. 3 and 7). Binding curves were fit to a simplified version of the single site binding equation that holds for our experimental conditions using Kaleidagraph version 4.0 (Synergy software)

\[
\theta = \frac{[L]}{K_{d,app} + [L]} \quad \text{(Eq. 1)}
\]

where θ represents the fraction of ligand binding sites filled, \(K_{d,app}\) is the apparent dissociation constant, and \([L]\) is the ligand concentration.

**DNA Relaxation and Supercoiling Assays**—pSG483, a derivative of pUC19 containing a unique Nb.BbvCI nicking site, was used for supercoiled and relaxed DNA substrates. Negatively supercoiled plasmid was purified from *E. coli* with a maxiprep kit (Macherey-Nagel). Relaxed plasmid was made using nick ligation by first nicking maxipreped plasmid DNA with Nb.BbvCI and then ligating with T4 DNA ligase. DNA supercoiling or relaxation assays (30 μl) were performed in a buffer containing 15 mM Tris-HCl, pH 7.5, 13% glycerol, 6 mM MgCl₂, 0.1 mg/ml BSA, 70 mM KCl, and 300 ng of (6 nM) nicked pSG483 as a DNA substrate. Varying amounts of protein were added to reaction mixtures (30 μl total volume) and equilibrated at 37 °C for 20 min, after which 60 units of ligase and 1 mM ATP were added. Reactions were allowed to incubate at 37 °C for an additional 30 min and then stopped by the addition of SDS (1% final concentration), EDTA (10 mM final concentration), and proteinase K (50 μg/ml final concentration) followed by incubation at 37 °C for 30 additional minutes. Reactions were analyzed by electrophoresis through 1.0% agarose gels (Invitrogen) with 1× TAE (40 mM Tris-acetate 1 mM EDTA) running buffer. Gels were run at 1.7 V/cm for 19–21 h, stained with ethidium bromide (EtBr), and visualized by UV trans-illumination.

To capture a gyrase holoenzyme “topology footprint,” we used an ATP-independent assay technique to prevent gyrase from supercoiling the plasmid DNA substrate. In these reactions, gyrase holoenzyme was incubated with relaxed plasmid and eukaryotic topoisoamerase IB, which relaxes DNA supercoils in the absence of ATP (22, 23). Holoenzyme writhes assays were performed in the presence and absence of 2 mM AMP-PNP using relaxed pSG483 plasmid and topoisomerase IB purified in-house from wheat germ (24).

**Topological Footprinting Assays**—The introduction of DNA writhe by various gyrase domains and subunits was assessed in a buffer containing 15 mM Tris-HCl, pH 7.5, 13% glycerol, 6 mM MgCl₂, 0.1 mg/ml BSA, 70 mM KCl, and 300 ng of (6 nM) nicked pSG483 as a DNA substrate. Varying amounts of protein were added to reaction mixtures (30 μl total volume) and equilibrated at 37 °C for 20 min, after which 60 units of ligase and 1 mM ATP were added. Reactions were allowed to incubate at 37 °C for an additional 30 min and then stopped by the addition of SDS (1% final concentration), EDTA (10 mM final concentration), and proteinase K (50 μg/ml final concentration) followed by incubation at 37 °C for 30 additional minutes. Reactions were analyzed by electrophoresis through 1.0% agarose gels (Invitrogen) with 1× TAE (40 mM Tris-acetate 1 mM EDTA) running buffer. Gels were run at 1.7 V/cm for 19–21 h, stained with ethidium bromide (EtBr), and visualized by UV trans-illumination.

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and 300 ng (6 nM) of DNA substrate; ATP was omitted to assess nucleotide-independent relaxation. Varying amounts of reconstituted holoenzyme were added to reaction mixtures, and reactions were allowed to proceed for 30 min at 37 °C. Time course reactions contained 4 mM ATP; for times longer than 30 min reactions were allowed to proceed for 30 min at 37 °C. Time course reactions contained 4 mM ATP; for times longer than 30 min reactions were allowed to proceed for 30 min at 37 °C. Time course reactions contained 4 mM ATP; for times longer than 30 min reactions were allowed to proceed for 30 min at 37 °C. Time course reactions contained 4 mM ATP; for times longer than 30 min reactions were allowed to proceed for 30 min at 37 °C. Time course reactions contained 4 mM ATP; for times longer than 30 min reactions were allowed to proceed for 30 min at 37 °C. Time course reactions contained 4 mM ATP; for times longer than 30 min reactions were allowed to proceed for 30 min at 37 °C. Time course reactions contained 4 mM ATP; for times longer than 30 min reactions were allowed to proceed for 30 min at 37 °C. Time course reactions contained 4 mM ATP; for times longer than 30 min reactions were allowed to proceed for 30 min at 37 °C. Time course reactions contained 4 mM ATP; for times longer than 30 min reactions were allowed to proceed for 30 min at 37 °C. Time course reactions contained 4 mM ATP; for times longer than 30 min reactions were allowed to proceed for 30 min at 37 °C. Time course reactions contained 4 mM ATP; for times longer than 30 min reactions were allowed to proceed for 30 min at 37 °C. Time course reactions contained 4 mM ATP; for times longer than 30 min reactions were allowed to proceed for 30 min at 37 °C. Time course reactions contained 4 mM ATP; for times longer than 30 min reactions were allowed to proceed for 30 min at 37 °C. Time course reactions were performed in triplicate. The ATP titration data were fit to the Michaelis-Menten model, 

\[
V = \frac{V_{\text{max}} [S]}{K_d + [S]},
\]

where \(V\) is the reaction rate, \(V_{\text{max}}\) is the maximum rate, \([S]\) is the total starting substrate concentration, and \(K_d\) is the Michaelis-Menten constant. The DNA titration data were fit to the Hill equation, 

\[
V = \frac{V_{\text{max}} [S]^h}{(K_d^h + [S]^h)},
\]

where \(V\) is the reaction rate, \(V_{\text{max}}\) is the maximum rate, \([S]\) is the total substrate concentration, \(h\) is the Hill coefficient, and \(K_d\) is the apparent dissociation constant.

**RESULTS**

**Control of DNA Wrapping by Negatively Charged E. coli GyrA**

**Tail**—The ability of gyrase to wrap large segments of DNA (~120–140 bp) has been well established for over 30 years (9). Wrapping is known to be a property of the GyrA subunit and its C-terminal domain (5, 6); however, biochemical studies of E. coli GyrA by Reece and Maxwell (5) have shown that the full-length protein is unable to introduce writhe into DNA on its own. Although this finding at the time did not elicit additional investigation, it is surprising in retrospect as several studies have shown that isolated GyrA CTDs are readily capable of wrapping DNA, in some instances by nearly a full turn (5–7, 25).

The dichotomy between the wrapping behaviors of E. coli GyrA and its CTD came to our attention when we found that MtbGyrA imparts writhe on its own as efficiently as its isolated CTD (see accompanying article (46)). In the course of investigating this distinction, we realized that one source of variation between the CTDs of the two species, and between bacterial GyrA proteins in general, resides in the extreme C terminus of the subunit (Fig. 1B). This element, referred to hereafter as the GyrA tail, is highly acidic in both E. coli and MtbGyrA (the...
predicted pI of each is ~3.0), but is significantly shorter in the latter. In other species, we found that the GyrA tail greatly varies in length, or is absent entirely (e.g. Borrelia burgdorferi and Thermotoga maritima). We might have dismissed these small differences as trivial were it not for a second source of variation that came to our attention; namely, every published study concerning the wrapping behavior of the isolated E. coli GyrA CTD has been conducted with constructs that lack a portion of the full-length domain, several of which include tail truncations (5, 6, 25).

To determine whether the tail plays a role in modulating wrapping by E. coli gyrase, we first examined its effect on the biochemical properties of the isolated CTD and of GyrA. Secondary structure predictions using PSIPRED indicate that the GyrA CTD tails are disordered (not shown), explaining why they have been either truncated or unobservable in the available crystal structures (6, 25, 26) (see also accompanying article (46)). We therefore created a series of tail deletions that lacked either an internal acidic segment or the entirety of the nonconserved region.

We initially assessed the ability of the CTD to wrap DNA using topology footprinting. In this assay, the amount of writh imparted by a protein is assessed by incubating varying amounts of the protein with nicked plasmid DNA. The subsequent addition of ligase seals the nicked plasmid, leaving the DNA with an “afterimage” of the number of supercoils originally constrained upon binding. Notably, the full-length E. coli GyrA CTD was completely unable to introduce any writh into plasmid DNA (Fig. 2A). By contrast, both the insert-less CTD and complete tail deletion exhibited the robust DNA wrapping activity seen by other groups. A similar examination of E. coli GyrA reproduced the lack of observable wrapping by the full-length protein as first noted by Reece and Maxwell (5) (Fig. 2B). As with the CTD alone, removal of the internal tail region resulted in extensive wrapping by the GyrA truncated construct (Fig. 2B), as did complete ablation of the tail (not shown).

**Regulation of DNA Binding by GyrA Tail**—Because removal of the GyrA tail actually led to a gain of wrapping function, we surmised that our alterations were not impairing the activity of the CTD, but rather eliminating an internal repressive element. The fact that the CTD responded to the deletions in a manner analogous to full-length GyrA in turn implied that at least part of the control was at the level of the CTD itself, and not simply due to an ability of GyrA to sequester the CTD from DNA in a tail-dependent manner.

To test this idea further, we set out to compare the abilities of the wild-type and truncated CTDs to bind DNA using fluorescence anisotropy. In this assay, short, fluorescently labeled DNA oligonucleotides were incubated with varying amounts of each purified E. coli CTD construct, and binding affinity was measured as a function of the change in the relative amount of circularly polarized light that is emitted by the sample (27). Analysis of the data from this assay showed clearly that both the tailless and the insert-less CTD constructs were competent to bind the DNA oligonucleotide with similar affinities (Kd values are as follows: tail-less 90 nM; insert-less, 42 nM; full-length, not determined).

**Effects of Tail on DNA Wrapping by Gyrase Holoenzyme**—Because the E. coli gyrase holoenzyme readily wraps DNA (28) and because GyrA alone is unable to do so (5), we reasoned that the binding of GyrB might be capable of relieving the repressive action of the CTD tail. An additional line of evidence support-
Impact of GyrA Tail on DNA Supercoiling by Gyrase—Given the marked effect on the control of DNA wrapping by the GyrA tail, we next assayed the impact of tail deletions on strand passage. To assess this function, we reconstituted tetrameric gyrase using either insert-less or wild-type GyrA, together with wild-type GyrB, and compared titrations of the mutant and wild-type enzymes in gyrase activity assays. We first looked at the ATP-dependent introduction of negative supercoils into relaxed plasmid DNA using native agarose gel electrophoresis over a range of holoenzyme concentrations. Notably, comparison of wild-type and insert-less gyrase showed that deletion of the CTD tail insert reduced the specific activity of supercoiling by more than 50-fold (Fig. 5A, upper). Deletion of the entire CTD tail produced a similar effect to that seen by the insert-less mutant (not shown). Because the insert-less GyrA protein used in this assay binds and wraps DNA extensively on its own (Fig. 2B) and because the same GyrB preparation was used for both the mutant and the wild-type supercoiling experiments, the most parsimonious explanation for this effect is that the GyrA tail is necessary for the robust activity normally displayed by the full-length E. coli holoenzyme.

To gain more insights into this reduction in catalytic ability, we asked whether the supercoiled DNA produced by the wild-type and insert-less enzymes was underwound to a similar extent. This property was assessed by running the same supercoiling reactions on agarose gels in the presence of 3 μg/ml chloroquine. Chloroquine is a weak intercalating agent that overwinds DNA to permit the separation of supercoiled topoisomers; at appropriate concentrations, chloroquine can help resolve negatively supercoiled species that would otherwise cluster in a single high mobility band on a native gel (32). Analysis of the topoisomer distribution by this method shows that the activity of the insert-less enzyme gave rise to DNA species that were much less negatively supercoiled than the products of wild-type gyrase (Fig. 5A, lower).

To discern whether the apparent reduction in supercoiling levels attained by the insert-less gyrase was due to a slow rate of enzyme turnover, which could prohibit the reaction from going to completion within our standard reaction time (30 min), we assayed supercoiling over a range of different periods. The reconstituted mutant E. coli gyrase was added to relaxed plasmid DNA and ATP at a concentration where complete supercoiling was seen in our enzyme titration experiments (20 nM) and incubated at 37 °C for 5, 10, 30, and 90 min; wild-type E. coli gyrase was compared as a control. Time points that exceeded 30 min were supplemented with additional ATP to prevent ATP-independent relaxation events from occurring, and the results of this assay were again visualized using agarose gel electrophoresis in the absence and presence of 3 μg/ml chloroquine. We found that even when given extremely long amounts of time, the insert-less E. coli gyrase was unable to negatively supercoil the plasmid DNA to the same extent as the wild-type enzyme (Fig. 5B). Interestingly, the degree of supercoiling manifested by the mutant approximated that seen for wild-type gyrase at the shortest time assayed (5 min), indicating that deletion of the tail did not simply slow the rate of strand passage. Rather, this element appears to have a direct and profound effect on the superhelical density “set point” of the enzyme.
In addition to negatively supercoiling DNA, gyrase can relax negatively supercoiled DNA in an ATP-independent manner (33). Although the physiological role of this reaction is unclear, it does provide another window into understanding the overall mechanism of the enzyme. Moreover, other type II topoisomerases such as topo II and topo IV fail to exhibit ATP-independent relaxation, suggesting that the unique DNA wrapping properties of gyrase may play a role in this activity. To assess the effect of the tail on the relaxation of negatively supercoiled DNA by gyrase, we titrated different amounts of either the insert-less mutant or wild-type gyrase against a fixed amount of DNA in the absence of ATP. After 30 min, the products of this reaction were visualized using native agarose gel electrophoresis. As with supercoiling, the insert-less mutant proved to be significantly less active (>10-fold) than wild-type enzyme. This finding indicates that either robust wrapping by the gyrase holoenzyme, the control of wrapping, or both is critical to its ATP-independent relaxation activity (Fig. 6).

Effect of the Tail on Gyrase ATPase Activity—Because GyrB and its ATPase status were the only factors previously known to variably modulate DNA wrapping by E. coli gyrase, we next asked whether the tail was an important factor in nucleotide turnover. To address this question, the ATPase activities of wild-type and insert-less E. coli gyrase were assessed using a malachite green colorimetric assay in which ATP hydrolysis is measured by phosphate release (34). We first conducted these measurements by incubating reconstituted gyrase with increasing amounts of ATP at a fixed concentration of sheared salmon-sperm DNA; the addition of DNA is known to dramatically stimulate the ATPase activity of the enzyme (Fig. 7A) (35). We then varied the amount of DNA present in the reaction while holding the initial concentration of ATP constant (Fig. 7B). Finally, we performed the reactions for different amounts of time using a fixed starting concentration of DNA and ATP. In all instances, the insert-less gyrase hydrolyzed ATP comparably to wild-type gyrase, demonstrating that alteration of the GyrA tail does not have an adverse effect on ATP turnover by gyrase. The findings further argue against the observed loss of supercoiling activity by the insert-less mutant as having arisen from simple inactivation of the enzyme. Instead, given our observation that gyrases lacking some or all of the tail are impaired for DNA wrapping and supercoil introduction in the context of the holoenzyme (but not with the isolated GyrA subunit or CTD), these data suggest that the tail is a linkage element that allows gyrase to

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efficiently couple ATP turnover to productive strand passage events.

**DISCUSSION**

Despite extensive study, the mechanisms used to modulate and tune topoisomerase function are still poorly understood. Here, we have uncovered a new control element that both promotes turnover efficiency and helps lock the set point for DNA supercoiling by an archetypal DNA remodeling enzyme, *E. coli* gyrase. Like any molecular machine, gyrase is composed of multiple moving parts and complementary activities that collaborate to support function. We have found that a nonconserved, acidic tail appended to GyrA regulates DNA binding and wrapping by an auxiliary domain at the C terminus of the subunit. The deletion of small internal segments of the tail, or its ablation, actually permits the CTD to engage and deform DNA, either on its own or in the context of GyrA, indicating that the tail is an autoinhibitory element (Figs. 2 and 3). It has been shown previously that GyrB can both restore and re-inhibit DNA wrapping by GyrA, depending on its ATPase status (29); we now find that this regulation depends on the presence of a fully intact GyrA tail (Fig. 4). Strikingly, removal of the tail decreases the rate and extent of DNA underwinding by gyrase, but does not significantly affect its ATP hydrolysis properties (Figs. 5 and 7). Hence, the tail helps to ensure that the consumption of ATP is tightly coupled to rapid and efficient strand passage.

How might the tail exert these effects physically? The finding that the negatively charged tail prevents the isolated CTD from binding DNA suggests that it acts in cis, perhaps by associating electrostatically with the rim of the domain, which is positively charged in all such domains imaged to date (6, 7, 25). This type of interaction could block DNA from accessing the CTD through steric occlusion; removal of small segments of the tail might prevent this interaction from forming stably. Alternatively, or in addition, the tail might help sequester the CTD away from DNA, perhaps by linking the domain to the rest of the GyrA subunit. The binding of GyrB could potentially relieve autoinhibition of the CTD directly, by engaging the tail to allow DNA wrapping, or allosterically, by inducing a conformational change in GyrA that promotes both CTD release and undocking of the tail to permit DNA engagement.

A scheme outlining these concepts is shown in Fig. 8. Importantly, it makes several predictions that are supported by various lines of experimental evidence. For instance, small x-ray scattering studies have suggested previously that the *E. coli* CTD may associate with the rest of GyrA in a manner that would prevent it from readily engaging a DNA bound to the active site region of the protein (36, 37). Our deletion studies demonstrate that the tail is not only critical for the GyrB-dependent modulation of DNA wrapping by gyrase, but that the extent of holoenzyme-induced wrapping is compromised when the tail is altered (Fig. 4). Given that partial or complete truncation of the tail enables robust wrapping by both the CTD and the GyrA on their own (Fig. 2), these data suggest that the presence of GyrB helps place the CTD in a productive conformation to bind DNA, introduce writhe, and correctly position a DNA segment in cis for strand passage. Direct tethering of the tail to

**FIGURE 7.** ATP hydrolysis by wild-type and insert-less *E. coli* gyrase. A, basal ATPase activity plotted as a function of starting ATP concentration in mM (x axis) and nmol of phosphate produced per minute (y axis). Data were fit to an apparent Michaelis-Menten model (R adj = 0.996; R full = 1.000). B, DNA stimulated ATPase activity plotted as a function of sheared salmon sperm DNA concentration in μM (x axis) and nmol of phosphate produced per minute (y axis). Data were fit to the Hill equation (R adj = 1.000; R full = 1.000). C, ATPase activity time course. Wild-type and insert-less *E. coli* gyrase ATPase activities are plotted as a function of nmol phosphate produced (x axis) and time in minutes (y axis). Phosphate produced during a 50-min time course is approaching the saturation point for the assay.
GyrB would link wrapping to the ATPase cycle and help ensure that turnover and strand passage are efficiently interconnected. Both coupling phenomena have been seen biochemically (30, 38–40), although the CTD tail has not been implicated in the process until now.

Because the GyrA tail is not conserved (Fig. 1), it is unlikely that the mechanism by which it controls E. coli gyrase will be universal. Indeed, differences in supercoiling between gyrase orthologs have been observed previously (41–43) (see also accompanying article (46)) and have been attributed to disparate factors such as alterations in GyrB sequence or CTD structure. These studies indicate that gyrase is not a monolithic entity and that there instead exists a range of gyrase activities that vary in their relative supercoiling efficiencies. They also suggest that rather than being the rule in terms of supercoiling efficiency, E. coli gyrase is a supercharged exception. It is presently unclear how the E. coli GyrA tail might selectively bind to different regions of gyrase to control holoenzyme conformation and function. It is also not understood how or why particular bacterial species adjust and select their respective topoisomerase retinue to achieve a particular level of steady-state supercoiling. Issues such as these are subjects for future investigations.

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REFERENCES
1. Schoeffler, A. J., and Berger, J. M. (2008) DNA topoisomerases: harnessing and constraining energy to govern chromosome topology. Q. Rev. Biophys. 41, 41–101
2. Gellert, M., Mizuuchi, K., O’Dea, M. H., and Nash, H. A. (1976) DNA
gyrase: an enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci. U.S.A. 73, 3872–3876
3. Forterre, P., Girbaldo, S., Gadelle, D., and Serre, M. C. (2007) Origin and evolution of DNA topoisomerases. Biochimie 89, 427–446
4. Kampranis, S. C., and Maxwell, A. (1996) Conversion of DNA gyrase into a conventional type II topoisomerase. Proc. Natl. Acad. Sci. U.S.A. 93, 14416–14421
5. Reece, R. J., and Maxwell, A. (1991) The C-terminal domain of the Escherichia coli DNA gyrase A subunit is a DNA-binding protein. Nucleic Acids Res. 19, 1399–1405
6. Brown, P. O., and Cozzarelli, N. R. (1979) A sign inversion mechanism for enzymatic supercoiling of DNA. Science 206, 1081–1083
7. Corbett, K. D., Schoeffler, A. J., Thomsen, N. D., and Berger, J. M. (2005) The structural basis for substrate specificity in DNA topoisomerase IV. J. Mol. Biol. 351, 545–561
8. Hsieh, T. J., Yen, T. J., Lin, T. S., Chang, H. T., Huang, S. Y., Hsu, C. H., Farh, L., and Chan, N. L. (2010) Twisting of the DNA-binding surface by a β-strand-bearing proline modules DNA gyrase activity. Nucleic Acids Res. 38, 4173–4181
9. McGuffin, L. J., Bryson, K., and Jones, D. T. (2000) The PSIPRED protein structure prediction server. Bioinformatics 16, 404–405
10. Crisona, N. J., Strick, T. R., Bensimon, D., Croquette, V., and Cozzarelli, N. R. (2005) A superhelical spiral in the DNA duplex outside the enzyme. Cell 115, 979–984
11. Ward, D., and Newton, A. (1997) Requirement of topoisomerase IV parE genes for cell cycle progression and developmental regulation in Caulobacter crescentus. Mol. Microbiol. 26, 897–910
12. Tretter, E. M., Lerman, J. C., and Berger, J. M. (2010) A naturally chimeric domain of DNA gyrase A adopts a DNA-bending β-pinwheel fold. Proc. Natl. Acad. Sci. U.S.A. 101, 7293–7298
13. Charvin, G., Bensimon, D., and Croquette, V. (2003) Single-molecule study of DNA unlinking by eukaryotic and prokaryotic type-II topoisomerases. Proc. Natl. Acad. Sci. U.S.A. 100, 22055–22059
14. Shuman, S. (1998) Vaccinia virus DNA topoisomerase: a model eukaryotic type IB enzyme. Biochim. Biophys. Acta 1400, 321–337
15. Champoux, J. J., and Dulbecco, R. (1972) An activity from mammalian cells that untwists superhelical DNA: a possible swivel for DNA replication (polyoma-ethidium bromide-mouse-embryo cells-dye binding assay). Proc. Natl. Acad. Sci. U.S.A. 69, 143–146
16. Duyan, W. S., Jendrisak, J. J., Hager, D. A., and Burgess, R. R. (1981) Purification and characterization of wheat germ DNA topoisomerase I (nick-closing enzyme). J. Biol. Chem. 256, 5860–5865
17. Heddle, J. G., Mitteiheiser, S., Maxwell, A., and Thomson, N. H. (2004) Nucleotide binding to DNA gyrase causes loss of DNA wrap. J. Mol. Biol. 337, 597–610
18. Dynan, W. S., Jendrisak, J. J., and Burgess, R. R. (1981) Purification and characterization of wheat germ DNA topoisomerase I (nick-closing enzyme). J. Biol. Chem. 256, 5860–5865
19. Tretter, E. M., and Berger, J. M. (March 28, 2012) Mechanisms for defining of the DNA-binding interface by transient electric dicthrom. J. Mol. Biol. 193, 555–569
20. McGuffin, L. J., Bryson, K., and Jones, D. T. (2000) The PSIPRED protein structure prediction server. Bioinformatics 16, 404–405
21. Lacowitz, J. R. (2006) Principles of Fluorescence Spectroscopy, Springer-Verlag New York Inc., New York
22. Tretter, E. M., and Berger, J. M. (March 28, 2012) Mechanisms for defining of the DNA-binding interface by transient electric dicthrom. J. Mol. Biol. 193, 555–569
23. Grodzicki, T., Przybylo, E., and Ledochowski, M. (2005) A naturally chimeric domain of DNA gyrase A adopts a DNA-bending β-pinwheel fold. Proc. Natl. Acad. Sci. U.S.A. 101, 7293–7298
24. Tretter, E. M., and Berger, J. M. (March 28, 2012) Mechanisms for defining of the DNA-binding interface by transient electric dicthrom. J. Mol. Biol. 193, 555–569
25. Hsieh, T. J., Yen, T. J., Lin, T. S., Chang, H. T., Huang, S. Y., Hsu, C. H., Farh, L., and Chan, N. L. (2010) Twisting of the DNA-binding surface by a β-strand-bearing proline modules DNA gyrase activity. Nucleic Acids Res. 38, 4173–4181
26. McGuffin, L. J., Bryson, K., and Jones, D. T. (2000) The PSIPRED protein structure prediction server. Bioinformatics 16, 404–405
27. Lacowitz, J. R. (2006) Principles of Fluorescence Spectroscopy, Springer-Verlag New York Inc., New York
28. Tretter, E. M., and Berger, J. M. (March 28, 2012) Mechanisms for defining of the DNA-binding interface by transient electric dicthrom. J. Mol. Biol. 193, 555–569
29. Grodzicki, T., Przybylo, E., and Ledochowski, M. (2005) A naturally chimeric domain of DNA gyrase A adopts a DNA-bending β-pinwheel fold. Proc. Natl. Acad. Sci. U.S.A. 101, 7293–7298
30. Tretter, E. M., and Berger, J. M. (March 28, 2012) Mechanisms for defining of the DNA-binding interface by transient electric dicthrom. J. Mol. Biol. 193, 555–569
31. Grodzicki, T., Przybylo, E., and Ledochowski, M. (2005) A naturally chimeric domain of DNA gyrase A adopts a DNA-bending β-pinwheel fold. Proc. Natl. Acad. Sci. U.S.A. 101, 7293–7298
32. Tretter, E. M., and Berger, J. M. (March 28, 2012) Mechanisms for defining of the DNA-binding interface by transient electric dicthrom. J. Mol. Biol. 193, 555–569
33. Grodzicki, T., Przybylo, E., and Ledochowski, M. (2005) A naturally chimeric domain of DNA gyrase A adopts a DNA-bending β-pinwheel fold. Proc. Natl. Acad. Sci. U.S.A. 101, 7293–7298
34. Tretter, E. M., and Berger, J. M. (March 28, 2012) Mechanisms for defining of the DNA-binding interface by transient electric dicthrom. J. Mol. Biol. 193, 555–569
35. Grodzicki, T., Przybylo, E., and Ledochowski, M. (2005) A naturally chimeric domain of DNA gyrase A adopts a DNA-bending β-pinwheel fold. Proc. Natl. Acad. Sci. U.S.A. 101, 7293–7298
36. Tretter, E. M., and Berger, J. M. (March 28, 2012) Mechanisms for defining of the DNA-binding interface by transient electric dicthrom. J. Mol. Biol. 193, 555–569
37. Grodzicki, T., Przybylo, E., and Ledochowski, M. (2005) A naturally chimeric domain of DNA gyrase A adopts a DNA-bending β-pinwheel fold. Proc. Natl. Acad. Sci. U.S.A. 101, 7293–7298
38. Tretter, E. M., and Berger, J. M. (March 28, 2012) Mechanisms for defining of the DNA-binding interface by transient electric dicthrom. J. Mol. Biol. 193, 555–569
39. Grodzicki, T., Przybylo, E., and Ledochowski, M. (2005) A naturally chimeric domain of DNA gyrase A adopts a DNA-bending β-pinwheel fold. Proc. Natl. Acad. Sci. U.S.A. 101, 7293–7298