Functional interactions between gyrase subunits are optimized in a species-specific manner

Received for publication, July 17, 2019, and in revised form, January 3, 2020. Published, Papers in Press, January 17, 2020, DOI 10.1074/jbc.RA119.010245

Daniela Weidlich and Dagmar Klostermeier

From the Institute for Physical Chemistry, University of Münster, Corrensstrasse 30, D-48149 Münster, Germany

Edited by Karin Musier-Forsyth

DNA gyrase is a bacterial DNA topoisomerase that catalyzes ATP-dependent negative DNA supercoiling and DNA decat- enation. The enzyme is a heterotetramer comprising two GyrA and two GyrB subunits. Its overall architecture is conserved, but species-specific elements in the two subunits are thought to optimize subunit interaction and enzyme function. Toward understanding the roles of these different elements, we compared the activities of Bacillus subtilis, Escherichia coli, and Mycobacterium tuberculosis gyrase and of heterologous enzymes reconstituted from subunits of two different species. We show that B. subtilis and E. coli gyrase are proficient DNA-stimulated ATPases and efficiently supercoil and decatenate DNA. In contrast, M. tuberculosis gyrase hydrolyzes ATP only slowly and is a poor supercoiling enzyme and decatenase. The heterologous enzymes are generally less active than their homolo- gous counterparts. The only exception is a gyrase reconstituted from mycobacterial GyrA and B. subtilis GyrB, which exceeds the activity of M. tuberculosis gyrase and reaches the activity of the B. subtilis gyrase, indicating that the activities of enzymes containing mycobacterial GyrB are limited by ATP hydrolysis. The activity pattern of heterologous gyrase is in agreement with structural features present: B. subtilis gyrase is a minimal enzyme, and its subunits can functionally interact with subunits from other bacteria. In contrast, the specific insertions in E. coli and mycobacterial gyrase subunits appear to prevent efficient functional interactions with heterologous subunits. Understanding the molecular details of gyrase adaptations to the specific physiological requirements of the respective organ- ism might aid in the development of species-specific gyrase inhibitors.

DNA topology affects vital cellular processes, such as a DNA replication and recombination (reviewed in Ref. 1) and gene expression (2, 3). In vivo, DNA topology is regulated by enzymes of the topoisomerase family (reviewed in Ref. 4), which catalyze the relaxation or introduction of negative and positive supercoils or resolve catenanes (reviewed in Ref. 5). Changes in DNA topology require cleavage of one or both strands of the DNA: type I topoisomerases cleave one strand of their DNA substrate, whereas type II topoisomerases cleave both strands (6). Type I topoisomerases are subdivided into type IA and IB according to the strand that remains covalently bound to the enzyme: type IA topoisomerases form a covalent tyrosyl-ester with the 5’-end of the cleaved DNA, type IB enzymes with the 3’-end (7–10). Type II topoisomerases bind covalently to the 5’-ends of the cleaved DNA (6). They are divided into type IIA and IIB subfamilies according to structural similarities (11).

The type IA topoisomerase reverse gyrase and the type IIA topoisomerase gyrase catalyze the introduction of positive or negative supercoils, respectively. The introduction of DNA supercoils is energetically disfavored, and both enzymes couple these reactions to ATP hydrolysis (reviewed in Refs. 12–14). Relaxation of DNA, on the other hand, is an energetically favor- able reaction. Type IB enzymes catalyze relaxation in an ATP- independent reaction through controlled rotation of the DNA (15–17), whereas type II topoisomerases relax DNA in an ATP- dependent reaction through a strand-passage mechanism (reviewed in Ref. 18).

Gyrase is a type IIA topoisomerase present in bacteria, some archaea, and plants that catalyzes DNA supercoiling and decat- enation of DNA in an ATP-dependent reaction (19). In the absence of ATP, gyrase is able to relax negatively supercoiled DNA. Most bacteria contain two type IIA topoisomerases, gyrase and topoisomerase IV (Topo IV).2 In bacteria, gyrase removes positive supercoils that accumulate ahead of replica- tion forks (20), whereas Topo IV decatenates replication intermediates (21). Mycobacteria (22) and other human pathogens, including Campylobacter jejuni, Helicobacter pylori (23), and Treponema pallidum (24), contain only one type IIA topoi- somerase, which has to perform both tasks in vivo (25).

The active gyrase heterotetramer is formed by the assembly of two GyrB subunits and two GyrA subunits (26, 27) (Fig. 1). GyrA consists of an N-terminal part, comprising a winged-helix domain (WHD), a tower domain, and a coiled-coil domain, as well as the C-terminal domain (CTD) (28–30). GyrA is a stable dimer in solution (28, 31). The dimer is stabilized by two protein-protein interfaces, formed by the WHDs and by the globular domains at the end of the coiled-coil domains of GyrA (28).

---

2 The abbreviations used are: Topo IV, topoisomerase IV; CTD, C-terminal domain (of GyrA); G-segment, gate segment; GHKL, GyrB-Hsp90-histidine/serine protein kinases-MutL; n.s., not significant; NTD, N-terminal domain; TOPRIM, topoisomerase-primase; WHD, winged-helix domain; kDNA, kin- etoplast DNA; ADPnP, 5’-adenyl-β,γ-imidodiphosphate.

This work was supported by Deutsche Forschungsgemeinschaft Grant KL1153/9-1. The authors declare that they have no conflicts of interest with the contents of this article.

This article contains supporting Methods, Tables S1–S6, and Figs. S1–S9.

1 To whom correspondence should be addressed: Institute for Physical Chemistry, University of Muenster, Corrensstrasse 30, D-48149 Muenster, Germany. Tel: 49-251-8323410; Fax: 49-251-29138; E-mail: dagmar.klostermeier@uni-muenster.de.

This is an Open Access article under the CC BY license.
Activities of heterologous gyrase

These interfaces are termed the DNA-gate and the C-gate, respectively. The WHDs contain the catalytic tyrosines for DNA cleavage and religation (6). The GyrB subunit harbors an ATPase domain of the GHKL superfamily (GyrB-Hsp90-histidine/serine protein kinases-MutL), a transducer domain, and a topoisomerase-prime (TOPRIM) domain (32, 33). Binding to GyrA is established by the interaction of the GyrB TOPRIM domains with the WHDs of GyrA, which leads to formation of the DNA-gate (28, 31, 34). GyrB dimerizes on binding of ATP or the nonhydrolyzable analog ADP-P to its ATPase domain (32, 35–37). In the gyrase heterotetramer, GyrB dimerization leads to formation of a third protein-protein interface, termed the N-gate (38–40). The N-gate operates as an ATP-dependent clamp that captures a DNA segment during supercoiling and decatenation (41).

Although the overall architecture of gyrase from different organisms is similar, structural studies have revealed subtle organism- or clade-specific differences in both subunits. The presence of these structural differences suggests a fine-tuned species-specific modulation of the functional interplay between GyrA and GyrB subunits and of DNA supercoiling and decatenation activities of gyrase. GyrB from Escherichia coli (and other Gram-negatives) contains a 170-amino acid insertion in its TOPRIM domain (34) that is absent in GyrB from Gram-positives, such as Bacillus subtilis, and in GyrB from Mycobacteria (Fig. 1 and Table 1). This insert forms a globular domain with an α/β fold and provides contacts with the coiled-coil domain of GyrA; it has been implicated in DNA binding and interdomain communication (34). GyrB from Mycobacterium tuberculosis contains a short insertion within the GHKL domain, termed the C-loop, which interacts with a highly negatively charged loop inserted into the tower domain of M. tuberculosis GyrA (42) (Table 1). This interaction might play a role in stabilizing M. tuberculosis gyrase in a resting state with a wide-open N-gate, which may explain the low ATPase activity of the enzyme (42) and might be related to its role in decatenation. GyrA from E. coli contains a 34-amino acid insertion of uncharacterized function in the coiled-coil domain that is not present in B. subtilis gyrase (29) or the mycobacterial enzyme (Table 1). It forms a small globular domain that packs against the coiled coil and the globular domains at their end that form the C-gate (34) and might contribute to C-gate stabilization. The CTDs of GyrA contain C-terminal tails that are species-specific and regulate the propensity of gyrase to wrap DNA and its supercoiling activity in different ways (43–46).

To shed light on the interdomain communication and the species-specific tuning of gyrase activities, we analyzed the DNA-stimulated ATPase, DNA supercoiling, and decatenation activities of B. subtilis, E. coli, and M. tuberculosis gyrase. We compared these activities with the activities of heterologous enzymes reconstituted from subunits from two different organisms. We show that B. subtilis and E. coli gyrase are proficient DNA-stimulated ATPases and efficiently supercoil and decatenate DNA. M. tuberculosis gyrase, on the other hand, exhibits a low ATPase activity that is not DNA-stimulated and shows low supercoiling and decatenation activities. Heterologous enzymes are generally less active than their homologous counterparts, indicating species-specific optimization of the functional interaction between subunits. The only exception is a heterologous gyrase reconstituted from mycobacterial GyrA and B. subtilis GyrB, which has increased activities compared with the homologous M. tuberculosis enzyme, and comparable activities with those of the B. subtilis enzyme. The activity pattern observed for heterologous gyrase can be explained by structural considerations: B. subtilis gyrase is a minimal enzyme, and its subunits can functionally interact with subunits from B. subtilis
from other organisms. For \textit{E. coli} gyrase and mycobacterial gyrase subunits, on the other hand, the species-specific insertions prevent efficient functional interactions with heterologous subunits.

\section*{Results}

\subsection*{Supercoiling activities of homologous gyrases}
To investigate the functional interaction of subunits from \textit{B. subtilis}, \textit{E. coli}, and \textit{M. tuberculosis}, we analyzed the supercoiling activity of homologous gyrases, assembled from GyrA and GyrB from the same organism, and heterologous gyrases harboring GyrA and GyrB from different organisms. Tetrameric gyrase was formed by incubating GyrA and GyrB for 3 min at 37 °C before starting the supercoiling reaction with ATP.

To be able to compare the activities of the different enzymes under identical conditions, we first identified buffer conditions that support supercoiling activity for all three gyrases. Buffer conditions previously used to monitor supercoiling activity of gyrase from \textit{B. subtilis}, \textit{E. coli}, and \textit{M. tuberculosis} differ: a representative buffer for measuring \textit{E. coli} gyrase activity is composed of 50 mM Tris/HCl, pH 7.5, 100 mM KCl, 10 mM MgCl\textsubscript{2}, 10% glycerol (compare Refs. 43, 47, and 48), and standard conditions for \textit{B. subtilis} gyrase are 50 mM Tris/HCl, pH 7.5, 100 mM KCl, 10 mM MgCl\textsubscript{2} (31, 37, 38, 49). For \textit{M. tuberculosis} gyrase, different buffers have been used before by different laboratories (42, 50–53) (see Supporting Methods). When we tested \textit{M. tuberculosis} gyrase activity in these buffers, we observed the highest activity in a buffer composed of 20 mM Tris/HCl, pH 7.5, 100 mM KOAc, 10 mM MgCl\textsubscript{2}, 35 μg/ml BSA, 1.2 mM DTT, 10% glycerol (Fig. S1), previously used by Blower et al. (53). We cannot exclude the possibility that \textit{M. tuberculosis} gyrase may show higher activity in other buffers. However, as \textit{B. subtilis} and \textit{E. coli} gyrase also showed robust activity in this buffer (Fig. S2), we performed all subsequent experiments in this buffer.

As a reference for the activities of heterologous gyrases, we next compared the supercoiling activity of the three homologous enzymes (Fig. 2a). At standard concentrations (100 nM gyrase, formed from 200 nM GyrA and 800 nM GyrB), \textit{B. subtilis} and \textit{E. coli} gyrase supercoiled relaxed DNA completely within 30 s. Titration experiments confirmed that GyrA is saturated with GyrB under these conditions (Fig. S3, A and B). Reactions at reduced gyrase concentrations (12.5 nM gyrase; Fig. S3C) show that both enzymes are highly processive. The \textit{E. coli} enzyme catalyzes DNA supercoiling slightly faster than \textit{B. subtilis} gyrase. Mycobacterial gyrase (100 nM, formed from 200 nM GyrA and 400 nM GyrB) reached the end point of the reaction after 15 min (Fig. 2a). Again, we confirmed that GyrA is saturated with GyrB under these conditions in titration experiments (Fig. S3, \textit{A and B}). The presence of intermediates with different levels of supercoiling at earlier time points indicates a limited processivity of this enzyme (Fig. 2A and Fig. S3, \textit{A and B}).

\subsection*{Supercoiling activities of heterologous gyrases}
Heterologous gyrases were formed by incubating GyrA from one organism with GyrB from another organism for 3 min at 37 °C prior to the supercoiling reaction. Reactions with gyrases formed by \textit{B. subtilis} \textit{and} \textit{E. coli} subunits were stopped after 5 min. Reactions containing one gyrase subunit from \textit{M. tuberculosis} were stopped after 30 min because of the low supercoiling velocity observed for \textit{M. tuberculosis} gyrase (Fig. 3A).

Heterologous gyrase containing GyrA from \textit{B. subtilis} (Gyr\textsubscript{As}) and GyrB from \textit{E. coli} (Gyr\textsubscript{Bc}) showed supercoiling

---

\begin{table}
\centering
\caption{Species-specific elements in \textit{B. subtilis}, \textit{E. coli}, and \textit{M. tuberculosis} gyrase subunits}
\begin{tabular}{llllll}
\hline
Domain Element & Tower & Coiled coil & CTD & ATPase & TOPRIM
\hline
\textit{B. subtilis} & -- & -- & Short & -- & --
\textit{E. coli} & -- & + & Long & -- & +
\textit{M. tuberculosis} & + & -- & Short & + & --
\hline
\end{tabular}
\end{table}

---

\textbf{Figure 2.} Supercoiling and decatenation by homologous gyrases. \textbf{A}, DNA supercoiling by \textit{B. subtilis}, \textit{E. coli}, and \textit{M. tuberculosis} gyrase. \textit{B. subtilis} and \textit{E. coli} gyrase completely supercoil relaxed DNA within 30 s. \textit{M. tuberculosis} gyrase needs 15 min to completely supercoil the DNA. B, decatenation of kDNA by \textit{B. subtilis}, \textit{E. coli}, and \textit{M. tuberculosis} gyrase. \textit{B. subtilis} and \textit{E. coli} gyrase decatenate and supercoil DNA; the decatenation is complete after 5 and 15 min, respectively. \textit{M. tuberculosis} gyrase decatenates DNA more slowly; the reaction is not complete after 60 min. Reactions were performed with 200 nM GyrA and 800 nM GyrB\textsubscript{Ec} (400 nM for GyrB\textsubscript{Mt}) in 20 mM Tris/HCl, pH 7.5, 100 mM KOAc, 10 mM MgCl\textsubscript{2}, 35 μg/ml BSA, 1.2 mM DTT, 10% glycerol at 37 °C and stopped at the indicated time points. Relaxed pUC18 was used at a concentration of 20 μg/ml, and the kDNA concentration was 12.5 μg/ml. Bs, \textit{B. subtilis}; Ec, \textit{E. coli}; Mt, \textit{M. tuberculosis}; rel, relaxed DNA; sc, negatively supercoiled DNA. Bands visible as relaxed DNA that are not supercoiled represent nicked DNA.
Activities of heterologous gyrases

Figure 3. Supercoiling and decatenation activity of homo- and heterologous gyrases. A, DNA supercoiling. Reactions were performed with 200 nM GyrA and 800 nM GyrB Bs or 400 nM GyrB Ec in 20 mM Tris/HCl, pH 7.5, 100 mM KOAc, 10 mM MgCl₂, 35 μg/ml BSA, 1.2 mM DTT, 10% glycerol at 37 °C and stopped after 5 min (if only B. subtilis or E. coli subunits were present) or 30 min (if one or both subunits were from M. tuberculosis). GyrA Ec shows the highest activity with its cognate GyrB Ec, slightly reduced activity with GyrB Bs, and strongly reduced activity with GyrB Mt. GyrA Mt shows similar activity with the cognate GyrB Ec and with GyrB Ec, but reduced activity with GyrB Mt. GyrA Mt shows highest activity with GyrB Ec, followed by GyrB Ec. Here, the lowest activity is reached with its cognate partner GyrB Ec. B, time dependence of DNA supercoiling by gyrase containing mycobacterial GyrA. Reactions were performed with 200 nM GyrA Mt and 400 nM GyrB in 20 mM Tris/HCl, pH 7.5, 100 mM KOAc, 10 mM MgCl₂, 35 μg/ml BSA, 1.2 mM DTT, 10% glycerol at 37 °C and stopped at the indicated time points. C, time dependence of DNA supercoiling by gyrase containing mycobacterial GyrB. Reactions were performed with 200 nM GyrA and 2000 nM GyrB, in 20 mM Tris/HCl, pH 7.5, 100 mM KOAc, 10 mM MgCl₂, 35 μg/ml BSA, 1.2 mM DTT, 10% glycerol at 37 °C and stopped after 5 and 30 min. Bs, B. subtilis; Ec, E. coli; Mt, M. tuberculosis; rel, relaxed DNA; sc, negatively supercoiled DNA. Bands visible as relaxed DNA that are not supercoiled represent nicked DNA.

activity. The presence of intermediates that are not (yet) fully supercoiled points to a reduced processivity of the heterologous enzyme compared with the homologous B. subtilis gyrase. Gyrase with GyrA Ec and GyrB from M. tuberculosis (GyrB Mt), on the other hand, showed only marginal activity. Gyrase harboring GyrA Ec showed supercoiling activity with GyrB Ec, but little activity with GyrB Mt. Gyrase containing mycobacterial GyrA (GyrA Ec), but little activity with GyrB Mt. Gyrase containing mycobacterial GyrA (GyrA Ec), showed supercoiling activity with GyrB Ec and GyrB Mt. Thus, GyrA and GyrB subunits from E. coli and B. subtilis can be interchanged, although gyrase with B. subtilis GyrB and E. coli GyrA shows higher supercoiling activity than the reverse mixture, in agreement with previous findings (54). GyrB Ec cooperates with either GyrA Ec or GyrA Mt, leading to supercoiling activity comparable to that of the homologous B. subtilis gyrase. In contrast, both heterologous gyrases with GyrB Ec were impaired compared with the homologous E. coli gyrase. GyrB Mt with GyrA Ec, or GyrA Ec also shows severely reduced supercoiling activity compared with the homologous M. tuberculosis gyrase.

To test whether the reduced activity is due to a lower affinity of the heterologous subunits and incomplete heterotetramer formation, we performed supercoiling reactions as a function of the GyrB concentration, keeping the concentration of GyrA constant (Fig. S4). For heterologous enzymes formed by B. subtilis and E. coli subunits, the reaction was complete after 1 min at 400 nM GyrB, lower than the 800 nM GyrB used for the single-time point reactions in Fig. 3 (Fig. S4A). The same behavior was observed for heterologous gyrases containing GyrA Ec and GyrB Mt or GyrB Ec (Fig. S4B). Thus, in these experiments, the GyrA subunit was saturated with GyrB. In contrast, an increase in the concentration of GyrB Mt led to an increase in the fraction of supercoiled DNA both in combination with GyrA Ec and with GyrA Mt, but the reaction did not reach completion even at the highest concentration (2000 nM; Fig. S4, B and C). Thus, the reduced activity of the heterologous GyrA Ec/GyrB Mt and GyrA Ec/GyrB Mt can in part be ascribed to a reduced assembly of heterotetramers (see below).

Next, we compared the time dependence of supercoiling for heterologous gyrases containing GyrA Mt, which had supercoiled DNA (almost) completely within 30 min (Fig. 3A). Whereas GyrA Mt/GyrB Mt, supercoiled DNA completely in 30 s to 1 min, GyrA Mt/GyrB Ec, needed 5 min for complete DNA supercoiling (Fig. 3B). Both heterologous enzymes thus catalyze DNA supercoiling faster than the homologous mycobacterial enzyme, which had completely supercoiled the DNA after 5–10 min, pointing to a gain of function. Finally, we also compared the time dependence of supercoiling for heterologous enzymes containing GyrB Mt at saturating concentrations of
GyrB (Fig. 3C). For these enzymes, GyrA was not saturated with GyrB under the standard conditions in Fig. 3A. The velocity and processivity for supercoiling was virtually identical for GyrA<sub>Bs</sub>/GyrB<sub>Mt</sub> and GyrA<sub>Ec</sub>/GyrB<sub>Mt</sub> and comparable with the velocity and processivity of the homologous mycobacterial enzyme.

**Homologous and heterologous gyrases reach different supercoiling end points**

To determine the degree of supercoiling of the reaction products generated by homo- and heterologous gyrases, we resolved the highly supercoiled species by agarose gel electrophoresis in the presence of 10 μg/ml chloroquine (Fig. S5). In the absence of chloroquine, highly supercoiled species migrate as one band with high electrophoretic mobility. Interkalation of chloroquine into DNA leads to a decrease in twist and increase in writhe. Negatively supercoiled DNA becomes more relaxed and migrates more slowly in the presence of chloroquine, whereas relaxed DNA becomes more positively supercoiled and migrates faster than in the absence of chloroquine (see controls). In the presence of chloroquine, highly negative supercoiled DNA species can thus be separated according to the degree of supercoiling.

The reaction products of mycobacterial gyrase showed decreased mobility in the presence of chloroquine. For the *B. subtilis* enzyme, the mobility was moderately reduced, and for *E. coli* gyrase only slightly. Thus, *E. coli* gyrase reaches the highest supercoiling density and mycobacterial gyrase the lowest. The heterologous gyrases also showed differences with respect to the level of supercoiling reached. Gyrase containing *E. coli* GyrB reached a high negative supercoiling density with its cognate GyrA<sub>Ec</sub>, but the supercoiling level was reduced strongly with the noncognate GyrA<sub>Bs</sub> and GyrA<sub>Mt</sub>. Gyrase containing *B. subtilis* GyrB showed a slightly lower level of supercoiling with GyrA<sub>Ec</sub> than with the cognate GyrA<sub>Bs</sub> with GyrA<sub>Mt</sub>, the degree of supercoiling was further reduced. GyrB<sub>Mt</sub> showed low levels of supercoiling with its cognate GyrA<sub>Mt</sub> and showed little or no supercoiling with GyrA<sub>Ec</sub> and GyrA<sub>Bs</sub>, respectively. Compared from the perspective of GyrA, GyrA<sub>Ec</sub> reaches the highest supercoiling density with its cognate GyrB<sub>Ec</sub>. With GyrB<sub>Bs</sub>, the final supercoiling density is reduced. With GyrB<sub>Mt</sub>, little supercoiling activity is observed. GyrA<sub>Bs</sub> also reaches the highest supercoiling density with its cognate partner, GyrB<sub>Bs</sub>; with GyrB<sub>Ec</sub>, the supercoiling density reached is lower; with GyrB<sub>Mt</sub>, little supercoiling activity is observed. GyrA<sub>Mt</sub> reaches only a low level of negative supercoiling with the cognate GyrB<sub>Mt</sub>.

Overall, both *E. coli* and *B. subtilis* gyrases supercoil DNA rapidly, but the *E. coli* enzyme reaches a higher degree of supercoiling. *M. tuberculosis* gyrase is the least efficient enzyme and reaches the lowest level of supercoiling. Heterologous enzymes containing *E. coli* and *B. subtilis* subunits show supercoiling activity. Enzymes containing mycobacterial GyrA show supercoiling activity with GyrB from all three organisms, whereas mycobacterial GyrB does not associate efficiently with the noncognate GyrA subunits from *E. coli* and *B. subtilis*, but shows supercoiling activity with GyrA subunits from all three species when provided under saturating conditions.

**Activities of heterologous gyrases**

In most prokaryotes, supercoiling and decatenation reactions are performed by gyrase and Topo IV, respectively. Mycobacteria possess gyrase as the only type IIA topoisomerase (22); the enzyme thus has to perform both activities in vivo (25). To compare the decatenation activities, we also monitored the time dependence of decatenation for the homologous enzymes (Fig. 2B). Whereas *B. subtilis* and *E. coli* gyrase completely decatenated kinetoplast DNA (kDNA) within 15 min, decatenation by *M. tuberculosis* gyrase was slower, and the reaction was not complete after 60 min. All homologous gyrases also (rapidly) supercoiled the decatenated minicircles.

For the heterologous gyrase, the decatenation reaction was stopped after 5 and 60 min, respectively, to take into account the different decatenation velocities of the three homologous enzymes (Fig. 3D). Whereas homologous *B. subtilis* gyrase exhibited robust decatenation activity, gyrase reconstituted from GyrA<sub>Bs</sub> and GyrB<sub>Ec</sub> showed no detectable decatenation after 5 min and only limited decatenation within 60 min; with GyrB<sub>Mt</sub>, no decatenation was observed. Mixing of GyrB<sub> Bs</sub> with GyrA<sub> Ec</sub> or GyrA<sub>Mt</sub> resulted in decatenation within 5 min. GyrA<sub>Ec</sub> with GyrB<sub>Bs</sub> showed the same robust decatenation activity as the homologous *E. coli* gyrase, whereas no decatenation was observed with GyrB<sub>Mt</sub>. GyrB<sub>Ec</sub> showed modest decatenation (and modest subsequent supercoiling) activity with GyrA<sub>Mt</sub>; decatenation was equal modest with GyrA<sub>Mt</sub>. Gyrase containing GyrA<sub>Mt</sub> and GyrB<sub>Ec</sub> showed the same low decatenation activity as the homologous mycobacterial enzyme; with GyrB<sub>Mt</sub>, the decatenation activity was similar to the robust activity of the homologous *B. subtilis* enzyme. GyrB<sub>Mt</sub> did not decatenate DNA in conjunction with either GyrA<sub>Ec</sub> or GyrA<sub>Bs</sub>.

Taken together, GyrA and GyrB subunits from *B. subtilis* and *E. coli* are partially interchangeable for decatenation activity. Mycobacterial GyrA can decatenate DNA with GyrB from all three organisms; the highest activity is reached with GyrB<sub>Bs</sub>. In contrast, mycobacterial GyrB cannot cooperate functionally with GyrA from *B. subtilis* or *E. coli*, which is due to inefficient heterotetramer formation. The activity pattern of heterologous enzymes is thus similar for DNA supercoiling and decatenation.

**DNA-stimulated ATPase activity**

To dissect intersubunit communication through the coupling of DNA binding to ATP hydrolysis, the steady-state ATPase activity of homologous and heterologous gyrases was examined in the absence and presence of pUC18 (Fig. 4, Table 2, and Table S1). These experiments were performed with an excess of GyrA over GyrB to ensure saturation of the ATPase (37). Original data and analyses with the Michaelis–Menten or Hill equation are shown in Fig. S6.

**Homologous gyrases**

Whereas *B. subtilis* and *E. coli* gyrase showed DNA-dependent ATPase activity with increased *k*<sub>cat</sub> and decreased *K*<sub>M, ATP</sub> values in the presence of DNA, mycobacterial gyrase did not show significant changes in *k*<sub>cat</sub> and *K*<sub>M, ATP</sub> in the presence of DNA (Fig. 4, A and B, Table 2, and Tables S1 and S2). *B. subtilis* GyrB showed DNA-dependent ATPase activity with increased *k*<sub>cat</sub> and decreased *K*<sub>M, ATP</sub> values in the presence of DNA.
Activities of heterologous gyrases

Figure 4. ATPase activity of homo- and heterologous gyrases. A, C, E, and G, $k_{\text{cat}}$ values for homologous and heterologous gyrases. B, D, F, and H, $k_{M}$ values for homologous and heterologous gyrases. $A$ and $B$, homologous gyrases; $C$ and $D$, heterologous gyrases with the $B. subtilis$ GyrB subunit; $E$ and $F$, heterologous gyrases with the $E. coli$ GyrB subunit. Values from 3–7 experiments are shown; error bars, S.D. Note that in $C$–$H$, the first two bars show the data for the homologous enzyme. See “Results” for more information. Experiments were performed with 0.5 $\mu M$ GyrA, 0.1 $\mu M$ GyrB, 0.1 $\mu M$ negatively supercoiled pUC18 at 37°C in 20 mM Tris/HCl, pH 7.5, 100 mM KOAc, 10 mM MgCl$_2$, 35 $\mu g$/ml BSA, 1.2 mM DTT, 10% (v/v) glycerol and ATP concentrations from 0 to 5 mM. See Fig. S6 and Table S1 for original data and $k_{\text{cat}}$ and $k_{M}$ values from individual experiments. Data shown here are summarized for enzymes with the same GyrB subunit. See Fig. S7 for a comparison of $k_{\text{cat}}$ and $k_{M}$ values for gyrases with the same GyrA subunit: $B$s, $B. subtilis$; $E$, $E. coli$; $M$, $M. tuberculosis$; n.s., not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 2
Michaelis–Menten parameters for homologous and heterologous gyrases, grouped according to the same GyrB subunit

<table>
<thead>
<tr>
<th>GyrA</th>
<th>GyrB</th>
<th>DNA</th>
<th>$k_{\text{cat}}$</th>
<th>$k_{M}$</th>
<th>$k_{\text{cat}}/k_{M}$</th>
<th>$n_{\text{Hill}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$s^{-1}$</td>
<td>$M$</td>
<td>$s^{-1}$ $M^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Bs</td>
<td>Bs</td>
<td>−</td>
<td>0.09 ± 0.01</td>
<td>0.66 ± 0.09$^*$</td>
<td>0.14 ± 0.02</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>Bs</td>
<td>Bs</td>
<td>+</td>
<td>0.56 ± 0.28</td>
<td>0.33 ± 0.10$^*$</td>
<td>1.70 ± 0.84</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>Ec</td>
<td>Bs</td>
<td>−</td>
<td>0.05 ± 0.02</td>
<td>0.88 ± 0.26</td>
<td>0.06 ± 0.02</td>
<td>NA</td>
</tr>
<tr>
<td>Ec</td>
<td>Bs</td>
<td>+</td>
<td>0.21 ± 0.04</td>
<td>0.45 ± 0.06</td>
<td>0.48 ± 0.08</td>
<td>NA</td>
</tr>
<tr>
<td>Mt</td>
<td>Bs</td>
<td>−</td>
<td>0.04 ± 0.006</td>
<td>1.2 ± 0.76</td>
<td>0.03 ± 0.005</td>
<td>NA</td>
</tr>
<tr>
<td>Mt</td>
<td>Bs</td>
<td>+</td>
<td>0.58 ± 0.09</td>
<td>0.49 ± 0.13</td>
<td>1.2 ± 0.19</td>
<td>NA</td>
</tr>
<tr>
<td>Ec</td>
<td>Ec</td>
<td>−</td>
<td>0.25 ± 0.14</td>
<td>0.44 ± 0.15</td>
<td>0.57 ± 0.31</td>
<td>NA</td>
</tr>
<tr>
<td>Ec</td>
<td>Ec</td>
<td>+</td>
<td>0.75 ± 0.17</td>
<td>0.12 ± 0.11</td>
<td>6.1 ± 1.4</td>
<td>NA</td>
</tr>
<tr>
<td>Bs</td>
<td>Ec</td>
<td>−</td>
<td>0.29 ± 0.10</td>
<td>0.65 ± 0.08</td>
<td>0.64 ± 0.22</td>
<td>NA</td>
</tr>
<tr>
<td>Bs</td>
<td>Ec</td>
<td>+</td>
<td>0.47 ± 0.14</td>
<td>0.47 ± 0.08</td>
<td>1.01 ± 0.29</td>
<td>NA</td>
</tr>
<tr>
<td>Mt</td>
<td>Ec</td>
<td>−</td>
<td>0.21 ± 0.05</td>
<td>0.43 ± 0.12</td>
<td>0.49 ± 0.13</td>
<td>NA</td>
</tr>
<tr>
<td>Mt</td>
<td>Ec</td>
<td>+</td>
<td>0.46 ± 0.17</td>
<td>0.46 ± 0.19</td>
<td>1.01 ± 0.37</td>
<td>NA</td>
</tr>
<tr>
<td>Mt</td>
<td>Mt</td>
<td>−</td>
<td>0.05 ± 0.01</td>
<td>0.96 ± 0.26$^*$</td>
<td>0.05 ± 0.01</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Mt</td>
<td>Mt</td>
<td>+</td>
<td>0.06 ± 0.01</td>
<td>0.52 ± 0.15</td>
<td>0.12 ± 0.01</td>
<td>NA</td>
</tr>
<tr>
<td>Bs</td>
<td>Mt</td>
<td>−</td>
<td>0.08 ± 0.01</td>
<td>0.76 ± 0.15$^*$</td>
<td>0.11 ± 0.02</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>Bs</td>
<td>Mt</td>
<td>+</td>
<td>0.12 ± 0.03</td>
<td>0.57 ± 0.06$^*$</td>
<td>0.21 ± 0.05</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>Ec</td>
<td>Ec</td>
<td>−</td>
<td>0.02 ± 0.018</td>
<td>0.66 ± 0.33</td>
<td>0.037 ± 0.027</td>
<td>NA</td>
</tr>
<tr>
<td>Ec</td>
<td>Ec</td>
<td>+</td>
<td>0.05 ± 0.015</td>
<td>0.80 ± 0.31</td>
<td>0.068 ± 0.019</td>
<td>NA</td>
</tr>
</tbody>
</table>

gyrase hydrolyzed ATP with a $k_{\text{cat}}$ value of 0.09 s$^{-1}$ in the absence and 0.56 s$^{-1}$ in the presence of DNA, corresponding to a 5.9-fold DNA stimulation ($p < 0.01$), in agreement with previous data (37, 55). For $E. coli$ gyrase, the unstimulated $k_{\text{cat}}$ was 0.25 s$^{-1}$. In the presence of DNA, this value increased 2.9-fold ($p < 0.001$) to 0.75 s$^{-1}$. Again, these values are in the range of values reported previously (56–58). $M. tuberculosis$ gyrase hydrolyzed ATP with $k_{\text{cat}} = 0.05$ s$^{-1}$ in the absence and 0.06 s$^{-1}$ in the presence of DNA. The $k_{\text{cat}}$ values are similar to values reported previously (42, 59). The DNA stimulation is modest (1.2-fold; $p < 0.05$); others have observed a stronger stimulation of ATP hydrolysis in the presence of DNA (42). The $k_{\text{cat}}$ values for $E. coli$ and $B. subtilis$ gyrase in the presence of DNA were similar ($p > 0.05$, n.s.; Table S4). $M. tuberculosis$ gyrase showed the lowest $k_{\text{cat}}$ values, both in the absence and presence of DNA. The $K_{M,\text{ATP}}$ value of $E. coli$ gyrase was lower than the value for
the B. subtilis enzyme, both in the absence and in the presence of DNA (Table S4; \( p < 0.05 \)). The decrease in \( K_{M, \text{ATP}} \) with DNA was 3.6-fold for E. coli gyrase (\( p < 0.001 \)), and 2.0-fold (\( p < 0.01 \)) for B. subtilis gyrase (Table S2). M. tuberculosis gyrase had the highest \( K_{M, \text{ATP}} \) value, both in the absence and presence of DNA, and the lowest decrease in the presence of DNA (1.8-fold, \( p < 0.05 \)). The \( K_{M, \text{ATP}} \) values are similar to values determined by others (59). Altogether, the catalytic efficiency \( k_{\text{cat}}/K_{M} \) in the presence of DNA is highest for the E. coli enzyme (6.1 s\(^{-1}\) mM\(^{-1}\); Table 2), followed by B. subtilis gyrase (1.7 s\(^{-1}\) mM\(^{-1}\)). Mycobacterial gyrase is a poor ATPase because of a lower turnover number and a higher \( K_{M, \text{ATP}} \); its catalytic efficiency \( k_{\text{cat}}/K_{M, \text{ATP}} \) is only 0.12 s\(^{-1}\) mM\(^{-1}\).

### Heterologous gyrase

Gyrases containing the GyrB\( _{B} \) subunit showed a prominent increase in \( k_{\text{cat}} \) in the presence of DNA with GyrA\( _{E} \) and GyrA\( _{M} \) (Fig. 4C, Table 2, and Tables S1, S2, and S5). The enzyme with GyrA\( _{E} \) showed a lower \( k_{\text{cat}} \), and the enzyme with GyrA\( _{M} \) showed a similar \( k_{\text{cat}} \) value compared with the homologous B. subtilis gyrase. All enzymes reached similar \( K_{M, \text{ATP}} \) values in the presence of DNA (Fig. 4D, Table 2, and Tables S1, S2, and S5). Whereas gyrase with GyrB\( _{B} \) and GyrA\( _{E} \) showed a reduced \( K_{M, \text{ATP}} \) in the presence of DNA (\( p < 0.05 \)), gyrase with GyrA\( _{M} \), did not (\( p > 0.05 \)). The catalytic efficiencies were 0.48 s\(^{-1}\) mM\(^{-1}\) (with GyrA\( _{B} \)) and 1.2 s\(^{-1}\) mM\(^{-1}\) (with GyrA\( _{M} \)), compared with 1.7 s\(^{-1}\) mM\(^{-1}\) for the homologous B. subtilis enzyme.

Heterologous gyrase containing GyrB\( _{E} \) showed different levels of DNA stimulation in combination with the three GyrA subunits (Fig. 4E and F, Table 2, and Tables S1, S2, and S5). The \( k_{\text{cat}} \) value in the presence of DNA did not significantly increase with GyrA\( _{M} \) (\( p > 0.05 \), n.s.) and only increased slightly with GyrA\( _{B} \) (1.6-fold; \( p < 0.05 \)), compared with a 2.9-fold increase for the homologous E. coli enzyme (\( p < 0.001 \)). The heterologous enzymes also did not show a decrease in \( K_{M, \text{ATP}} \) in the presence of DNA (\( p > 0.05 \), n.s.), whereas the homologous E. coli gyrase exhibited a 3.6-fold decrease (\( p < 0.01 \)). The catalytic efficiency of 6.1 s\(^{-1}\) mM\(^{-1}\) for E. coli gyrase decreases to 1.0 s\(^{-1}\) mM\(^{-1}\) for the two heterologous variants.

The turnover numbers \( k_{\text{cat}} \) for heterologous gyrase containing GyrB\( _{M} \) were modest, as for the homologous M. tuberculosis enzyme (Fig. 4G, Table 2, and Tables S1, S2, and S5), and showed only a small increase (1.4–2.2-fold) in the presence of DNA. The enzyme with GyrA\( _{E} \) showed no decrease in \( K_{M, \text{ATP}} \) with DNA (\( p > 0.05 \), n.s.; Fig. 4H, Table 2, and Tables S1, S2, and S5); the enzyme with GyrA\( _{B} \) had a slightly decreased \( K_{M, \text{ATP}} \) in the presence of DNA (\( p < 0.05 \)). The catalytic efficiency of the enzyme with GyrA\( _{B} \) (\( k_{\text{cat}}/K_{M} = 0.21 \) s\(^{-1}\) mM\(^{-1}\)) exceeded the efficiency of the homologous M. tuberculosis enzyme (\( k_{\text{cat}}/K_{M} = 0.12 \) s\(^{-1}\) mM\(^{-1}\)) slightly, and the enzyme with GyrA\( _{E} \) had the lowest value with \( k_{\text{cat}}/K_{M} = 0.07 \) s\(^{-1}\) mM\(^{-1}\).

Although the ATPase activity is provided by the GyrB subunit, it is also informative to compare heterologous enzymes containing the same GyrA subunit, combined with GyrB subunits from the different organisms (Fig. S7 and Tables S3 and S6). Gyrase containing GyrA\( _{B} \) shows a DNA-stimulated ATPase activity with a prominent increase in \( k_{\text{cat}} \) and decrease in \( K_{M, \text{ATP}} \) with its cognate GyrB\( _{B} \) subunit. In combination with GyrB\( _{E} \) and GyrB\( _{M} \), the DNA-induced changes in \( k_{\text{cat}} \) and \( K_{M, \text{ATP}} \) are small or not significant. In contrast, GyrA\( _{E} \) shows a DNA-dependent increase in \( k_{\text{cat}} \) with all three GyrB subunits. The \( K_{M, \text{ATP}} \) value is decreased in the presence of DNA when GyrB is from E. coli or B. subtilis (\( p < 0.01 \), \( p < 0.05 \)); with GyrB\( _{M} \), the \( K_{M, \text{ATP}} \) value is not DNA-dependent. Finally, GyrA\( _{M} \) shows no DNA-dependent increase in \( k_{\text{cat}} \) or decrease in \( K_{M, \text{ATP}} \) with GyrB\( _{E} \). With GyrB\( _{B} \), \( k_{\text{cat}} \) is slightly DNA-dependent; with GyrB\( _{M} \), both \( k_{\text{cat}} \) and \( K_{M, \text{ATP}} \) are slightly DNA-dependent.

In summary, GyrB\( _{B} \) and GyrB\( _{E} \) are both robust ATPases. GyrB\( _{B} \) cooperates most efficiently with its cognate GyrA subunit and with mycobacterial GyrA, but also with GyrA\( _{E} \). GyrB\( _{E} \) cooperates with GyrA from all three organisms, but shows little coupling of ATP hydrolysis and DNA binding when it is paired with a noncognate GyrA. GyrB\( _{M} \) is a poor ATPase with its cognate GyrA subunit, as well as with GyrA\( _{B} \) or GyrA\( _{E} \). The homologous enzyme shows moderate stimulation of its ATPase activity by DNA. Paired with GyrA\( _{M} \), the catalytic efficiency is higher than for the homologous enzyme; with GyrA\( _{E} \), it is lower. Overall, the subunits from B. subtilis and M. tuberculosis appear to be more similar than E. coli and M. tuberculosis in terms of DNA-dependent ATP hydrolysis.

### Subunit interaction and heterotetramer formation

The activity data of homo- and heterologous gyrase (Fig. 3) indicate that GyrB\( _{B} \) and GyrB\( _{E} \) functionally interact with all other GyrA subunits, although to different extents. Thus, these subunits must also interact physically. Gyrase reconstituted from GyrB\( _{M} \) and GyrA\( _{B} \), or GyrA\( _{E} \), is the only case where little supercoiling or decatenation activity is detected. These enzymes also do not show DNA stimulation of ATP hydrolysis. We have shown above that heterotetramers are formed under the conditions used in the activity tests between subunits from the same organism, as well as in heterologous enzymes formed from E. coli and B. subtilis subunits and heterologous enzymes containing GyrA\( _{M} \) (Figs. S3, S4, A and B). In contrast, the activity of heterologous gyrase formed by GyrB\( _{M} \) and GyrA\( _{B} \), or GyrA\( _{E} \), increases when the GyrB concentration is increased (Fig. S4, B and C), pointing to a reduced interaction between these subunits and incomplete heterotetramer formation under conditions of the supercoiling and decatenation reaction.

To further test whether the mycobacterial subunits can interact with B. subtilis and E. coli subunits, we performed complementation assays of homologous B. subtilis and E. coli gyrase by an excess of GyrB\( _{M} \) and of M. tuberculosis gyrase by an excess of B. subtilis and E. coli GyrB (Fig. 5, A and B). The supercoiling activities of B. subtilis and E. coli gyrase were not affected by a 4- or 10-fold excess of GyrB\( _{M} \). However, the relaxation activity of B. subtilis gyrase was inhibited by mycobacterial GyrB, indicating that GyrB\( _{M} \) can compete with GyrB\( _{B} \) for the interaction with GyrA\( _{B} \) (Fig. S5A). For E. coli gyrase, no inhibition by mycobacterial GyrB was detected, indicating that GyrB\( _{M} \) and GyrA\( _{E} \) do not interact. In the reverse experiment with mycobacterial gyrase and an excess of GyrB\( _{B} \) or GyrB\( _{E} \) (Fig. 5B), the supercoiling activity of mycobacterial gyrase was
Activities of heterologous gyrases

Figure 5. Interactions between gyrase subunits. A, inhibition of B. subtilis and E. coli gyrase (200 nM GyrA, 200 nM GyrB; 5-min incubation at 37 °C for supercoiling reactions, 30 min for relaxation reactions) by a 4- and 10-fold excess of GyrBMt. rel, relaxed DNA; sc, negatively supercoiled DNA. B, complementation of mycobacterial gyrase (200 nM GyrA, 200 nM GyrB; 30-min incubation at 37 °C for supercoiling reactions, 60 min for relaxation reactions) with GyrBMt or GyrBEc generates relaxation activity. rel, relaxed DNA; sc, negatively supercoiled DNA. C, size-exclusion chromatograms of mixtures of 4 μM GyrBMt with 0.8 μM GyrABs (red), GyrAEC (green), or GyrAMt (blue). Below the chromatogram, the SDS-PAGE analysis of individual fractions is shown; elution regions of complexes are indicated by the colored boxes. mAU, milli-absorption units. D, size-exclusion chromatograms of mixtures of 4 μM GyrAMt with 0.8 μM GyrBBs (red), GyrBEc (green), or GyrBMt (blue). Below the chromatogram, the SDS-PAGE analysis of individual fractions is shown; elution regions of complexes are indicated by the colored boxes. Note that the blue chromatograms in C and D (GyrAMt and GyrBMt) as well as the SDS-PAGE analysis of the fractions for this run shown below are identical. Bs, B. subtilis; Ec, E. coli; Mt, M. tuberculosis.

not altered. This is expected, as any heterologous enzymes formed would also show supercoiling activity. In contrast, the negligible relaxation activity of mycobacterial gyrase was increased in the presence of excess heterologous GyrB, demonstrating that GyrAMt can capture GyrBBs and GyrBEc to generate a heterologous enzyme with relaxation activity.

To test directly whether cognate and noncognate subunits interact physically, we performed pulldown assays with His-tagged GyrA subunits immobilized on Ni2⁺-nitrilotriacetic acid–Sepharose (Fig. S8). However, conclusions on subunit interactions were not possible due to the significant nonspecific binding of GyrA and GyrB to the chromatographic material. Also, the His-tag at the N-terminus of GyrA is close to the GyrA/GyrB interface and may interfere with subunit interaction. We therefore analyzed the formation of heterologous gyrases containing one mycobacterial subunit by size-exclusion chromatography with 4 μM GyrA and 0.8 μM GyrB (Fig. 5, C and D), reflecting the concentration ratios used in ATPase assays (Fig. 4). The retention times of the individual GyrB and GyrA subunits from the three different organisms were determined in control experiments (Fig. S9). All GyrB subunits eluted at ~12 ml; the GyrA subunits eluted between 9.5 ml (Bs and Ec) and 10.5 ml (Mt). The elution of the gyrase heterotrimer overlaps with the elution of the GyrA dimer. Therefore, we used the shift in elution volume of GyrB from 12 ml (free GyrB) to 10 ml (GyrB bound to GyrA) as a readout for complex formation and co-elution of GyrB and GyrA. To directly detect GyrB in fractions from size-exclusion chromatography, we analyzed the protein content of individual fractions by SDS-PAGE (Fig. 5, C and D). GyrBMt co-eluted with GyrAMt as well as with
GyrABs; no co-elution was observed with GyrAEc (Fig. 5C). Thus, GyrBMt forms heterotetramers with GyrABs, but not with GyrAEc, under these conditions. Both GyrBMt and GyrBBs co-eluted with GyrAMt (Fig. 5D), in agreement with the observed subunit interaction in titration experiments (Fig. S4B). GyrAMt and GyrBEc have similar electrophoretic mobilities and cannot be separated by SDS-PAGE. In this case, we therefore had to interpret the chromatogram directly. Compared with the chromatograms of the individual subunits (Fig. S9), it is evident that a large fraction of GyrBEc elutes as free GyrB (peak at ∼12 ml), leaving only a small fraction of GyrBEc to co-elute with GyrAMt (at ∼10 ml; Fig. 5D). From these experiments, we can therefore conclude that GyrAMt forms heterotetramers with GyrBBs, but not (or to a lesser extent) with GyrBEc under these conditions.

Altogether, the interaction studies show that mycobacterial GyrA can physically interact with B. subtilis and (to a lesser extent) with E. coli GyrB. Similarly, mycobacterial GyrB interacts with GyrABs, but not to the same extent with GyrBEc.

**Discussion**

Within the set of gyrases we have chosen for comparison in this study, B. subtilis gyrase is a “minimal” gyrase: it contains none of the insertions present in E. coli and mycobacterial gyrase (see Figs. 1 and 6), and its GyrA subunit has a short version of the C-tail. Differences of B. subtilis gyrase activities from the activity of M. tuberculosis gyrase must be related to the DEEE loop in GyrAMt and the C-loop in the ATPase domain of GyrB. Differences from E. coli gyrase, on the other hand,
must be caused by the uncharacterized insert in the coiled-coil region of GyrAEC by the insertion in the TOPRIM domain of GyrBEC characterized previously (34), and/or by the longer C-tail of E. coli GyrA. A comparison of E. coli and M. tuberculosis gyrases is more complex, as these enzymes differ with respect to a number of elements: the DEEE- and C-loops, the long and short C-tails of the GyrA CTDs, the insert in the coiled coil, and the insert in the TOPRIM domain.

Homologous enzymes differ in catalytic activities

Both E. coli and B. subtilis gyrase show DNA-stimulated ATPase activity with a prominent decrease in $K_{M_{ATP}}$ and increase in $k_{cat}$ in the presence of DNA. The DNA-stimulated $k_{cat}$ values for E. coli and B. subtilis gyrase are similar; these enzymes also catalyze DNA supercoiling and decatenation with similar velocities. In contrast, M. tuberculosis gyrase is the slowest ATPase; its ATPase activity shows little DNA stimulation. Furthermore, it is the slowest supercoiling enzyme and the slowest decatenser of the three gyrase homologs studied here. This observation is in contrast to previous studies, which reported similar supercoiling activities of M. tuberculosis and E. coli but a higher decatenser activity of mycobacterial gyrase compared with the E. coli enzyme (25). The discrepancy might be due to different reaction conditions used for these enzymes in previous work, whereas we used identical buffers for all three gyrase homologs in this work. Overall, the low supercoiling and ATPase activities of mycobacterial gyrase are consistent with the timescales reported previously (42, 60). Altogether, gyrase from B. subtilis and E. coli show similar efficiencies in DNA-stimulated ATP hydrolysis, DNA supercoiling, and decatenation, whereas M. tuberculosis gyrase catalyzes all three reactions with much lower efficiency. Whereas the E. coli and (to a lower extent) B. subtilis enzymes are evolutionarily optimized for high catalytic efficiencies supporting rapid cell division, mycobacteria as much more slowly growing species might be able to afford a less efficient gyrase. Mycobacterial gyrase might thus constitute an evolutionary compromise to provide both functions provided by the only type IIA topoisomerase in mycobacteria.

The final level of supercoiling is higher for E. coli gyrase than for B. subtilis gyrase, whereas M. tuberculosis gyrase reaches the lowest supercoiling level. These differences may reflect different supercoiling levels of genomic DNA of these bacteria in vivo. Such a connection has been demonstrated for E. coli and Salmonella typhimurium, where the higher superhelical density of genomic DNA in E. coli compared with S. typhimurium has been linked to different levels of supercoiling reached by the respective gyrases (46). The supercoiling densities of the mycobacterial and B. subtilis genomic DNA in vivo are unknown, precluding a comparison of supercoiling levels in vivo with end points reached in vitro. The high negative supercoiling density of DNA in E. coli might explain why the mycobacterial gyrase does not relax negatively supercoiled plasmids isolated from E. coli.

It has been shown previously that the end point of the supercoiling reaction is determined by the C-tail following the CTD of GyrA (43, 45, 46). B. subtilis and M. tuberculosis GyrA both have a short C-tail, whereas the C-tails of E. coli and S. typhimurium GyrA are longer. The effect of the C-tail has been analyzed in E. coli and M. tuberculosis GyrA (43, 44), in B. subtilis GyrA (45), and in E. coli and S. typhimurium GyrA (46). The end point of the supercoiling reaction is indeed different between the short-tail enzymes from B. subtilis and M. tuberculosis gyrase on one hand and the long-tail enzyme from E. coli gyrase on the other hand. However, supercoiling end points also differ between the short-tail enzymes from B. subtilis and M. tuberculosis gyrase (this work), and between the long-tail gyrases from E. coli and S. typhimurium (46). Thus, the regulation of the supercoiling end point must be more complex and likely involves elements other than just the C-terminal tail.

Heterologous enzymes are less efficient than their homologous counterparts

In general, all heterologous gyrases studied here are less efficient than their homologous counterparts in DNA-stimulated ATP hydrolysis, DNA supercoiling, and decatenation, pointing to an evolutionarily optimized functional interaction in the cognate pairs. Functional and hence physical exchange between E. coli and B. subtilis subunits is (at least partially) possible. Both heterologous enzymes show DNA-stimulated ATPase activity as well as supercoiling and decatenation. Notably, GyrBEC and GyrBBS show similar rate constants of ATP hydrolysis in these enzymes, which are coupled productively to the action of the noncognate GyrA subunit. Interestingly, the heterologous enzyme with higher supercoiling and decatenation activities, GyrAEC/GyrBES, shows ATP hydrolysis rates similar to those of the homologous B. subtilis enzyme, whereas the inverse enzyme GyrABS/GyrBEC with lower supercoiling and decatenation activities has a reduced ATPase activity compared with the homologous E. coli enzyme. In addition, the ATPase activity of the heterologous GyrAEC/GyrBES enzyme with the higher supercoiling activity is reduced, both in the absence and presence of DNA, but stimulated by DNA. In contrast, the inverse GyrABS/GyrBEC enzyme has a higher $k_{cat}$ but hydrolysis is barely stimulated by DNA, which points to reduced coupling. On a structural level, the different activities show that GyrBBS tolerates the insertion in the coiled-coil domain of GyrAEC, whereas the presence of the insertion in the TOPRIM domain of GyrBEC has adverse consequences for the functional interaction with GyrABS. Thus, the insert in GyrABS may play merely a structural and/or stabilizing role, whereas the insertion in the TOPRIM domain in GyrBEC exerts a functional role in interdomain communication (34), which is optimized for the cooperation with its cognate GyrA, but not with B. subtilis GyrA.

Previous studies of heterologous gyrases reconstituted from E. coli and B. subtilis subunits also report activity. Orr et al. (61) and Gubarev et al. (54) detected activity for gyrase reconstituted from GyrAEC and GymbS, whereas the inverse combination of GyrABS and GyrBEC leads to inactive enzyme. This is in qualitative agreement with the observations we present here, although the inverse GyrABS/GyrBEC enzyme still shows appreciable supercoiling activity, possibly due to the different buffer conditions and subunit ratios used and due to longer reaction times. Two other studies comparing the activities of heterologous enzymes reconstituted from E. coli and S. typhimurium (St) or Xanthomonas albilineans (Xa) subunits (46, 62) also observed
supercoiling activity only for one combination: the heterolo-
gous enzymes containing the GyrB<sub>Ec</sub> subunit in combination
with either GyrA<sub>St</sub> or GyrA<sub>XA</sub> yielded a functional enzyme. In
contrast, the heterologous enzymes reconstituted from GyrA<sub>Ec</sub>
and GyrB<sub>St</sub> or GyrB<sub>XA</sub> were supercoiling-deficient. E. coli and S.
typhimurium GyrA and GyrB are highly similar, and both
contain the insertions in the tower and TOPRIM domains char-
acteristic of gyrase from Gram-negatives. The <i>X. albilineans</i>
enzyme is more distant in sequence from <i>E. coli</i> gyrase: the
GyrA and GyrB subunits also contain the insert in the tower
and TOPRIM domains but additionally feature two insertions
within the GyrA CTD and a short insertion in the ATPase
domain of GyrB. How these structural differences are related to
the differences in activities is unclear.

In contrast to the partial compatibility of <i>B. subtilis</i> and
<i>E. coli</i> subunits, neither <i>E. coli</i> nor <i>B. subtilis</i> GyrA cooperate
efficiently with mycobacterial GyrB, setting GyrB<sub>Mt</sub> apart.
Titration experiments show that the subunits interact, but even
under saturating conditions, the supercoiling activity is slow
and limited by the slow ATP hydrolysis by GyrB<sub>Mt</sub>. On the
other hand, <i>M. tuberculosis</i> GyrA functions both in combina-
tion with <i>E. coli</i> and <i>B. subtilis</i> GyrB, indicating that the rapid
DNA-stimulated hydrolysis of ATP by GyrB<sub>Ec</sub> and GyrB<sub>Bs</sub>
can be coordinated with the action of GyrA<sub>Mt</sub>. Furthermore, gyrase
reconstituted from GyrA<sub>Mt</sub> and GyrB<sub>Bs</sub> is the only example
where the combination of noncognate subunits leads to a gain
of function compared with the homologous counterpart: the
heterologous enzyme shows a higher DNA-stimulated ATPase
activity as well as higher supercoiling and decatenation activi-
ties than the homologous mycobacterial enzyme. This behavior
further supports the notion that mycobacterial gyrase is not
optimized for maximum activity and catalytic efficiency.

**Structural reasons for the functional interplay between
heterologous gyrase subunits**

The activity pattern (Fig. 6) observed for the heterologous
gyrase shows that the insertion in the TOPRIM domain of
<i>E. coli</i> GyrB constitutes no or only a minor hindrance for the
functional interaction with GyrA<sub>Mt</sub> or GyrA<sub>Bs</sub> respectively.
Examination of the structural data available reveals that the
presence of the insert in <i>E. coli</i> GyrB and of the DEEE-loop in
GyrA<sub>Mt</sub> is compatible without causing steric hindrance (Fig. 6).
<i>B. subtilis</i> GyrA does not contain any elements that could steri-
cally interfere with the insert in GyrA<sub>Ec</sub>. On the other hand, the
insertion in the coiled-coil domain in <i>E. coli</i> GyrA does not
interfere with a functional interaction with GyrB<sub>Bs</sub>, but pre-
vents the cooperation with GyrB<sub>Mt</sub>. Again, the lack of insertions
in <i>B. subtilis</i> GyrB explains why GyrB<sub>Bs</sub> can tolerate the inser-
tion in GyrA<sub>Ec</sub> in the heterologous enzyme. In the recently
reported cryo-EM structure of mycobacterial gyrase, the
ATPase domains of the two GyrB<sub>Mt</sub> subunits are folded back,
such that they interact with the DEEE-loop in GyrA<sub>Mt</sub> through
the C-loop inserted into the ATPase domain (42). Although
such an arrangement would bring the C-loop in GyrB<sub>Mt</sub> closer
to the insert in <i>E. coli</i> GyrA, no steric hindrance between these
elements is evident from the structural model (Fig. 6). The con-
ventional subunit arrangement captured in the structures of
<i>Thermus thermophilus</i> (39) and <i>E. coli</i> gyrase (63) or Saccharo-
myces cerevisiae topoisomerase II (64) also does not predict a
clash between the C-loop in GyrB<sub>Mt</sub> and the insertion in
GyrA<sub>Ec</sub> (Fig. 6). Although the C-loop of one GyrB<sub>Mt</sub> subunit
would lead to a steric hindrance with one of the CTDs, this
effect does not appear to be detrimental for activity, as the het-
erologous GyrA<sub>Ec</sub>/GyrB<sub>Mt</sub> enzyme and the homologous myco-
bacterial gyrase supercoil and decatenate DNA despite this
potential clash. On the other hand, it is conceivable that the
longer C-tail of <i>E. coli</i> GyrA may cause a more severe interfer-
ce and prevent the interaction between GyrA<sub>Ec</sub> and GyrB<sub>Mt</sub>.

The only clear exception to the generally observed loss of
function is the heterologous gyrase reconstituted from GyrA<sub>Mt</sub>
and GyrB<sub>Bs</sub>, which shows higher supercoiling and decatenation
activities than the homologous mycobacterial enzyme and
activity comparable with that of <i>B. subtilis</i> gyrase. GyrB<sub>Mt</sub> also
shows similar catalytic efficiencies in ATP hydrolysis with
GyrA<sub>Mt</sub> and GyrA<sub>Bs</sub>, indicating that GyrA<sub>Mt</sub> and GyrA<sub>Bs</sub>
are closely related and functionally interchangeable. The only dif-
ference between these subunits is the DEEE-loop present in
GyrB<sub>Mt</sub>. In its absence, neither supercoiling nor decatenation
activities are affected.

**Conclusions**

We show here that, in contrast to <i>B. subtilis</i> and <i>E. coli</i>
gyrase, mycobacterial gyrase is a very inefficient enzyme that
shows slow, DNA-independent hydrolysis of ATP and low
supercoiling and decatenation activities. Whereas <i>B. subtilis</i>
gyrase is a minimal version of gyrase with high enzymatic effi-
ciency both in supercoiling and decatenation, <i>E. coli</i> gyrase is a
specialized and highly efficient supercoiling enzyme, but a less
efficient decatenase. Mycobacterial gyrase, on the other hand,
is not evolutionarily optimized for enzymatic efficiency, neither
in supercoiling nor in decatenation. Instead, it represents a
compromise with a broader range of activities in vivo to meet
the physiological requirements of mycobacteria. The species-
specific variations between GyrA and GyrB subunits lead to
optimized functional cooperation of gyrase subunits with their
cognate partners and limit the compatibility and functional
cooperation with noncognate subunits. Whereas the GyrB<sub>Bs</sub>
subunit is functionally promiscuous and cooperates with GyrA<sub>Ec</sub>
and GyrA<sub>Mt</sub>, GyrB<sub>Mt</sub> shows a preference for <i>E. coli</i>
GyrA, presumably because of the interaction between the two
insertions in the GyrB and GyrA subunits that optimize inter-
subunit communication and coupling. The species-specific
insertions lead to a reduced stability of the mycobacterial gyrase
heterotetramer and interfere with complex formation of
GyrB<sub>Mt</sub> with GyrA<sub>Ec</sub>. GyrB<sub>Mt</sub> does not support rapid supercoiling
in combination with noncognate GyrA subunits. In con-
trast, GyrA<sub>Mt</sub> can functionally cooperate both with <i>B. subtilis</i>
and with <i>E. coli</i> GyrB. The heterologous enzymes reach higher
supercoiling and decatenation activities than the homologous
mycobacterial enzyme. Presumably, the slow hydrolysis of ATP
by mycobacterial GyrB is a limitation for the topoisomerase
activities of mycobacterial gyrase, which can be overcome by
the cooperation with faster-hydrorlizing GyrB subunits in het-
erologous enzymes. Understanding the molecular details of
individual adaptations of gyrase to the physiological require-
Activities of heterologous gyrases

ments of the respective organism might provide a basis for the development of species-specific gyrase inhibitors.

Experimental procedures

Protein production and purification

All proteins were produced recombinantly in E. coli BL21(DE3). Cells were grown in Luria-Bertani medium at 37 °C to OD_{600} = 0.6 and gene expression was induced with 0.2 mM isopropyl-β-D-1-thiogalactopyranoside for 4 h at 30 °C. Cells were harvested by centrifugation and resuspended in lysis buffer (GyrA<sub>mt</sub>, GyrB<sub>mt</sub>, GyrA<sub>ec</sub>, and GyrB<sub>ec</sub>): 50 mM Tris/HCl, pH 7.5, 1 M NaCl, 10 mM MgCl<sub>2</sub>; GyrB<sub>ex</sub>: 50 mM Tris/HCl, pH 7.5, 1 M NaCl, 10% glycerol; GyrB<sub>mt</sub>: 20 mM Tris/HCl, pH 8.0, 100 mM NaCl, 10% glycerol, 2 mM β-mercaptoethanol, and supplemented with protease inhibitor (cOmplete™, Mini, EDTA-free protease inhibitor mixture, Roche) and DNase I (only for GyrA purifications), and disrupted in a microfluidizer. Crude extracts containing GyrA were dialyzed overnight against 50 mM Tris/HCl, pH 7.5, 10 mM MgCl<sub>2</sub>, 2 mM β-mercaptoethanol (buffer A) and applied to a 5-ml heparin-sepharose column equilibrated with the same buffer. Proteins were eluted from the heparin column with a 100-ml gradient to 2M NaCl in buffer B (GyrBBs and GyrBM<sub>ex</sub>) and proteins were eluted from the heparin column equilibrated with the same buffer. Proteins were eluted with a 100-ml gradient from 200 mM NaCl to 2 M NaCl in buffer A. Fractions containing GyrA were pooled, concentrated with Vivaspin columns, and applied to an S200 column equilibrated in buffer A. Fractions containing GyrA were pooled, concentrated, flash-frozen in liquid nitrogen, and stored at −80 °C.

Crude extracts from cells overproducing GyrB were supplemented with ammonium sulfate to 50% saturation, incubated on ice for 30 min, and centrifuged. The pellet was dissolved in 50 ml of buffer B (GyrB<sub>bs</sub> and GyrB<sub>ex</sub>) or a 50-ml gradient to 50% buffer B (GyrB<sub>mt</sub>). Fractions containing GyrB were pooled, concentrated with Vivaspin columns, and applied to a S200 column equilibrated in buffer A. Fractions containing GyrB were pooled, concentrated, flash-frozen in liquid nitrogen, and stored at −80 °C.

Determination of protein concentrations

Protein concentrations were determined photometrically from the absorption at 280 nm, using the following extinction coefficients: GyrA<sub>mt</sub>, 42,750 M<sup>−1</sup> cm<sup>−1</sup>; GyrB<sub>mt</sub>, 52,720 M<sup>−1</sup> cm<sup>−1</sup>; GyrA<sub>ex</sub>, 52,260 M<sup>−1</sup> cm<sup>−1</sup>; GyrB<sub>ex</sub>, 71,740 M<sup>−1</sup> cm<sup>−1</sup>; GyrA<sub>ec</sub>, 48,250 M<sup>−1</sup> cm<sup>−1</sup>; GyrB<sub>ec</sub>, 60,850 M<sup>−1</sup> cm<sup>−1</sup>.

Preparation of relaxed plasmid

Negatively supercoiled pUC18 was obtained from E. coli XL1 blue transformed with pUC18 using the QIAprep Spin Mini-prep Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Relaxed pUC18 was purified from reactions containing 100 nM negatively supercoiled pUC18, 400 nM GyrA, 1600 nM GyrB in 50 mM Tris/HCl, pH 7.5, 100 mM KCl, 50 mM NaCl, 10 mM MgCl<sub>2</sub> using the Promega Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI) with subsequent ethanol precipitation.

Supercoiling and decatenation

DNA supercoiling and decatenation activity of gyrase was tested with relaxed pUC18 or kDNA (Inspiralis, Norwich, UK). For supercoiling reactions, 200 nM GyrA and 800 nM GyrB<sub>bs</sub> or 400 nM GyrB<sub>mt</sub> (if not specified otherwise) were incubated with 20 nM pUC18 and 1.5 mM ATP in buffer containing 20 mM Tris/HCl, pH 7.5, 100 mM K<sub>2</sub>CO<sub>3</sub>, 10 mM MgCl<sub>2</sub>, 35 μg/ml BSA, 1.2 mM DTT, 10% (v/v) glycerol. After preincubating the samples for 3 min at 37 °C, reactions were started with ATP and stopped with 0.5% (w/v) SDS and 12.5 mM EDTA, pH 8.0, at different time points. The decatenation activity of gyrase was tested under the same conditions, using 12.5 μg/ml kDNA as a DNA substrate. Reaction products were separated on 1.3% (w/v) agarose gels in TEP buffer (36 mM Tris, 36 mM NaH<sub>2</sub>PO<sub>4</sub>, 1 mM EDTA, pH 8.0; 2.6 V/cm, 3.5 h).

Steady-state ATPase activity

Steady-state ATPase activity was measured in a coupled enzymatic assay as described (37) with 0.5 μM GyrA, 0.1 μM GyrB, 0.1 μM negatively supercoiled pUC18 (if present) at 37 °C in 20 mM Tris/HCl, pH 7.5, 100 mM K<sub>2</sub>CO<sub>3</sub>, 10 mM MgCl<sub>2</sub>, 35 μg/ml BSA, 1.2 mM DTT, 10% (v/v) glycerol, and ATP concentrations from 0 to 5 mM. Data were analyzed with the Michaelis–Menten or Hill equation to obtain k<sub>cat</sub>, KM, and k<sub>cat</sub>/KM values. Reaction velocities of homologous B. subtilis gyrase (in the absence and presence of DNA), of M. tuberculosis gyrase (in the absence of DNA), and of heterologous gyrase formed by GyrA<sub>mt</sub> and GyrB<sub>mt</sub> (in the absence and presence of DNA) did not follow Michaelis–Menten behavior but required description with the Hill equation, giving Hill coefficients of ~2 (Table 2 and Table S1). Errors reflect S.D. from 3–7 independent experiments.

The errors σ for the -fold change x and y in k<sub>cat</sub> and K<sub>M</sub> in the presence of DNA and for k<sub>cat</sub>/K<sub>M</sub> were propagated from the errors σ<sub>x</sub> and σ<sub>y</sub> of the individual values a and b according to Equation 1,

\[
\sigma \left( \frac{a}{b} \right) = \sqrt{\sigma_a^2 \cdot \left( \frac{1}{b^2} \right)^2 + \sigma_b^2 \cdot \left( -\frac{a}{b^2} \right)^2} \tag{Eq. 1}
\]

where x = k<sub>cat</sub>+/DNA/k<sub>cat</sub>−DNA, y = K<sub>M</sub>+/DNA/K<sub>M</sub>−DNA, and k<sub>cat</sub>/K<sub>M</sub> = k<sub>cat</sub>+/DNA/k<sub>cat</sub>−DNA. k<sub>cat</sub>/K<sub>M</sub> denote the turnover number in the absence and presence of DNA, respectively, and K<sub>M</sub>−DNA and K<sub>M</sub>+/DNA denote the K<sub>M</sub> in the absence and presence of DNA. p values were calculated from two-sample t tests using the hypothesis testing routine of OriginPro 2019.
Analytical size-exclusion chromatography

The formation of homologous and heterologous gyrases was analyzed on a calibrated S200 10/300 GL column (GE Healthcare, Freiburg, Germany) in 20 mM Tris/HCl pH 7.5, 100 mM KCl, 10 mM MgCl2, 10% (v/v) glycerol with 4 μM GyrA and 0.8 μM GyrB at 25 ºC. Protein elution was monitored via the absorbance at 280 nm, and fractions were analyzed for GyrA and GyrB by 10% SDS-PAGE.

Author contributions—D. W. investigation; D. W. and D. K. visualization; D. W. and D. K. methodology; D. W. and D. K. writing-original draft; D. W. and D. K. writing-review & editing; D. K. conceptualization; D. K. and D. W. formal analysis; D. K. supervision; D. K. funding acquisition; D. K. project administration.

Acknowledgments—We thank Jessica Guddorf for excellent technical assistance, and Frederic Collin, Airat Gubaev, and Markus Rudolph for comments on the manuscript.

References


Activities of heterologous gyrases
Activities of heterologous gyrases


