Escherichia coli condensin MukB stimulates topoisomerase IV activity by a direct physical interaction

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In contrast to the current state of knowledge in the field of eukaryotic chromosome segregation, relatively little is known about the mechanisms coordinating the appropriate segregation of bacterial chromosomes. In *Escherichia coli*, the MukB/E/F complex and topoisomerase IV (Topo IV) are both crucial players in this process. Topo IV removes DNA entanglements following the replication of the chromosome, whereas MukB, a member of the structural maintenance of chromosomes protein family, serves as a bacterial condensin. We demonstrate here a direct physical interaction between the dimerization domain of MukB and the C-terminal domain of the ParC subunit of Topo IV. In addition, we find that MukB alters the activity of Topo IV in vitro. Finally, we isolate a MukB mutant, D692A, that is deficient in its interaction with ParC and show that this mutant fails to rescue the temperature-sensitive growth phenotype of a *mukB*$^-$ strain. These results show that MukB and Topo IV are linked physically and functionally and indicate that the activities of these proteins are not limited to chromosome segregation but likely also play a key role in the control of higher-order bacterial chromosome structure.

bacterial chromosome segregation | mass spectrometry

During cell division, chromosomes must be faithfully replicated and segregated. Structural maintenance of chromosomes (SMC) proteins and their non-SMC accessory partners play a crucial role in this process (1, 2). In eukaryotes, SMC proteins function as heterodimers and can be grouped into three categories: cohesin (SMC1/SMC3), condensin (SMC2/SMC4), and a DNA repair complex (SMC5/SMC6) (1, 2). In contrast, most prokaryotes contain a single SMC protein that functions as a homodimer (3, 4).

Escherichia coli MukB was the first member of the SMC protein family to be discovered (5). In spite of its limited sequence homology with other SMC proteins, MukB shares a five-domain structure common to all SMC family members (6). Its globular N- and C-terminal domains combine to form a bipartite ATP binding cassette ATPase "head" domain, which is connected to a smaller globular dimerization or "hinge" domain by a rod-shaped antiparallel coiled coil (Fig. 1*A*) (6–14). In addition to its structural similarity to canonical SMC proteins, MukB shares a common function with prokaryotic SMCs. Both *E. coli mukB*$^-$ strains and *smc*$^-$ strains from *Bacillus subtilis* and *Caulobacter crescentus* show temperature-sensitive colony formation and an increase in the number of anucleate cells at the permissive temperature, suggesting a deficiency in chromosome segregation (5, 7, 15–19). Moreover, a convincing array of experiments has demonstrated that MukB functions as a condensin in vitro and in vivo (20–28).

As is the case for bacterial SMC proteins, two non-SMC accessory proteins, MukE and MukF, are required for full MukB function (29, 30). Null mutations in the *mukE* or *mukF* genes, which are encoded within the same operon as MukB, result in a phenotype indistinguishable from that of a *mukB*$^-$ mutation (31). Like most non-SMC subunits of the eukaryotic and other prokaryotic SMC complexes, these accessory proteins associate with the MukB head domain (30).

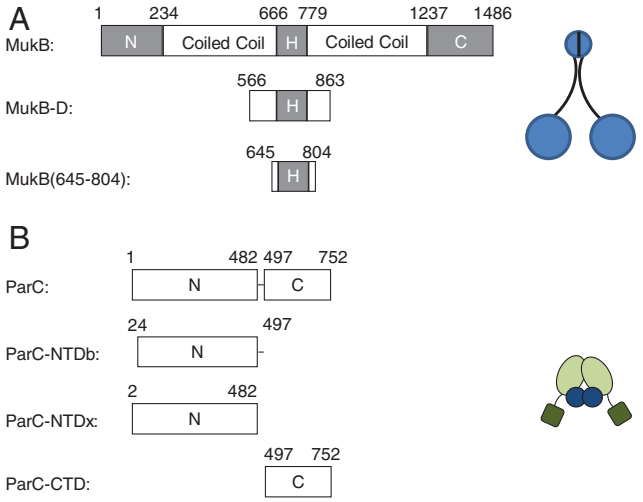

Fig. 1. Schematic views of MukB and Topo IV. (*A Left*) Domain structure of MukB and truncated constructs used. (*Right*) Cartoon representation of a MukB homodimer. Head domains are represented by blue circles and the coiled coils by black lines. The dimerization domains are represented as blue half-circles. (*B*, *Left*) Domain structure of ParC and truncated constructs used. (*Right*) Cartoon view of Topo IV heterotetramer. N- and C-terminal domains of ParC are represented by light green ovals and dark green squares, respectively; ParE is represented by dark blue circles. The hinge, N-, and C-terminal domains are indicated by the appropriate initial.

In eukaryotes, it is well established that the SMC complexes interact transiently with binding partners in addition to their stably associated, non-SMC accessory subunits, including the condensin partners Cti1 and PARP-1-XRCC1 and the cohesin partner Wap I (32–34). In contrast, apart from MukF/E, only one potential binding partner for MukB has been identified. The acyl carrier protein (ACP) copurifies with MukB, which also appears in a proteomic screen for ACP binding partners

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(7, 23, 35). However, the physiological importance of this interaction is not clear (7, 23, 35). Nevertheless, considering the complexity of *E. coli* chromosome organization and dynamics, we viewed it as likely that MukB, one of the central proteins in chromosome segregation, would cooperate with additional protein factors to achieve its cellular functions.

Using affinity purification-mass spectrometry (36), we show here that MukB interacts with ParC, a subunit of topoisomerase IV (Topo IV). In *E. coli*, ParC, the DNA binding and cleavage subunit, combines with ParE, the ATPase subunit, to form a functional $ParC_2ParE_2$ heterotetramer (Fig. 1*B*) (37–39) that resolves the catenated chromosomes created during replication (40–43). We further present evidence that the presence of MukB leads to a stimulation of both the relaxation and decatenation activities of Topo IV. Finally, we demonstrate that a MukB mutant with reduced affinity for ParC fails to rescue a *mukB*$^-$ mutation in vivo. This demonstration of a physical and physiologically important interaction between MukB and Topo IV provides a mechanism for communication between two major players in bacterial chromosome organization. These observations also suggest that cross-talk between SMC complexes and other cellular factors is not restricted to eukaryotes.

Results

Identification of Binding Partners for MukB. Because the hinge domain of SMC proteins is thought to have important functions in addition to dimerization (44–46), we initially sought to identify binding partners for a truncated version of MukB containing this domain and a portion of the coiled coil (MukB-D; residues 566–863) (44). In these initial pull-down experiments (see *SI Text*), we identified ParC, one of the two subunits of Topo IV, as a binding partner for MukB-D. As Topo IV is an essential enzyme for chromosome segregation (37, 47), this observation promoted our investigation into the role of the interaction of its ParC subunit with MukB.

To test the physiological relevance of this interaction, we carried out proteomic assays using isolated MukB complexes from *E. coli* strain KAT1. In this strain (48), the chromosomal coding sequence for MukB has been replaced by GFP-tagged MukB, resulting in the expression of MukB-GFP under the control of the endogenous promoter. MukB-GFP-containing complexes were immunoprecipitated from cell lysates with a specific anti-GFP antibody (36). The isolated protein complexes for KAT1 and the wild-type W3110 control were subjected to SDS-PAGE and mass spectrometry (MS) analysis (Fig. 2*A* and Fig. S1). As expected, ParC was immunoprecipitated, along with previously identified MukB binding partners MukE, MukF, and ACP (Fig. 2*A* and Fig. S1). The positive identification of ParC by MS was supported by good sequence coverage (41%) and an extremely low expectation value (5E-10) and was further confirmed by tandem MS sequencing of four unique peptides (Fig. S1). Taken together, these results strongly suggest an interaction between ParC and MukB-D.

A Direct Physical Interaction Between ParC and MukB. To establish whether the interaction between MukB-D and ParC is direct or indirect, His_6-tag pull-down assays were carried out with *E. coli* cell lysates containing overexpressed proteins. Untagged ParC is not retained on a Ni-NTA column in the absence of His_6-MukB-D (Fig. 2*B*, *Left*); however, untagged ParC is clearly retained in the presence of His_6-MukB-D (Fig. 2*B*, *Left*). This result demonstrates that MukB-D interacts directly with full-length ParC.

To rule out any possible influence of the His_6 tag on the observed interaction, we switched the His_6 tag onto ParC. In addition, MukB-D was substituted with intact MukB to study the effect of MukB coiled-coil and head domains for ParC binding. As expected, untagged MukB was not retained on the Ni-NTA column in the absence of His_6-ParC but was retained in its

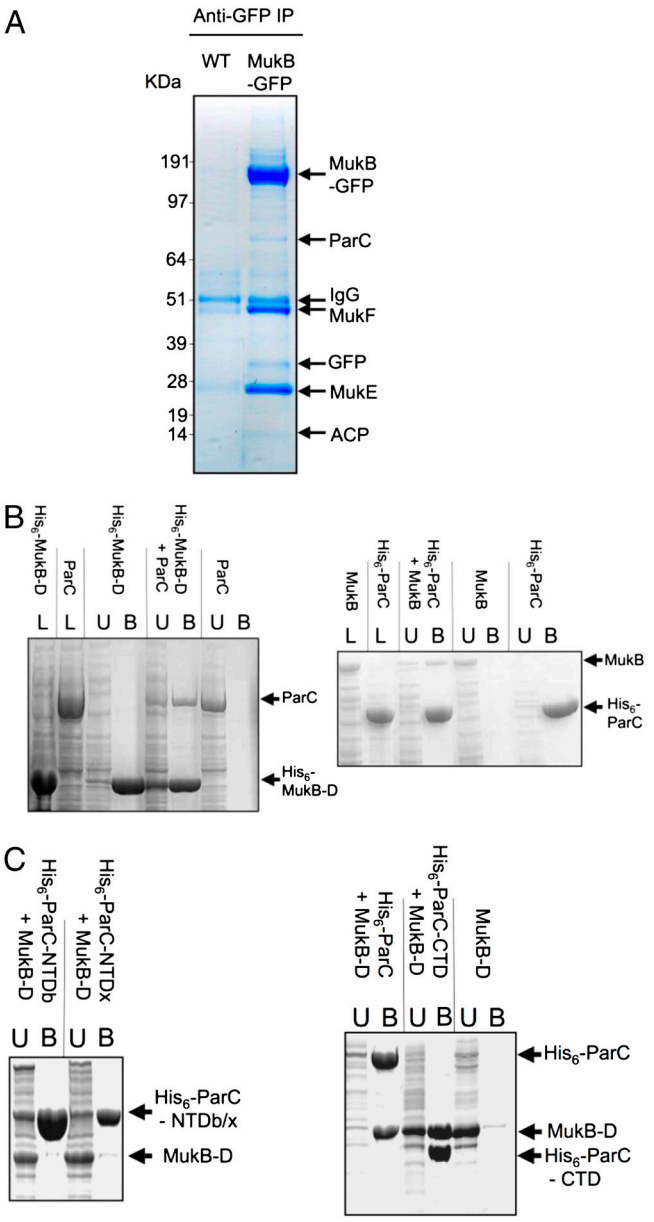

Fig. 2. MukB interacts with ParC in vivo and in vitro. (*A*) Isolation of MukB associated proteins via the GFP tag. The proteins were visualized by Coomassie blue stain and identified by mass spectrometry. (*B Left*) His_6-MukB-D (566–863) and untagged full-length ParC (1–752). (*Right*) His_6-ParC (1–752) and untagged full-length MukB (1–1,486). (*C*) Identification of the domains responsible for the interaction between MukB and ParC. Untagged MukB-D (566–863) with His_6-ParC-NTDb (24–497) or His_6-ParC-NTDx (2–482) (*Left*). Untagged MukB-D (566–863) with His_6-ParC (1–752) or His_6-ParC-CTD (497–752) (*Right*). The lane labels for parts *B* and *C* are as follows: L: lysate; B: bound to the Ni-NTA resin; U: unbound.

presence (Fig. 2*B*, *Right*). This observation confirms that full-length ParC is able to interact with full-length MukB. To our surprise, this interaction remained stable even at high concentrations of salt (Fig. S2*A*), suggesting that the binding between MukB and ParC is robust even under stringent conditions.

To determine whether the MukB-ParC interaction is affected by the presence of ParE, we carried out pull-down experiments with the three proteins together. His_6-ParE pulls down untagged MukB in the presence of untagged ParC (Fig. S2*B*). Similarly, His_6-ParC pulls down untagged MukB-D in the presence of untagged ParE (Fig. S2*C*). These results suggest that MukB does not interfere with the ParC-ParE interaction and Topo IV formation.

We also investigated the location of the protein–protein interface between MukB and ParC. Pull-down experiments were carried out with untagged MukB-D and a series of His_6-tagged ParC truncation constructs (Fig. 1*B*). ParC consists of an N-terminal domain (NTD) and a C-terminal domain (CTD) connected by a short linker region (49). Constructs lacking the ParC C-terminal domain (ParC-NTDb and ParC-NTDx) (49) showed no binding activity toward MukB-D (Fig. 2*C*, *Left*). In contrast, untagged MukB-D was retained with Ni-NTA beads in the presence of His_6-tagged ParC C-terminal domain (ParC-CTD) (Fig. 2*C*, *Right*).

To determine the binding affinity and stoichiometry of the complex between ParC and MukB, we carried out isothermal titration calorimetry experiments by titrating ParC against MukB-D at 25 °C (Fig. S3). The interaction between MukB-D and ParC is exothermic ($\Delta H = -8.0$ kcal/mol) with a K_d of approximately 0.4 μM. A similar K_d (0.5 μM) was observed for the interaction between MukB-D and ParC-CTD (Fig. S3). Finally, we measured the binding affinity of ParC for a truncated version of the MukB hinge domain, MukB (645–804). Although this construct lacks all but two heptads of the coiled-coil region (50), it also binds to ParC with similar affinity to that of MukB-D ($K_d = 0.5$ μM) (Fig. S3), suggesting that little, if any, of the adjacent coiled-coil domain is required for ParC binding. Taken together, these results suggest that residues 645–804 of MukB and 497–752 of ParC are necessary and sufficient for the observed physical interaction.

Interestingly, the observed stoichiometry for binding of MukB-D to full-length ParC is about 0.5 (one MukB-D dimer to two ParC dimers). When ParC-CTD, which lacks the dimerization domain of ParC, is used, the observed stoichiometry is 1.0 (Fig. S3). This relationship indicates that each subunit of a MukB dimer is capable of binding to a monomer of ParC (through a single CTD) and that an intact ParC dimer cannot contact both subunits of the MukB dimer simultaneously. These observations suggest that it is possible for a ParC homodimer (or a $ParC_2ParE_2$ heterotetramer) to bridge the hinge domains of neighboring MukB dimers.

MukB Stimulates Topo IV Activity. Topo IV belongs to the type IIA topoisomerase family and is homologous to both DNA gyrase and eukaryotic topoiosmerase II (Topo II) (51, 52). In vitro, the heterotetramer formed by ParC and ParE shows a broad range of activities, from relaxing supercoiled DNA substrates to unlinking catenated DNA rings (37–39, 53, 54). In *E. coli* cells, the primary role of Topo IV is to resolve catenated chromosomes, which form during the replication of the closed circular genome (40–43).

To assess the effect of MukB on the activity of *E. coli* Topo IV, we turned to both DNA relaxation and decatenation assays. During electrophoresis, relaxed plasmids migrate more slowly than supercoiled plasmids, allowing these topoisomers to be separated on an agarose gel. When the concentration of Topo IV is held constant, the fraction of relaxed topoisomers formed increases with increasing MukB concentration (Fig. 3*A*). This observation shows that MukB is able to stimulate the relaxation activity of Topo IV in a dose-dependent manner. We also performed a time-course experiment to determine the effect of MukB on the kinetics of the Topo IV-catalyzed DNA relaxation reaction. In the presence of MukB, a larger fraction of the DNA is partially or completely relaxed at early time points in the reaction (Fig. 3*B*). Similarly the reaction reaches completion significantly earlier in the presence of MukB than in its absence (Fig. 3*B*), demonstrating that MukB enhances the rate of Topo IV-catalyzed DNA relaxation.

The standard assay to measure the decatenation activity of type II topoisomerases uses kinetoplast DNA (*k*DNA) as substrate (55). *k*DNA is a network of interlocked and covalently closed minicircles (~2.5 kb) (56). Prior to decatenation by Topo IV, *k*DNA will remain in the wells during electrophoresis

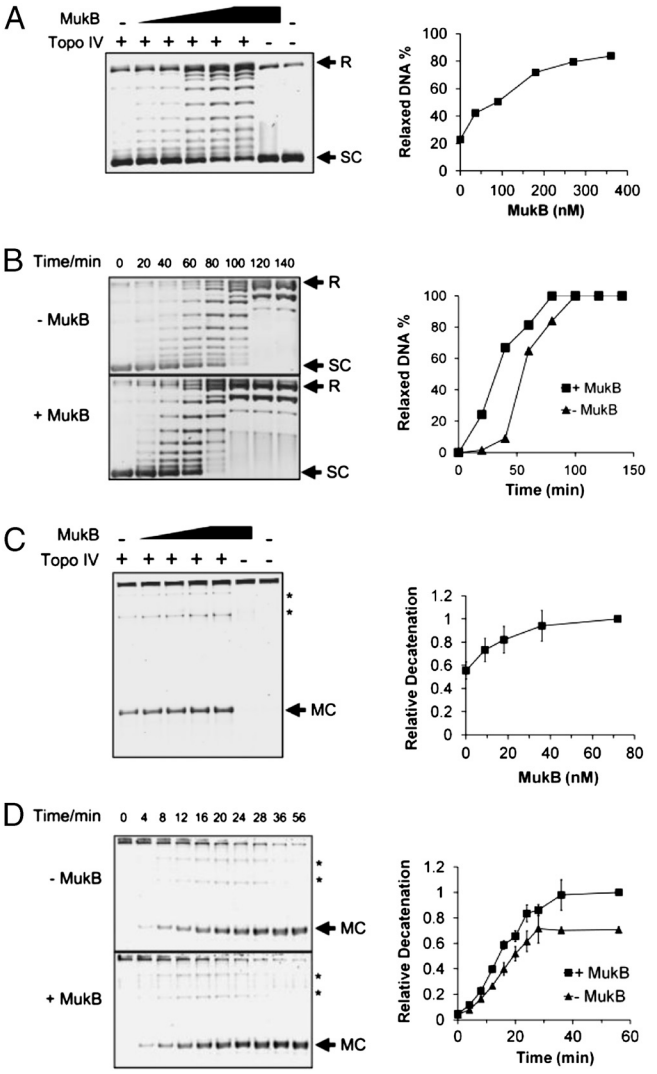

Fig. 3. MukB specifically stimulates the relaxation and decatenation activities of Topo IV. The position of supercoiled plasmids is indicated by SC, and the position of completely relaxed plasmids is indicated by R. Between them are incompletely relaxed topoisomers. Excess MukB is used to drive complex formation at experimentally accessible concentrations of Topo IV. (*A*) Topo IV (2.7 nM) is incubated with negatively supercoiled pBR322 (100 ng) at 30 °C for 30 min, along with MukB at various concentrations (lanes 1–6: 0, 36, 90, 180, 270, and 360 nM, respectively). (*B*) In the absence or presence of MukB (39 nM), Topo IV (3.0 nM) is incubated with pBR322 (100 ng) at 30 °C. The reactions were quenched at different time points (indicated above the lanes). (*C*) Topo IV (0.67 nM) with various concentrations of MukB (lanes 1–5: 0, 9.0, 18, 36, and 72 nM, respectively), was incubated with *k*DNA (180 ng) at 30 °C for 12 min. The unreacted *k*DNA network is retained in the wells due to its large molecular weight. The position of decatenated minicircles is indicated by MC. The asterisks (*) indicate the incompletely decatenated products. A representative agarose gel is shown. (*D*) Topo IV (0.74 nM) was incubated with *k*DNA (180 ng), in the absence or presence of MukB (39 nM). The reactions were quenched at different time points (indicated above the lanes) and loaded onto a single agarose gel for quantification.

due to its high molecular weight. In the presence of Topo IV, decatenated minicircles will enter the agarose gel. The band intensity of these minicircles can then be used to quantify the decatenation activity of Topo IV. MukB increases the amount of decatenated DNA minicircle products in the presence of Topo IV in a modest, but dose-dependent manner (Fig. 3*C*). Similarly, we also quantified the effect of MukB in time-course experiments (Fig. 3*D*). Again, MukB has a modest but reproducible stimulating effect on the rate of Topo IV-catalyzed decatenation and on the

amount of minicircle product (ca. 35% greater in the presence of MukB).

Because full-length MukB affects the supercoiling and condensation of DNA (20, 21, 23, 24), we also probed the ability of MukB-D, which lacks the DNA-binding head domain and much of the coiled coil (44) to stimulate Topo IV function. As with intact MukB, we observe that MukB-D is sufficient to stimulate the DNA decatenation and relaxation activities of Topo IV (Fig. S4 *A* and *B*), although it stimulates the relaxation reaction less efficiently than the full-length protein. As MukB-D does not interact with DNA at the concentrations tested here (44), this result suggests that MukB-dependent stimulation of Topo IV activity occurs is not due to MukB-induced changes in DNA supercoiling or condensation.

To examine the specificity of MukB's effect on the activity of Topo IV, control experiments were performed with Human Topo II, the eukaryotic homolog of *E. coli* Topo IV. MukB fails to stimulate the relaxation activity of Topo II in the relaxation assay and may even inhibit its decatenation activity (Fig. S4 *C* and *D*). Similarly, MukB-D has no effect on the rates of these Topo II catalyzed reactions (Fig. S4*E*). These results provide confirmation that the effect of MukB on Topo IV activity occurs through a direct physical interaction.

A MukB Mutant with Reduced Affinity for ParC. To assess the importance of this interaction for MukB function, we conducted an alanine scan of surface acidic residues in the MukB hinge domain. As the surfaces of the relevant domains of MukB and ParC are highly acidic and basic, respectively, we reasoned that electrostatic attractions would be important in this interaction. Thirteen Asp or Glu residues were mutated to Ala (Fig. 4*A*), separately or in tandem, and the resulting MukB mutants were assayed for their ability to rescue the temperature-sensitive growth phenotype of the *mukB*$^-$ strain SH7718 when expressed from a low-copy number plasmid under the control of the native *mukB* promoter (57). Two of the 12 mutant proteins, MukB-D692A, and MukB-A686-689, in which E688 is replaced with Ala, failed to rescue the growth at the restrictive temperature (37 °C; Fig. 4*B*). These residues form a common locus on the surface of the MukB hinge domain (Fig. 4*A*).

We chose the point mutant, MukB-D692A, for further characterization. As expected, the D692A mutation affected neither the structure nor the dimerization state of the MukB hinge domain (Fig. S5 *A*–*C*), nor did it affect the DNA-binding ability of full-length MukB (Fig. S5*D*). However, in pull-down experiments with ParC, significantly less ParC is retained on the Ni-NTA column in the presence of His_6-MukB-D-D692A than in the presence of wild-type His_6-MukB-D (Fig. 4 *C* and *D*). Thus, the D692A mutation leads to impaired ParC binding by MukB, suggesting that this residue is at the ParC-MukB interface. Taken together, these observations suggest the MukB-ParC interaction is required for complete MukB function in vivo.

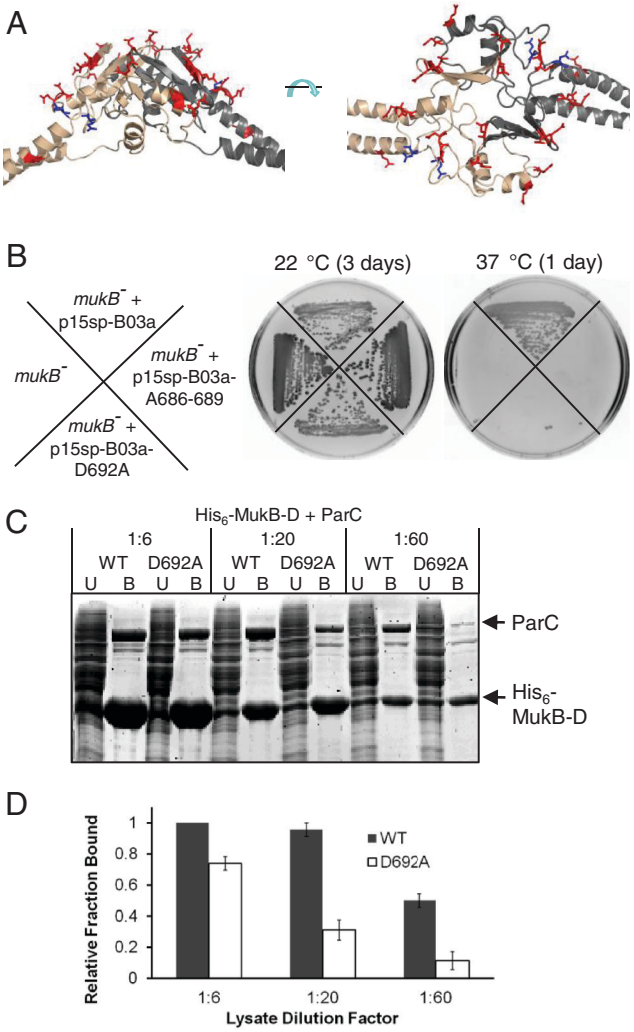

Fig. 4. MukB mutants fail to complement *mukB*$^-$ mutation. (*A Left*) Side view of MukB-D dimer. Positions at which Ala substitutions led to the most severe growth defects are shown in blue, whereas those at which Ala substitution had no obvious effect on growth are shown in red. For clarity, one monomer of MukB-D is shown in tan, whereas the other is shown in gray. (*Right*) Top view of MukB-D dimer. (*B*) The *mukB*$^-$ null cells were transformed with the p15sp-B03a plasmid containing either wild-type, D692A, or Ala686-689 MukB. All cells exhibited normal growth at 22 °C (*Middle*). However, both D692A and Ala686-689 MukB cells showed severe temperature sensitivity at 37 °C (*Right*). (*C*) MukB-D-D692A shows reduced binding with ParC. Both the wild-type MukB-D and MukB-D-D692A mutant were assayed for binding to ParC using the pull-down assay. ParC binding is clearly impaired for MukB-D-D692A. U: unbound, B: bound to the Ni-NTA resin. (*D*) Quantification of the binding data from *C*.

Discussion

Both the *E. coli* condensin MukB and Topo IV are crucial for chromosome segregation, as a single point mutation in either can cause a segregation-deficient phenotype (5, 37). In spite of their similarly impaired chromosome segregation phenotypes, the two proteins play different roles in the process. ParC acts in a complex with ParE to form Topo IV, which disentangles topologically linked daughter chromosomes. In contrast, MukB's effect on chromosome segregation is a result of its condensin activity (20–25). Nonetheless, we have demonstrated that MukB and the ParC subunit of Topo IV are coupled by a direct physical and functional interaction. Moreover, a point mutation at the apparent ParC-binding interface of MukB abrogates the ability of the mutant protein to rescue a *mukB*$^-$ mutation, suggesting that this interaction is essential for the activity of the MukBEF complex in vivo.

This interaction between MukB and ParC appears to be mediated by electrostatic interactions between the C-terminal domain of ParC and the globular dimerization domain of MukB. MukB mutants in which E688 or D692 have been replaced with Ala fail to rescue a *mukB*$^-$ mutation, and the D692A mutation impairs the ParC-binding activity of MukB. Both residues are strictly conserved in all MukB proteins for which sequence information is available. Conversely, in the accompanying manuscript (58), Marians and co-workers demonstrate that mutations at two highly conserved Arg residues in the CTD of ParC similarly abrogate complex formation between MukB and ParC.

To our knowledge, ParC is the only protein demonstrated to interact with the hinge region of a prokaryotic SMC protein. However, several examples of non-SMC accessory factors that

interact with the hinge domain of eukaryotic SMC proteins have been reported. In fission yeast, the essential protein Cti1 interacts with the hinge domain of Cut3/SMC4 and may play a supporting role in the DNA repair function of condensin in interphase cells (32). Similarly, in budding yeast, the hinge region of the Smc5/Smc6 heterodimer interacts with the non-SMC accessory proteins Nse5 and Nse6 (59). Finally, also in budding yeast, the cohesin accessory protein Pds5 appears to associate with the SMC hinge domain, perhaps mediating an interaction between the head and hinge domains of SMC1 (60). SMC conformations consistent with direct head-hinge interactions have also been observed for yeast SMC complexes (10).

In contrast to MukB, numerous examples of proteins with physical and/or functional interactions with ParC have been reported, including the DNA polymerase III holoenzyme, FtsK, MreB, and SeqA (61–64). Marians and co-workers have clearly shown functional significance for the interactions of Topo IV with the first of these three binding partners (61, 62, 64). Moreover, these workers have proposed an attractive model for their roles in the activation of Topo IV in the center of the *E. coli* cell immediately prior to cytokinesis, thus ensuring that the daughter chromosomes are unlinked before cell division. The significance of the interaction with SeqA is less clear.

Just as cell division and decatenation of sister chromosomes are coordinated in the bacterial cell cycle, it may also be the case that chromosome condensation, which results in the overall movement of daughter chromosomes from the cell center to the cell quarter positions, must be coordinated with chromosome decatenation. However, the modest enhancement of the Topo IV decatenation activity by MukB suggests this is not the sole function of the physical interaction between the two proteins.

Unlike ParC, MukB does not appear to be localized to the replication fork (48, 65). Instead, MukB is found consistently at the quarter and eighth positions of living *E. coli* cells, with the exception of newborn cells under slow growth conditions, when it is found at the cell center (65). Intriguingly, ParC foci are occasionally observed to occupy a position closer to the quarter positions than to the centrally located replication factory (62), perhaps suggesting colocalization of ParC and MukB foci during a discrete portion of the cell cycle. Alternatively, MukB may associate with a smaller pool of ParC that is not sequestered at the replication fork. Consistent with this notion, although high levels of Topo IV activity appear to be restricted to the cell center following DNA replication, low levels of Topo IV activity are observed in the nucleoid throughout the cell cycle (62). Indeed, the relaxation activity of Topo IV may be required for efficient MukB-dependent DNA packaging.

Eukaryotic condensin complexes also appear to interact functionally with Topo II, the eukaryotic homologue of Topo IV. It has been reported that the Drosophila non-SMC condensin subunit Barren (CAP-H) interacts with Topo II, stimulating its activity (66). Consistent with this report, depletion of the Condensin I non-SMC subunit CAP-D2 leads to an alteration of the spatial organization of Topo II in both Drosophila and humans, along with a change in CAP-H levels or localization (67, 68). Moreover, Topo II appears to be improperly localized in mitotic chromosomes from SMC-2 or SMC-4 depleted cell extracts (for a review, see ref. 69). Finally, Graumann and co-workers have shown that the overexpression of Topo IV partially rescues *smc*$^-$ defects in *B. subtilis* (70). Given the functional similarities between MukB and prokaryotic SMC proteins, it is attractive to propose that this genetic interaction is also the result of a direct physical interaction.

Finally, as the two subunits of Topo IV are not colocalized throughout the bacterial cell cycle (62), there may be an important functional interaction between MukB and ParC that is independent of Topo IV catalytic activity. The most likely role for such an interaction is in the chromosome scaffold. In eukaryotes, Topo II and condensin SMC subunits are abundant components of histone-depleted mitotic chromosomes (69). Similarly, the MukBEF complex remains stably associated with the *E. coli* chromosome following cell lysis, suggesting that this complex also serves a scaffolding role that organizes the bacterial chromosome into a higher-order structure (71). Electron microscopy studies have revealed that MukBEF complexes can aggregate to form rosettes (72), and high-resolution structural studies suggest that this process may occur through MukF/E mediated bridging of MukB dimers (73).

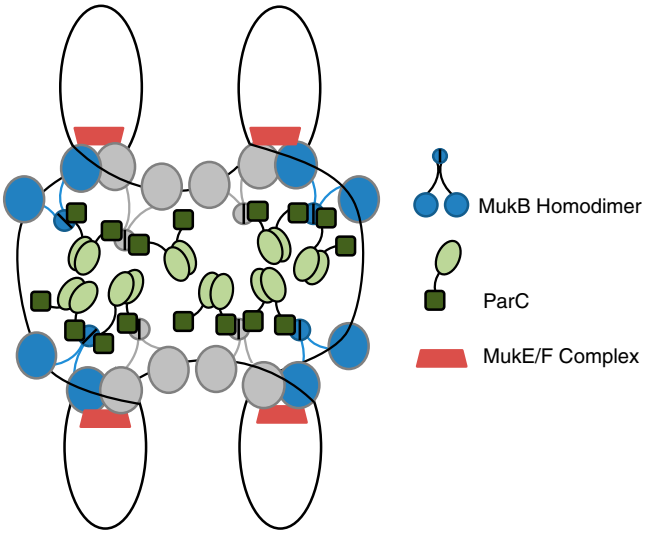

Fig. 5. Schematic model for MukB-ParC interactions within chromosome scaffold. MukB and ParC cartoons are the same as in Fig. 1; MukB homodimers are presented alternately as blue and gray to facilitate visualization of inter- and intradimer contacts. MukE/F complex is represented as rose-colored trapezoid. ParC dimers bridge MukB hinge domains, whereas the MukE/F complex bridges head domains in an interdimer fashion (73). Other models for bridging MukB homodimers are possible; indeed, bridging between different MukB dimers at the head and hinge ends of the molecule may facilitate the formation of higher-order structure. For simplicity, only ParC homodimers are shown; but ParE may also associate with ParC.

Our observation of a robust interaction between MukB and ParC suggests that Topo IV is also a component of this higher-order chromosome architecture in γ-proteobacteria. Indeed, as each dimer of MukB appears to be capable of binding two equivalents of the $ParC_2$ dimer (or $ParC_2ParE_2$ heterotetramer), our results raise the possibility Topo IV may bridge the hinge regions of MukB while MukE/F bridge the head domains, providing two major sites of interaction for the formation of an ordered array (Fig. 5). Thus, in addition to their individual roles in chromosome segregation, the interaction between *E. coli* condensin MukB and Topo IV is likely to be important for the organization of the chromosome into a higher-order structure.

Methods

Experimental details are available in *SI Text*.

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