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The role of MukE in assembling a functional MukBEF complex

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Abstract

The MukB-MukE-MukF protein complex is essential for chromosome condensation and segregation in *Escherichia coli*. The central component of this complex, the MukB protein, is related functionally and structurally to the ubiquitous SMC (structural maintenance of chromosomes) proteins. In a manner similar to SMC, MukB requires the association of two accessory proteins (MukE and MukF) for its function. MukF is a constitutive dimer that bridges the interaction between MukB and MukE. While MukB can condense DNA on its own, it requires MukF and MukE to ensure proper chromosome segregation. Here, we present a novel structure of the *E. coli* MukE-MukF complex, in which the intricate crystal packing interactions reveal an alternative MukE dimerization interface spanning both N- and C-terminal winged helix domains of the protein. The structure also unveils additional cross-linking interactions between adjacent MukE-MukF complexes mediated by MukE. A variant of MukE encompassing point mutations on one of these surfaces does not affect assembly of the MukB-MukE-MukF complex and yet cannot restore the temperature sensitivity of the *mukE::kan* strain, suggesting that this surface may mediate critical protein-protein interactions between MukB-MukE-MukF complexes. Since the dimerization interface of MukE overlaps with the region of the protein that interacts with MukB in the MukB-MukE-MukF complex, we suggest that competing MukB-MukE and MukE-MukE interactions may regulate the formation of higher order structures of bacterial condensin.

Keywords

MukE; MukF; kleisin; MukB; chromosome segregation

Introduction

The ubiquitous SMC (structural maintenance of chromosomes) proteins play critical roles in virtually every chromosome transaction.¹ SMC are large proteins with canonical Walker A and Walker B motifs at their N- and C-terminal domains that associate to form a bipartite ATPase known as the head domain. The two halves of the head domain are connected by a long helical insertion disrupted in the middle of the protein by a hinge domain that reverses the orientation of the helical region, defining a long antiparallel coiled-coil region of about 50 nm. SMC proteins dimerize through the hinge domain adopting their characteristic V-shaped architecture.^{2,3} Together with SMC-associated proteins, SMC form a variety of

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PDB accession codes

The coordinates and structure factors of the *E. coli* MukEF complex have been deposited in thePDB under the accession code 3LCQ.

complexes with specific functions in sister chromatid cohesion (cohesin), chromosome condensation (condensin), DNA repair and gene regulation.^{4,5} While the nature and number of SMC-associated proteins required for function differ in each complex, one of them often belongs to a superfamily of proteins known as kleisins that includes SMC-associated proteins such as human Rad21 and *Saccharomyces cerevisiae* Scc1.⁶ Kleisins bridge the interaction between the different components of the SMC complex and mediate the formation of higher-order structures identified as rosettes or filaments.⁵

In contrast to eukaryotes that contain multiple SMC genes with specialized functions, bacteria encode a single SMC polypeptide that is essential for chromosome condensation.⁷ Bacterial SMC also rely on their association with accessory proteins ScpA and ScpB to perform their function in chromosome condensation and partitioning.^{8,9} A subset of γ -proteobacteria including *Escherichia coli* does not encode homologs of SMC, ScpA or ScpB; however they encode the functionally related MukB, MukF and MukE proteins.^{10,11} Defects in any of the *muk* genes cause abnormal localization of nucleoids, anucleate cell formation, temperature-sensitive colony formation and hypersensitivity to the DNA gyrase inhibitor novobiocin, strongly suggesting that the MukB-MukE-MukF complex, often referred to as MukBEF, plays a central role in chromosome condensation and partitioning.^{11,12} MukB shares the characteristic architecture of the SMC proteins,^{3,13} and MukF has been classified as a kleisin based on sequence and structural analysis.¹⁴

Notably, while the ScpA and ScpB subunits inhibit the ATPase activity of *Bacillus subtilis* SMC,¹⁵ MukE and MukF stimulate the ATPase activity of *E. coli* MukB.¹⁶ However, since MukB is a significantly slower ATPase than *B. subtilis* SMC, their regulated ATPase activities (~18 ATP molecules per minute for the *B. subtilis* SMC holocomplex and ~6 ATP molecules per minute for *E. coli* MukBEF) are strikingly similar despite the apparent opposite effects of the accessory subunits of the complex.^{15–17} The ATPase activity of these complexes is essential to condense DNA.^{16,18} It has been proposed that ATP binding by SMC leads to engagement of the head domains, while ATP hydrolysis results in the release of DNA.¹⁵ Therefore, by inhibiting its ATPase activity, ScpA/ScpB ensures stable binding of the SMC holocomplex to DNA. The MukBEF complex condenses long DNA molecules more efficiently than MukB on its own, presumably due to its enhanced ATPase activity.¹⁶ Overexpression of MukB rescues the chromosome condensation defects associated with the loss of the *mukF* and *mukE* genes but it neither reduces the production of anucleate cells nor rescues the temperature sensitivity of *muk*-deficient cells,¹⁹ suggesting that MukF and MukE function at maintaining chromatin architecture rather than chromosome condensation. In support of this idea, association of MukE and MukF to MukB mediates the formation of higher-order MukBEF structures that could act as chromatin scaffolds.²⁰

Two distinct MukBEF complexes referred to as saturated and unsaturated complexes can be isolated from cells, but only the latter binds DNA.²¹ MukE and MukF also form two stable complexes in the absence of MukB, in which either one dimer or two dimers of MukE associate to a dimer of MukF, suggesting that binding and dissociation of the MukE subunit could regulate the opening of the MukBEF ring to either entrap or release DNA.²² The recent structures of the *E. coli* MukE:MukF (MukEF) and *Haemophilus ducreyi* MukBEF complexes have provided insight into the specific inter-subunit interactions that buttress these complexes.¹⁷ The saturated MukEF complex adopts a Y-shaped structure, with the N-terminal winged helix domain (N-WHD; residues 1–103) and the helical bundle (121–292) of MukF accounting for the stem of the Y and the middle region of MukF (292–328) embedded in a MukE dimer accounting for each arm of the Y.¹⁷ Both arms of the MukEF complex can interact with one head domain of the MukB dimer simultaneously to form a closed ring structure; however one of the MukEF arms is forced to detach upon ATP-mediated engagement of the two MukB heads.¹⁷

In this work, we present the structure of a novel crystal form of the *E. coli* MukEF complex. This structure unveils an alternate dimerization interface in MukE involving both the N- and C-terminal WHDs (winged-helix domains) of the protein and recreates the intricate network of protein-protein interactions that presumably mediates the formation of the higher-order MukBEF assemblies identified by Matoba *et al.*²⁰ Protein variants encompassing mutations in either the dimerization interface of MukE or the region of MukE that mediates the interaction between adjacent MukEF complexes are predominantly monomers in solution and cannot rescue the temperature-sensitivity for colony formation associated to the lack of *mukE*. Therefore, we propose a model for the formation of higher-order structures where competing MukB-MukE and MukE-MukE interactions could promote MukBEF oligomerization.

Results and Discussion

Structure of the *E. coli* MukEF complex

We had shown previously that a homogeneous MukE:MukF complex (MukEF) with a 4:2 stoichiometry can be assembled by mixing purified proteins.²² Crystals of this heterohexameric complex were grown in Na/K phosphate and belonged to the P6₅22 space group with unit cell dimensions of $a = b = 149.9 \text{ \AA}$, $c = 738.6 \text{ \AA}$, $\alpha = \beta = 90^\circ$, $\gamma = 120^\circ$ (Table 1). Molecular replacement using a MukEF heterotrimer [Protein Data Bank (PDB) ID 3EUH] located two independent protomers (I and II) in the asymmetric unit. Although only two protomers were found by molecular replacement, a third protomer (III) could be readily identified upon inspection of the electron density maps. Protomers I and III associated to form the characteristic Y-shaped hexameric MukEF complex (Fig. 1a), while protomer II formed a complete complex through crystal symmetry. Similarly to the MukEF structure previously solved (PDB ID 3EUH), the C-terminal regions of both proteins were disordered in our crystal structure. Indeed, only residues Thr7-Arg327 (MukF), Met10-Ile213 (proximal MukE) and Met10-Glu199 (distal MukE) could be positioned in our electron density maps.

Failure to find the protomer III in the molecular replacement searches could have been due to the relative orientation between MukE and MukF within the complex that was significantly different from that of the other two protomers (Fig. 1b). Superimposition of the helical bundles of the three protomers found in our crystal onto those of the previously solved MukEF structure revealed that residue Lys293 of MukF functions as a hinge allowing the reorientation of the middle region of MukF (residues 294 to 328) and, consequently, that of the MukE dimer with respect to the dimerization region of MukF (residues 1–292). Despite the different orientation of the middle regions of MukF, the overall organization of the MukEF complex was virtually identical with that of the MukEF structure reported previously.¹⁷

Dimerization of MukE

Purified *E. coli* MukE self-associates to form dimers and we have previously proposed that only the dimeric form of MukE interacts with MukF.²² Based on the structure of the *E. coli* MukEF complex,¹⁷ and by analogy to the structure of *Mycobacterium tuberculosis* ScpB (MtScpB, Ref. ²³), it had been proposed that interaction of the N-WHD of MukE mediates dimerization (Fig. 2). However, while dimerization of MtScpB occludes $\sim 1,400 \text{ \AA}^2$ /molecule from the solvent (Fig. 2a), association of two MukE monomers through their N-WHD only occludes $\sim 700 \text{ \AA}^2$ /molecule from the solvent (Fig. 2b). This value is significantly smaller than the average solvent-excluded area for bona fide dimers,²⁴ therefore, the N-WHD alone is unlikely to support dimerization of MukE in the absence of MukF.

The arrangement of MukEF protomers in our crystal revealed extensive interactions between MukEF complexes that were not found on the previous crystal structure of this complex.¹⁷

In particular, distal MukE molecules in adjacent MukEF dimers interact through a reciprocal interface that is chiefly hydrophobic and occludes $\sim 1,000 \text{ \AA}^2$ /molecule from the solvent (Fig. 3a and b). Since our structure was determined at modest resolution (Table 1), we wanted an independent method to assess whether this interaction was the true dimerization interface of MukE or a crystallographic artifact. To this end, we mutated two (Leu38Ser and Leu41Ala) or four (Leu38Ser, Leu41Ala, Phe185Ser and Arg186Glu) key residues in this reciprocal interface and analyzed the oligomeric state of these MukE variants (MukE-LL and MukE-LLFR, respectively) using analytical ultracentrifugation.

Sedimentation velocity data revealed that the MukE-LL variant was predominantly a dimer in solution (Fig. 3c). Sedimentation equilibrium data collected for MukE-LL at loading concentrations of 3.0–33 μM were also consistent with an essentially monodisperse and dimeric MukE having a best-fit molecular mass of $50 \pm 1 \text{ kDa}$ (Supplementary Fig. 1). Based on these experiments we infer that dimerization affinity is smaller than 0.05 μM , thus revealing that this variant forms a tighter dimer than wild-type MukE ($K_d = 18 \mu\text{M}$).²² Conversely, the MukE-LLFR variant existed predominantly as a monomer in solution (Fig. 3d). At the lowest protein concentration assayed (13 μM), only monomers were observed, but the proportion of dimers increased at higher protein concentrations (Fig. 3d). At concentrations above 100 μM , traces of larger species consistent with tetramer formation were possibly present (data not shown). Analysis of sedimentation equilibrium data modeled in terms of reversible monomer-dimer equilibrium returned a K_d of $450 \pm 70 \mu\text{M}$ (Supplementary Fig. 2), which is an order of magnitude weaker than the K_d previously reported for wild-type MukE. We concluded that the surface encompassing both the N-WHD and the C-WHD, rather than the N-WHD alone, mediates the dimerization of MukE in the absence of MukF.

Additional interaction surfaces in MukE

The structure also revealed additional interactions between MukEF complexes mediated by MukE. Two MukEF protomers related by the dimerization of their distal MukE molecules interact with the C-WHD of the proximal MukE molecule in another MukEF protomer to form a three-way interaction among MukEF protomers (Fig. 4a). This interaction is mediated primarily by polar interactions between MukE molecules (Fig. 4b). We mutated the residues that mediate this three-way interaction (Asp196Ala, Arg198Ala, Glu199Ser and Arg202Ser) to generate the MukE-DRER variant of MukE. Unexpectedly, sedimentation velocity experiments indicated that MukE-DRER also had an apparently weaker dimerization K_d than wild type MukE (Fig. 4c). However, the distribution of species at increasing sample concentrations suggested that dimerization of MukE-DRER was not as severely impaired as for the MukE-LLFR variant (compare Fig. 3d and 4c). Analysis of the sedimentation equilibrium data for this variant was complicated by the formation of even higher order species at high protein concentrations (Supplementary Fig. 3 and data not shown), thereby preventing us from estimating a dimerization K_d for the MukE-DRER mutant.

The residues of MukE that mediate this three-way interaction reside in the last α -helix of the C-WHD (shown in red in Fig. 2b). This helix is not part of the dimerization interface of MukE; however, the loop preceding it (residues Phe187-Asp195) contributes to the stabilization of the MukE dimer (Fig. 3b). Therefore, substitutions in this region may indirectly affect the stability of the MukE dimer.

Interacting surfaces in MukE are important to assemble a functional MukBEF complex

Since defects in either one of the *muk* genes cause temperature-sensitivity for colony formation,¹¹ we subsequently used the AZ5450 strain (YK1100 *mukE::kan*) to assay

whether these variants of MukE could restore growth at nonpermissive temperatures. We assayed colony formation of the AZ5450 strain harboring the pAG8009 (wild-type MukE), pAG8354 (MukE-LLFR), pAG8473 (MukE-LL) and pAG8355 (MukE-DRER) plasmids at both permissive (22 °C) and nonpermissive (37 °C) temperatures (Fig. 5a). As expected, the plasmid encoding wild-type MukE restored growth of the AZ5450 strain at both temperatures. The plasmid encoding MukE-LL also restored growth of the AZ5450 strain at the restrictive temperature, strengthening the idea that mutation of these two residues does not affect the function of MukE (Fig. 5a). Conversely, the plasmids encoding the MukE-LLFR and MukE-DRER variants only supported growth at the permissive temperature (Fig. 5a), suggesting that integrity of these two surfaces is important for MukE function.

The dimerization interface of MukE partly overlaps with the surface that mediates the interaction with MukB and MukF in the MukBEF complex (Fig. 5b and Supplementary Fig. 4). Hence, it is possible that the MukE-LLFR and MukE-DRER variants did not rescue the temperature-sensitivity defect of the AZ5450 strain because they had lost the ability to bind MukF or MukB. Indeed, sedimentation velocity analysis revealed that MukE-LLFR does not interact with MukF (Fig. 6), which in turn abrogates the interaction between MukF and MukB. Conversely, both MukE-LL and MukE-DRER interacted with MukF in a manner similar to that of wild-type MukE, as judged by their sedimentation velocity $c(s)$ profiles at various loading ratios of MukE-LL or MukE-DRER to MukF (Fig. 6 and data not shown).

The residues mutated in the MukE-DRER variant (Asp196, Arg198, Glu199 and Arg202) do not overlap with the surface of MukE that mediates the interaction with MukB; however, Val191, Arg192 and Ala193 interact with MukB in the context of the MukBEF complex and are adjacent to the residues mediating the MukE three-way interaction (Supplementary Fig. 4). Hence, we entertained the possibility that the defects of the MukE-DRER variant could result from its inability to interact with MukB. We used dynamic light scattering and size exclusion chromatography to monitor the formation of the MukBEF complex using MukE and MukE-DRER, as well as the MukE-LLFR variant as a negative control. As expected, dynamic light scattering revealed that MukE and MukE-DRER, but not MukE-LLFR, interact with MukF (Fig. 7a). In the presence of ATP and Mg^{2+} , MukB showed a broad distribution centered at a radius of 65 nm. However, the peak was displaced toward larger sizes (~95 nm) when both MukE and MukF were present in the mixture (Fig. 7b). The same effect was observed when the complex was assembled with MukE-DRER (Fig. 7b), indicating that this variant of MukE interacts with MukF and MukB.

Due to its mass and shape, the MukB dimer is excluded from a Superdex200 column (GE Healthcare), whereas MukF, MukE and the MukEF complex are within the separation range of the column.²² Therefore, we assessed complex formation by monitoring the presence of MukE and MukE-DRER, along with MukF, in the excluded volume of the column. When MukB and MukF were incubated with stoichiometric amounts of MukE or the MukE-DRER variant in the presence of ATP and Mg^{2+} , the Superdex200 elution profiles revealed the presence of two species corresponding to the MukBEF and MukEF complexes (Supplementary Fig. 5). Conversely, when the MukBEF complex was assembled with the MukE-LLFR variant, three species were obtained corresponding to the MukBF complex and free MukF and MukE (Supplementary Fig. 5). These results are in good agreement with previously published data indicating that MukF can interact with MukB even in the absence of MukE²⁵.

Since MukE-DRER interacts with MukF and MukB in a manner similar to that of wild-type MukE, we concluded that the temperature-sensitivity phenotype associated to the MukE-DRER variant must be due to a disruption of the protein-protein interactions mediated by this interface of MukE beyond those required to assemble the MukBEF complex. To our

knowledge, this is the only variant of MukE ever identified that differentially affects these two functions of the protein.

Model for the assembly of higher-order MukBEF structures

Two structures of the MukEF complex bound to the head domain of MukB from *H. ducreyi* have been determined.¹⁷ These structures reveal that each arm of the heterohexameric MukEF complex can interact with one MukB head domain, however ATP-mediated head engagement of MukB forces one of the MukEF complexes to detach generating an asymmetric MukBEF condensin molecule (Fig. 8(i–iii)).¹⁷ In this asymmetric complex the C-WHD of MukF is bound to one of the MukB heads while the middle region of MukF (339–352) adopts an extended conformation and interacts extensively with the other head domain of MukB. The distal MukE molecule also stabilizes the MukBEF complex, though its role is probably minor as the interaction between the distal MukE and MukB is only maintained in one of the two complexes found in the asymmetric unit of the crystal structure (PDB ID 3EUK).¹⁷

The structure of the MukEF complex reported here unveils the multiple interaction interfaces of MukE. Interestingly, the region of MukE that interacts with MukB in the MukBEF structure partly overlaps with the region that we have identified as responsible for MukE dimerization (Fig. 3 and 5b), suggesting that competing MukB and MukE interactions through the alternate dimerization surface could facilitate the detachment of one of the MukEF arms from the condensin complex. This, in turn, implies that the free MukEF arm on the active MukBEF condensin could mediate the interaction of multiple MukBEF complexes to form higher-order structures (Fig. 8(iii–v)). Most importantly, this structure of MukEF unveils a surface in MukE that is dispensable to assemble the MukBEF heterohexameric complex and yet it is essential for cell survival at restrictive temperatures (Fig. 4a). This surface mediates intricate MukE-MukE interactions that could explain why the MukBEF complexes are more prone to form multimers than the MukB homodimer,²⁰ underscoring the critical role of MukE on the formation of these supramolecular structures.

The ATPase activity of MukBEF is important for DNA condensation and segregation presumably by enhancing cycles of MukB head engagement/disengagement and promoting the reeling activity of the MukBEF complex (Fig. 8).^{16,17} Accordingly, mutations in the middle region of MukF, which has been shown to be important for the interaction with MukE and the ATP-dependent detachment from MukB,¹⁷ confer temperature sensitivity and interfere with the focal localization of MukB, which in turn causes cell growth and division defects.²⁶ Interestingly, the only apparent defect of the MukE-DRER variant is its increased tendency to form high-molecular weight oligomers (Supplementary Fig. S3).²² It has been previously shown that excess MukEF destabilizes DNA binding by MukBEF.²¹ Therefore, it is possible that MukE-DRER may inhibit the activity of the mutant MukB-(MukE-DRER)-MukF complex by either altering its ATPase activity and/or preventing head disengagement. This, in turn, would affect DNA binding by the complex and lead to the chromosome condensation/segregation defects observed for this variant of MukE (Fig. 5).

Collectively, this work demonstrates that mutations in areas of MukE that are dispensable for the interaction with MukF and MukB confer temperature sensitivity for colony formation. To our knowledge, this is the first variant of MukE that can separate interaction with MukB and MukF from the assembly of a functional MukBEF complex. Hence, we propose that the MukE-mediated interactions seen in our crystal structure replicate those that orchestrate the assembly of supramolecular MukBEF structures. It is unclear at this point how the ATPase and DNA binding activities of MukBEF are affected by impairing the formation of these higher-ordered structures; however, this work provides a platform to

dissect the myriad of interactions that fine-tune the formation of a functional MukBEF condensin complex.

Materials and Methods

Cloning, protein purification and crystallization

Full-length MukF (pAG8011) and MukE (residues 10–234, pAG8009) were overproduced and purified to assemble the MukEF complex. We had previously shown that deletion of the nine N-terminal residues of MukE does not affect protein oligomerization or complex formation with MukF.^{17,22} Therefore, we used a truncated version of MukE encompassing residues 10–234, rather than full-length MukE, due to its improved solubility. The histidine-tagged MukE and MukF proteins were purified using a HiTrap Ni-chelating affinity column and remaining impurities were eliminated by ionic exchange using Q Sepharose HP (MukE) or MonoQ 10/100 (MukF) columns from GE Healthcare as described earlier.²² Pure MukE and MukF were concentrated and stored at 4 °C in 20 mM Tris (pH 8), 100 mM NaCl, 1.4 mM β -mercaptoethanol and 5% (v/v) glycerol (storage buffer). The MukEF complex (MukF:MukE, 2:4) was assembled as previously described.²² Crystals of the complex were grown by mixing equal volumes of protein (2 mg/mL) and a crystallization solution containing 0.78–0.9 M Na/K phosphate, 25 mM MgSO₄ and 5% (v/v) methyl-2,4-pentanediol at 20 °C. Optimal crystals were stabilized in the crystallization solution supplemented with 10% (v/v) ethylene glycol and, subsequently, cryo-protected by increasing the ethylene glycol concentration in the solution to 25% (v/v) prior to flash-freezing them in liquid nitrogen.

Data collection, structure determination and refinement

Crystals were mounted with the c axis (738.6 Å) almost parallel with the rotation axis to minimize overlap during data collection (Table 1). A complete data set diffracting to approximately 3.7 Å resolution was collected at the X29B beam line in National Synchrotron Light Source, Brookhaven National Laboratory (Upton, NY). Data were indexed, processed and merged using HKL2000.²⁷ The initial phases were determined by molecular replacement using PHASER²⁸ and the structure of the *E. coli* MukEF complex as a searching model (PDB ID: 3EUH). The structure was refined by alternating cycles of manual building in Coot with standard protocols in REFMAC and phenix.refine.^{29–31} The final model has over 92% of residues were in favored regions, and 0.1% in the disallowed regions, of the Ramachandran Plot as judged by MolProbity.³²

Mutagenesis and complementation assays

The MukE-LLFR (MukE-L38S/L41A/F185S/R186E), MukE-DRER (MukE-D196A/R198A/E199S/R202S) and MukE-LL (MukE-L38S/L41A) variants of MukE were derived from a pET15b plasmid encoding MukE (pAG8009) by site-directed mutagenesis using a QuikChangeTM kit (Stratagene). Sequences of the mutants were verified by DNA sequencing (MOBIX Laboratory, McMaster University). All variants were overproduced and purified similarly to wild-type MukE. The AZ5450 strain (YK1100 *mukE::kan*) was a kind gift of Prof. Sota Hiraga.¹¹ Plasmids pAG8009 (MukE), pAG8354 (MukE-LLFR), pAG8473 (MukE-LL) and pAG8355 (MukE-DRER) were transformed into the AZ5450 strain and grown at 37 and 22 °C for 20 and 72 h, respectively, to test the ability of the MukE variants to restore the temperature sensitivity of the *mukE::kan* strain.

Sedimentation velocity

Sedimentation velocity experiments were conducted at 20.0 °C on a Beckman Coulter Proteome XL-I analytical ultracentrifuge using the absorbance optical detection system.

MukE-LLFR and MukE-DRER samples were studied at loading concentrations ranging from 10 to 60 μM , whereas MukE-LL samples were studied at loading concentrations ranging from 16 to 250 μM . Two-channel, 3-mm path-length sector-shaped cells were usually used for concentrations above 50 μM (100 μL), otherwise samples were loaded into two-channel, 12-mm path-length sector-shaped cells (400 μL). We acquired 100 scans at intervals of 7 min and rotor speeds of 50 krpm with absorbance data collected as single-absorbance measurements at 280 nm using a radial spacing of 0.003 cm. Data were analyzed in SEDFIT 11.9b in terms of a continuous $c(s)$ distribution.³³ The solution density ρ was measured experimentally at 20.0 °C on a Mettler Toledo DE51 density meter and used to determine the precise glycerol concentration. The viscosity η was calculated using the program SEDNTERP 1.09,³⁴ as were values for the partial specific volumes v of the mutant proteins. The $c(s)$ analyses were carried out using an s range of 0–5 with a linear resolution of 100 and confidence levels (F-ratio) of 0.68. In all cases, excellent fits were observed with r.m.s.d. values ranging from 0.0033 to 0.0041 absorbance units. Sedimentation coefficients were corrected to standard conditions at 20.0 °C in water, $s_{20,w}$.

Sedimentation equilibrium

Sedimentation equilibrium experiments were conducted at 4.0 °C on a Beckman Optima XL-A analytical ultracentrifuge. MukE-LLFR and MukE-DRER samples were studied at loading concentrations ranging from 15 to 120 μM . We used two-channel, 3-mm path-length sector shaped cells for concentrations above 100 μM (35 μL), otherwise samples were loaded into six-channel, 12-mm path-length sector shaped cells (135 μL). Data were acquired at 14, 18, 22 and 26 krpm, as an average of four absorbance measurements at 250 and 280 nm using a radial spacing of 0.001 cm. Sedimentation equilibrium at each rotor speed was achieved within 60 h. Samples of MukE-LL were studied at loading concentrations ranging from 6 to 33 μM with data collected in a similar fashion at 9, 14, 19 and 24 krpm. In this case, however, absorbance data were also collected at 230 nm for the lowest concentrations studied. In all cases data collected at different speeds, loading concentrations and wavelengths were analyzed globally in terms of a monomer-dimer self-association in SEDPHAT 8.01,³⁵ with the implementation of mass conservation. Partial specific volumes v were calculated based on the amino acid composition in SEDNTERP 1.09,³⁴ as were the extinction coefficients at 280 nm. Values of the extinction coefficient at 250 and 230 nm were determined as part of the fitting parameters in SEDPHAT. Errors in the equilibrium constants were determined using the method of F-statistics with a confidence level of 95%. Samples of MukE-LL were studied at loading concentrations ranging from 3 to 33 μM with data collected in a similar fashion at 9, 14, 19 and 24 krpm. In this case, however, data were analyzed globally using SEDPHAT 8.01 in terms of a single ideal solute. Errors in the best-fit molecular mass were determined using the method of F-statistics with a confidence level of 95%. We note that an analysis of the individual data sets demonstrated that the best-fit molecular mass did not vary with the loading concentration.

MukBEF complex formation

Frozen stocks of MukF, MukE, MukE-LLFR and MukE-DRER were separated on a Superdex 200 column (GE Healthcare) pre-equilibrated with storage buffer to remove aggregates, while MukB was purified fresh prior to every experiment. The MukBEF complexes (MukB, MukE and MukF, 1:2:1) were assembled by pre-incubating MukB in assembly buffer [20 mM Tris (pH 8), 250 mM NaCl, 1.4 mM β -mercaptoethanol, and 10% (v/v) glycerol] supplemented with 5 mM AMPPnP and 10 mM MgCl_2 for 2 h at 4 °C, followed by addition of stoichiometric amounts of MukE and MukF and overnight incubation at 4 °C. Samples were spun at 10,000g for 10 min to remove protein aggregates just prior to dynamic light scattering measurements on a Nano-S Zetasizer (Spectra Research Corporation) using a 12- μL cuvette.

MukBEF complexes assembled as described above using either MukE or the MukE-LLFR and MukE-DRER variants were resolved on a Superdex 200 column (GE Healthcare) pre-equilibrated with assembly buffer supplemented with 20 mM MgCl₂. We collected 250 µL fractions and resolved them on 4–15% SDS-polyacrylamide gels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

SMC proteins	SMC are ubiquitous proteins that play central roles in chromosome condensation, pairing of sister chromatids, chromosome partitioning, DNA repair and gene regulation. They are characterized by a V-shaped architecture that is able to entrap DNA upon association with specific SMC-associated proteins such as kleisins
kleisins	a conserved family of proteins characterized by their ability to interact directly with SMC protein dimers. They receive their name from the Greek word for closure (kleisimo) and contain at least one winged-helix domain. Members of this family include the Scc1 subunit of cohesin, the CAP-H subunit of condensin, the ScpA subunit of bacterial SMC complex and MukF of the <i>E. coli</i> MukB-MukE-MukF (MukBEF) complex
muk genes	<i>E. coli</i> does not encode an SMC protein or any SMC accessory proteins. However, three <i>muk</i> genes (<i>mukB</i> , <i>mukE</i> and <i>mukF</i>) have been found to be important for chromosome condensation and segregation in <i>E. coli</i> . <i>muk</i> genes are named after the Japanese word mukaku (meaning “anucleate”) because <i>muk</i> mutants produce anucleate cells and are temperature-sensitive for colony formation. Based on its architecture and function, the MukBEF complex is assumed to be the <i>E. coli</i> analog of the bacterial SMC complex

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Figure 1.

Architecture of the MukEF complex. (a) Orthogonal views of the MukEF complex formed by protomer I (pink to purple) and protomer III (green to blue). Molecules are color-coded by polypeptide chain and labeled as follows: F (MukF), E_p (MukE proximal to the helical bundle of MukF) and E_d (MukE distal to the helical bundle of MukF). An illustrative cartoon with the disordered regions of the structure indicated with brackets is added for reference. (b) C^α -trace superimposition of MukF molecules from protomers I, II and III (this work) shown in orange, red and yellow, respectively, and the two MukF molecules (blue and purple) in the 3EUH structure.¹⁷ The pivotal residue Lys293 and the secondary structure elements immediately preceding (α_9) and following (α_{10}) this residue labeled for reference.

Figure 2. Comparison of the ScpB and MukE dimers. (a) Ribbon diagram of the *MScpB* dimer (PDB ID 2Z99) colored as a rainbow from the N-terminus (blue) to the C-terminus (red). (b) Ribbon diagram of the MukE dimer (PDB ID 3EUH) colored as in (a). N-WHD and C-WHD are indicated.

Figure 3.

Dimerization interface of MukE. (a) Ribbon diagram of two adjacent MukEF protomers interacting through their distal MukE monomers (E_d). Orthogonal views of the complex, as well as a descriptive cartoon are shown for clarity. Each MukEF protomer is shown with the MukF subunit shown in a dark shade of blue or green, the proximal MukE monomer (E_p) shown in a lighter shade and the distal MukE monomer (E_d) shown as a semi-transparent surface. (b) Detailed view of the reciprocal interactions that mediate the interaction between the two MukE molecules with side chains shown as sticks in the same color-coding as in (a). (c) Continuous $c(s)$ distributions obtained from sedimentation velocity data collected at 50 krpm, for the MukE-LL variant at loading concentrations of 16 μ M (green), 31 μ M (blue) and 63 μ M (red). (d) Continuous $c(s)$ distributions obtained from sedimentation velocity data collected at 50 krpm, for the MukE-LLFR variant at loading concentrations of 13 μ M (green), 26 μ M (blue) and 59 μ M (red). A 3-mm path-length cell was used at 59 μ M.

Figure 4.

Additional interacting surfaces in MukE. (a) Cylinder diagram of the interaction between three MukEF protomers. The two protomers on the top (shown as in Figure 3) interact through a reciprocal hydrophobic interface on the distal MukE molecules of each protomer. The MukF (brown) and proximal MukE (E_p, orange) subunits of a third protomer (brown-orange-yellow) interact with the distal MukE (E_d) molecules of the other two protomers (blue and green surfaces) through polar interactions. (b) Detailed view of the specific residues that participate on the three-way knot with side chains shown as sticks in the same color-coding as in (a). (c) Continuous $c(s)$ distributions obtained from sedimentation velocity data collected at 50 krpm, for the MukE-DRER variant at loading concentrations of 12 μ M (green), 25 μ M (blue) and 50 μ M (red). A 3-mm path-length cell was used at 50 μ M.

Figure 5.

Integrity of the interacting surfaces is required for MukE function. (a) Colony-formation assays of the AZ5450 strain (YK1100 *mukE::kan*) harboring an empty pET15b plasmid (empty) as well as, pET15b plasmids encoding the following: wild-type MukE, MukE-DRER (DRER), MukE-LLFR (LLFR) and MukE-LL (LL). Growth at permissive (left) and restrictive (right) temperatures is shown. (b) Surface representation of *E. coli* MukE with residues involved in MukE dimerization shown in yellow, residues mediating additional MukEF interactions shown in purple, residues involved in MukB-MukE association shown in blue and those involved in both MukE dimerization and MukB association shown in green. The specific residues mutated in MukE-LLFR and MukE-DRER are labeled in blue and black, respectively. A complete list of residues involved in these interactions is shown in Supplementary Fig. 3.

Figure 6.

MukE-LL, but not MukE-LLFR, interacts with MukF. Continuous $c(s)$ distributions obtained from sedimentation velocity data collected at 50 krpm, for the 2:4 stoichiometric mixtures of MukF:MukE for MukE-LLFR (blue), MukE-DRER (green) and MukE-LL (red) at MukF loading concentrations of 10 μ M (blue) or 13.4 μ M (green, red). The F_2E_4 complex observed for MukE-DRER has a sedimentation coefficient the same as that of the complex formed with the wild-type MukE. Interestingly the complex formed with the MukE-LL variant has a slightly larger sedimentation coefficient indicative of a slightly more compact structure.

Figure 7.

The DRER surface in MukE is dispensable to assemble the MukBEF complex. (a) Dynamic light scattering profiles of wild-type MukF (dark gray), wild-type MukE (light gray) and the MukEF complexes assembled with MukE (red), MukE-DRER (green) and MukE-LLFR (blue). (b) Dynamic light scattering profiles of wild-type MukB (dark gray) and the MukBEF complexes assembled with MukE (red), MukE-DRER (green) and MukE-LLFR (blue). (See Supplementary Fig. 5 for an alternate method to monitor the formation of MukB:MukE:MukF and MukB:(MukE-DRER):MukF complexes).

Figure 8.

Model of the association of condensin complexes. The MukB dimer can bind two MukEF complexes simultaneously (i); however, one arm of the complex detaches upon ATP-mediated head engagement (ii). The free MukEF arm could then either bind another MukB dimer (iii) or interact with additional condensin complexes through MukE-MukE interactions (iii-v), suggesting that MukBEF supramolecular structures could form by a variety of MukE-MukE interactions as described in Figs. 3 and 4. The MukB dimer is shown in red, with ATP molecules shown as black dots. The MukF dimer is shown in blue, and the MukE dimer is shown as a green horseshoe.

Table 1

Data collection and Refinement Statistics

Data Collection	
Space group	P6 ₅ 22
Cell Dimensions	
a, b, c (Å)	149.89, 149.89, 738.55
α , β , γ (°)	90, 90, 120
Wavelength (Å)	1.0809
Resolution (Å) ^a	20–3.6 (3.73–3.6)
Completeness (%) ^a	97.3 (99.7)
Redundancy	3.5 (3.4)
R _{merge} (%) ^a	10.4 (52.9)
I/ σ (I)	9.7 (1.4)
Solvent content (%)	70
Refinement	
Resolution (Å)	20–3.7
Number of reflections (work)	52,638
Number of reflections (test)	2,633
R _{work} / R _{free} (%)	23.93 / 28.67
Number of atoms	
Protein	17,081
Solvent	2
r.m.s.d.	
Bond lengths (Å)	0.004
Bond angles (°)	0.784
Ramachandran Analysis (%)	
Most favored	92.23
Additionally allowed	7.62
Disallowed	0.15

^aData in the highest resolution shell are shown in parentheses.