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The Role of MukE in Assembling a Functional MukBEF Complex

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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 (*s*tructural *m*aintenance of *c*hromosomes) 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.

Introduction

The ubiquitous SMC (*s*tructural *m*aintenance of *c*hromosomes) 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 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

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Abbreviations used: PDB, Protein Data Bank; MtScpB,

Table 1. Data collection and refinement statistics

Data collection	
Space group	$P6_522$
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)
$I/\sigma(I)$	9.7 (1.4)
Solvent content (%)	70
Refinement	
Resolution (Å)	20–3.7
Number of reflections (work)	52,638
Number of reflections (test)	2633
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

[a] Data in the highest-resolution shell are shown in parentheses.

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