Structures of the MukBEF Complex Mediating Chromosome Compaction

The MukB–MukE–MukF complex is a key mediator of chromosome condensation in a subset of bacteria. We determined the structure of the MukE–MukF subcomplex and the ATPase domain of MukB bound to ATPγS and the MukF's C-terminal winged helix domain (C-WHD). MukE and MukF form an elongated frame structure with the two MukF's C-WHDs dangling from the two distal ends due to the preceding flexible linker. We found that only one MukF's C-WHD can bound to the two ATP-bound, dimerized MukB ATPase domains. The detachment of the C-WHD from the ATPase domain is ATP-dependent and the intact flexible linker.

Chromosome condensation is an essential process for faithful chromosome partitioning and segregation during the cell division in all domains of life. By reducing the volume of the newly synthesized DNA duplexes, this process facilitates the resolution of the replicated chromosomes and prevents chromosome severing during cytokinesis [1]. In a subset of bacteria including *Escherichia coli*, the MukB–MukE–MukF complex plays a critical role in chromosome condensation. MukB belongs to the structural maintenance of chromosome (SMC) family proteins that are the critical players not only in chromosome condensation but also in sister-chromatid cohesion, gene dosage compensation, DNA recombination and repair [2]. Electron microscopic images show that, like other SMC proteins, MukB is a V-shaped homodimer with ~50 nm-long long coiled-coil arms ending with two identical ATPase head domains that together assemble composite active sites [3]. ATP-binding induced engagement of the two MukB head domains could result in a large closed ring-like structure transiently, which is opened up by the following ATP-hydrolysis that drives the two head domains apart. MukE and MukF are non-SMC subunits that have been demonstrated to be as essential as MukB [4], although their functional relationship with MukB has been unknown. In most bacteria, if not all, the SMC–ScpA–ScpB complex plays an equivalent role of the MukB–MukE–MukF complex. How the SMC subunit interacts with the non-SMC subunits to form a functional holocomplex has been unknown, and thus how the three subunits mediate chromosome condensation at the molecular level was virtually unknown.

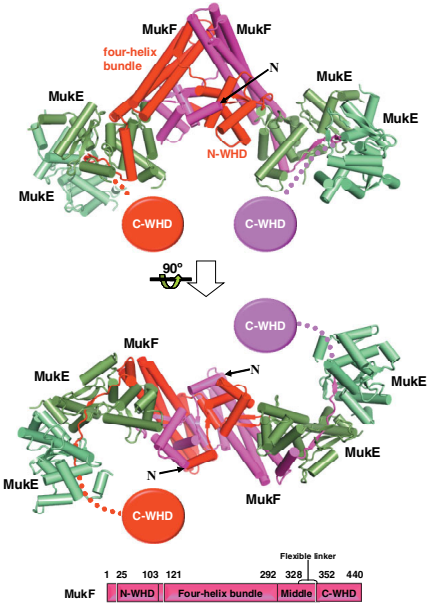

Figure 1
The dimeric structure of MukEF in two different orientation. The invisible C-terminal parts of MukF and C-WHDs are represented as dots and spheres, respectively. The sequence diagram of MukF's primary structure is shown at the bottom.

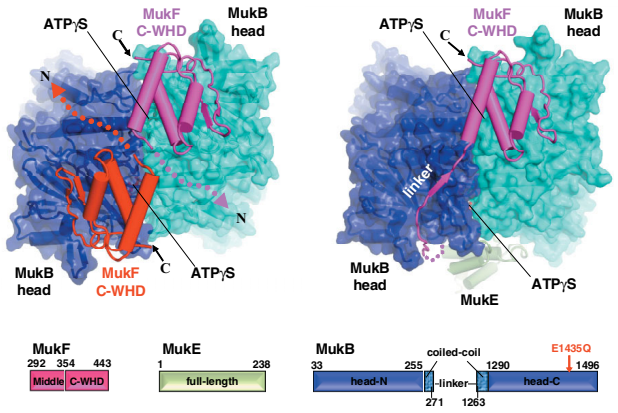

Figure 2
The structures of MukB heads bound to ATPγS and the C-terminal fragment of MukF and MukE. Two different crystal forms were obtained which contained the symmetric or asymmetric dimer of the quaternary complex, respectively. (*Left*) The symmetric complex in which two MukF's C-WHDs (red and magenta) are bound to MukB heads (blue and cyan). (*Right*) The asymmetric complex in which only one MukF's C-WHD is bound to MukB heads. Note the displacement of one MukF C-WHD by the flexible linker. ATPγS is invisible in this orientation. Sequence diagrams of the protein constructs used for crystallization are shown at the bottom.

As a big step toward the detailed and conclusive view of how bacterial chromosome condensation, partitioning and segregation are achieved, we have determined the full architecture of the MukB–MukE–MukF complex (=MukBEF), provided by the crystal structures of the MukE–MukF complex and two different quaternary complexes between a MukF fragment, full-length MukE and MukB head bound to ATPγS [5]. The revealed structure of the MukE–MukF complex (MukEF) is a largely elongated S-shaped dimeric framework with the C-terminal winged-helix domains (C-WHDs) of MukF dangling from the two distal ends (Fig. 1). Dimeric MukB was shown to bind to MukEF through the intersubunit interaction between MukB head and MukF C-WHD to form tripartite closed ring structures. Astonishingly, the two different quaternary complexes revealed that one of the two bound MukF C-WHDs is forced to detach by the ATP-mediated engagement of MukB heads (Fig. 2). These observations imply that the closed ring-like structures of the MukBEF condensin can be transiently opened by ATP binding and then re-sealed by reassociation of the two domains upon subsequent ATP hydrolysis. This critical observation not only explains adequately the functional role of the non-SMC subunit and the indispensable role of the ATP-mediated engagement/disengagement of the SMC subunits, but also hints at an ATP-dependent gating mechanism by which chromosome fibers are loaded and entrapped within the condensin rings. Although it has been only implicitly suggested that the SMC-based condensins may encircle different parts of chromosomal DNA within their ring structures and thus counterbalance the entropic volume expansion of chromosome (= chromosome condensation), how the ring structures could be opened and closed has been totally enigmatic. Now, our comprehensive study provides a clue that the transient dissociation between MukB head and MukF C-WHD may serve as the gateway into the condensin rings. Given the structural and biochemical similarity, the SMC–ScpA–ScpB condensin is likely to function similarly as the MukB–MukE–MukF condensin.

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