HHS Public Access

Author manuscript

J Mol Microbiol Biotechnol. Author manuscript; available in PMC 2016 February 17.

Published in final edited form as:

J Mol Microbiol Biotechnol. 2014 ; 24(0): 371–383. doi:10.1159/000369099.

MukBEF, a chromosomal organizer

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Abstract

Global folding of bacterial chromosome requires the activity of condensins. These highly conserved proteins are involved in various aspects of higher order chromatin dynamics in a diverse range of organisms. Two distinct superfamilies of condensins have been identified in bacteria. The SMC-ScpAB proteins bear significant homology to eukaryotic condensins and cohesins and are found in most of presently sequenced bacteria. This review focuses on the MukBEF/MksBEF superfamily, which is broadly distributed across diverse bacteria and is characterized by low sequence conservation. The prototypical member of this superfamily, the *Escherichia coli* condensin MukBEF, continues to provide critical insights into the mechanism of the proteins. MukBEF acts as a complex molecular machine that assists in chromosome segregation and global organization. The review focuses on mechanistic analysis of DNA organization by MukBEF with the emphasis on its involvement in formation of chromatin scaffold and plausible other roles in chromosome segregation.

Keywords

condensins; MukB; MksB; condensin; bacterial chromosome; chromosome structure; chromosome scaffold; Pseudomonas aeruginosa

Preface: Chromatin scaffold and global folding of the chromosome

The problem of chromatin folding is often described as the need to pack a very long chromosomal DNA into the confines of a tiny cell. Indeed, a single human cell carries 2 meters of DNA, whereas a typical bacterial chromosome, whilst admittedly shorter, about few mm, fits inside a proportionally smaller 1 μm cell. The resulting DNA packing density is about the same in bacteria and eukaryotic cells suggesting that at least some key features of chromosome organization are, perhaps, also conserved across kingdoms of life.

Besides bulk packing, chromosome structure must also meet even more pressing challenges of maintaining at least partial order in DNA without compromising its accessibility to information processing machinery. This must be accomplished in the face of thermal motion, which randomizes spatial arrangement of DNA, and DNA unwinding activity of

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The authors have no conflict of interests to declare.

various polymerases, which introduce supercoils and promote intertwining of DNA strands [Alexandrov et al., 1999; Schvartzman and Stasiak, 2004].

The scaffold model of the chromosome (Fig. 1) seeks to meet these demands by postulating that the chromosome is split into a series of giant loops, which are anchored to proteinaceous matrix at their base [Johnson et al., 2005; Pettijohn, 1996; Saitoh et al., 1995]. The model was initially inspired by electron microscopy observations of gently lysed bacterial [Kavenoff and Bowen, 1976] and eukaryotic [Paulson and Laemmli, 1977] cells and later substantiated by cell biology and genetic tests. Notable among those were the findings that isolated bacterial chromosomes require multiple DNA breaks for complete relaxation and that supercoiling-driven assembly of pre-recombination complexes occurs only for rather closely spaced DNA fragments [Higgins et al., 1996; Sinden and Pettijohn, 1981; Worcel and Burgi, 1972]. This line of reasoning builds upon realization that supercoiling is a global property of DNA and, therefore, supercoils should readily equilibrate throughout the entire molecule unless some DNA fragments are attached to a larger structure. By anchoring bases of DNA loops to a scaffold, the molecule can be subdivided into a set of topologically insulated fragments, known as topological domains. The most reliable current estimates reveal that the *E. coli* chromosome is split into multiple, about 10 kb domains that are dynamically and stochastically distributed through the chromosome [Deng et al., 2004; Postow et al., 2004].

The scaffold model offers an attractive balance of order and disorder. Whereas most of the DNA remains accessible to information processing enzymes, the sparsely set scaffold attachment sites should suffice when a force needs to be applied to DNA during chromosome segregation. This arrangement also offers the most effective way to control the size of the chromosome or separate topological domains [Marko and Trun, 1998]. The biggest challenge of the model is to explain location of the scaffold attachment sites.

The issue seems trivial at the first glance. Indeed, numerous proteins display DNA bridging activity and are expected to stabilize DNA loops inside the cell (reviewed in [Browning et al., 2010; Dillon and Dorman, 2010; Johnson et al., 2005; Luijsterburg et al., 2006; Rimsky and Travers, 2011]). Should the reaction follow the random collision mechanism, however, the size of the loops would be small, around few hundred bp, with chances of obtaining larger loops rapidly decaying with increasing DNA separation [Levene et al., 2013]. This pattern is at odds with the predictions of the scaffold model. This suggests a contribution of nonrandom mechanisms to selection of scaffold attachment regions, SARs. An easy way to accomplish that would be by employing a sequence specific DNA binding protein whose binding sites are dispersed throughout the chromosome. This idea, however, does not readily agree with the dynamic and stochastic nature of SARs. Moreover, sequencing of eukaryotic SARs did not reveal any conserved motifs beyond these regions being intergenic and AT-rich [Mirkovitch et al., 1984]. Apparently, a novel mechanism needs to be devised to explain the sparse organization of the SARs. A plausible such mechanism is beginning to emerge from studies of condensins.

Condensins were discovered over the span of few years in several diverse organisms [Cobbe and Heck, 2004; Graumann and Knust, 2009; Gruber, 2011; Reyes-Lamothe et al., 2012;

Rybenkov, 2009]. Distinct lines of inquiry led to their discovery. One of those lines employed fractionation of the poorly soluble frog chromatin scaffold, which revealed DNA topoisomerase II and condensins as the major protein components of the scaffold [Earnshaw et al., 1985; Saitoh et al., 1994]. The first discovered condensin was MukBEF, which emerged from an elegant screen for *E. coli* proteins involved in partitioning of chromosomes but not necessarily plasmids [Hiraga et al., 1989; Niki et al., 1991]. Independently, condensins and cohesins were discovered in *Saccharomyces ceverisiae* as the proteins required for chromosome segregation [Strunnikov et al., 1995; Strunnikov et al., 1993]. Yet another line of research identified condensins in frog oocytes as the soluble factors responsible for chromosome condensation during cell division [Hirano and Mitchison, 1994]. The convergence of the multiple approaches likely reflects the central role of condensins in many aspects of the higher order chromosome dynamics and must be kept in mind when deducing their mechanism.

Architecture of MukBEF

Three families of condensins have been identified so far in bacteria. The SMC-ScpAB condensins are found in vast majority of sequenced bacteria. The core SMC (structural chromosome maintenance) subunit of the complex shares high degree of homology to archeal and eukaryotic condensins [Britton et al., 1998; Cobbe and Heck, 2004; Mascarenhas et al., 2002; Soppa et al., 2002]. The second family, MukBEF, is found in enterobacteria and several other related orders of γ-*Proteobacteria* [Hiraga, 2000; Niki et al., 1991; Yamanaka et al., 1996]. The name of the complex stands for *mukaku*, anucleate, to mark its phenotype. Recently, a third family of condensins, MksBEF (MukBEF-like SMCs), was identified in diverse proteobacteria [Petrushenko et al., 2011]. These proteins have the same operon organization as MukBEF but display virtually no sequence homology to it. One or more members of MksBEF family typically coexist with SMC-ScpAB or MukBEF and augment rather than substitute their function. These last two families will be the focus of this review. Bacteria also harbor other SMC-like proteins, including SbcCD [Connelly et al., 2003] and RecN [Reyes et al., 2010], which function in DNA recombination and repair but do not seem to contribute to chromosome partitioning.

MukBEF is the sole condensin in *E. coli* [Hiraga, 2000; Petrushenko et al., 2011]. All three subunits of the protein are encoded in the operon *smtA-mukF-mukE-mukB* together with the unrelated gene *smtA* [Yamanaka et al., 1995, 1996]. Mutational inactivation of MukBEF results in chromosome disorganization, decondensation and cutting, anucleate cell formation (even at permissive temperatures) and severe decline in colony formation at temperatures above 25°C [Niki et al., 1991; Sawitzke and Austin, 2000; Wang et al., 2006; Yamanaka et al., 1996] presumably due to chromosome decondensation [Sawitzke and Austin, 2000; Weitao et al., 1999]. Similar defects are observed in condensin-deficient strains of *Bacillus subtilis* and *Caulobacter crescentus* indicating that these proteins play the same role inside the cell [Britton et al., 1998; Jensen and Shapiro, 1999]. The severity of *mukB* phenotype is notable given its modest copy number, about 400 MukBEFs per growing cell [Kido et al., 1996; Petrushenko et al., 2006b]. For comparison, the copy number of “traditional” nucleoid proteins, such as HU, HNS or FIS, often exceeds 10,000 [Johnson et al., 2005]. This

observation lends support to the notion that MukBEF serves as a part of the chromosome scaffold rather than a bulk DNA packing protein [Saitoh et al., 1995].

At the heart of MukBEF is MukB, a member of the SMC protein family [Cobbe and Heck, 2004; Graumann and Knust, 2009; Gruber, 2011; Rybenkov, 2009]. MukB consists of two globular, the N- and C-terminal domains connected by two long α-helices with a hinge region in between (Fig. 2A and [Melby et al., 1998; Niki et al., 1992]). The N- and C-terminal domains, which contain the Walker A and Walker B motifs, respectively, fold into a single globular head domain with the ATP binding site located on its surface [Woo et al., 2009]. In solution, MukB dimerizes via the hinge domain to form a distinctive V-shaped molecule where two long coiled-coils protruding from the hinge terminate in globular head domains [Matoba et al., 2005; Melby et al., 1998; Niki et al., 1992]. The head domains themselves can associate via the shared ABC-type ATP binding site leading to ATP-modulated formation of protein rings or macromolecular assemblies [Matoba et al., 2005; Woo et al., 2009]. The DNA binding site is located on the positively charged hinge-proximal side of the head domain and spreads over its sides ([Woo et al., 2009] and Fig 3A).

Because of the sheer length of the coiled-coil region (~ 50 nm in MukB), no crystal structures of a full length condensin has emerged so far. This leaves open the question about connectivity within the complex. It is generally assumed that the ATP-binding sites of MukB heads face each other within the V-shaped dimer and, by extension, the protein acts as an ATP-modulated protein ring. The opposite arrangement, however, is also conceivable, in which case formation of multimeric structures would be expected (Fig. 2B). Notably, experimental evidence strongly supports existence of multimeric MukBEFs in vitro and in vivo [Badrinarayanan et al., 2012b; Petrushenko et al., 2006b] and, furthermore, biochemical studies suggest that multimerization of MukBEF is essential for its function [Cui et al., 2008].

The other two subunits, MukE and MukF, form a stable complex with each other and dynamically associate with MukB [Petrushenko et al., 2006b; Woo et al., 2009; Yamazoe et al., 1999]. MukEF does not show any DNA binding activities of its own but modulates MukB-DNA interactions [Cui et al., 2008; Petrushenko et al., 2006b]. MukF serves as a kleisin that interacts with MukB heads and links MukE to the complex [Fennell-Fezzie et al., 2005; Woo et al., 2009]. Both purified and reconstituted MukEF form an elongated complex $MukE_4F_2$ [Gloyd et al., 2007; Petrushenko et al., 2006b]. MukF and MukE also form a complex $MukE_2F_2$ but only at substoichiometric levels of MukE [Petrushenko et al., 2006b].

The structural basis for this ambiguity lies in the unusual organization of MukF. MukF consists of two winged-helix domains (WHD) connected by a long unstructured linker peptide [Woo et al., 2009]. The N-terminal WHD provides a dimerization interface whereas the C-terminal WHD associates with MukB head via mostly hydrophobic interactions on its hinge-distal side. The long unstructured linker serves as a docking site for MukB and MukE and is perfectly structured in their presence. The MukE binding stretch of the linker can asymmetrically bind two MukE monomers. Within the crystal, MukE occupies space immediately atop of the DNA binding site of MukB (Fig. 3A) which could conceivably lead

to DNA displacement. However, no direct contacts between MukE and MukB were observed, indicating that the entire assembly could swivel away.

In this arrangement, MukF acts essentially as a tether that ensures proximity of all globular domains but does not impose a rigid structure, Therefore, the complex can assemble and be at least partially functional even in the absence of some of the subunits. It remains unclear whether the two MukEs in the $MukE_2F_2$ complex are bound to the same or different MukFs. However, the former possibility seems more likely given the propensity of MukE for self-association. Notably, a recent study revealed essential mutations in MukE that seem unrelated to DNA binding activities of MukB (Fig. 3B), suggesting that the protein plays a distinct role of its own [She et al., 2013]. Although some of these mutations were proposed to fall on a novel MukE-MukE interface [Gloyd et al., 2011], others face solvent in all available crystal structures of MukBEF.

MukEF and MukB interact with each other in a dynamic ATP-modulated manner both in vitro and in vivo [Badrinarayanan et al., 2012b; Petrushenko et al., 2006b; Woo et al., 2009]. Association between MukB and MukEF is stimulated in the presence of calcium and magnesium [Yamazoe et al., 1999]. When reconstituted in vitro, MukB and MukEF produce two complexes, $MukB_2$-$Muk(E_2F)$ and $MukB_2$-$Muk(E_2F)_2$ [Petrushenko et al., 2006b]. Whereas $MukB_2$-$Muk(E_2F)$ is stable under a range of conditions, $MukB_2$-$Muk(E_2F)_2$ can be only observed at low salt and in the presence of magnesium. The $MukB_2$-$Muk(E_2F)_2$ complex is similarly disrupted in the presence of ATP [Woo et al., 2009]. The structural basis for this dynamics lies in steric hindrances that develop upon ATP-mediated dimerization of MukB heads. Whilst each MukB monomer can associate with a C-terminal WHD of MukF the room for only one remains upon MukB dimerization [Woo et al., 2009].

Notably, the $MukB_2$-$Muk(E_2F)_2$ complex proved to be completely inert in DNA binding whereas DNA remodeling activities of $MukB_2$-$Muk(E_2F)$ seem indistinguishable from those of $MukB_2$ [Petrushenko et al., 2006b]. This suggests that alterations in the composition of the complex could be coupled to loading of the protein onto DNA. This idea is consistent with the observation that the ATPase activity of MukB is stimulated by MukEF but not DNA [She et al., 2013; Woo et al., 2009]. This reveals that MukEF but not DNA helps stabilize the transition state of the MukB ATPase and thereby activate conformational changes, which, in turn, modulate interaction of the protein with DNA. Furthermore, real time single molecule fluorescence studies of MukBEF in live cells indeed revealed two populations of the complexes [Badrinarayanan et al., 2012b]. The presumably DNA-bound stationary population consists of oligomers of $MukB_4$-$Muk(E_2F)_2$ complexes, whereas $MukB_2$-$Muk(E_2F)_2$ complexes were mobile and apparently DNA-free. The generality of this phenomenon remains unclear since MukBEF is so far the only condensin for which variation in composition was reported. For comparison, the *B. subtilis* condensin operates at SMC_2-ScpAB composition [Burmann et al., 2013].

A lingering issue in this context is the oligomeric state of the active MukBEF. Hydrodynamic methods clearly show that the protein exists as a $MukB_2$-$Muk(E_2F)$ complex [Petrushenko et al., 2006b]. This, however, is at odds with high dimerization propensity of MukEF [Petrushenko et al., 2006b; Woo et al., 2009]. Indeed, purified MukEF always exists

in its dimeric form, Muk$(E_2F)_2$. What precludes MukF dimerization when it is a part of MukBEF? Several possibilities must be considered. Perhaps the dimeric complex has a short life time or its dissociation constant could be too high in vitro. The complex could conceivably be stabilized inside the cell through its interaction with DNA or other cofactors. Alternatively, the N-terminal WHD domain of MukF could be engaged in the as yet unidentified secondary binding site on MukBEF. Such arrangement would not be unlike that for the SMC-ScpAB complex, where the N- and C-terminal domains of the kleisin ScpA associate, respectively, with the neck and head regions of the SMC subunit [Burmann et al., 2013].

The hinge domain of MukB is a rather small compact globule from which two coiled coils emanate at almost a 90° angle to each other [Ku et al., 2010; Li et al., 2010a]. The two halves of the hinge could conceivably be separated if a twisting or peeling stress is applied to it from the head via coiled coils. The coiled coils of MukB are not contiguous but include several interruptions [Weitzel et al., 2011]. In these so called knuckle regions, one of the α-helices of the coiled coil buckles out of the register to produce a short three-helix bundle. These knuckles might serve as bends or flexure points; however, their functional significance is unknown.

Perhaps the most striking feature of the hinge is its association with ParC, a subunit of DNA topoisomerase IV [Hayama and Marians, 2010; Li et al., 2010b]. The interface is located on the outward side of the hinge and is comprised mostly of charged amino acids [Vos et al., 2013]. Although this association is rather weak, it seems essential. Mutations in ParC or MukB that disrupt their interface are detrimental for the cell. In fact, one of the long known temperature sensitive mutations in ParC, G725D, maps close to this interface [Vos et al., 2013]. The interaction is specific to the C-terminal domain (CTD) of ParC; no association with DNA gyrase is detected. Because the interaction is fairly weak, it is unclear whether ParC should be viewed as a part of MukBEF complex or a transient cofactor. The presence of MukB stimulates intramolecular but not intermolecular reactions of topo IV [Hayama et al., 2013] by neutralizing inhibitory effects of CTD on some of the topo IV reactions [Vos et al., 2013]. Specifically, MukB stimulates topo IV-catalyzed relaxation of negatively supercoiled DNA but has no effect on relaxation of positive supercoils or decatenation and promotes DNA knotting [Hayama et al., 2013]. How topo IV affects reactions of MukBEF is yet to be determined.

ATP plays a central role in coordinating MukB interactions with its accessory subunits and DNA [Cui et al., 2008; Woo et al., 2009]. The ATPase site of MukB is homologous to that of other SMC proteins with readily identifiable Walker A and B regions, the signature motif and the D-loop (Fig. 3C). A number of mutations in the ATPase site have been described. Some of them are particularly useful in conformational analysis. In particular, the E1407Q mutation disrupts ATP hydrolysis but not binding and stabilizes the ATP-sandwiched MukB head [Woo et al., 2009]. In contrast, the D1406A mutation is deficient in ATP binding and head engagement and S1366R (in C-motif) precludes head engagement but not ATP binding or association with MukEF [Hirano et al., 2001; Woo et al., 2009]. The Walker A K40I mutation is expected to fail in ATP binding [Hirano et al., 2001; Woo et al., 2009], whereas the K40D S41G was deficient in DNA bridging but not binding [Petrushenko et al., 2010].

A recurrent theme in these studies is that ATP serves as a conformational switch (Fig. 4). ATP binding promotes MukB loading onto DNA, whereas ATP hydrolysis helps to displace it. Accordingly, ATPase deficient mutant E1407Q displayed markedly longer life time within stationary foci in live cells than its ATPase proficient counterpart whereas the ATP binding mutant D1406A failed to associate with foci [Badrinarayanan et al., 2012b]. The effect is even more pronounced with the *P. aeruginosa* MukBEF-like protein MksBEF. MksB binding to DNA significantly declines in the presence of ATP, whereas ATPase rate is negatively affected by DNA [Petrushenko et al., 2011].

In contrast to its functional role, structural perturbations caused by ATP are yet to be established. Clearly, ATP binding and hydrolysis lead to major conformational changes within the dimeric MukB head. What is less clear is whether or not these changes involve disassociation of the dimer. The evidence in favor of disengagement is based upon electron microscopy studies, which reveal both V- and I-shaped MukB molecules [Matoba et al., 2005; Melby et al., 1998] as well as sizing studies of ATPase mutants [Hirano et al., 2001; Woo et al., 2009]. The argument against such disengagement stems from the comparison with the ABC transporters, the only other known class of ABC type ATPases. The ATPase unit of the transporters is believed to stay intact throughout the enzymatic cycle using, instead, the generated energy to agitate the transmembrane domain [Oldham et al., 2008]. This view recently found strong support from crystallography studies of the SMC-like protein Rad50 [Lammens et al., 2011]. ATP hydrolysis was shown to cause a massive transversal motion within the dimeric Rad50 head that was tilting the coiled coils but did not disrupt the interface between the two heads. Such tilting motion could conceivably be occurring in MukB and used to transmit the conformational change along the coiled coils. Whether or not this change leads to conformational rearrangement at the hinge or its disassociation is unknown.

DNA organization: binding, bridging and condensation

MukBEF displays a complex set of DNA reshaping activities that befit its role in global chromosome organization. We review these activities below with the focus on the presumed ability of MukBEF to produce chromatin scaffold while also pointing out observations that do not quite fit into this scheme and suggest an even richer mechanism.

All DNA binding activities of the complex reside in its SMC subunit, MukB, whereas MukEF regulates it and does not bind DNA on its own [Petrushenko et al., 2006b]. MukB binds linear and circular DNA equally well and has only slight preference, if at all, to supercoiled DNA [Petrushenko et al., 2006a]. This underscores the importance of physical association between the protein and DNA. Curiously, MukB binds phage single stranded DNA with high affinity. The significance of this interaction has not been explored. Perhaps it reflects preferred binding to DNA crossings which has been also reported for other SMC proteins [Akhmedov et al., 1998; Kimura and Hirano, 1997]. Fluorescence microscopy and cell biology studies find MukB in complex with the chromosome [Badrinarayanan et al., 2012b; She et al., 2007], which reveals that its primary substrate is double stranded DNA.

When overproduced in living cells [Wang et al., 2006] or mixed in vitro with long phage DNA [Chen et al., 2008], MukB induces DNA condensation. Such condensation cannot be explained by DNA binding alone and implies through-space DNA bridging events. Similarly, condensation of single DNAs stretched using magnetic tweezers involves DNA looping [Cui et al., 2008]. Notably, DNA condensation proceeds slowly on single DNAs, suggesting that this is not the primary mechanism of DNA compaction. In contrast, DNA bridging events between separate DNAs are highly efficient and might be limited by collision frequency [Petrushenko et al., 2010]. Moreover, a single MukB can apply only a weak force, < 0.4 pN, to DNA during condensation, which further argues for the random collision mechanism of DNA condensation.

A single MukB has a short life time on DNA. However, it can be stabilized by further protein binding [Cui et al., 2008]. MukB binding to DNA is highly cooperative, with the Hill coefficient of 3. As a result, the protein binds DNA according to the zipper mechanism, much like for DNA annealing, when the slow nucleation step is followed by a fast propagation of the protein cluster (Fig 5A). DNA condensed by such clusters displays significant resilience to applied forces, which might become handy if the protein is indeed involved in chromosome segregation.

The nucleation-propagation mechanism could conceivably serve as a sensor for finding a suitable DNA binding site. Indeed, an ideal scaffold protein is expected not to interfere with information processing enzymes but, rather, stay in the shades and bind only silent DNA. Such silent sites are likely to be only sparsely bound by other proteins and would offer a good landing spot for MukB clusters. If, on the other hand, a need arises for the bound DNA, MukB clusters could be readily removed, owing to low stability of the monomers, one protein at a time with the help of chromatin remodeling activity of DNA helicases or polymerases. In this sense, the protein behaves as a macromolecular clamp that associates with sparsely populated DNAs.

Dynamics of DNA bridging is also consistent with this mechanism. Mixing order experiments using magnetic bead pull-down assay [Petrushenko et al., 2010] revealed that DNA binding and bridging are two distinct events in the reaction cycle of MukB and that bridging occurs most efficiently between a preformed, DNA-bound MukB cluster and a fragment of protein-free DNA (Fig. 5B). Similar to the case of DNA condensation, the formed bridges initially display low stability but are quickly strengthened by propagation of the bridge.

Both DNA binding and bridging can occur in the absence of any nucleotide. Both reactions, however, are accelerated in the presence of ATP. Owing to the high cooperativity of the reaction, the effect is quite dramatic, indicating that ATP can be used to regulate association of MukB with DNA. Importantly, ATP plays a role of a conformational switch here rather than an energy cofactor since effects of ATP could be reproduced by chelating magnesium [Cui et al., 2008]. This is not unlike MukB-MukEF interaction, which is similarly affected by inclusion of ATP and removal of magnesium [Petrushenko et al., 2006b; Woo et al., 2009]. Apparently, the two treatments stabilize the same conformation of MukB. This feature remains a poorly understood curiosity of MukBEF. Perhaps it is somehow related to

the high affinity of MukF for another divalent cation, calcium [Yamazoe et al., 1999], and reflects a dormant ability of MukBEF to serve as an environmental sensor.

Taken together, the activities of MukB offer a plausible view of how chromatin scaffold could be organized ([Cui et al., 2008] and Fig. 5C). Owing to its DNA clamping activity, MukB is expected to bind random regions of DNA that do not participate in other cellular activities and relocate to a different place when needed. Given its low intracellular level, MukB may not be able to cover the entire chromosome but probably spreads from the initial binding site to the neighboring DNA loops via a series of bridging events. This binding pattern should minimize non-productive use of the protein that does not result in bridges and offers an excellent example of self-organization in complex systems.

Another attractive aspect of this mechanism is the prediction that chromosomes are condensed without any external force, which could be damaging to chromosome integrity. Indeed, the bridges are predicted to form through capture of randomly colliding protein-free DNA fragments. Thus, by activating MukB through the use of cofactors, the cell can modulate the density of bridges and, therefore, the overall compactness of the chromosome. Once formed, however, the bridges become resilient to external forces and can be used to pull chromosomes apart if needed. This scaffolding mechanism appears generic and might be shared by other chromosome organizing proteins.

Chirality of MukB-DNA interactions

When examined using the topo-2 coupled DNA knotting assay, all tested condensins displayed propensity for stabilization of right handed DNA loops [Kimura et al., 1999; Petrushenko et al., 2006a; Stray et al., 2005]. Importantly, the topology of the generated knots is such that it rules out existence of a plectoneme, i.e. the interwound superhelix between the loops. Such plectoneme spontaneously forms in supercoiled DNA owing to the rules of DNA mechaniscs [Vologodskii and Cozzarelli, 1994] and dramatically alters the topological complexity of the topoisomerase reaction [Wasserman and Cozzarelli, 1991], The fact that such plectonemes were not observed reveals that the proteins do not generate freely diffusing DNA supercoils but rather stabilize right handed DNA crossings at the base of the loops (Fig. 6A). In contrast, the accompanying DNA supercoiling varied from protein to protein. For MukB, the generated supercoils are negative, which is the opposite to what is expected given the right handed chirality of the loops [Petrushenko et al., 2006a]. Apparently, MukB unwinds DNA upon binding, which might explain its high affinity for single stranded DNA.

Magnetic tweezers analysis of DNA bridging revealed high preference of MukB for right handed crossings (Fig. 6B and [Petrushenko et al., 2010]). This is consistent with chirality of DNA loops detected using knotting assay and further implicates DNA bridging as the chirality discriminating reaction step. The structural basis of such discrimination is unclear. Indeed, MukB is a homodimer and, therefore, is expected to be achiral or exist as a racemic mixture of enantiomers. This should result, in turn, in a racemic mixture of DNA loops. The proteins could conceivably acquire chirality due to posttranslational modifications present in one of the subunits of the protein. This explanation, however, seems stretched given that

overproduced MukB was employed in the experiments and that chirality of DNA looping is conserved across kingdoms of life. A more plausible explanation postulates that the protein somehow senses the double helical nature of the DNA- perhaps via DNA unwinding- and amplifies it by preferentially stabilizing right handed crossings. Yet another possibility envisions binding of two DNAs to the same MukB head on two distinct surfaces of the protein (Fig. 6C). Such binding would be chiral simply by the virtue of stereochemistry of the binding site.

The chirality of condensin-DNA interactions might impose it onto other aspects of higher order chromosome packing and might be ultimately responsible for chiral appearance of the chromosomes as observed using light microscopy [Hochstrasser et al., 1986]. Evolutionary advantage of such anisotropy, however, is unclear. This anisotropy could become mechanistically significant in the light of the recently discovered interaction between MukB and ParC, a subunit of DNA topoisomerase IV [Hayama and Marians, 2010; Li et al., 2010b]. As outlined below, this interaction might help recruit the protein to a unique DNA substrate.

Beyond scaffold: Recruit and be recruited

The chromatin scaffolding activity of MukB does not recuperate all of its intracellular functions nor does it even require a contribution from MukE and MukF. This suggests other roles for the protein besides maintenance of the chromosomal size. A direct test confirmed this conjecture by revealing that MukEF is essential even in cells with constitutively condensed chromosomes [Wang et al., 2006]. This additional function is likely related to focal subcellular localization of MukBEF.

Similar to other bacterial condensins [Lindow et al., 2002; Minnen et al., 2011; Volkov et al., 2003], MukBEF forms clusters in the middle of the nucleoids [Ohsumi et al., 2001; She et al., 2007]. This location changes from the middle of the short cells to the ¼ and ¾ positions in cells with replicating chromosomes. MukE appears to play the primary role in driving such localization as was revealed in a recent random mutagenesis study. All point loss-of-function mutations in MukE disrupted focal localization of MukBEF but not its interaction with DNA ([She et al., 2013] and Fig. 3B). A similar loss of focal localization was observed upon disruption of the linker region in MukF, which tethers MukE to MukB, and inducer-triggered degradation of MukE in live cells [Shin et al., 2009]. Taken together, these data point to a certain separation of functions within MukBEF. While MukB is responsible for DNA organization, MukE ensures its targeting to specific cellular addresses and MukF links the two together and perhaps coordinates their activities by modulating ATP turnover by MukB (Fig. 5C).

Neither MukB nor MukE can form clusters in the absence of the other components of the complex [Ohsumi et al., 2001; She et al., 2007]. Apparently, both the DNA binding activity of MukB and the positioning activity of MukE are required for their formation. Accordingly, MukBEF found in the clusters consists of multimers of the DNA proficient $MukB_4$-$Muk(E_2F)_2$ complex [Badrinarayanan et al., 2012b]. In contrast, the diffused fraction of MukBEF has composition $MukB_2$-$Muk(E_2F)_2$. The coexistence of the mobile and stationary

fractions was also observed for the *B. subtilis* condensin SMC-ScpAB, in which case the mobile and stationary fractions of condensin was composed of SMC and SMC-ScpAB complexes, respectively [Kleine Borgmann et al., 2013a; Kleine Borgmann et al., 2013b]. It should be noted that intracellular dynamics of MukBEF composition needs further elucidation, since ultracentrifugation studies of *E. coli* cell extracts did reveal coexistence of $MukB_2$-$Muk(E_2F)_2$ and $MukB_2$ complexes but not of $MukB_4$-$Muk(E_2F)$ [She et al., 2013].

Subcellular localization of MukBEF is similar to that of *oriC*, the origin of chromosomal replication [Danilova et al., 2007]. This similarity is functionally significant since depletion of MukB or MukE leads to the loss of *oriC* localization whereas repletion of the protein restores the foci [Adachi et al., 2005; Badrinarayanan et al., 2012a]. The loss of *oriC* localization is accompanied by disorganization of the entire chromosome and the failure to relocate the chromosome arms into the opposite halves of the cell. Thus, recruitment of MukBEF towards the origin region is essential for chromosome segregation in *E. coli*. Of note, repletion of MukBEF leads to rapid restoration of MukBEF foci followed by slow repositioning of the chromosome, suggesting that formation of the protein clusters could be prerequisite to spatial chromosome organization [Badrinarayanan et al., 2012a].

The force responsible for positioning of MukBEF is unknown. Perhaps it involves a possibly indirect interaction with an origin proximal region as was found for SMC-ScpAB. In *B. subtilis* and *Streptococcus pneumoniae*, condensin is enriched in the vicinity of the replication origin and this enrichment is dependent on the presence of the *parABS* operon [Gruber and Errington, 2009; Minnen et al., 2011]. ParAB*S* encodes a bacterial mitotic apparatus, which consists of two proteins, Par and ParB, and a DNA element, *ParS*, which serves as a ParB binding site (reviewed in [Mierzejewska and Jagura-Burdzy, 2012; Szardenings et al., 2011]). ParAB*S* is located close to *oriC* of many bacteria but is completely absent from the genome of *E. coli* K12. SMC binding is enriched in the vicinity of Par*S*, and moving the *parABS* operon away was sufficient to relocate SMC towards chromosomal arms as well. Clearly, ParAB*S* recruits SMC towards the origin region. Which system recruits MukBEF to *oriC* is unknown.

Besides being recruited to its site of action, MukBEF appears to serve as a recruiter itself. A weak interaction between MukB and ParC was recently discovered using co-immunoprecipitation [Hayama and Marians, 2010; Li et al., 2010b]. This interaction is sufficiently selective to discriminate between topo IV and DNA gyrase [Vos et al., 2013] and sufficiently strong to recruit a subset of topo IVs to the quarter positions inside the cell [Nicolas et al., 2014]. Mutations that disrupt MukB-ParC interface adversely affect such localization and *E. coli* viability [Hayama and Marians, 2010; Li et al., 2010b].

Mechanistic understanding of the benefits of PaC-MukB interaction is still lacking. The primary effect of MukB on topo IV is to derepress its activity on negatively supercoiled DNA [Vos et al., 2013]. It seems tempting to conclude then that MukB helps topo IV in removal of negative supercoils. This idea, however, does not readily explain the findings that suppressors of MukB deficiency map to topoisomerase mutations that increase rather than decrease negative supercoiling [Adachi and Hiraga, 2003; Sawitzke and Austin, 2000]. Among alternative- or, rather, additional- possibilities, we favor the one that envisions

recruitment of topo IV to a hazardous, hard-to-find substrate. Such a substrate could be the right handed pre-catenane nodes that might be generated behind replication fork if topoisomerases are unable to keep up with its rapid progression (Fig. 6C and [Postow et al., 2001; Wang, 2009]). Accumulation of such nodes would entangle the daughter chromosomes and could preclude their segregation into opposite halves of the cell potentially leading to a collapse of the segregation machine. Topo IV is the only enzyme capable of resolving such interlinks. Although it performs this reaction efficiently in vitro it could conceivably be slow locating this substrate on its own within the cellular milieu.

In this view, increased negative DNA supercoiling should alleviate the need for topo IV via two mechanisms. First, DNA supercoiling strongly favors DNA unlinking [Alexandrov et al., 1999; Rybenkov et al., 1997] and, thereby, promotes decatenation of newly made sister chromatids. Second, increased negative supercoiling would decrease the rate with which positive supercoils are generated by DNA polymerase and preclude accumulation of pre-catenanes [Alexandrov et al., 1999; Postow et al., 2001].

Concluding remarks

MukBEF functions at the heart of several cellular processes that ensure chromosome replication, segregation and global organization.

MukBEF acts as a mobile, dynamic complex with distinct contribution from its subunits. MukB is responsible for DNA binding and reshaping. MukE is responsible for recruitment of MukBEF to the protein clusters in the middle of nucleoids. MukF physically links MukB and MukE and also coordinates ATP hydrolysis and DNA binding.

MukB displays macromolecular clamping and bridging activities, which, taken together, should result in formation of a chromatin scaffold. These activities could be shared by other chromosome organizing proteins. What makes MukBEF unique is its recruitment and regulation. MukBEF could also serve as a platform for recruitment of additional components. MukB-ParC interaction could be an example of such recruitment.

The nature of MukBEF clusters at the quarter positions remains unknown. A possibility worth exploring is that these clusters are chromosome replication and segregation factories, of which MukBEF is an integral part.

Functional and structural interactions between MukBEF and DNA topoisomerases were reported in numerous, seemingly unrelated studies. These coincidences are difficult to ignore and implicate MukBEF as a key guardian of the topological integrity of the chromosome.

Acknowledgment

This work was supported by Grants 1049755 from the National Science Foundation and AI094124 from the National Institutes of Health.

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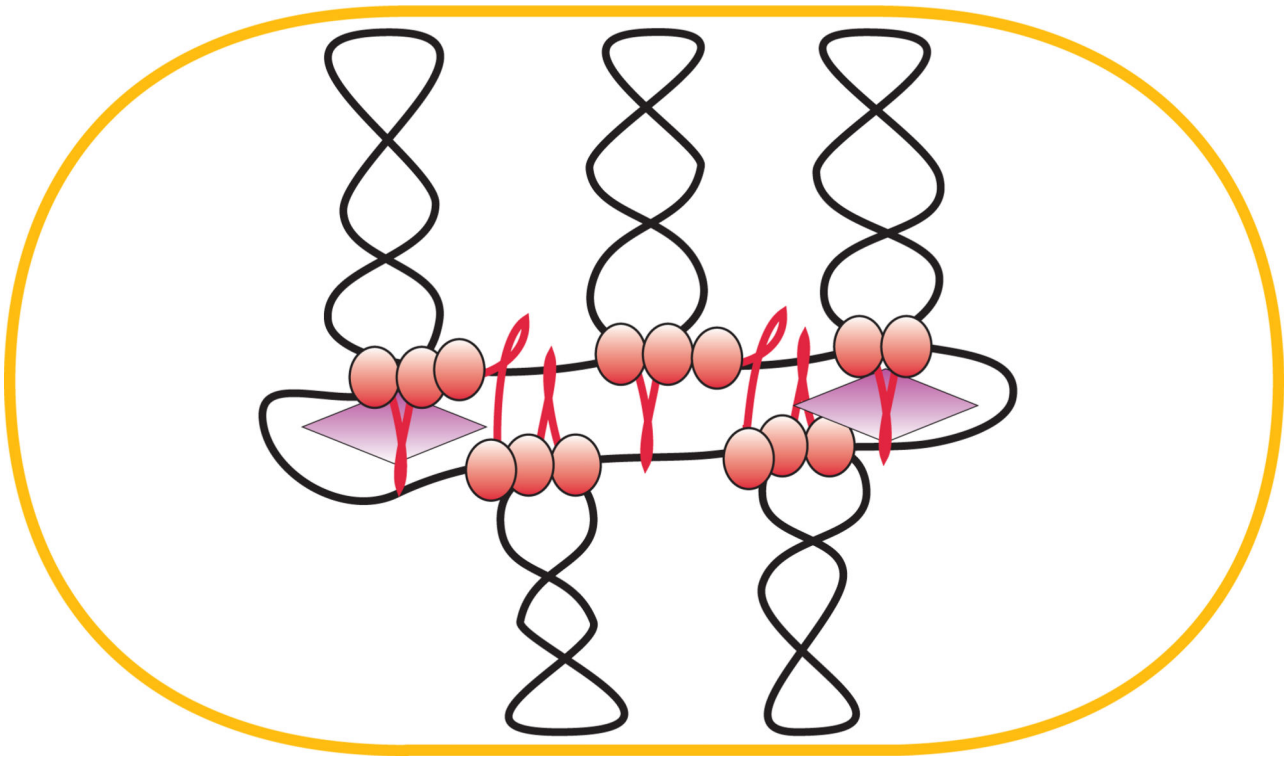

Figure 1.

A scaffold model of the chromosome. The giant loop architecture of the chromosome is stabilized by multiple attachments to the cellular matrix. The matrix does not have to be continuous but is likely to be linked to the chromosome segregation machinery. The chromosome is further stabilized by DNA supercoiling and numerous nucleoid associated proteins, NAPs, which bend and bridge DNA (not shown).

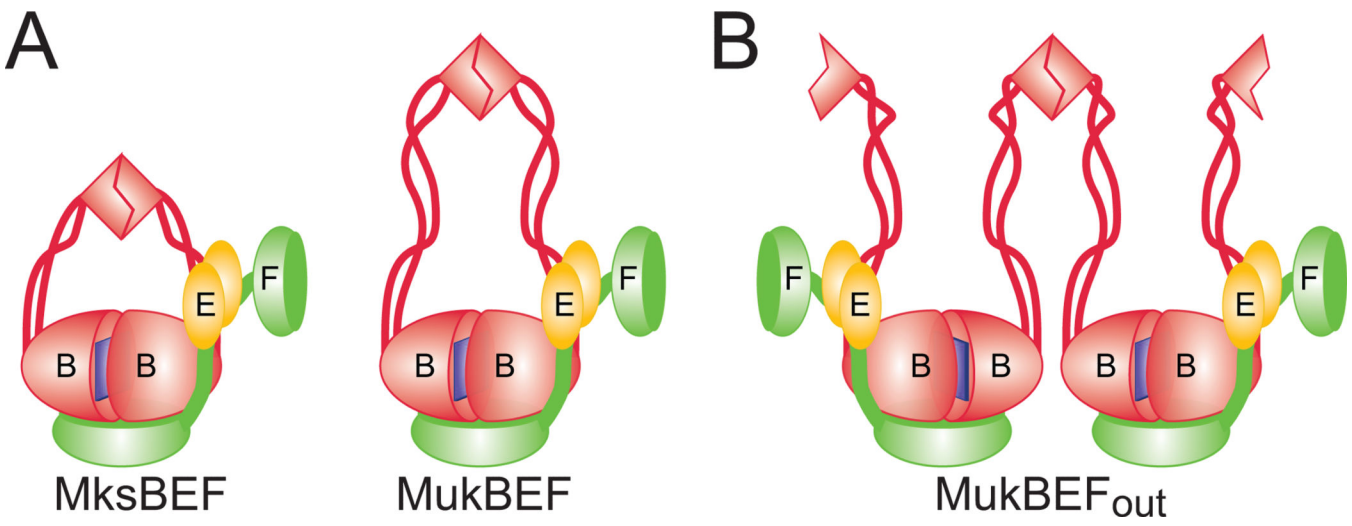

Figure 2. Architecture of MukBEF

(A) Organization of MukBEF and MksBEF. ATP-mediated dimerization of MukB head creates a high affinity DNA binding site. The head can accommodate a single C-terminal WHD of MukF. N-terminal WHD of MukF is prone to further dimerization. With the exception of the shorter coiled coils, MksBEF is predicted to form a similar structure to MukBEF.

(B) A possible organization of MukBEF if dimerization interfaces of its hinge and head domains face away from each other.

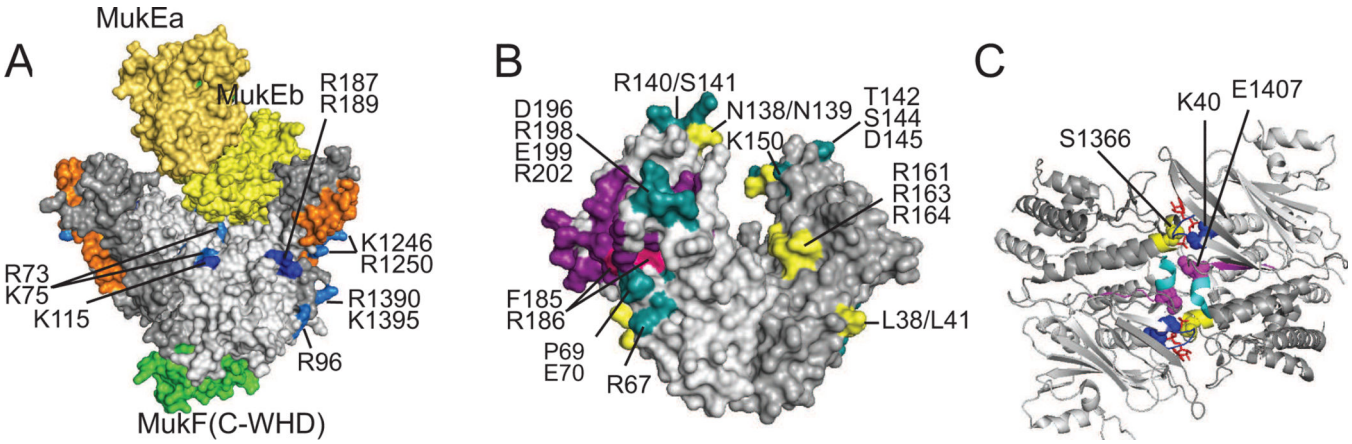

Figure 3.

Architecture of crystallized MukBEF fragments. (A) Crystal structure of dimerized MukB head in complex with a fragment of MukF and a dimer of MukE (PDB 3EUK). The N- and C-terminal domains of MukB are shown in light and darker grey, respectively, MukF is green and the two MukEs are in two shades of yellow. The stems of the long coiled coils are shown in orange. Amino acids involved in DNA binding are blue (essential) or light blue (reduced affinity). Amino acids are numbered according to their position in the *E. coli* MukB. Note that no MuB-MukE contacts hold MukE above MukB head, where it blocks the DNA binding site.

(B) Crystal structure of MukE (PDB 3EUH) with known mutations. The two MukE monomers are shown in two shades of gray. Purple marks the interface with MukF. Also shown are non-essential amino acids (yellow), essential amino acids found on the MukE-MukF interface (red) and essential amino acids elsewhere (green).

(C) Organization of the ATPase site (PDB 3EUK). The dimeric MukB head viewed from the top with two bound ATPγS (red). Shown are the conserved ATPases regions, including Walker A (blue), C-motif (signature; yellow), Walker B (magenta) and D-loop (cyan). The N- and C-terminal domains are shown in light and darker shades of grey.

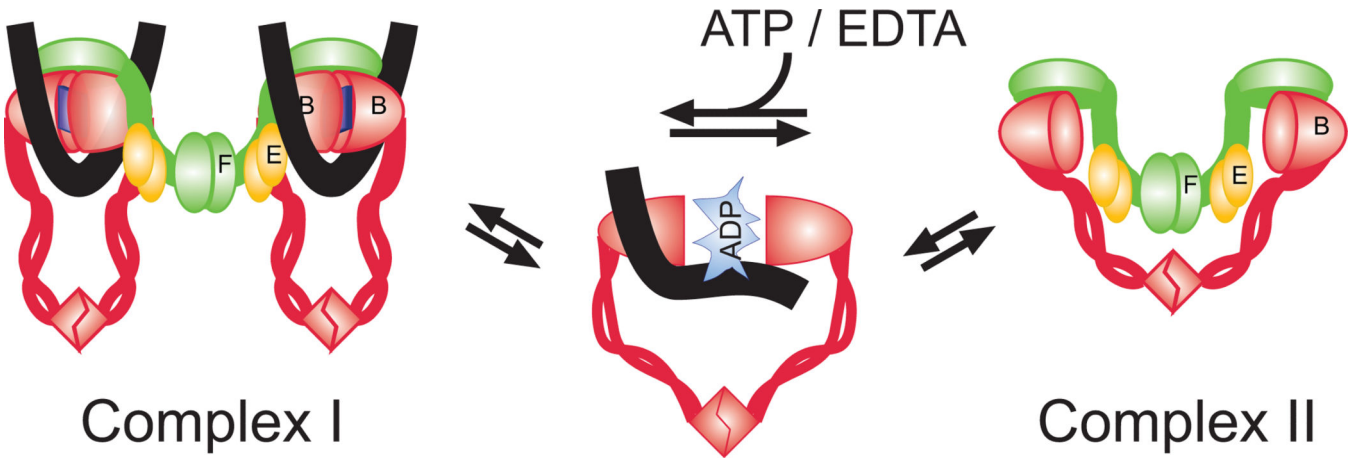

Figure 4.

A schematic view on ATP-mediated regulation of DNA binding and architecture of MukBEF. ATP binding promotes engagement of MukB heads, which creates a high affinity DNA binding site and is accompanied bybinding of DNA (black lines) and formation of the $MukB_2$-$Muk(E_2F)$ complex (Complex I). ATP hydrolysis promotes dissociation of the heads (or perhaps another functionally identical conformational change), which disrupts the DNA binding site and forces dissociation of DNA and promotes formation of the $MukB_2$-$Muk(E_2F)_2$ complex (Complex II).

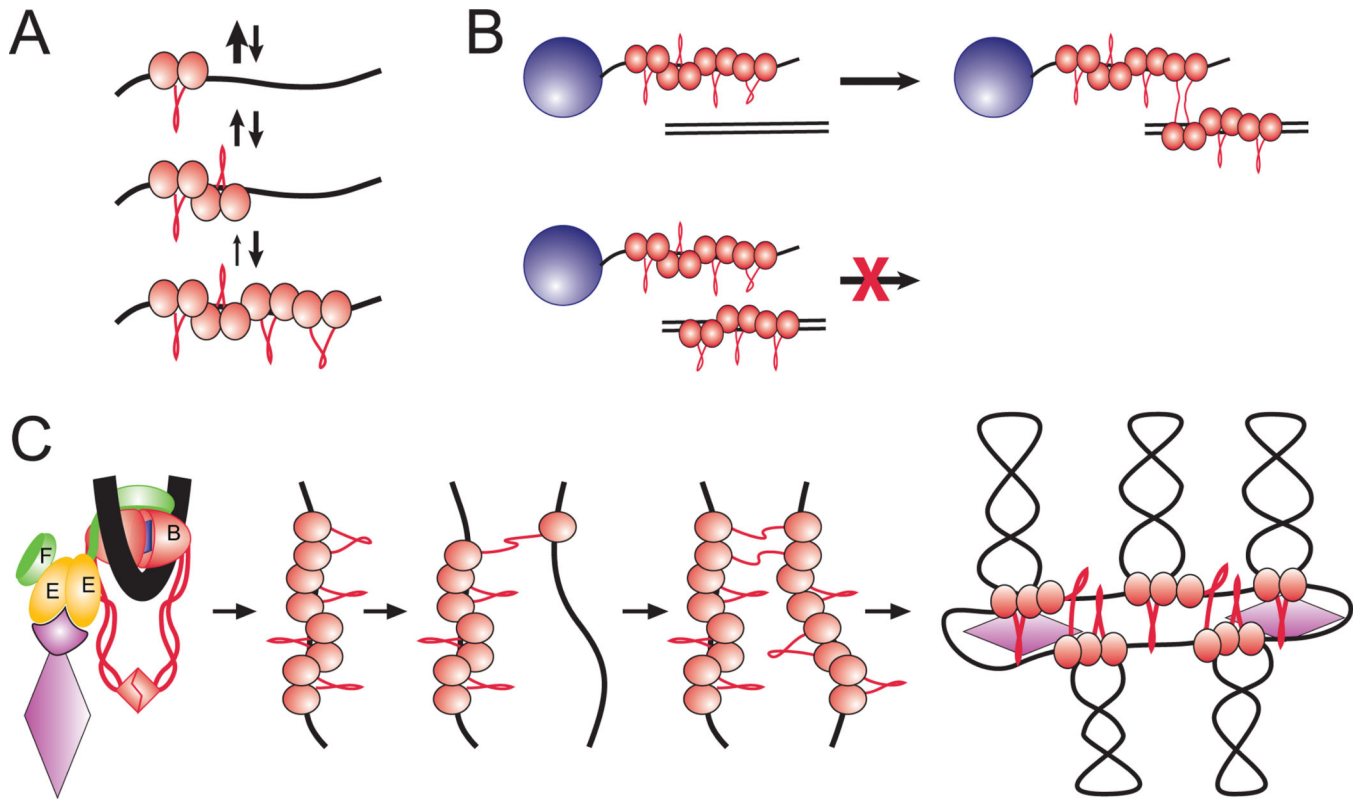

Figure 5. DNA scaffolding activity of MukBEF

(A) Nucleation-propagation mechanism of DNA binding. A single MukB forms only a short-lived complex with DNA. The binding, however, is highly cooperative, and each subsequent bound protein stabilizes the cluster through nearest neighbor interactions.

(B) Bridging occurs between DNA bound MukB clusters and naked DNA.

(C) Proposed mechanism of chromosome organization. The protein is postulated to be recruited to its initial binding site with the help of the regulatory subunits. The protein binds the target site via the clamping mechanism described in (A). Once the clamp is formed, the MukB cluster attempts bridging with randomly colliding DNA segments. Finding a sufficiently long stretch of naked DNA allows stable bridging and further spreading of the scaffold.

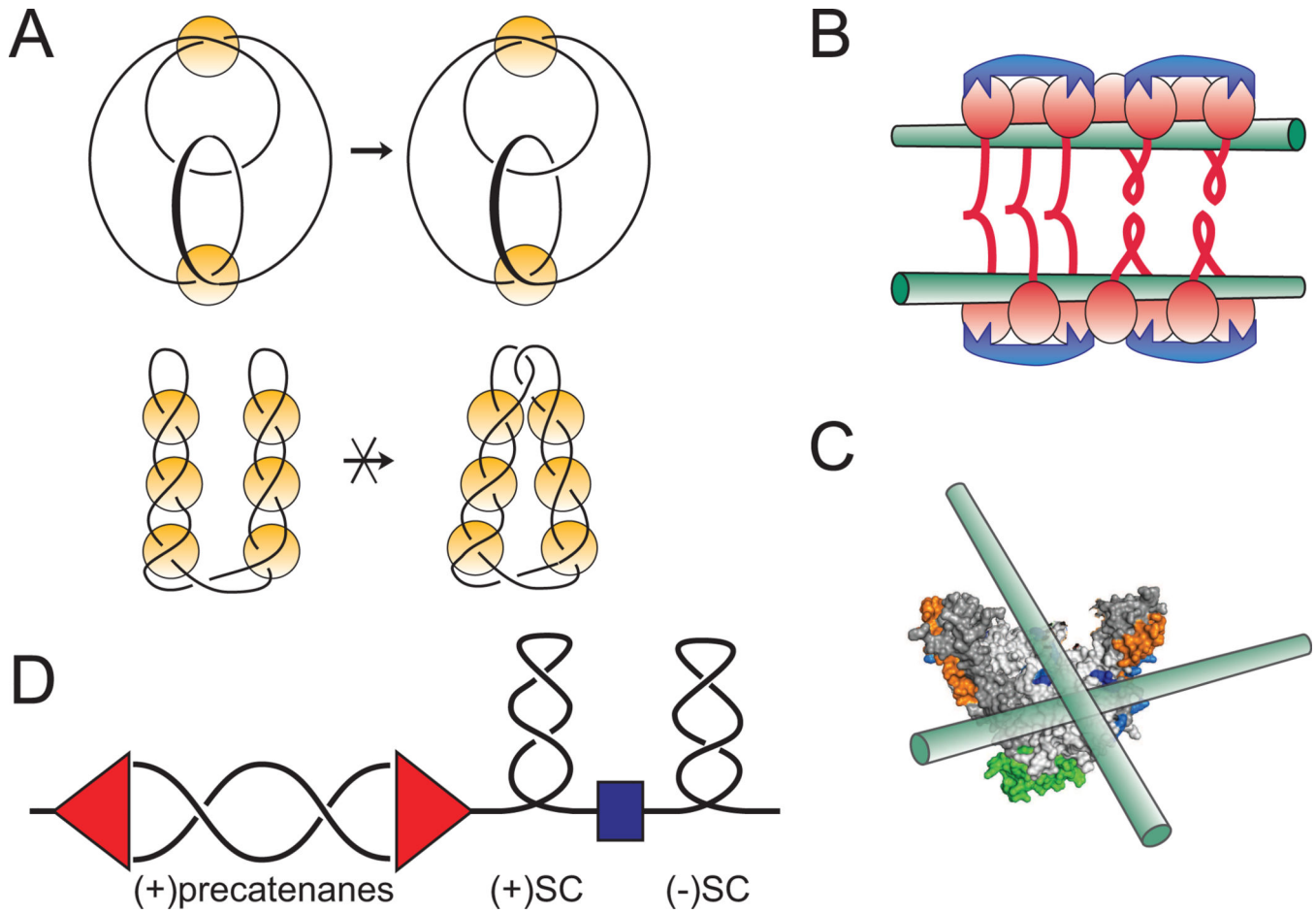

Figure 6. Chirality of MukB-DNA interactions

(A) Knotting patterns expected on looped and interwound DNA substrates in topo-2 coupled assay. Knots are often classified according to the minimal number of crossings in a projection of the knot onto a flat surface [Rolfsen, 1976]. The simplest knots, trefoils, contain three irreducible crossings and can exist as either right- or left-handed enantiomers. Condensins promote formation of (+) trefoils, which reveals right-handed DNA looping (top diagram). In contrast, topo-2 reaction on an interwound superhelix would produce twist family knots with multiple irreducible crossings (bottom diagram). Yellow circles depict proteins that capture DNA crossings that were either spontaneously formed in the interwound DNA superhelix (bottom) or comprise the base of protein-induced DNA loops (top).

(B and C) The high preference of MukB for right handed crossings could be explained by chiral binding of DNA to different MukBs from the same cluster (B) or the same MukB head (C).

(C) Pre-catenane formation around replication fork. Progression of the replication fork (red) induces waves of positive supercoiling, whose propagation is limited by domain boundaries (blue). Negative supercoiling ahead of the fork is restored by DNA gyrase. Failure to achieve that results in positive supercoiling which migrates into the replicated region in the

form of pre-catenanes. Recruitment of topo IV to this region might facilitate their removal and further progression of DNA replication.