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Combing Chromosomal DNA Mediated by the SMC Complex: Structure and Mechanisms

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Genome maintenance requires various nucleoid-associated factors in prokaryotes. Among them, the SMC (Structural Maintenance of Chromosomes) protein has been thought to play a static role in the organization and segregation of the chromosome during cell division. However, recent studies have shown that the bacterial SMC is required to align left and right arms of the emerging chromosome and that the protein dynamically travels from origin to *Ter* region. A rod form of the SMC complex mediates DNA bridging and has been recognized as a machinery responsible for DNA loop extrusion, like eukaryotic condensin or cohesin complexes, which act as chromosome organizers. Attention is now turning to how the prototype of the complex is loaded on the entry site and translocated on chromosomal DNA, explaining its overall conformational changes at atomic levels. Here, we review and highlight recent findings concerning the prokaryotic SMC complex and discuss possible mechanisms from the viewpoint of protein architecture.

1. Introduction

The relative simplicity of the structure of bacterial cells has significantly contributed to our understanding of many complex biological systems. Yet in bacteria, the principles of chromosome organization have not fully understood. Bacteria maintain the genetic materials as a compact nucleoid through the action of many nucleoid-associated proteins (NAPs),[1] but the chromosome is free in the cytoplasm and not enclosed in a compartmentalized space. Recent advances, such as chromosome conformation capture experiments including Hi-C and ChIA-PET techniques, have provided detailed information that allows to obtain a picture of chromosomal domains within the whole nucleoid, as well as the position of specific proteins attached to the intact chromosome.[2]

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Now studies on bacterial nucleoid structure have moved forward to elucidating the mechanism by which proteins actively individualize newly synthesized circular chromosomes preventing entanglements.

Independently from abundant NAPs which stabilize local DNA structures, the SMC protein with the regulatory subunits, ScpA and ScpB, is involved in the large-scale organization of the bacterial chromosome (**Figure 1**A).[3–7] Most bacteria including *Bacillus subtilis* have the SMC subunit that shares hom`ology with eukaryotic SMC molecules. The SMC homodimer has a rod shape with ≈50 nm helical arms.[8] The ScpAB subcomplex then forms an asymmetrical bridge between distal tips of the SMC dimer (Figure 1A).[9,10] Cells lacking any of the genes encoding these three subunits are inviable under fast-growth conditions, however the mutants are viable under slow-growth conditions and produce anucleate cells to some extent.[11] SMC-ScpAB is recruited to a site adjacent to the replication origin in a manner dependent on the Spo0J/ParB partitioning protein bound to bacterial centromeric *parS* sites.[12,13] Therefore, the complex is essential for the separation of the newly replicated origin regions.[14–17] It has been suggested that the SMC ATPase activity drives conversion of the complex into an active ring conformation, which in turn facilitates its targeting to the Spo0J coated DNA region (Figure 1B).

2. Secrets of the Coiled-Coil Arm in SMC Proteins

What is the role of the long coiled-coil arm of SMC? Recently, the Gruber's group has tackled this challenging question and succeeded in constructing a structural model of a full-length SMC protein (**Figure 2**A). First, Bürmann et al. have clarified that the SMC coiled-coil length faithfully reflects a helical periodicity between the two juxtaposed arms.[18] Even among prokaryotic SMC proteins, the primary sequence of the coiled-coil region is not conserved, whereas the length is evolutionarily conserved in several groups, in which contact area between arms appears in multiples of 91 amino acids (Figure 2A). Second, Diebold-Durand et al. have reported an archaeal SMC structure in combination with in vivo cysteine crosslinking and crystallography.[19] The modelled dimer has the appearance of reverse action forceps and contains the expected continuous antiparallel coiled

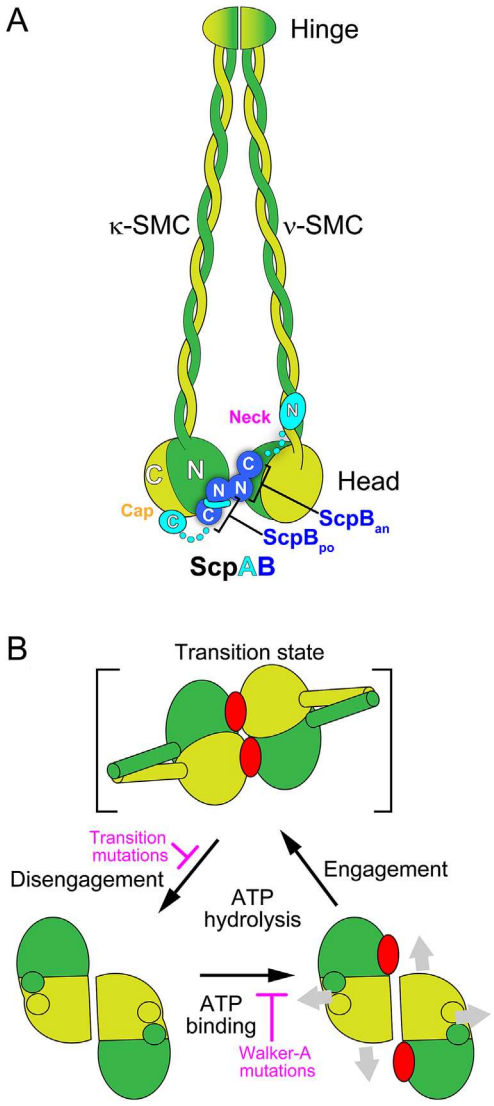

Figure 1. Schematic architecture of SMC-ScpAB and the SMC ATPase cycle. A) The SMC monomer is formed by folding its peptide chain backward through anti-parallel coiled-coil interaction. The N- and C-terminal regions fold together into an ATP-binding cassette (ABC)-type ATPase "head" domain at one end.[67] At the distal end, the middle section of the peptide chain forms a "hinge" domain that mediates SMC dimerization. The ScpA subunit, which belongs to the kleisin family,[68] comprises an N-terminal domain (NTD) and C-terminal winged-helix domain (WHD) at its tips.[9,10] ScpB homodimerizes through its N-terminal WHDs and uses its two C-terminal WHDs to aid binding to the ScpA middle region.[10] Among the two subunits of ScpB within the subcomplex, the one close to the ScpA NTD is referred to as ScpB$_{an}$ ("an" for anterior) and the other is referred to as ScpB$_{po}$ ("po" for posterior).[10] The ScpA NTD binds to the "neck" region of one SMC protomer (termed ν-SMC), whereas its C-terminal domain (CTD) binds to the "cap" region of the other (κ-SMC).[9] Thus, the functional SMC full complex is thought to exhibit an asymmetrical configuration due to the bridging of the ScpAB subcomplex. B) Two ATP molecules (red) are bound to the SMC head domains. Engagement is dimerization of the ATP-bound form, and disengagement is the dissociation after ATP hydrolysis. Movements of the head domain for engagement are shown by gray arrows based on crystal structures.[19,24] Walker A mutations block the ATP binding step, whereas transition state mutations apparently hold the intermediate state by decreasing the rate of ATP hydrolysis.

coils in a juxtaposed orientation (Figure 2B). Different from numerous hydrogen bonds observed between the eukaryotic SMC2/4 arms in the region beneath their hinge heterodimer [20], the prokaryotic SMC arm is supported by pinpoint contacts, mainly at seven positions, maintaining the arm's linearity (Figure 2C). In addition, an irregular coiled-coil structure, termed joint, is observed near the head domain (Figure 2D). The model also suggests that the two arms force the head domains into a non-engaged position (Figure 1A), and argues that their subsequent engagement mechanically opens the inter-arm space. This resting mode is also observed in complexes containing the ABC-type ATPase domain.[21] The two arms probably constantly search for suitable contacts within the restricted range to avoid repulsion between residues having the same electropotential charge. These results also provide valuable insights into how a pair of linear structures is aligned dynamically but within a cross-linkable range, over a length of approximately 50 nm.

3. Twisting and Opening Leading to the Asymmetrical Hinge Dimer

To date, a variety of SMC hinge structures from different species have been reported, clarifying the specific folding and conserved basic potential surface.[20,22,23] Overall, there are two types of structure observed. One is a symmetrical dimer with rod-like juxtaposed coiled coils,[20] and the other is a dimer with coiled coils protruding in almost opposite directions[22] (**Figure 3**A). Moreover, a recently solved bacterial hinge exhibits an asymmetrically oriented dimeric structure with a half-opened interface.[24] Structural comparison among twisted hinge structures[22,25] suggests that the dimer resolves one of the interfaces to expose the conserved basic surface (Figure 3B). This motion is expected when the direction of the coiled-coil domain is changed after head-head engagement; therefore, the hinge domain works as a bimodal switch. The asymmetrical hinge with widely separated coiled coils is structurally relaxed, as the start site of the C-terminal helix of the coiled coil is stably anchored to the domain by its conserved hydrophobic residues (Figure 3A). The hinge structure with juxtaposed coiled coils is a rather constrained form. Close juxtaposition of the arms creates distortions on their joints of the hinge domain, thereby forcing the anchoring residues to be unplugged and resulting in a symmetrical dimer fold.

4. Potential Dual Regulation by ScpB

Mechanistic details on the ScpAB subcomplex are largely obscure. Different from ScpA, ScpB is not directly involved in formation of the tripartite ring.[26] However, ScpB is essential for triggering the SMC ATPase activity and loading the complex onto chromosomes.[10,27] One of the key questions is how the SMC is dynamically regulated by these factors. The binding of the ScpA NTD to the SMC neck has been already reported,[9] and probably this feature is common among all the SMC complexes.[28] However, binding of the ScpA NTD to the neck region is also negatively regulated by the ScpB$_{ap}$ CTD

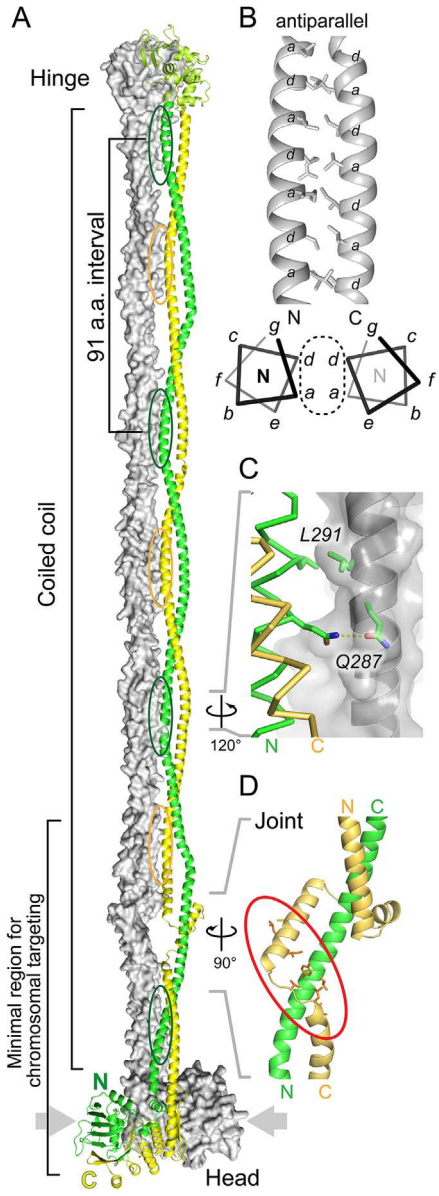

Figure 2. Dimer model of the full-length *Pyrococcus yayanosii* SMC. A) Two protomers with longitudinal alignment of the coiled coils are represented by cartoon and a gray surface. The PDB coordinate is based on supplemental data of the paper.[19] The N-terminal and C-terminal halves, and the middle hinge region of the cartoon are displayed in green, yellow and lime, respectively, as color-coded in Figure 1A. Local contact areas between the two coiled-coil arms, based on crosslinking experiments, are roughly denoted by the green and orange circles. Each circle color is derived from either helix of one coiled-coil arm. Gray arrows show expected motion of the head domain for engagement. B) A typical anti-parallel coiled-coil is characterized by a heptad repeat labeled *abcdefg*, in which the internal hydrophobic residues are located at the *a* and *d* positions side-by-side. The knobs-into-holes packing of two anti-parallel helices results in a mixed layer at the two positions. C) A close-up view of a contact point between *B. subtilis* SMC coiled-coil arms (PDB: 5NMO). The crystal structure shows that Gln287 and Leu291 in one arm interact their counterparts in the other arm through van der Waals contacts and a hydrogen bond, respectively. D) A close-up view of the *B. subtilis* SMC joint (PDB: 5NMO). At this site, the coiled-coil register is interrupted, and some hydrophobic residues (red oval) cluster on the surface, but are hidden by dimerization of the arms. These drawings were created using Pymol.

(Figure 3C).[24] Mutations in the ScpA NTD, which do not affect ScpB binding, can control binding to the neck, affect the ATPase rate of the whole complex, and impact on the its overall conformation. ScpB is an essential requirement for chromosome entrapment by the complex.[27] Therefore, it is possible that the negative regulation by ScpB is linked to the stage of DNA loading. Interestingly, a mutation in mice is found in a similar location in the putative NTD of Caph2^{nes}, a kleisin subunit of condensin II.[29] This hypomorphic mutation causes tissue-specific defects in cell proliferation and maintenance of ploidy, potentially being explained by the compromised loading of SMC by the ScpA mutants in vivo.[24] A regulator of the eukaryotic cohesin complex, Pds5, is also known to have a dual function, possibly because it controls the open state of the cohesin ring at the neck of the SMC1 subunit.[30,31] Considering the relative spatial configuration of Pds5 within the cohesin complex, inhibition of kleisin's NTD binding by the nearest subunit might be a common regulation theme.[32,33]

5. The Spo0J/ParB Based Looping of DNA Including *parS*

In *B. subtilis*, the chromosomal partition protein Spo0J/ParB forms discrete foci, which colocalise with the origin of replication region.[34–37] There are ≈1000 Spo0J per the cell, which is a great excess over the eight known *parS* binding sites.[34,35,38] One explanation for this difference is that Spo0J has been shown to associate with several kilobases of DNA that flank its specific binding sites (*parS*) in vitro.[39] However, such extensive spreading is accomplished by a limited number of dimers in cell (≈20 per *parS* site), suggesting long-range bridging, which is capable of DNA loop formation.[40,41] A recent study of the C-terminally truncated *Helicobacter pylori* Spo0J, crystallized with DNA containing the *parS* sequence, has provided a structural basis for DNA bridging.[42] Each of the Spo0J monomers binds to half of the palindromic *parS* site through its central DNA binding domain. Then, two of the dimeric Spo0J on DNA oligomerize by their NTDs in both *cis* and *trans* (**Figure 4**A). Mutations of the arginine residues in a highly conserved patch, RRXR of the NTD, lead to severe spreading defects.[40,43] These data provide a structural basis for the formation of a Spo0J-*parS* cluster that can bridge and trap large DNA loops.

6. Recruitment of the SMC Complex by the Spo0J-*parS* Complex

Spo0J is critical for enriching SMC complexes near the replication origin in some bacteria.[12,13,37,44] Previously SMC has been shown to be able to be crosslinked to Spo0J in vivo, indicating protein-protein interactions.[12] Minnen et al. have explained static and dynamic features of the SMC complex, distinguishing the initial targeting and the subsequent relocation to other regions of the chromosome.[45] They found that, as established by subcellular localization studies, targeting of the complex is strictly dependent on SMC head-head engagement (see explanations of each step of the ATP cycle, Figure 1B). This

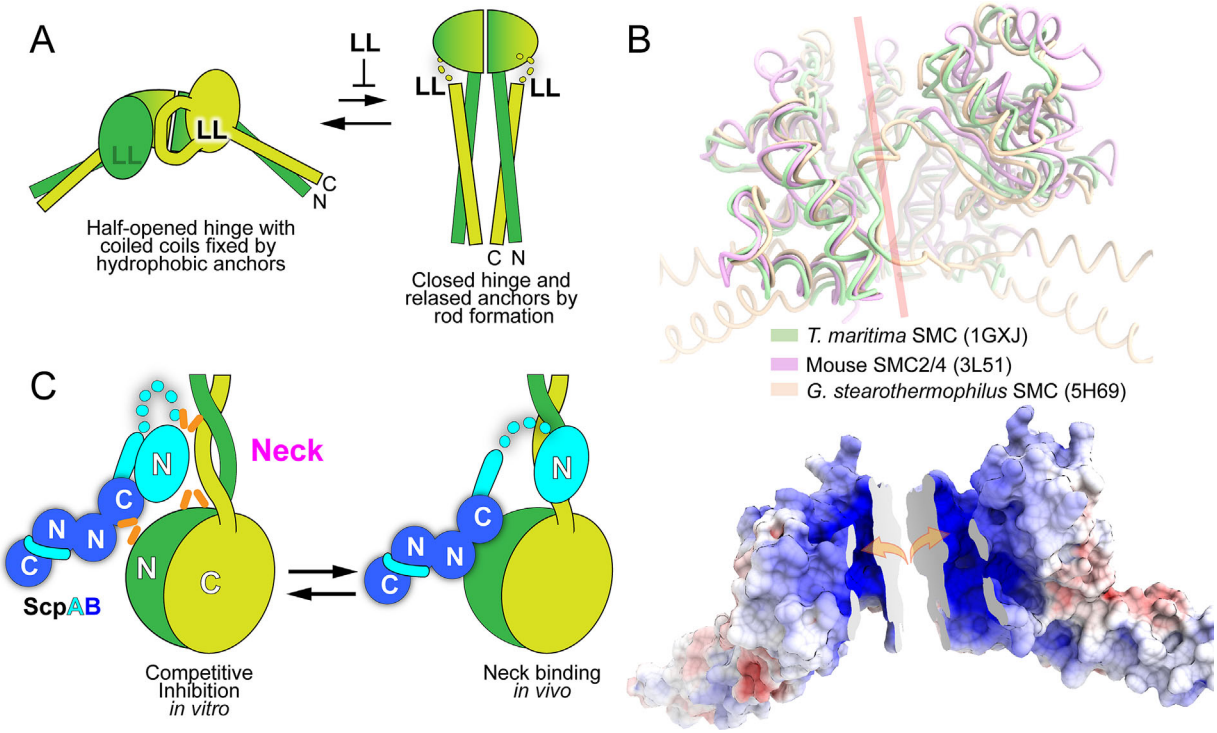

Figure 3. Local conformational changes at the two poles of the rod structure. A) Relaxed and constrained forms of the SMC hinge. The bimodality is dependent on anchoring terminal hydrophobic residues (LL) of the coiled-coils arm to the hinge domain. B) (Upper) Superimposed α-carbon representation of three different hinge dimers with a half-opened interface. These asymmetrical structures from different species have a similar extent of twist, which is generated by intramolecular relaxation. (Lower) Cut-open molecular surface of the *Geobacillus stearothermophilus* SMC hinge dimer showing the electrostatic potential. These drawings were created using CueMol. C) In vitro, steric hindrance is expected between ScpAB and the head domain (left). In contrast, *in vivo*, the ScpA NTD is partially deformed without release from the neck, suggesting that structural rearrangements in ScpAB must occur around the neck region (right).

means that proper geometry of the coiled-coil arms by the engagement is a structural prerequisite for this step. Such SMC localization phenotypes are dependent on each step of the ATPase reaction cycle, and a similar classification is also utilized for a study of dynamic behavior of Soj at the stage of DNA replication initiation.[46] Minnen et al. have also found a minimal region including the coiled coil for targeting to chromosomal ParB-*parS*. The most likely site for interaction with Spo0J is the head-proximal joint region. Conservation of some hydrophobic residues on the surface of this region strongly suggests that this might be the candidate site (Figure 2D). Cells harboring DNA bridging-deficient Spo0J mutants have an abnormal nucleoid morphology and the mutant proteins also show attenuation of chromosome entrapment by the SMC complex.[27,40] However, evidence of stable interactions between these proteins has not been reported yet, therefore, involvements of their hidden interfaces might be considered in the targeting process.

7. Loop Extrusion and Arm Alignment for Bacterial Chromosome Organization

A previous deep sequencing study of the circular *Caulobacter crescentus* chromosome has clarified that SMC coordinates alignment of the left and right DNA arms which connect the *oriC* and *Ter*.[16] Rudner and coworkers have shown that the SMC loading depends on Spo0J and the *parS* sequence in *B. subtilis*.[14] Recently, using cells containing only a single *parS* site, the same group has chased progression of the SMC complex by ChIP-seq and examined the chromosome architecture by time-resolved Hi-C.[47] The authors concluded that the coordinated alignment is directed by continuous loading of SMC complexes at *parS* rather than sequential loading of new SMC complexes at the leading edge of the juxtaposed DNA and then the complexes move down to the *Ter* region (Figure 4B). The movement of the complex is replication-independent and its apparent translocation rate is ≈50 kb/min, almost the same order of rate as the replication fork,[48–50] and quite fast compared with a yeast condensin (≈3.6 kb/min).[51] The SMC translocation is slower on either arm, when an ectopic *parS* site is introduced, and severely inhibited by head-on transcription.[14,37,47] Tran et al. reported that such conflict defect likely creates an irregular enlargement of the DNA loop that causes SMC stalling or dissociation from either chromosomal arm.[37] The asymmetrical enrichment of SMC is also consistent with a model in which two associated SMC rings independently hold a single DNA duplex and contact each together.[47,52] Wang et al. also proposed a coordinated release of the two rings coupled with the *Ter* region, beyond which the progression stops.[47]

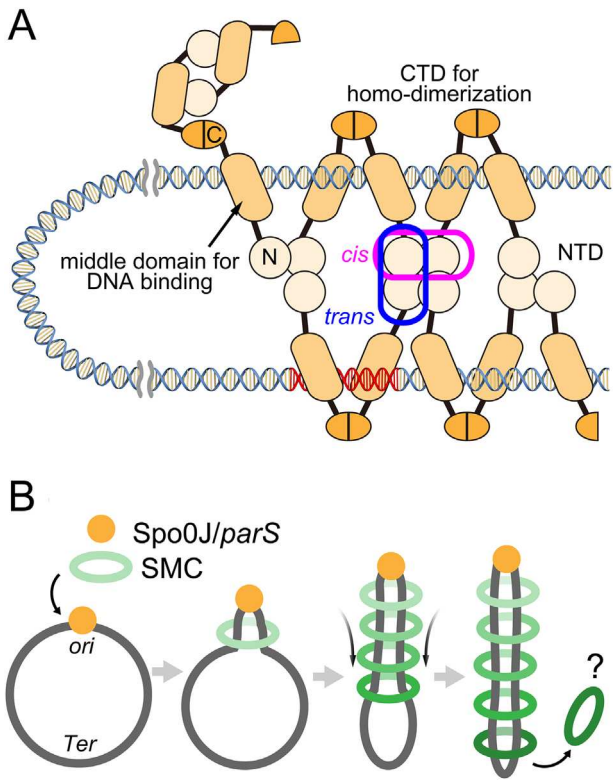

Figure 4. A) A schematic model of chromosomal DNA looping by Spo0J oligomerization. Spo0J consists of three domains, NTD, CTD and the intervening middle domain. Initially, the middle domain is specifically bound to one of *parS* site (red), and then NTD oligomerizes into a tetrameric configuration through bridging interactions both *in cis* (magenta circle) and *trans* (blue circle). Non-specific spreading to flanking DNA is also supported by dimerization of the CTD. The oligomerization might be terminated by self-dimerization of the NTD and the middle domain, based on the *Thermus thermophilus* Spo0J structure.[69] B) Loading and translocation of the SMC complex at origin proximal Spo0J/parS site. Recent Hi-C data[16,37] suggest that the SMC complex is actively extruded juxtaposing left and right arms of the circular chromosome. Whether one or two ring of the complex is utilized is still unclear.

However, results from these studies have raised a number of fundamental questions concerning protein behavior. For example, what is the energy source for the translocation? As most of SMC complexes exhibit weak ATPase activity in vitro, differently from active DNA translocators,[24,53] it is a mystery as to how such a high speed is maintained, even if the chromosomal DNA relatively moves against SMC complexes tethered on other platform. Moreover, alignment of the arms is not possible beyond the region surrounding the replication terminus. What is the barrier (or mechanism) at the *Ter* region that is able to disrupt the alignment and/or halt the progression of SMC complexes? This question is intimately connected to the issue of whether the system utilizes one or two topological protein rings. The outstanding results reported by Wang et al. are the first step towards fully recapitulating the behavior of SMC complexes.

8. Overall Transition and DNA loading Based on DNA Loop Extrusion

From the viewpoint of protein stability, a recent report has discussed a possible structural transition of the SMC-ScpAB complex.[24] The complex folds into a rod shape capable of tolerating locally unfavorable conformations in the hinge and head regions. That is, the overall structure is maintained by a summation of the stability earned by coiled-coil juxtaposition and the instability at the constrained hinge and the loosened structural region near the ScpA NTD. ATP binding and head-head engagement induces loss of the juxtaposition, which is in turn utilized for relaxation of the hinge domain and the formation of a stable interaction between the ScpA NTD and the ScpB CTD. This explains why in vitro purified SMC-ScpAB complexes have asymmetrical and separated arms.[10,24] However, unfavorable conformations of the ScpA NTD remain in the complexes in *B. subtilis*[24] (Figure 3C). There might be a mechanism through which the overall structure overrides the negative regulation of ScpB.

Gruber and coworkers have proposed a series of mechanical actions performed by the SMC complex for DNA loading (**Figure 5**).[19] In their model, the closed-rod form of the complex initially prevents DNA entry by being in a resting conformation, in which the two head domains are separated. Then, the ATP hydrolysis possibly causes the formation of a widely open rod, as an intermediate form just for a short time. Switching between the two modes initiates scanning of specific binding sites within the Spo0J-*parS* cluster, and catches the DNA loop with the help of basic residues in the hinge domain. During this step, specific binding to Spo0J might prolong the unstable intermediate state. Distinct from the large intra-arm space, Gruber et al. also postulate the presence of another small chamber, which is formed by the SMC head domains and the ScpAB subcomplex for storing two DNA strands of a loop. Alternation between capture and merging results in stepwise additions of DNA strands to the small chamber to produce a growing DNA loop. Structures of the engaged form of the head domain reveal that a C-terminal peptide of the domain reaches across the dimer interface.[24,54] In this scenario, the peptide could work as a non-return valve for the DNA merging. This proposed mechanism is based on a one-directional loop harvest by the SMC complex, explaining the relative progression by extruding DNA. However, the mechanism that dictates the direction of the loop harvest is not clear yet. If the basic surface of the hinge domain mainly controls capturing DNA strands, the directionality could be explained by the asymmetrical hinge structure with biased basic electrostatic potential. If so, binding of ScpAB might control asymmetrical head-head disengagement, in turn, resulting in biased opening of the rod form of the complex creating the asymmetrical hinge. Despite the elasticity of the arm at the engagement, how the SMC arms would be rigid enough to let DNA strands move by their peristalsis toward the head domain? These questions await answers and should be addressed in the future.

9. Origin of Archaeal SMC Complex

An accurate genome segregation mechanism must operate also in cells belonging to the archaeal domain. Archaeal genomes

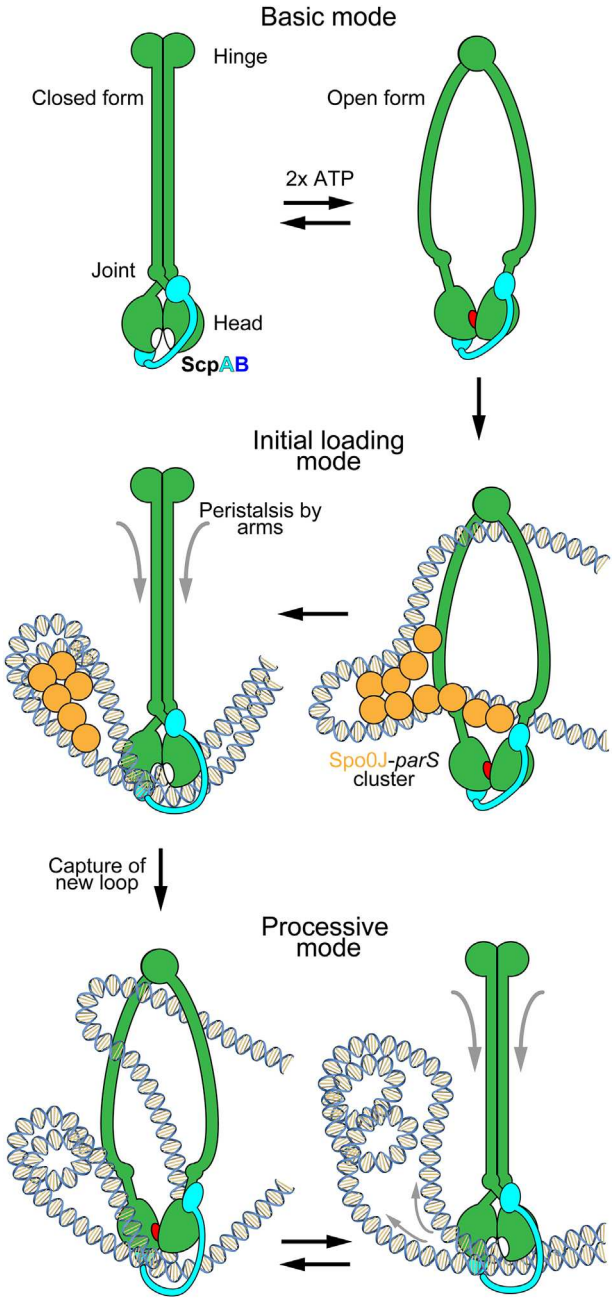

Figure 5. Models for chromosomal loading and loop extrusion by the prokaryotic SMC complex. Basic mode. The action of the SMC protein, proposed by the Gruber's group, is mainly based on the two forms that are converted using the energy from ATP hydrolysis.[19] The schematic represents the two modes of the complex, with ScpB being omitted for clarity. Initial loading mode. Through head-head engagement the complex opens up the inter-arm space, and then captures a DNA loop containing a Spo0J-*parS* cluster within the space. The closure of the arms delivers the captured DNA to an additional chamber, which is mainly created by the kleisin subunit ScpA under the head domains, holding the DNA. Processive mode. Subsequently, another round of ATP hydrolysis ignites the capture of DNA loops by the hinge domain and drives the merging with DNA previously loaded into the small chamber, generating a larger DNA loop.

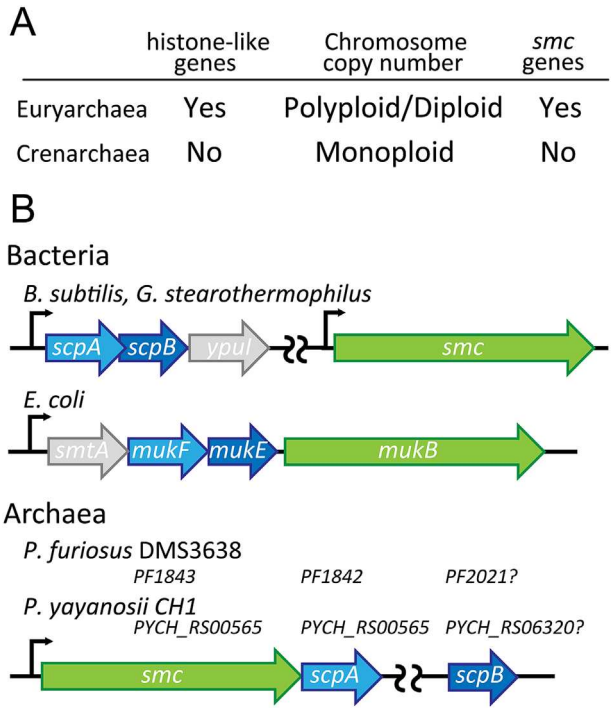

Figure 6. SMC subunits in archaeal species. A) Relationship between chromosome copy number and presence of the *smc* gene in the archaeal phyla (based on[55–57]). B) Examples of genomic contexts of the *smc* gene cassette and relevant genes in bacteria and archaea.

consist of a circular chromosome and possible extrachromosomal elements. Euryarchaeal species contain multiple chromosome copies, and harbor genes for archaeal histone homologs, whereas the crenarchaeal genera contain a single chromosome and lack histone genes.[55–57] These observations suggest a potential correlation between chromosome copy number and the origin of archaeal histones, which are only found in Euryarchaeota. It is also likely that the archaeal SMC mediates chromosome segregation. Interestingly, *smc* genes are commonly found in euryarchaeal species, but not in crenarchaeal families characterized to date (**Figure 6**A). This might suggest that SMC is required for maintenance of multiple chromosome copies or individualization of each replication origin. In Crenarchaeota, proteins bearing no sequence homology to bacterial SMC might be responsible for organization and segregation of chromosomes. However, this is a field that awaits investigations, as no information is currently available. Among the euryarchaeal species, however, the locations of genes encoding the two subunit ScpA and ScpB are somehow different from those observed in bacteria (Figure 6B). In most bacteria, both genes are tandem arranged, and *scpA* is always found upstream of *scpB*. The euryarchaeal *scpA*, however, is found downstream of *smc*, and all the annotated cognate *scpB* genes are distantly located. Therefore, further studies will be required to assess the involvement of ScpB in the archaeal SMC complex. It will also be crucial to understand to what extent the basic mechanisms of condensin-based chromosome organization are shared among archaea. Intuitively, when 30 or more

chromosomes are packed within the small volume of an archaeal cell,[58] the need for a DNA condensation strategy becomes more stringent.

10. Conclusions and Perspectives

Recent advances in bacterial cytology have elucidated the organization of chromosome domains as well as the positional information of regulatory factors. Hi-C results of the small *Mycoplasma pneumoniae* chromosome (≈0.8 Mb) also revealed maintenance of a similar symmetry between the two arms.[59] On the other hand, subsets of proteobacteria, including *Escherichia coli,* do not have SMC. Instead, they encode the MukB protein and its cognate factors MukE and MukF.[60–62] *E. coli* lacks a ParAB partition system and its chromosome is not maintained as juxtaposed as in *B. subtilis*.[63] However, *E. coli* condensin MukBEF with TopoIV promotes timely segregation of newly replicated DNA molecules.[64] Thus, there is no single unified system for chromosome organization and segregation in bacteria.

It is also important to note that the SMC loading phenotype revealed by Hi-C data is highly dependent on Spo0J and *parS*. However, *spo0J* mutants do not display severe defects in chromosome segregation as compared to *smc* mutants. Therefore, the growth defect of *smc* mutants is apparently independent of the zipping action itself. Inactivation of any subunit of the SMC complex in *B. subtilis* results in massive accumulation of sister chromosomes interlinked at the replication origin.[11,65] Thus, accurate resolution of new replication origins might be more significant as the main function of the SMC complex. Nonetheless, unlike the abundant protein H-NS that is capable of forming loop,[66] what do SMC proteins recognize in the Spo0J-bound DNA platform? What mechanism does ignite SMC complexes to move along DNA towards the *Ter* region by dissociation of the preformed specific contacts? Many exciting questions remain to be addressed in the future.

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Conflict of Interest

The authors have declared no conflict of interest.

Keywords

chromosome condensation, condensin, loop extrusion, SMC complex, ScpAB, Spo0J, *parS*

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