At the heart of the chromosome: SMC proteins in action

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Abstract | Structural maintenance of chromosomes (SMC) proteins are ubiquitous in organisms from bacteria to humans, and function as core components of the condensin and cohesin complexes in eukaryotes. SMC proteins adopt a V-shaped structure with two long arms, each of which has an ATP-binding head domain at the distal end. It is important to understand how these uniquely designed protein machines interact with DNA strands and how such interactions are modulated by the ATP-binding and -hydrolysis cycle. An emerging idea is that SMC proteins use a diverse array of intramolecular and intermolecular protein–protein interactions to actively fold, tether and manipulate DNA strands.

Walker A and Walker B motifs
A pair of nucleotide-binding motifs that is commonly found in most, if not all, nucleotide-binding proteins.

Coiled-coil motif
A rod-like structural motif found in many proteins that is formed by two long α-helices twisted around each other. Parallel arrangements of the two helices are much more common than antiparallel arrangements.

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Structural maintenance of chromosomes (SMC) proteins are now recognized as one of the most fundamental classes of proteins that regulate the structural and functional organization of chromosomes from bacteria to humans[1,2]. Since their discovery more than a decade ago, two features of SMC proteins have strongly attracted the attention of chromosome biologists. The first is the various chromosome functions in which they participate. A series of genetic and cell-biology studies have demonstrated that SMC proteins have crucial roles in chromosome segregation (during both mitosis and meiosis), chromosome-wide gene regulation and recombinational repair. It is anticipated that more functions will be added to this still-expanding list.

The second intriguing feature of SMC proteins is their unique protein architecture. Although SMC proteins were initially suspected to be 'chromatin motors'[3], accumulating lines of evidence indicate that they represent a completely novel type of protein machine that functions as dynamic linkers of the genome. Despite substantial efforts, we have only had a glimpse of the mechanisms of action of this class of chromosomal ATPases. Here, I focus on the mechanistic aspects of SMC proteins, and discuss recent progress in the field as well as crucial questions that need to be addressed in the future (for reviews on the *in vivo* functions of SMC proteins, see REFS 4–7).

Architecture of SMC proteins

General architecture. SMC proteins are large polypeptides (1,000–1,300 amino acids) with a unique domain organization. Two canonical nucleotide-binding motifs, known as the Walker A and Walker B motifs, are located separately at the N-terminal and C-terminal domains, respectively. Between the two motifs are two long coiled-coil motifs that are connected by a non-helical sequence. The possibility for antiparallel folding of the long coiled-coil motifs was first suggested by Saitoh *et al.*[8]; subsequent electron-microscopy and biochemical studies[9–11] have established that an SMC monomer folds back on itself through antiparallel coiled-coil interactions, creating an ATP-binding 'head' domain at one end and a 'hinge' domain at the other. Two monomers associate with each other at the hinge domain to form a V-shaped molecule (FIG. 1a).

The length of each arm is ~50 nm, which is equivalent to ~150 bp of double-stranded DNA (dsDNA). The conformation of SMC dimers is highly flexible, and a wide range of structures is detectable by electron microscopy, including open-V, closed-V and ring-like molecules[9] (FIG. 1b). Although the majority of purified SMC proteins are present as free dimers in solution, rare examples of multimer formation have been reported. For example, recent studies have revealed rosette-like structures in which 4–8 dimers associate with each other, probably through their head domains[12,13]. It remains to be determined, however, whether these are functionally relevant structures.

Most, if not all, bacterial genomes contain a single *smc* gene whose product forms a homodimer. In eukaryotes, there are at least six different SMC proteins that form heterodimers in specific combinations. The SMC1–SMC3 pair constitutes the core of the cohesin complex that mediates sister-chromatid cohesion[5], whereas SMC2–SMC4 is a component of the condensin complex that is essential for chromosome assembly and segregation[4]. The remaining two SMC proteins, SMC5 and SMC6, whose sequences are substantially

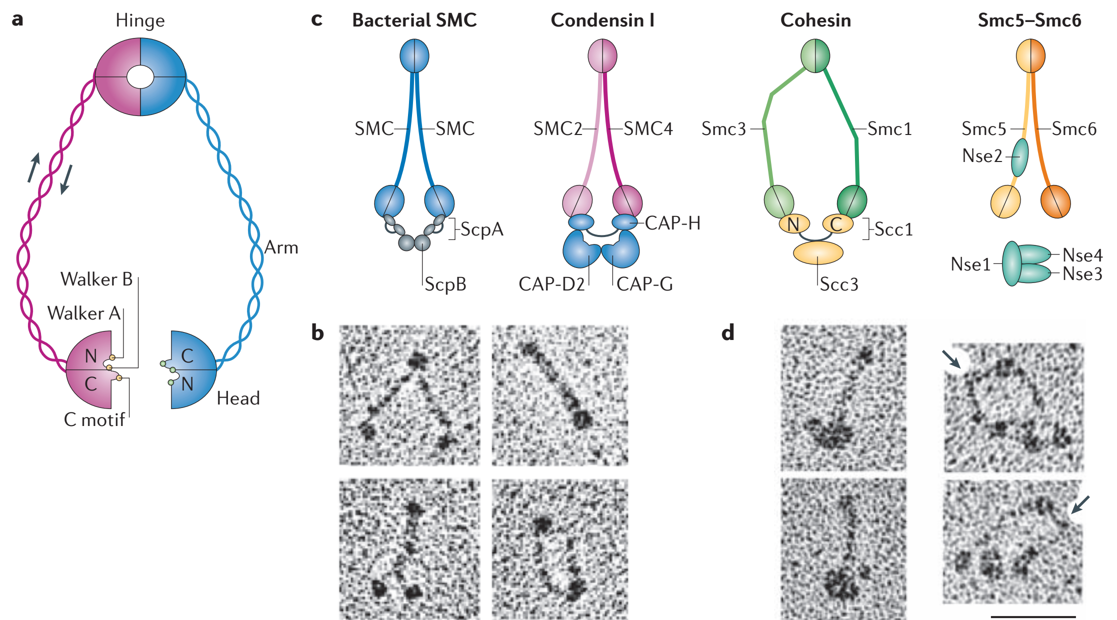

Figure 1 | **The architecture of SMC proteins and SMC–protein complexes. a** | The basic architecture of a structural maintenance of chromosomes (SMC) protein dimer. Each SMC subunit self-folds by antiparallel coiled-coil interactions (the antiparallel arrangement is indicated by the arrows) to form a hinge domain at one end and an ATP-binding head domain at the other. A hinge–hinge interaction between two subunits mediates dimerization and produces a V-shaped molecule. **b** | Electron micrographs of the *Bacillus subtilis* SMC (BsSMC) homodimers show a wide variety of conformations[9]. Bar, 50 nm. Figure reproduced with permission from REF. 9 © (1998) Rockefeller University Press. **c** | SMC–protein complexes in bacteria and eukaryotes. The bacterial SMC–protein complex is composed of an SMC homodimer and two non-SMC subunits; ScpA (kleisin) and ScpB. An SMC2–SMC4 heterodimer functions as the core of condensin I (and condensin II; not shown). The CAP-H subunit of condensin I belongs to the kleisin family, whereas the CAP-D2 and CAP-G subunits contain HEAT repeats. An Smc1–Smc3 heterodimer functions as the core of the cohesin complex, which contains two non-SMC subunits, Scc1 (also known as Mcd1 or Rad21) and Scc3 (also known as SA). Scc1 is a member of the kleisin protein family. An Smc5–Smc6 heterodimer functions as part of a yet-to-be-named complex that contains at least four non-SMC subunits (Nse1–4). **d** | Examples of rotary-shadowed images of condensin I and cohesin purified from human tissue culture cells[14]. Note the striking difference in the arm conformation between condensin I (left panels) and cohesin (right panels). A sharp kink in one of the coiled-coil arms of SMC3 is indicated by the arrow. Bar, 50 nm. Figure reproduced with permission from REF. 14 © (2002) Rockefeller University Press.

divergent from those of SMC1–4, form a third complex that has been implicated in DNA-repair and checkpoint responses[6]. Each of the dimers further associates with a distinct set of non-SMC regulatory subunits to form a functional complex (FIG. 1c).

Interestingly, although both cohesin and condensin display the two-armed structure that is characteristic of SMC proteins, their conformations are remarkably different, as judged by electron microscopy. The hinge domain of condensin is closed and the coiled-coil arms are placed close together. Three non-SMC subunits of condensin form a subcomplex and bind to one (or both) of the head domains, forming a 'lollipop-like' structure[14,15] (FIG. 1d, left panels). By contrast, the hinge of cohesin is wide open and the arms are spread apart. Non-SMC subunits of cohesin apparently bridge the two head domains, creating a ring-like structure[14] (FIG. 1d, right panels). Subunit–subunit interaction assays independently revealed the ring-shaped configuration of cohesin, and explained it at a molecular level by demonstrating that the N- and C-terminal domains of Scc1 bind to the head domains of Smc3 and Smc1, respectively[10]. Likewise, bacterial SMC (MukB in *Escherichia coli*) dimers associate with non-SMC subunits through their head domains[12,16–18]. Substantial lines of evidence suggest that the non-SMC subunits modulate the catalytic cycle of SMC proteins as well as their intramolecular and intermolecular interactions, as discussed below.

Head structure. The C-terminal domain of SMC proteins contains a highly conserved sequence that is characterized by an LSGG(E/Q)(K/R) motif[8]. A closely related sequence, often referred to as the signature motif (or the C motif), is found in a large family of ATP-binding cassette (ABC) ATPases including ABC transporters and the double-strand break (DSB)-repair protein Rad50. Recent structural studies demonstrated that ATP binding to the SMC head domains drives the formation of a nucleotide-sandwich dimer[19,20] (FIG. 2a), as has been shown for Rad50 (REF. 21) and ABC transporters[22,23]. In these structures, ATP binds to a pocket formed by the Walker A and Walker B motifs from one SMC subunit, and makes a contact with the C motif from the second subunit. Mutational analyses of these key residues in the *Bacillus subtilis* SMC protein (BsSMC) support the idea that head–head engagement is essential for ATP hydrolysis; a mutation in the Walker A

Signature motif
(C motif). An amino-acid-sequence motif that is highly conserved among the ABC-ATPase superfamily, which includes ABC transporters, Rad50 and SMC proteins. This motif is not required for ATP binding but is essential for its hydrolysis.

ABC transporters
A large family of transmembrane ATPases that mediate the active translocation of a diverse range of small molecules in and out of cells and organelles. The functional unit of an ABC transporter is composed of two transmembrane domains and a pair of ATP-binding cassette (ABC) domains.

Rad50
The ATPase core subunit of the MRN (Mre11–Rad50–Nbs1) complex that has a crucial role in double-strand break repair. Rad50 shares many structural similarities with SMC proteins, including its ATP-binding head domains and its antiparallel coiled coils.

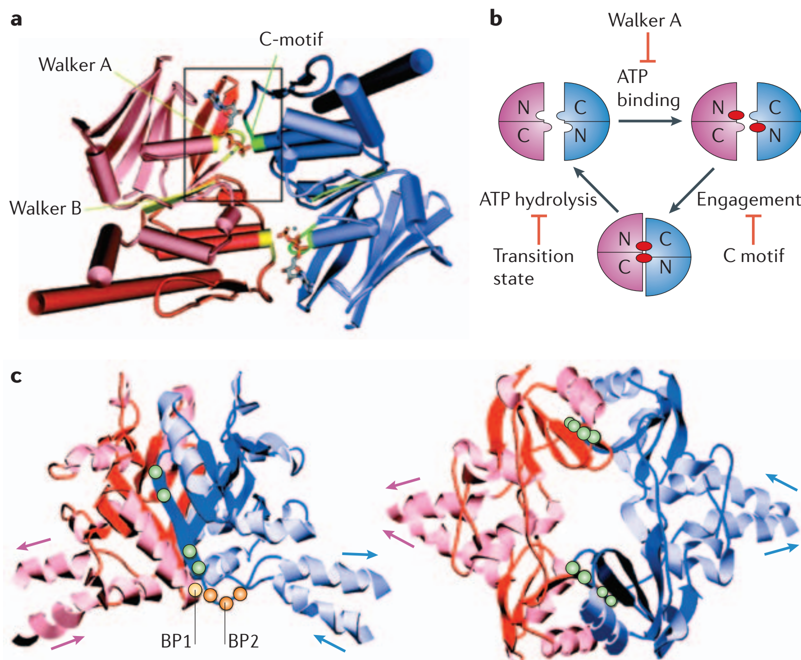

Figure 2 | **Structure and action of SMC protein subdomains. a** | Crystal structure of a nucleotide-sandwich dimer of the *Saccharomyces cerevisiae* structural maintenance of chromosomes (SMC) protein SMC1 head domains[19]. A pair of ATPγS (a slowly hydrolysable analogue of ATP) molecules (indicated in orange and blue) is sandwiched between the Walker A and B motifs of one domain and the C motif of the other. One of the ATPγS molecules surrounded by the three motifs is boxed. **b** | The SMC-ATPase cycle. Binding of ATP (red circles) to the head domains induces their engagement, and the subsequent hydrolysis of ATP triggers their disengagement. The head–head engagement might occur either intramolecularly within a dimer or intermolecularly between different dimers. A mutation in the Walker A motif blocks ATP binding, whereas a mutation in the C motif specifically prevents engagement. A transition-state mutation stabilizes the engaged state by slowing down ATP hydrolysis. **c** | Crystal structure of a homodimer of the *Thermotoga maritima* SMC hinge domains[10]. A view from the side is shown on the left, whereas a view from the top (opposite to the surface from which the two coiled coils protrude) is shown on the right. The locations of the key DNA-binding residues (basic patch (BP)1 residues (yellow) and BP2 residues (orange))[28] and of the Gly residues that are essential for dimerization (green)[11] are shown. The structures in parts **a** and **c** are reproduced with permission from REF. 2 © (2005) Annual Reviews.

motif abolishes ATP binding, whereas a mutation in the C motif allows ATP binding but blocks head–head engagement and ATP hydrolysis[24]. A substitution of the Glu residue in the Walker B motif with a Gln residue (often referred to as a transition-state mutation) stabilizes head–head engagement by slowing down ATP hydrolysis[16]. So, the three mutations offer a powerful set of tools for dissecting the mechanochemical cycle of SMC proteins (FIG. 2b; see below).

Given the heterodimeric nature of eukaryotic SMC proteins, it had been thought that the engagement of SMC head domains always occurred in a heterotypic fashion (for example, SMC1–SMC3, but not SMC1–SMC1 or SMC3–SMC3). It was therefore surprising to find that two SMC1 head domains had homodimerized in a protein crystal[19]. Although this could just be an artefact of protein crystallization, the possibility cannot be excluded that it reflects the capacity of SMC1 head domains from neighbouring complexes to interact with each other and to form large protein assemblies.

Transition-state mutation
A specific point mutation in the Walker B motif that stabilizes the engagement of two ABC domains by suppressing the hydrolysis of ATP molecules that are sandwiched between them.

Zinc-hook domain
A folding domain that is created at one end (apex) of the antiparallel coiled-coil arm of Rad50. A zinc ion bridges two hook domains and mediates their dimerization.

Hinge structure. The amino-acid sequence of the SMC hinge domain is unique to this class of proteins and is distinct from the zinc-hook domain of Rad50 (REF. 25). There is no indication so far that zinc or any other specific cations are required for hinge-mediated dimerization of SMC proteins. A crystal structure of the SMC hinge domain from the bacterium *Thermotoga maritima* shows that a hinge monomer is composed of two domains that display pseudo-twofold symmetry[10] (FIG. 2c). In this structure, the N-terminal region of one monomer associates with the C-terminal region of the same monomer, forming an antiparallel coiled coil. Dimerization is achieved primarily by β-sheet interactions between the monomers, producing a doughnut-shaped structure that protrudes two coiled-coil arms in opposite directions (FIG. 2c). This crystal structure provides compelling evidence that each arm of an SMC dimer is composed of an intra-subunit coiled coil rather than an inter-subunit coiled coil (FIG. 1a).

Unlike the head–head engagement, which is dynamically regulated by ATP binding and hydrolysis, the hinge–hinge interaction is very strong and occurs independently of ATP. A study with BsSMC showed that mutations in conserved Gly residues that are located at the dimerization interface destabilize the hinge-mediated dimerization[24]. Substitution of the four Gly residues in different combinations induces progressive changes in the sedimentation properties of BsSMC, which is indicative of the gradual opening of the hinge. The most severe mutant, known as DDDD, completely disrupts the dimerization, producing single-armed monomers[11]. A similar mutational study using mammalian SMC1 and SMC3 head domains showed that the Gly residues in both subunits need to be mutated to disrupt the heterotypic hinge–hinge interaction[26]. Given that the hinge sequences of SMC5 and SMC6 deviate substantially from those of SMC1–4 and BsSMC, their dimerized structure is predicted to be different from the hinge of the 'canonical' SMC proteins. Nonetheless, a recent report identified amino-acid residues that, when mutated, abolish the stable dimerization of SMC5 and SMC6 (REF. 27). As discussed below, it has become increasingly clear that the SMC hinge domain is not a simple dimerization domain; instead, it functions as an essential determinant of dynamic interactions between DNA and SMC proteins[11,28].

Hypothetical actions of SMC proteins

As already discussed, SMC proteins adopt a unique and unprecedented structure, in which a central hinge domain connects two long coiled-coil arms, each having a 'sticky' ATP-binding head at their distal ends. From a mechanistic point of view, this architecture of SMC proteins is of great interest, and indicates that their mechanisms of action might involve a diverse and dynamic array of intramolecular and intermolecular interactions (FIG. 3). In principle, two types of head–head engagement can be considered. If the two head domains within a dimer bind to each other (intramolecular engagement), it would result in the formation of a ring-shaped or a closed V-shaped molecule (FIG. 3a).

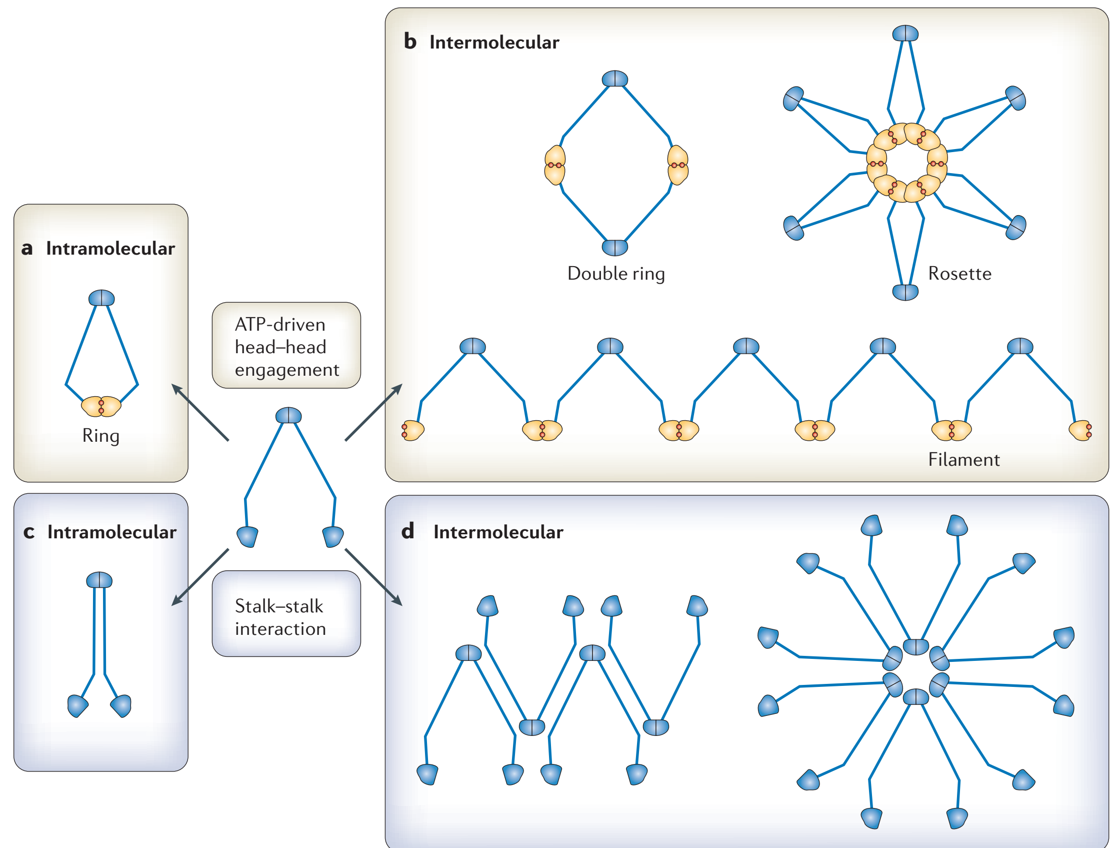

Figure 3 | **Hypothetical actions of SMC proteins.** The unique architecture of structural maintenance of chromosomes (SMC) proteins indicates potential actions that might involve a diverse array of intramolecular and intermolecular protein–protein interactions. ATP binds to the head domains and drives their engagement. ATP-bound head domains are indicated in yellow, whereas ATP-free head domains are indicated in blue. **a** | If engagement occurs intramolecularly between the two head domains within an SMC dimer, it would lead to the formation of a ring or a closed-V structure. **b** | If engagement occurs intermolecularly between the head domains from different SMC dimers, it would result in the formation of a variety of structures: such as a double-ring, rosette or filament structure. **c**,**d** | Interactions between coiled-coil arms (stalks) might create a different set of conformations, possibly in an ATP-independent manner. Again, such interactions would occur either intramolecularly (**c**) or intermolecularly (**d**).

If head–head engagement occurs between different dimers (intermolecular engagement), then it would create a variety of structures: such as double-sized rings, filaments and rosette-like structures (FIG. 3b). It is also possible that the long coiled-coil domains (stalks) involve protein–protein interactions, conceivably in an ATP-independent manner. Again, such interactions could occur either intramolecularly (FIG. 3c) or intermolecularly (FIG. 3d).

Currently available lines of evidence indicate that intermolecular protein–protein interactions might occur in the presence of DNA, but are barely detectable in its absence[24]. Even though it is tempting to speculate that all SMC dimers and complexes share a basic mechanism of action, they are also structurally and functionally differentiated to support their unique roles in chromosome dynamics. I will first describe the basic enzymology of bacterial SMC proteins and then discuss eukaryotic SMC complexes, such as condensin and cohesin, which have more complex and sophisticated actions.

Bacterial SMC proteins: understanding the basics

The mechanochemical cycle of SMC proteins. The enzymology of SMC proteins has been best studied using BsSMC as the model system. A BsSMC homodimer interacts with both single-stranded DNA (ssDNA) and dsDNA, and displays an intrinsic (that is, DNA-independent) ATPase activity as well as a DNA-stimulated ATPase activity[24,29]. When hinge-mediated dimerization is disrupted, the resulting single-armed monomer completely loses its capacity to interact with ssDNA and dsDNA[11].

Experiments with truncated constructs demonstrated that the head domain is not essential for basal DNA binding, and that the hinge-mediated dimerization of two coiled-coil arms of a certain length is a minimum requirement for BsSMC to interact with DNA (Supplementary information S1 (box)). Accordingly, ATP has little, if any, effect on DNA binding by full-length BsSMC, as judged by standard gel-shift assays. However, when a transition-state mutation (Glu1118Gln in BsSMC) is introduced that stabilizes head–head engagement by slowing down ATP hydrolysis, ATP-stimulated DNA binding becomes

readily detectable[16]. The use of non-hydrolysable or slowly hydrolysable analogues of ATP only partially mimics such nucleotide-stimulated DNA binding. The poor response of SMC ATPases to nucleotide analogues is reminiscent of that of many ABC transporters[22,30]. So, although cryptic under standard experimental conditions, it is probable that the ATPase cycle of SMC proteins has an important role in their dynamic interaction with DNA by modulating the cycle of head–head engagement and disengagement.

A recent study has shown that the hinge domain of BsSMC has a crucial function in modulating its mechanochemical cycle[28]. The hinge domain has two positively charged 'basic patches' (BP1 and BP2) that are located near the dimerization interface in a symmetric manner: each of them is composed of a crucial Lys residue (Lys565; BP1) from one subunit and three consecutive Lys residues (Lys666–Lys667–Lys668; BP2) from the other subunit (FIG. 2c). BP2, but not BP1, is essential for an initial interaction with DNA. Although this 'sitting' mode of DNA binding does not require head–head disengagement (FIG. 4a, stage 1), it leads to the opening of the arms by triggering hydrolysis of ATP that is bound to the head domain, which is located ~50 nm away from the hinge domain (FIG. 4a, stage 2). This conformational change would allow the crucial Lys residue of BP1 to be exposed, leading to its stable interaction with DNA by a 'hooking' mode (FIG. 4a, stage 3). Although neither the sitting nor the hooking mode requires ATP, ATP binding has a negative effect on the hooking mode because head–head disengagement is a prerequisite for this mode of DNA binding.

Once binding is achieved by the hooking mode, ATP binding has a positive effect on the interaction of the SMC dimer with DNA by driving the engagement between the liberated head domains. Such engagement could occur either within a dimer ('trapping'; FIG. 4a, stage 4) or between different dimers ('gathering'; FIG. 4a, stage 5), thereby bringing two different DNA strands or two segments of a single DNA strand together. Protein–protein crosslinking and dominant-negative ATPase-suppression experiments showed that different SMC dimers indeed interact with each other in the presence of DNA[16,24,28]. Even though the scenario described here remains speculative and needs to be tested further, it nicely explains the 'mechanistic logic' of the two-armed structure of SMC proteins and provides a basic framework for our understanding of the seemingly complex actions of SMC proteins.

Interestingly, a recent study using atomic force microscopy has provided evidence for another example of long-distance communications through coiled-coil arms in the action of Rad50 (REF. 31). By contrast to SMC proteins, the dimerization of Rad50 is primarily mediated by its head domains (in part through their interactions with Mre11), whereas the interaction between the coiled-coil apices (also known as the zinc hooks[25]) is flexible. In the absence of DNA, the two apices associate with each other intramolecularly, forming a ring-shaped molecule (FIG. 4b, stage 1). When DNA interacts with the head domains, the intramolecular apex–apex interaction are weakened (FIG. 4b, stage 2), allowing the liberated apices to interact with those of another complex bound to a different DNA molecule (FIG. 4b, stage 3). The role of the ATP-binding and -hydrolysis cycle in this series of conformational changes, if any, is not understood. So, although the initial DNA-interaction sites might be different between Rad50 and SMC proteins, the proposed modes of long-distance communication between the two ends of a long molecule share a remarkable similarity. It is of great interest to compare and contrast the mechanistic actions of the two different classes of DNA-linking protein machines that have ABC-ATPase domains.

A tight gate-keeping mechanism. BsSMC interacts specifically with two non-SMC subunits, ScpA and ScpB, both *in vivo* and *in vitro*[16,17,32–34]. ScpA belongs to a superfamily of SMC-interacting proteins, known as kleisins, which includes the Scc1 subunit of the eukaryotic cohesin complex[35]. It has been shown that both the N- and C-terminal domains of kleisins contain a common folding motif known as the winged helix[19,36], and that the C-terminal winged-helix domain binds directly to the SMC head domain[19] (FIG. 5a). ScpB also contains two winged-helix motifs and dimerizes through its C-terminal half[37]. ScpB binds to the head domain of BsSMC only in the presence of ScpA, and the two proteins cooperate to stabilize BsSMC head–head engagement by suppressing its ATPase activity[16].

Taken together, these results indicate that ATP, ScpA and ScpB constitute a 'triple locking' system that ensures the tight regulation of the engagement–disengagement cycle (FIG. 5b). Like the role of ATP, the contribution of ScpA–ScpB to the function of BsSMC can be either negative or positive. For example, the stabilization of intramolecular head–head engagement in solution prevents arm opening and blocks stable dsDNA binding. Once BsSMC binds to dsDNA, however, the subsequent action of ScpA–ScpB stabilizes the dsDNA binding by preventing head–head disengagement[16]. Such a tight gate-keeping mechanism is probably important to support the timely loading and unloading of BsSMC–ScpA–ScpB, a complex that that would otherwise cause abortive compaction of the nucleoid.

Structural and mechanistic parallels between the coiled-coil arms of SMC proteins and the transmembrane domains of ABC transporters have been discussed previously[2,38]. In short, conformational changes in the ABC-ATPase domains are transmitted to the substrate-specific domains, and vice versa. Among ABC transporters, the maltose transporter MalK is unique in that it has a non-transmembrane domain that constrains the engagement–disengagement cycle of ABC domains[39]. The relative location of this C-terminal regulatory domain of MalK is reminiscent of the location of the equivalent domain in ScpA–ScpB (or kleisins in general), implicating an additional mechanistic parallel between the two classes of ABC ATPases (FIG. 5c). An emerging theme is that a pair of ABC domains functions as a receiver, transmitter and modulator of conformational changes of a protein machine that supports vectorial transport either of small molecules across the membrane (in the case of ABC transporters), or of DNA strands into and out of a coiled-coil loop (in the case of SMC proteins).

Atomic force microscopy
A scanning-microscopy technique that allows imaging of the surface of a sample at atomic resolution by measuring repulsive forces between a probing tip and the sample. It is possible to collect a series of time-resolved images under aqueous and physiological conditions.

Kleisins
A conserved family of proteins that directly interact with SMC protein dimers. Members of this family include the Scc1 subunit of cohesin, the CAP-H subunit of condensin and the ScpA subunit of the bacterial SMC complex.

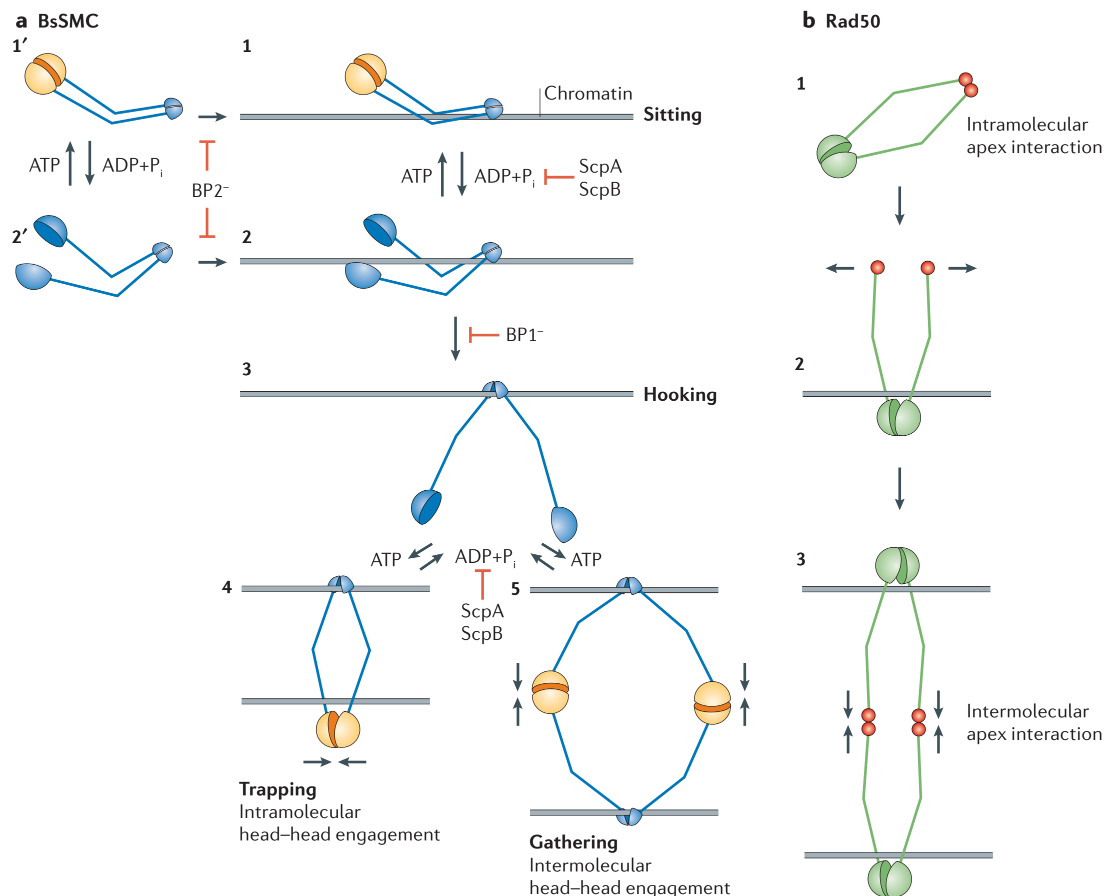

Figure 4 | **Comparison between SMC proteins and Rad50. a** | Proposed conformational changes of the *Bacillus subtilis* structural maintenance of chromosomes (BsSMC) induced by DNA binding[28]. BsSMC undergoes a DNA-independent ATPase cycle that regulates the engagement and disengagement of the two head domains within a dimer (stages **1′** and **2′**). ATP-bound head domains are indicated in yellow, whereas ATP-free head domains are indicated in blue. An initial interaction of BsSMC with DNA (the 'sitting' mode) occurs at the hinge domain, guided by two coiled-coil arms (stage **1**). This interaction triggers DNA-stimulated ATP hydrolysis and the disengagement of the head domains (stage **2**), thereby allowing the coiled-coil arms to open fully (stage **3**). Mutations in the basic patch 2 ($BP2^-$) prevent the initial stage of BsSMC–DNA interaction regardless of the presence (from stage **1′** to **1**) or absence (from stage **2′** to **2**) of ATP. The opening of the hinge domain makes the basic patch 1 (BP1) accessible to double-stranded DNA, leading to a stable DNA interaction by a 'hooking' mechanism (stage **3**). A mutation in BP1 ($BP1^-$) prevents this hooking mode of DNA binding, but not the initial sitting mode of binding. Subsequent ATP binding drives head–head engagement either intramolecularly by a 'trapping' mode (stage **4**) or intermolecularly by a 'gathering' mode (stage **5**). A number of other variations in intermolecular engagement are possible, including filaments and rosettes (see FIG. 3b). ScpA–ScpB suppresses the ATPase activity of BsSMC and modulates its function either negatively or positively. For example, the stabilization of intramolecular head–head engagement would prevent arm opening and block stable DNA binding (stage **1**). On the other hand, once BsSMC interacts with DNA, the subsequent action of ScpA–ScpB would stabilize the DNA binding by preventing head–head disengagement[16] (stage **4** or **5**). **b** | Proposed conformational changes of Rad50 upon DNA binding[31]. The dimerization of Rad50 is primarily mediated by its head domains, in part through their interactions with Mre11 (not shown). In the absence of DNA, the apices of the two coiled-coil arms (indicated by red circles) associate with each other intramolecularly, forming a ring-shaped molecule (stage **1**). When DNA interacts with the dimerized head domains, the intramolecular apex–apex interaction is loosened, making a molecule with the coiled-coil arms protruding in parallel (stage **2**). This conformational change now allows the liberated apices to interact with those of a different Rad50 molecule, thereby contributing to the tethering of two DNA duplexes (stage **3**). The role of ATP binding and hydrolysis in this mechanism is unknown.

Condensins: folding individual DNA duplexes

Condensins are five-subunit complexes that have a central role in chromosome assembly and segregation during mitosis and meiosis in eukaryotic cells[4] (FIG. 1c). In vertebrates, an SMC2–SMC4 heterodimer constitutes the core of two types of condensin complexes, condensin I and condensin II (REF. 40).

Cooperative DNA binding by SMC2–SMC4 dimers. The SMC2–SMC4 dimer seems to bind to dsDNA in a cooperative manner[15,41]. ATP has little, if any, effect on dsDNA binding, which indicates that the cooperative dsDNA binding might be mediated by an ATP-independent stalk–stalk interaction rather than by an ATP-dependent head–head interaction.

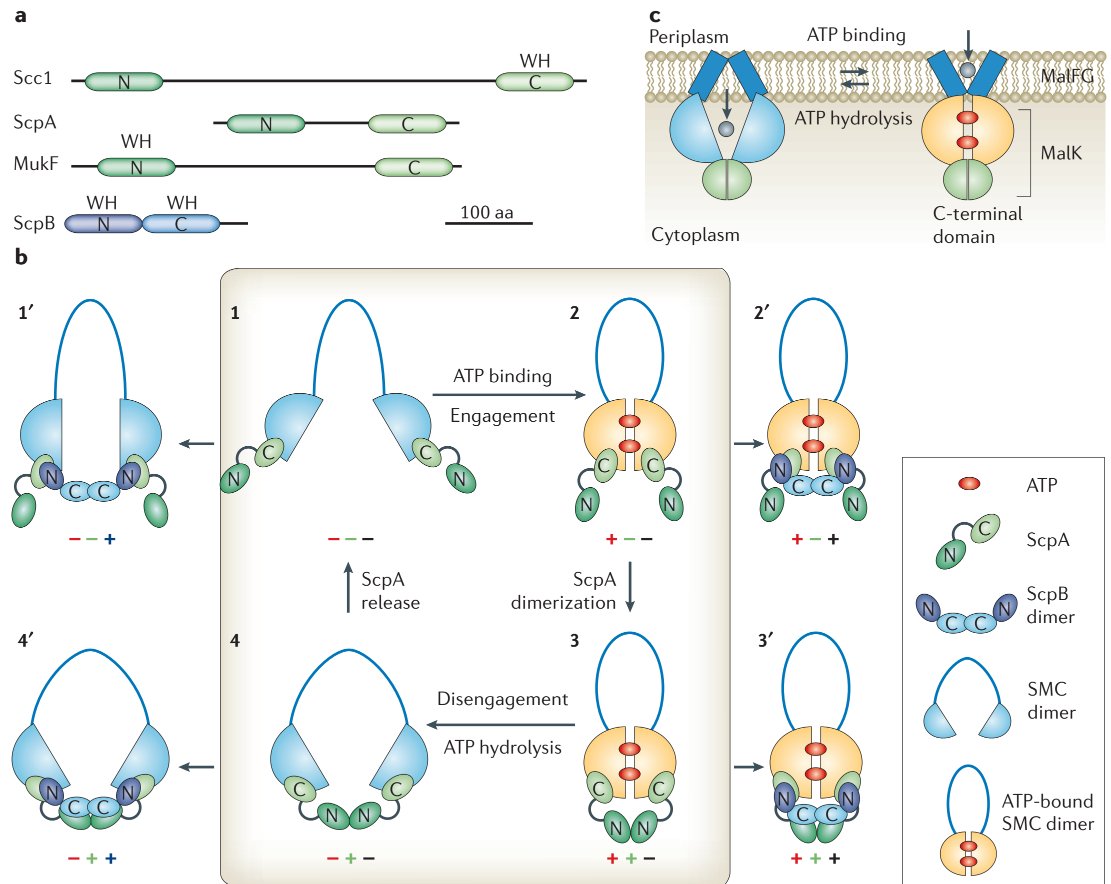

Figure 5 | **A gate-keeping role of the kleisin subunits in SMC protein actions. a** | Domain organization of kleisins and related molecules. Each member of the kleisin family (Scc1, ScpA and MukF) is composed of conserved N-terminal and C-terminal domains and a highly divergent central domain. The C-terminal domain of Scc1 (REF. 19) and the N-terminal domain of MukF[36] contain a common folding motif known as the winged helix (WH). So, the two domains of ScpA probably form winged helices. Crystallization of the first two-thirds of *Chlorobium tepidum* ScpB showed that it is composed of tandem winged helices[37]. **b** | A 'triple locking' mechanism of structural maintenance of chromosomes (SMC) protein action modulated by ATP binding, ScpA and ScpB. Based on the co-crystal structure of yeast Smc1 and Scc1 (REF. 19), an ScpA monomer is predicted to associate with an SMC head through the C-terminal domain of ScpA (stage **1**). When ATP binding drives the engagement of two SMC head domains (stage **2**), ScpA dimerizes, possibly through its N-terminal domain, and stabilizes the engaged state[16] (stage **3**). Subsequent ATP hydrolysis (stage **4**) releases the ScpA–ScpA interaction, returning the complex to the original state (stage **1**). ScpB, which is already dimerized in solution, bridges the SMC head domains in a ScpA-dependent manner (stages **1′**–**4′**). This could occur through the interaction of ScpB with the C-terminal domain of ScpA[36]. Unlike the action of ScpA, the action of ScpB is apparently ATP independent[16]. At each stage, the locking and unlocking actions of the three factors are shown by + and –, respectively. For example, stage **3′** (+++) represents the most tightly locked stage. **c** | The architecture of an ABC transporter[39]. The maltose transporter MalFGK$_2$ is composed of two transmembrane subunits, MalF and MalG (MalFG in the figure), and two copies of the ABC subunit MalK. Unlike many other ABC domains, MalK has a C-terminal extension that constrains its dimerization at the cytoplasmic side. The structural and functional contribution of this C-terminal regulatory domain to maltose transport might be analogous to that of ScpA to SMC protein regulation (for simplicity, the maltose-binding protein that is essential for this transport system is omitted in this figure). Maltose is shown as a grey circle.

Electron microscopy studies showed that under saturated conditions SMC2–SMC4 can assemble into two different nucleoprotein structures — long flexible filaments and ring-shaped 'doughnuts'[42]. The dimer can also convert complementary ssDNAs into dsDNA[43] by dynamic protein–protein interactions[44]; similar activity has been found in BsSMC[29]. In contrast to BsSMC, the SMC2–SMC4 dimer has only a very weak ATPase activity, which is not stimulated in the presence of either ssDNA or dsDNA[41,45].

Type I topoisomerase
A type of DNA topoisomerase that changes the topology of DNA by nicking and rejoining one strand of the DNA double helix.

ATP-dependent supercoiling and looping. The holocomplex of *Xenopus laevis* condensin I, which is composed of SMC2–SMC4 and three non-SMC subunits (FIG. 1c), displays DNA-stimulated ATPase activity *in vitro*[46]. Two functional assays showed that the holocomplex can induce positive superhelical tension in dsDNA in an ATP-hydrolysis-dependent manner. In the first assay, the complex converts relaxed circular DNA into positively supercoiled DNA in the presence of a type I topoisomerase[46]. The supercoiling activity is not

supported by the SMC2–SMC4 heterodimer alone[42,45]; it requires non-SMC subunits that are phosphorylated in a mitosis-dependent manner[47]. This observation is consistent with the idea that the activity *in vitro* might directly contribute to mitotic chromosome assembly and condensation *in vivo*. A similar activity has been detected in a condensin fraction purified from *Caenorhabditis elegans* embryos[48] that is predicted to be composed of condensin II, which contains a different set of non-SMC subunits. In the second assay, the condensin I complex converts nicked circular DNA into a positively knotted form of DNA (that is, a positive three-noded knot, also known as a trefoil) in the presence of a type II topoisomerase[49]. This second activity indicates that condensin I not only introduces positive supercoils into DNA, but that it also has the capacity to organize (at least) two supercoils into an ordered, solenoidal form.

The visualization of the supercoiling reaction by electron spectroscopic imaging suggests that a single condensin complex might be able to introduce two DNA supercoils, possibly by a 'wrapping' mechanism[50]. Most recently, nanomanipulation of a single DNA molecule using magnetic tweezers has shown that condensin I can physically compact DNA in an ATP-hydrolysis-dependent manner[51]. The compaction reaction occurs in a highly dynamic and reversible fashion, and requires a relatively high concentration of proteins. This behaviour would be best explained by the cooperative actions of multiple condensin I complexes on single DNA molecules. If positive supercoiling of DNA involves part of this compaction reaction, one might expect that different templates of single DNA molecules, either negatively or positively supercoiled, might display different kinetics during compaction. However, this was not observed, at least under the current experimental set-up[51].

A working model for the action of condensin. Although the currently available data are fragmentary and difficult to integrate fully with each other, they have begun to reveal the highly dynamic actions of condensin. Assuming that condensin shares some, if not all, of its basic properties with BsSMC, a working model for the action of condensin is proposed here. In this model, a closed form of condensin makes its initial contact with chromatin in a sitting mode (FIG. 6, stage 1). Subsequent hydrolysis of bound ATP facilitates opening of the arms, which leads to a more stable interaction with chromatin through the hooking mode (FIG. 6, stage 2). In the following steps, two different scenarios could be considered. In the first scenario, ATP-driven head–head engagement between different condensin molecules might support the ordered assembly of a filamentous structure, which creates and traps superhelical tension of DNA in itself (FIG. 6, stage 3). Alternatively, local wrapping of DNA followed by head–head engagement within individual complexes might lead to the formation of chiral loops (FIG. 6, stage 3′). These structures could be further organized into a rosette-like configuration (FIG. 6, stage 4), whose helical stacking, possibly facilitated by stalk–stalk interactions, leads to the assembly of a prometaphase chromatin fibre (FIG. 6, stage 5). Finally, coiling of this prometaphase fibre might convert it into a metaphase chromatid (FIG. 6, stage 6).

It is anticipated that this series of events is achieved by the combined actions of protein–protein interactions and superhelical tension of DNA that is accumulated within the nucleoprotein structure. Although admittedly highly speculative, this model is consistent with cytological observations that condensin subunits associate first with a peripheral region of prophase chromatin[52] and become progressively enriched at the central axis of metaphase chromatids[40,52–54]. The cooperative actions of several condensin molecules outlined here could also help to explain a spreading mechanism used by the *C. elegans* dosage-compensation complex[55], a condensin-like complex that is specifically recruited to X-chromosomes and which reduces their gene expression by half.

Clearly, further extensive studies are required to test, or refine, this and other models of the action of condensin. In particular, it will be important to determine to what extent individual condensin complexes can support one or any of the reactions observed, and how cooperative interactions between several condensin complexes might contribute to a higher level of organization and stabilization. It is also important to keep in mind that the physiological substrate of condensin is a chromatin fibre rather than a naked strand of DNA. The gigantic size of the complex and its density on chromosomes need to be taken into account to draw a complete molecular picture (Supplementary information S2 (figure)). Finally, the specific role of non-SMC subunits, especially that of the two HEAT repeat subunits[40,56], in condensin function remains to be addressed. Because the HEAT repeats form a solenoidal, spring-like structure[57], it is possible that this motif confers additional structural flexibility on condensin-mediated assembly of higher-order chromosome structures.

Cohesins: linking sister DNA duplexes

Cohesins are four-subunit complexes with a central role in holding sister chromatids together during mitosis and meiosis in eukaryotic cells[5] (FIG. 1c). The SMC1–SMC3 heterodimer constitutes the core of the cohesin complex.

The ring model for the action of cohesin. A model for the action of cohesin, which is often referred to as a 'ring' or 'embrace' model, has been proposed by Nasmyth and colleagues (FIG. 7a) based on our knowledge of the molecular architecture of the complex[10] and the key regulatory event, which involves the proteolytic cleavage of the kleisin subunit Scc1 (REF. 58). Biochemical dissection[10] and electron microscopy data[14] are consistent with the idea that Scc1 bridges the head domains of Smc1 and Smc3, thereby forming a tripartite ring-like structure (FIG. 1c). According to this model, individual cohesin complexes embrace two DNA duplexes within their coiled-coil space to hold the sister chromatids together until metaphase. Proteolytic cleavage of Scc1 by separase at the onset of anaphase triggers the opening of the ring, and thereby promotes the separation of the two sister chromatids.

Type II topoisomerase
A type of DNA topoisomerase that changes the topology of DNA by breaking and rejoining both strands of the DNA double helix.

Electron spectroscopic imaging
An electron-microscopy technique that provides both structural and analytical information on the basis of energy losses of an electron beam. The mapping of phosphorus allows the visualization of the path of DNA within a nucleoprotein complex. The technique is also used to measure the mass of the complex and to determine the stoichiometric relationship of protein and DNA components within the complex.

Magnetic tweezers
An experimental set-up that allows nanomanipulation of a single DNA molecule tethered to a paramagnetic bead. By measuring end-to-end extension of the DNA molecule to which a fixed force is applied, one can monitor the process of DNA compaction and decompaction in real time.

HEAT repeat
A ~30 amino-acid-repeat motif that is found in a number of proteins with diverse functions. It was named after four proteins in which the repeat was originally detected (huntingtin, elongation factor 3, the regulatory A subunit of protein phosphatase 2A and TOR1).

Separase
A cysteine protease that promotes sister-chromatid separation at the onset of anaphase by cleaving the Scc1 subunit of cohesin.

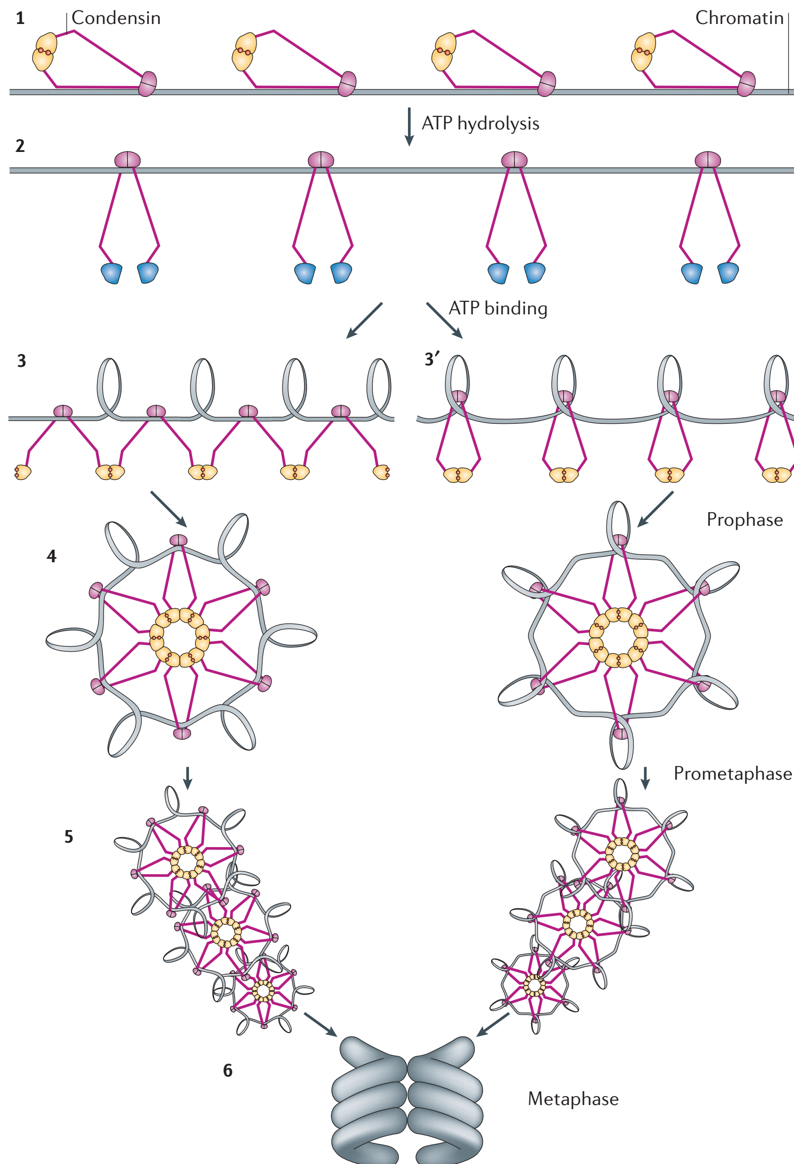

Figure 6 | **A working model for the action of condensin.** Condensin might first interact with chromatin in a closed form (stage **1**). This interaction would trigger hydrolysis of ATP (ATP is depicted as small red circles) bound to the head domain, thereby opening the arms and establishing chromatin binding by a 'hooking' mode (stage **2**). ATP-bound head domains are indicated in yellow, whereas ATP-free head domains are indicated in blue. At the next stage, intermolecular head–head engagement could assemble a nucleoprotein filament in which positive superhelical tension is trapped (stage **3**). Alternatively, a chiral loop might be produced by intramolecular head–head engagement (stage **3′**). Subsequent protein–protein interactions would then assemble a rosette-like structure (stage **4**), the stacking of which leads to the formation of a prometaphase chromatin fibre (stage **5**). The final assembly of a metaphase chromatid might involve the helical coiling of the prometaphase fibre (stage **6**). Although not shown here, it is likely that the initial binding stage also involves cooperative protein–protein interactions that are mediated by coiled-coil arms. Also not shown are the non-structural maintenance of chromosomes (non-SMC) protein subunits of condensin, which might make additional structural contributions to the dynamic and flexible assembly of mitotic chromosomes.

The model further predicts that the establishment of sister-chromatid cohesion is accomplished naturally when a replication fork passes through the cohesin ring that is preloaded during the G1 phase of the cell cycle. Consistent with the model, artificial cleavage of cohesin subunits *in vivo* causes the dissociation of cohesin from chromatin and the loss of sister-chromatid cohesion[59]. Most recently, a biochemical assay has been developed to purify replicated circular minichromosomes from yeast cells[60]. The cohesin complex bound to these minichromosomes is released by the proteolytic cleavage of its subunits *in vitro*. Conversely, the linearization of DNA causes the dissociation of cohesin from the minichromosomes, implicating that the interaction between cohesin and chromatin involves a topological linkage.

Biochemical studies of SMC1–SMC3 and cohesin. Despite the simplicity and elegance of the ring model, biochemical analyses of purified cohesin *in vitro* are less advanced compared with those of bacterial SMC proteins and condensin components. The purified complex has a modest affinity for DNA or chromatin, and no ATP-dependent activity has been reported so far[44,61,62]. Importantly, two notable differences between cohesin and condensin have been reported in parallel experiments. First, cohesin stimulates the intermolecular catenation of closed circular DNA in the presence of a type II topoisomerase, whereas condensin directs the intramolecular knotting in the same assay[61]. This observation underscores the distinct cellular functions of the two complexes, and is consistent with the idea that cohesin functions as an intermolecular DNA crosslinker, whereas condensin functions as an intramolecular DNA crosslinker[63]. Second, unlike the SMC2–SMC4 dimer or the BsSMC dimer, the SMC1–SMC3 dimer does not support the re-annealing of ssDNA[44]. The physiological significance of this observation is less clear, but it indicates that protein–protein interactions between different cohesin complexes, if any, might be much weaker than those between condensin complexes. In any case, direct evidence for the ring model remains to be gathered from *in vitro* reconstitution studies that use purified components.

One potential difficulty that is associated with the biochemical analysis of cohesin might be that the interaction of this complex with DNA is regulated more tightly than that of condensin. For example, the loading of cohesin onto chromatin *in vivo* requires a separate Scc2–Scc4 complex[64], although such a specialized loading factor has not yet been identified for condensin. The Scc2–Scc4 complex could in fact regulate the catalytic cycle of cohesin, as has been suggested from genetic studies testing the behaviour of ATPase-defective SMC mutants *in vivo*[65,66]. So, the inclusion of Scc2–Scc4 might be required to reconstitute efficient cohesin–DNA interactions *in vitro*.

Static rings or dynamic rings? Future experiments need to address at least two important predictions from the ring model. First, the original model predicts that the interaction of cohesin with DNA or chromatin is purely topological. This mode of binding might be true for cohesin, but clearly not for condensin or the bacterial SMC proteins. For example, the SMC2–SMC4 dimer and BsSMC dimer can bind to DNA without ATP-driven head–head engagement or without non-SMC

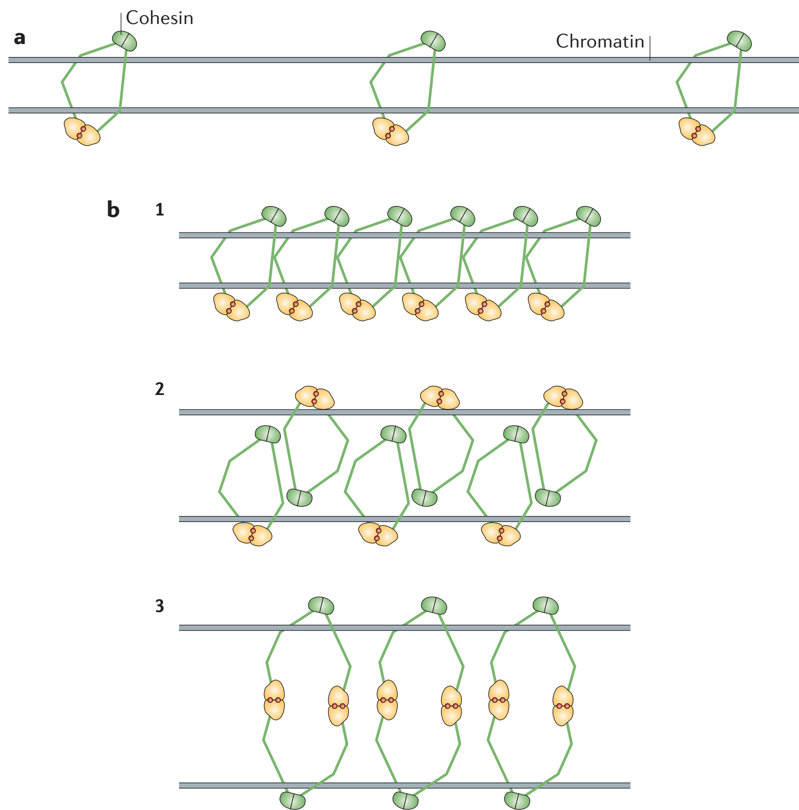

Figure 7 | **Potential actions of cohesin. a** | The original ring model in which individual cohesin complexes embrace two sister chromatids within their coiled-coil arms[10]. **b** | Alternative models for the action of cohesin. Each of the cohesin complexes embraces two chromatids as in part **a**, but stalk–stalk interactions between multiple cohesins assemble a higher-order structure that stabilizes cohesion (model **1**). Alternatively, each cohesin encircles only one chromatid, and stalk–stalk interactions help establish cohesion between the two chromatids (model **2**). Finally, ATP-driven head–head engagement might occur between different cohesin complexes and produce a cohesion-proficient structure (model **3**). To emphasize the central role of head–head engagement in structural maintenance of chromosomes (SMC) protein actions, the non-SMC subunits of cohesin are not shown. It is certainly possible, however, that they have active roles in holding sister chromatids together.

Prereplication complex
A protein complex that assembles at replication origins from late mitosis through to the G1 phase. The assembly of this complex is a prerequisite for the initiation of DNA replication in S phase.

subunits that might cause 'closure' of the coiled-coil arms. It remains to be tested whether the specific hinge-mediated DNA binding, as revealed for BsSMC, might be applicable to the action of cohesin (and condensin).

Second, the original ring model predicts that a single cohesin complex embraces two sister chromatids together within its coiled-coil arms. This is more difficult to test. The currently available evidence does not exclude the possibility that multiple cohesin complexes interact with each other to assemble a higher-order structure (for example, a double ring or a helical filament) that is proficient for cohesion. Recent evidence indicates that cohesin might associate with silenced chromatin (at the *HMR* locus in yeast) in a manner that is different from predictions derived from the original model[67]. Likewise, it is also possible that cohesin supports cohesion at centromeric and arm regions in mechanistically distinct ways, as the density and targeting mechanisms of cohesin at the two regions seem to differ substantially[68–71]. Some examples of alternative arrangements of cohesin are shown in FIG. 7b. Given the versatile and flexible structures of cohesin and its contributions to a diverse array of chromosomal functions, it would not be surprising to find that the complex uses a number of different variations in its action[72].

Another important question in the field is how cohesin-mediated cohesion might be established and modulated during DNA replication and other DNA transactions. In bacteria, SMC proteins might be loaded onto ssDNA regions created behind the replicative helicase during DNA replication[28]. Because there has been no sign that cohesin has a strong binding preference for ssDNA, such a model cannot be directly applied to eukaryotic species. In fact, the loading of cohesin (and its loading factor Scc2) on chromatin in *X. laevis* cell-free extracts depends on the assembly of the prereplication complex, but not on the initiation of DNA replication[73,74]. In *Saccharomyces cerevisiae*, cohesin binds to chromatin during the G1 phase even before the prereplication complex assembles on replication origins[75].

Even though a number of replication-related factors have been identified that are required for the proper establishment of cohesion *in vivo*, it remains completely unknown how they might contribute to coupling replication to cohesion at a mechanistic level. Moreover, accumulating lines of evidence show that the interaction of cohesin with chromatin is remarkably dynamic and must be coordinated with other chromosomal activities such as active transcription[76–80] and DSB repair[81,82]. In the future, it will be essential to develop an experimental system in which the functional coupling between cohesion and the other DNA transactions can faithfully be reconstituted *in vitro*.

Conclusions and perspectives

Recent advances in biochemistry, genetics, electron microscopy, structural biology and biophysics have just begun to reveal the unique architecture of SMC proteins, and their highly dynamic and flexible actions. A common theme is that SMC proteins are ATP-modulated molecular linkers of the genome that actively fold, tether and manipulate DNA strands. Central to the actions of SMC proteins are ATP binding and hydrolysis by the ABC-like head domains, which regulate closing and opening, respectively, of the gigantic V-shaped molecules. It has become increasingly clear that the central hinge domain has an equally important role in modulating the mechanochemical cycle of SMC proteins. A number of basic questions remain to be answered, however. For example, how many DNA duplexes (or segments) do individual SMC complexes interact with simultaneously? Exactly how is the mechanical cycle of SMC proteins coupled to their catalytic cycle? How crucial are intermolecular protein–protein interactions in the action of SMC proteins? The emerging high-resolution approaches that allow us to visualize and to monitor protein actions in real time (that is, time-resolved atomic force microscopy and single-molecule nanomanipulation) will be instrumental in addressing these questions. The use of chromatin templates will also be one of the big challenges in the field. The crucial comparison of ABC transporters, Rad50 and

SMC proteins will continue to enhance our understanding of the mechanics of this class of ubiquitous ATPases.

It will also be important to dissect the structural and functional variations among different SMC protein machines. Different SMC dimers display different biochemical properties *in vitro*. Distinct sets of non-SMC subunits confer additional levels of functional divergence to individual complexes. Whereas genetic and biochemical data are consistent with a gate-keeping role for the kleisin subunits, specific functions of other non-SMC subunits are completely unknown. The less-conserved subunits of condensin and cohesin are apparently unique to eukaryotes and could have more active roles, rather than the currently postulated regulatory roles in the dynamic organization of chromosomes.

Finally, it will be of great interest to study the mechanics of SMC proteins from an evolutionary point of view. The origin of SMC proteins precedes that of histones, and most, if not all, bacterial and archaeal species probably use SMC proteins as fundamental organizers of their genomes. Evolutionarily, the differentiation and functional specialization of SMC protein machines might parallel the changes of DNA polymerases, RNA polymerases and DNA-repair machineries, that would have contributed to modifying the size, content and dynamics of an organism's genome. It is therefore reasonable to anticipate that comparative analyses of SMC proteins might shed new light on the mechanism of genome evolution from the perspective of higher-order chromosome architecture and dynamics.

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