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Catching DNA with hoops—biophysical approaches to clarify the mechanism of SMC proteins

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Structural maintenance of chromosome (SMC) complexes are central regulators of chromosome architecture that are essential in all domains of life. For decades, the structural biology field has been debating how these conserved protein complexes use their intricate ring-like structures to structurally organize DNA. Here, we review the contributions of single-molecule biophysical approaches to resolving the molecular mechanism of SMC protein function.

Genomes of different organisms vary greatly in size, from a million to one-hundred billion base pairs, but they all share the challenge of needing to be squeezed into a micron-sized cell that is many orders of magnitude smaller than the length of the DNA. The spatial organization of the genome within cells is an intriguing scientific question that is currently of high interest. SMC protein complexes are the key players in the spatiotemporal organization and maintenance of DNA from bacteria to humans and are essential for many chromosomal processes such as compaction, chromosome segregation, DNA repair, and gene regulation[1–3].

SMC protein complexes have a unique structural organization characterized by a ring shape consisting of three proteins along its circumference: two SMC proteins complemented by a kleisin subunit (**Fig. 1a**). The main part of the SMC subunits involves an ~50 nm long antiparallel coiled coil that connects a hinge domain on one end with ATPase heads on the other. The SMC heads are ABC transporter ATPases with canonical Walker A and Walker B motifs. All SMC rings associate with different subunits and cofactors to form functional complexes[4] (**Fig. 1b**,**c**).

The ring-like structure is highly conserved and thus of vital importance for the function of SMC proteins. Prokaryotes have only a single type of SMC complex. The well-characterized BsSMC in *Bacillus subtilis*, for example, contains a homodimer of SMC proteins and the kleisin protein ScpA, whereas subfamilies of γ-proteobacteria, such as *Escherichia coli*, have an SMC complex called MukBEF (**Fig. 1b**). The structure of MukBEF deviates slightly from those of the other SMC complexes in that the MukF kleisin domain forms dimers that permit the formation of multimers of SMC complexes[5]. Deletion or mutation of Smc or MukBEF leads to severe chromosomal defects, including disruption of nucleoid structure and failure to segregate sister chromatids[6–9].

In eukaryotes, the SMC complex has evolved to three types of protein complexes that are all essential but that have different, partially overlapping functions: cohesin, condensin, and Smc5–Smc6 (**Fig. 1c**). Cohesin is responsible for faithful chromosome segregation during cell division, as it holds sister chromatids together while they align under the tension of the mitotic spindle[10] (**Fig. 2a**). Most of the cohesin is removed from the chromosome arms in prophase, but some stays bound at centromeres until the onset of anaphase when its kleisin, Scc1, is cleaved by separase to release the cohesion[10,11]. In addition, cohesin plays an important role in gene expression (**Fig. 2b**, recently reviewed in ref. 12). Condensin is the main mediator of mitotic chromosome assembly (**Fig. 2a**). Most eukaryotes have two condensin complexes, condensin I and condensin II, that work together to ensure proper DNA compaction and segregation[13]. Like cohesin, condensin has nonmitotic chromosome functions, such as gene regulation, dosage compensation, and DNA-damage response and repair[3,14,15]. Finally, the Smc5–Sm6 complex is the least well-understood SMC complex. While it is needed for double-strand-break repair, it also has a role in chromosome segregation[16–19].

SMC proteins in chromosome organization

The spatial organization of the genome is a topic of intense current investigation[20]. Genome-mapping studies have provided ample evidence for topological domains and loop formation. Exactly how such loops are established and stabilized is still unclear, but SMC proteins are the main candidates for directing these processes. A topological embrace of DNA, in which the SMC complex encompasses one or more DNA molecules, is thought to be the functional basis of the ring-shaped SMC complexes, and this unique principle has been the starting point for many studies of their molecular mechanism[21–24].

The classic and most simple model for chromosome organization by SMC complexes is that random DNA–DNA cross-links are established by trapping DNA inside the SMC ring[25,26]. Using condensin, DNA compaction could be achieved by grabbing two DNA strands and connecting them inside the condensin ring (**Fig. 2c**). Linking could be accomplished by a single SMC ring or by two mutually interacting SMC rings. The same principle can be applied to cohesin in the context of loop formation and sister-chromatid cohesion. A stochastic nonspecific linking does not explain how chromosomes arrange into elongated loop structures instead of an entangled, random-blob arrangement of mutually cross-linked sister chromatids. To

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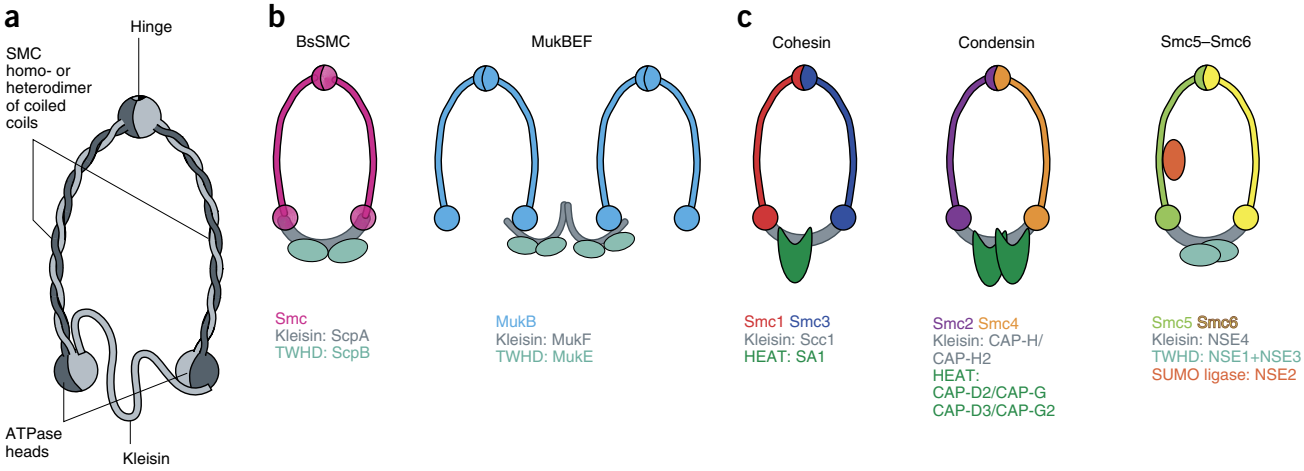

Figure 1 Architecture of SMC complexes. (**a**) General architecture of SMC complexes. SMC complexes consist of two SMC proteins that are connected at the hinge. In prokaryotes, the complex is a homodimer, whereas eukaryotic complexes are heterodimeric. The opposite ends of the SMC proteins, or heads, possess ATPase activity. A kleisin subunit completes the ring. (**b**) Overview of prokaryotic SMC complexes. (**c**) Overview of eukaryotic SMC complexes. Names of the human proteins are listed.

test whether crosslinking is sufficient to compact DNA into chromosomes, a computer-simulation study modeled chromosome compaction as stochastic, pairwise bonding between condensin molecules that connect distant DNA sites[27]. This pairwise-interaction model condensed the DNA accurately and matched the Hi-C data, thereby indicating that this simple model can go a long way to explain basic features of DNA compaction.

Recently, an alternative 'loop-extrusion' model has gained attention within the field[28–30] (**Fig. 2d**). In this model, an SMC protein binds DNA, initiates formation of a loop, and translocates DNA through its ring to form an extended DNA loop[31,32]. Such a principle could be employed by condensin to compact DNA into mitotic chromosomes or by cohesin to establish loop formation in topologically associating domains. For example, cohesin might halt and anchor the loop when it encounters two CTCF sites. Alipour and Marko first simulated a 1D model with condensin as a loop-extruding enzyme machine that employs two DNA-binding sites per protein[29]. The assumption was that each binding site moves away from the other along the DNA in an ATP-hydrolysis-dependent manner that drives the extrusion of a loop. The authors found that under certain association and dissociation conditions, two possible outcomes could result: either the formation of loops of variable sizes with gaps in between or the formation of a stack of proteins anchoring a single loop. Two independent studies recently applied this model on a larger scale[31,33]. Although these reports modeled general 'extrusion factors', the authors speculated that these factors could be cohesin molecules. Sanborn *et al.* assumed that each SMC extruder would cease extruding upon recognition of a CTCF motif of the correct directionality[33], leading to the formation of stable loops in a manner that is consistent with the experimental Hi-C data that accompanied the modeling study. A second analysis by Fudenberg *et al.* reached the same conclusion[31]. Yet another large-scale study used parameters from experimental analyses to model DNA compaction with condensin as the loop-extruding factor[34]. These simulations showed either loops separated by gaps or tightly stacked loop arrays, depending on the parameters employed. The authors showed that one condensin per 10–30 kb can generate loop sizes consistent with those seen in Hi-C assays. Loop extrusion by condensin was also shown to be able to compact chromatin into the dense structure characteristic of sister chromatids[35].

Although the random-cross-linking and loop-extrusion mechanisms represent the two dominant models, variations on these themes have been proposed throughout the years, including clustering-, translocation-, and supercoiling-based models[36–40]. Many questions

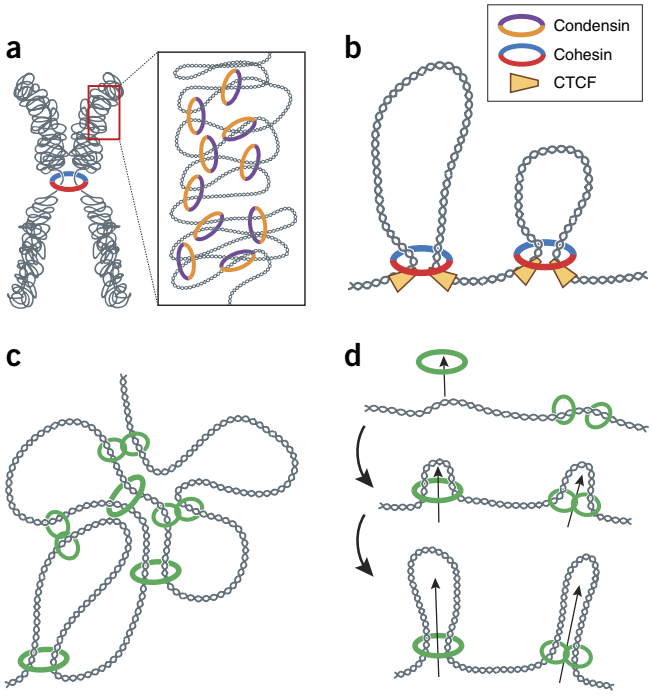

Figure 2 Mechanisms of SMC-complex function. (**a**) Schematic depicting the central biological functions of cohesin and condensin. Condensin compacts the DNA into mitotic chromosomes, whereas cohesin holds sister chromatids together at metaphase. Molecules are not drawn to scale. (**b**) Cohesin acts as a boundary element for topologically associating domains that are defined by CTCF-binding sites. (**c**) The random cross-linking model. An SMC complex links DNA together by trapping two DNA strands inside its ring. Looping can be accomplished either by a single SMC complex or by two interacting SMC complexes. (**d**) The loop-extrusion model. DNA gets trapped inside one or two SMC complexes and is subsequently extruded to form a DNA loop.

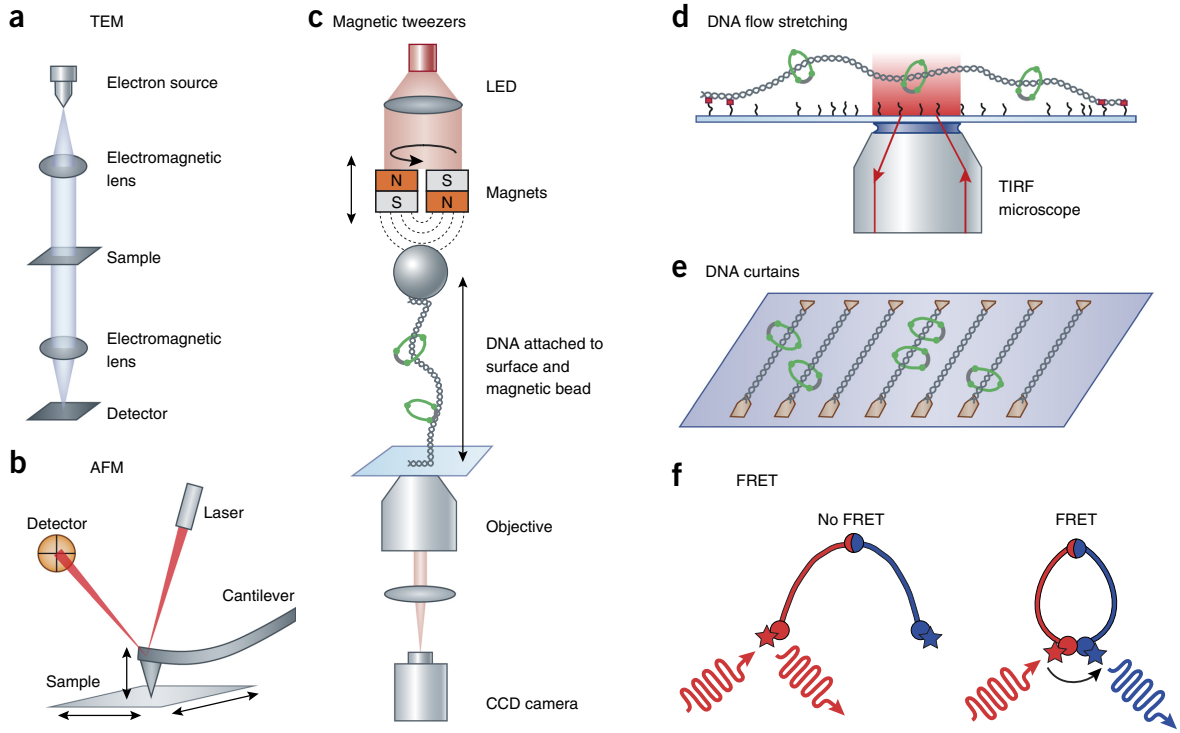

Figure 3 Experimental setups of single-molecule approaches. (**a**) Transmission EM (TEM). (**b**) Atomic-force microscopy (AFM). (**c**) Magnetic tweezers. (**d**) DNA flow stretching. (**e**) DNA curtains. (**f**) Fluorescent resonance energy transfer (FRET).

remain to be addressed in order to begin unraveling the mechanism of the SMC protein function, as we are still in the dark about how SMC complexes interact with DNA. For example: What conformational changes occur within SMC complexes? What is the role of ATP binding and hydrolysis? What are the dynamics of loading and unloading? Where are SMC complexes loaded, what drives their processivity, and how do they know when to stop? How does cohesin recognize CTCF orientation? And if all of these questions can be addressed: Is this mechanism the same for all SMC proteins? How do cohesin, condensin, and Smc5–Smc6 differ? How do eukaryotic SMC proteins differ from their prokaryotic counterparts, and to what extent do their functional mechanisms differ between organisms? Despite numerous cellular and biochemical studies in the past decades, there is a need for new approaches to address the many fundamental questions that remain. As these questions are largely mechanistic in nature, we feel that single-molecule biophysical techniques are uniquely suited to this purpose[41].

Excitingly, in the last five years, much progress has been made on the purification of several SMC complexes, enabling researchers to perform more *in vitro* analyses[42]. Although virtually impossible to deduce from bulk experiments, the mechanical properties of SMC protein complexes can be probed with various biophysical techniques at the level of individual molecules and are of particular interest from a biophysical perspective. SMC rings must withstand cellular forces generated during various stages of the cell cycle, such as segregation, and thus must be strong and stable in their association with DNA[43]. External forces can be applied and probed with methods such as magnetic tweezers (**Fig. 3c**), optical tweezers, and atomic force microscopes. The two most common techniques used to visualize SMC complexes at the single-molecule scale are transmission electron microscopy (TEM, **Fig. 3a**) and atomic force microscopy (AFM, **Fig. 3b**). Visualization of protein–DNA interactions is also possible with optical techniques such as DNA flow stretching[44] (**Fig. 3d**) and DNA curtains[45] (**Fig. 3e**), techniques that rely on visualizing a stretched DNA molecule with fluorescence microscopy. With fluorescent resonance energy transfer (FRET), the interaction between two molecules, or two sites within the same molecule, can be investigated[46] (**Fig. 3f**). Some of the advantages and limitations of these techniques are summarized in **Table 1**. In the following sections, we review results obtained by applying these approaches to SMC protein complexes.

Single-molecule imaging of SMC complexes

Owing to their large, multisubunit architecture, SMC complexes are difficult to purify, and structural information is difficult to obtain[47]. Although parts of SMC subunits have been crystalized, crystal structures of full SMC complexes are not yet available (for a recent review on crystallography, see ref. 47). Accordingly, most of the information that we have on global SMC architecture is derived from real-space imaging techniques such as AFM and EM.

TEM can yield high-quality images using low-wavelength electrons (**Fig. 3a**). An electron source emits electrons that are focused into a thin beam that hits the sample, which is stained with, for example, heavy metals for increased contrast. Whereas some electrons are scattered, most travel through, creating a 'shadow image' of the sample. Potential artifacts can be introduced during the sample preparation when transferring proteins from solution to air to vacuum, a challenge that recently has largely been overcome using cryo-EM[48] (which, to the best of our knowledge, has not yet been applied to SMC complexes).

In AFM, a sharp tip at the end of a cantilever scans the surface of the sample, oscillating near its resonance frequency (**Fig. 3b**). The oscillation of the tip is altered as the tip interacts with the sample, and the resultant deflection is detected by a photo diode. This information is then translated into a topological image with nanometer resolution.

Table 1 Advantages and limitations of single-molecule techniques

Single-molecule imaging techniques	TEM	High-resolution (near atomic level) imaging	Surface technique, imaging in vacuum, static snapshots, potential artifacts in sample preparation and contrast enhancement
	AFM	High-resolution (nm-scale) imaging of molecules in air or in liquid, no need for labeling	Surface technique, static snapshots
	High-speed AFM	Observing dynamics with nm-scale resolution in liquid. Acquisition of videos at a rate of up to ~20 images per second	Surface technique
Force spectroscopy	Magnetic tweezers	Controlled application of force and torque, accurate measurement of DNA end-to-end distance	No visualization of proteins acting on DNA
	Optical tweezers	Controlled measurements of force and DNA end-to-end distance	Low throughput
Fluorescent imaging techniques	DNA flow stretching	Visualization of fluorescently labeled proteins on stretched, immobilized DNA	Limited optical resolution
	DNA curtains	Visualization of many DNA molecules in parallel (high throughput)	Limited optical resolution
	FRET	Sensitive measurements of local dynamics within proteins	Incorporation of fluorescent tags at position of interest can be challenging, limited size range (up to ~10 nm)

Conventional AFM can be used to take h+igh-resolution static 'snapshots' of molecules on a surface. Thanks to recent technical advances, it is now also possible to observe the motion of single molecules in real time with high-speed AFM, which can acquire images at a video rate of 20 images per second[49,50]. In AFM, there is no need to label or stain the sample, but a fundamental limitation of both EM and AFM is that proteins need to be bound to a surface for visualization. We note that some caution is needed when interpreting images from EM and AFM reports, as these techniques dry the molecules, which can potentially trap them in nonphysiological conformations.

Despite these caveats, imaging techniques have provided a number of valuable insights into the structure of SMC subunits, the shape and dynamics of full SMC complexes, and their interaction with DNA. Specifically, researchers have attempted to classify the shape of the SMC dimers and the complexes using the letter system depicted in **Figure 4a**. This system is of interest as the deduced shape may directly relate to SMC complex function: interaction between the heads will close the loop; interaction between the heads and hinge may indicate an intermediate for loading; stiff rods could indicate that the SMC dimers are clamped onto DNA, etc. So far, the imaging efforts have yielded widely scattered results for different species of SMC complexes under varying conditions.

The first images of SMC proteins appeared in the early nineties, when bacterial MukB dimers were visualized with low-angle rotary-shadowing EM[51]. This study was the first report of globular structures (heads and hinge) separated by coiled-coil segments, thus establishing a key step in determining the structure of SMC proteins. Several years later, higher-resolution EM imaging of MukB and BsSmc dimers revealed another crucial characteristic of SMC proteins: the antiparallel arrangement of the coiled coils that brings the C and N termini together at the head[52]. EM studies also showed that MukE and MukF bind to the MukB heads[53]. MukB dimers and BsSMC dimers were mostly observed in I-shaped and V-shaped conformations[51–56] and occasionally in Y or O shapes[57] (**Fig. 4b**). Similar I- and V-shaped conformations were later found for the full BsSmc–ScpAB complex[58,59].

One of the unanswered questions for SMC proteins is whether they mutually interact and cooperate. Interestingly, MukBEF complexes were shown to form either fiber-form multimers or rosette shapes[53]. Similar rosette structures were also observed in liquid AFM for BsSMC[55], whereas multimers were detected with dry AFM[57]. After incubation with plasmid DNA, MukB complexes were shown to form large networks that appeared to consist of many catenated plasmids[60]. Such clusters, however, appear to be a much less prominent feature of eukaryotic SMCs. A live-cell imaging study used superresolution photo-activated localization microscopy (PALM) to probe the architecture of MukBEF complexes *in vivo*[61]. Despite their different molecular weights, all of the subunits showed the same diffusion coefficient, indicating that they were moving as a unit. Single-molecule fluorescent-particle tracking estimated a stoichiometry of 4:4:2 molecules for MukB:MukE:MukF, and functional units apparently consist of 8–10 such MukBEF complexes.

Importantly, the first EM studies on eukaryotic SMC complexes confirmed that cohesin and condensin share the same head-coiled-coil-hinge structure[62]. An equally important finding was that the antiparallel coiled coils of cohesin Smc1–Smc3 dimers are intramolecular, thus folding back on themselves, and not two SMC proteins mutually coiled together along their entire length[63]. Imaging of individual Smc1 or Smc3 proteins revealed that each protein forms an elongated structure with a globular structure on both sides of the coiled coil, revealing that cohesin therefore consists of one Smc1 arm and one Smc3 arm that are connected at the hinge.

Dimers and holocomplexes of cohesin and condensin have been imaged for a variety of species. Budding yeast Smc1–Smc3 dimers formed in the presence of ATP were reported to be in both V and O shapes (**Fig. 4b**), whereas mutants deficient in ATP binding showed less head engagement, suggesting that ATP binding influences the interaction between the cohesin heads[64,65]. An AFM study in liquid reported I-shaped cohesin dimers, and the authors suggested that both coiled coils were mutually intertwined within this I-shaped structure[66]. Interestingly, deacetylated cohesin showed a higher occurrence of V- and Y-shaped Smc1–Smc3 dimers, suggesting that modifications, such as acetylation, influence the orientation. The same might be possible for condensin[62]. The majority of full cohesin complexes of both human and yeast was found to be in a V, O, or Y shape[63,65,67] (**Fig. 4b**). In some cases, kinks in the coiled coils were

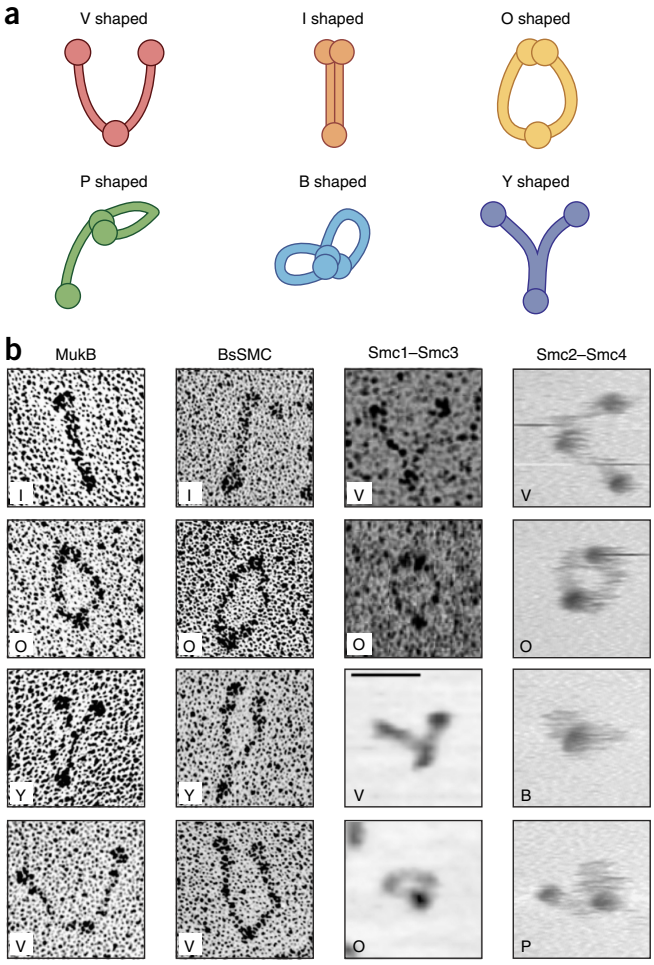

Figure 4 SMC complex configurations. (**a**) The shapes of the SMC complexes, which are of crucial importance as they probably relate to their function, are classified according to a letter system. (**b**) Captured images of various SMC complexes showing differences and similarities among species and visualization techniques. Highest-quality representative images were selected for the following complexes: MukB and BsSMC, EM images adapted from ref. 52; Smc1–Smc3, EM images (top two panels) from ref. 63; dry AFM images (bottom two panels) from ref. 64; Smc2–Smc4 dimers, stills from high-speed AFM movies obtained from ref. 69. The letter in each panel indicates the letter shape shown in **a**.

observed. Several groups have attempted to visualize the interaction of condensin with DNA. With electron spectroscopic imaging[68], *Xenopus* condensin was seen to interact with plasmid DNA in an ATP-hydrolysis-dependent manner[39]. Remarkably, the DNA appeared to be wrapped around the heads only when ATP was present, thereby leading to the proposal that condensin creates supercoils by wrapping DNA around the ATPase heads[39].

It is likely that SMC complexes can assume different conformations depending on the function and stage in the cell cycle and that these conformational changes are dynamic. Condensin Smc2–Smc4 dimers imaged with high-speed AFM in liquid at physiological conditions indeed revealed complexes that switched between various conformations over time[69]. Dimers were observed to switch between V, O, B, and P shapes, whereas I-shaped conformers were not detected. Though the existence of the head–hinge interaction has been predicted, this observation is the sole report of B and P shapes so far[70,71]. Furthermore, this study revealed that the coiled coils are flexible, with a persistence length of only ~4 nm[69], demonstrating that condensin has the structural flexibility to change conformation and engage in a chromatin embrace. Cohesin imaged with high-speed AFM showed that the coiled coils were flexible and that the molecules change their configuration within imaging time, although no quantification was provided[66].

Among all SMC complexes, the architecture and function of Smc5–Smc6 is the least well studied. Indeed, to our knowledge, there has not been any imaging or single-molecule study of the Smc5–Smc6 complex. Visualization of this complex and its arrangement of subunits would greatly aid our understanding of its structure, but the purification of a clean and complete complex remains a challenge to such studies[19,23].

In summary, the abundance of imaging studies has not yielded a uniform conformation of SMC complexes, but rather has revealed conformers that vary among groups and species as well as among imaging techniques and sample-preparation methods. In fact, these studies have established that these flexible complexes can adopt many different conformations.

Force spectroscopy with magnetic tweezers

The reorganization of DNA by SMC proteins can be studied in real time using single-molecule tweezers. With optical tweezers, beads are trapped by a laser, and the force and displacement of the trapped bead, for example, DNA displacement in response to a protein, can be measured. In the context of SMC proteins, however, only magnetic tweezers have been employed. Magnetic tweezers are exceptionally suited to apply a force clamp on a molecule, monitor changes in DNA length upon protein binding, and study DNA supercoiling induced by SMC complexes[72]. In these studies, a DNA molecule is tethered between a surface and a magnetic bead (**Fig. 3c**), and an external magnet is used to manipulate the bead and thereby the tethered molecule. Rotation and vertical movement of the magnets apply torque and force, respectively. Note that in this technique, the readout is the *z* position of the bead, whose precision permits very accurate measurement of the DNA end-to-end length. A limitation of conventional magnetic-tweezers techniques is that the proteins acting on DNA cannot be visualized.

Magnetic tweezers have been used to monitor the end-to-end distance of a DNA molecule as it is shortened by the compacting action of SMCs (**Fig. 5a**). A pioneering study with condensin holocomplex extracted from mitotic *Xenopus laevis* cells showed that compaction and decompaction occurred in large steps (±70 nm) upon the addition of ATP. Compaction was not observed in the absence of ATP, and only very weak compaction was seen when condensin from interphase cells was used[73]. Although no compaction was observed in the absence of ATP, condensin did interact with DNA in an ATP-independent fashion. Applying forces >10 pN reversed compaction. Similar results were obtained in a recent magnetic-tweezers study on the *Saccharomyces cerevisiae* complex[74] (**Fig. 5b**) that examined how the rate of compaction depends on protein concentration, ATP concentration, and applied force. Compaction was found to be reversible with high-salt concentrations, but condensin remained bound, indicating topological loading. Interestingly, both magnetic-tweezers studies on eukaryotic condensin failed to detect a supercoiling activity for condensin that was previously detected by biochemical studies[37–39].

The *E. coli* MukB dimer similarly showed compaction of DNA against low forces with steps of ~70 nm[75]. Addition of the subunits MukE and MukF decreased the rate of compaction. The authors argued that MukB formed clusters that could resist forces up to 10 pN. ATP had no effect on compaction rate but shortened the lag time before initiation of compaction. Two DNA molecules were attached

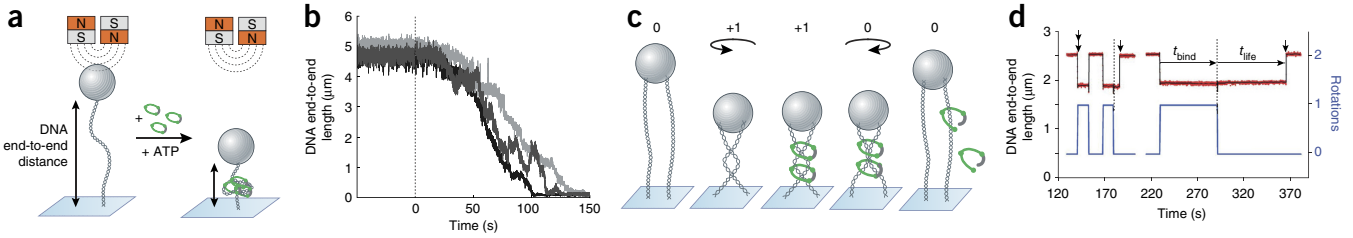

Figure 5 SMC-mediated DNA compaction visualized by magnetic tweezers. (**a**) The basic principle of the magnetic-tweezers assay to monitor DNA compaction. A DNA molecule is stretched between a magnetic bead and a surface. Upon addition of condensin, the end-to-end distance of the DNA decreases as the DNA is compacted. Adapted from ref. 74. (**b**) DNA compaction by the *S. cerevisiae* condensin complex in the presence of ATP. DNA end-to-end length decreases as it is compacted by the addition of condensin. Different shades of gray represent different DNA molecules in a single experiment. Adapted from ref. 74. (**c**) Schematic representation of the time course of a magnetic-tweezers DNA-bridging experiment performed in the presence of MukB (see text). DNA bridges were generated by introducing rotation (+1) to magnetic beads attached to two DNA molecules. Subsequently, the bead was untwisted to zero rotations (0) to attempt to remove the bridges. (**d**) DNA end-to-end length (red) decreased as the magnets made one turn (blue) and reverted to the initial value in the absence of protein (double arrow). In the presence of MukB, a delay in this recovery was observed (arrows, t_{life}), which is attributed to a MukB-induced bridge that was released after a given time interval (t_{life}).

to one magnetic bead to examine the ability of MukB to form a bridge between two DNA molecules (**Fig. 5c,d**)[76]. Interestingly, the probability that an SMC complex would form a bridge increased in the presence of ATP and decreased for an ATPase mutant.

Surprisingly, budding yeast's Smc1–Smc3 dimer (thus, not a full cohesin complex) was sufficient to compact DNA in a step-wise manner (130-nm steps) as well[67]. This compaction was not dependent on ATP and was still observed when a variant lacking the ATPase heads was used, but not when the hinge was replaced.

We note that all of the step sizes reported in these SMC-induced DNA-condensation studies are strikingly large (70–200 nm). Although a detailed step analysis in a recent study called for caution in interpreting step size by magnetic-tweezers assays that employ low forces[74], it is clear that the steps observed for SMC proteins are much larger than those of typical DNA-translocating motor proteins such as helicases, translocases, or polymerases, which typically move in one-base pair increments[77–80]. In fact, these large steps are similar to or even larger than the size of the SMC complexes themselves, which are a maximum of 70 nm along their longest axis[62]. A similar size suggests conformational changes at the scale of the full SMC complex itself, whereas larger sized steps are puzzling, despite the fact that they are consistently observed in different studies. Such large steps may involve the concerted action of multiple SMC complexes or bursts of fast sequential steps of a single SMC complex. We note that protein aggregation can also reduce the end-to-end distance of a DNA molecule in magnetic tweezers, thus calling for caution in interpreting results. However, further analysis of the mechanism underlying step size is clearly a direction of future research.

Fluorescent imaging techniques

The interaction between SMC complexes and DNA can be visualized using fluorescent-imaging approaches in which both the DNA and the protein of interest are fluorescently labeled. In flow-stretching experiments, a linear DNA molecule is stretched out along a PEGylated glass slide, and SMC complexes may bind to regions along the DNA (**Fig. 3d**). With the DNA-curtain technique, DNA is attached to freely diffusing lipids that, upon applying a flow, diffuse toward microfabricated barriers to form 'curtains' (**Fig. 3e**). An advantage of DNA curtains is that many DNA molecules can be visualized in parallel, thus making it easier to build statistics in these single-molecule experiments. The drawbacks of both techniques include limited optical resolution (typically >300 nm) and the fact that conformational changes, such as compaction, are difficult to observe when the DNA is fixed at both ends.

Using single-molecule imaging on flow-stretched DNA, fluorescently labeled BsSMC complexes were observed to display two types of behavior: static binding and 1D Brownian diffusion[40] (**Fig. 6a**). At higher concentrations, clusters of BsSMC were able to compact the DNA against the flow on a single tethered curtain (**Fig. 6b**). ATP had only a marginal influence on the compaction rate, whereas non-SMC subunits ScpA and ScpB reduced clustering on the DNA substrate. Interestingly, a headless mutant also showed local bending of the DNA. The authors suggested that the ATPase domains are required

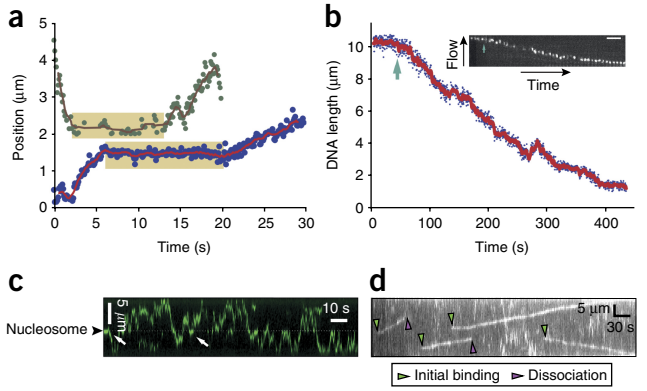

Figure 6 SMC motion on flow-stretched DNA. (**a**) Time course profile of the position of BsSMC moving along DNA. The motion of BsSMC switches between static binding (brown box) at a single spot and random 1D diffusion along the DNA. Adapted from ref. 40. (**b**) Time course of DNA end-to-end length while BsSMC compacts DNA. At high concentrations, BsSmc complexes cluster and compact DNA. The kymograph is obtained from the quantum-dot-labeled end of a DNA molecule (see inset) that is being compacted. Adapted from ref. 40. (**c**) Kymograph (position along DNA versus time) of a fluorescently labeled cohesin complex whose motion is tracked along DNA. Obstacles (in this case nucleosomes, black arrowhead) restrict random diffusion. Cohesin is observed to transiently pause at the nucleosome (white arrows), but it is able to subsequently diffuse past it. Adapted from ref. 81. (**d**) Kymograph showing the linear motor action of condensin as complexes first bind (green arrowheads) and subsequently traverse the DNA in an ATP-dependent manner over long distances (>10 kb). Pink arrowheads indicate protein dissociation. Adapted from ref. 86.

for cooperative clustering, whereas single BsSMC dimers might bend the DNA, thereby producing local DNA compaction.

Two studies of cohesin revealed a similar diffusive behavior for motion along DNA. In a DNA-curtain study, *Schizosaccharomyces pombe* cohesin showed a diffusion constant of 3.8 ± 0.2 $\mu m^2/s$ in 500 mM salt[81], which is similar to that of human cohesin on flow-stretched DNA (1.7 ± 0.1 $\mu m^2/s$)[82]. These values correspond well to an *in vivo* estimate of the cohesion diffusion rate (3.0 ± 0.2 $\mu m^2/s$)[83]. Both studies found that neither ATP nor a loading complex were necessary for cohesin loading and diffusion. Cohesin remained associated with DNA at high-salt concentrations, consistent with biochemical experiments and highly suggestive of a topological-embrace model[84].

Both studies also probed cohesin's ability to diffuse past obstacles of various sizes. DNA-bound obstacles of up to ~10 nm in size could be bypassed without problems, but complexes >20 nm could not be overcome. Cohesin occasionally paused upon encountering a nucleosome, but could diffuse over it (**Fig. 6c**). Interestingly, the majority of cohesin failed to bypass the transcriptional regulator CTCF, which serves as a boundary element *in vivo*[82]. Both the bacterial DNA translocase FtsK and the T7 RNA polymerase could push the cohesin ring along the DNA. Although eukaryotic cohesin would not encounter these bacterial complexes *in vivo*, it does indicate that cohesin can, in principle, be displaced by polymerases.

A third study probed the dynamics of *Xenopus* cohesin on flow-stretched DNA[85]. In contrast to the above reports, these authors claim that cohesin diffusion is dependent on both ATP and the cohesin-loading complex Scc2–Scc4. The movement they observed was consistent with random diffusion rather than active linear translocation. The presence of Wapl–Pds5 (required for cohesin removal in prophase) was found to reduce cohesin's diffusional motion, an effect that was antagonized by cohesin acetylation.

Recently, a DNA-curtain study showed that the *S. cerevisiae* condensin complex is a mechanochemical molecular motor that translocates on DNA[86] (**Fig. 6d**). Translocation was ATP dependent, persisted for very long distances (>10 kb), and showed an average velocity of ~60 base pairs per second. Strikingly, condensin was able to cotranslocate a second DNA molecule along the DNA curtains. These findings demonstrate that condensin has a DNA-translocating motor domain, which is an essential component for DNA compaction in a mechanism such as loop extrusion. Although loop extrusion is most often discussed in the context of cohesin, eukaryotic condensin is so far the only SMC protein for which motor activity is reported.

Single-molecule FRET techniques have also been used to study the dynamics of SMC complexes. The spatial proximity of two fluorescently labeled sites with distinct excitation and emission spectra can be determined with FRET. This principle relies on the energy transfer by excitation of one fluorophore (donor) to the nearby second fluorophore (acceptor). The efficiency of this transfer is strongly dependent on the distance between the donor and acceptor, making this technique a very sensitive tool to study inter- and intramolecular interactions for distances of up to ~10 nm. Incorporation of the suitable fluorescent tags into the proteins of interest at the position of choice can, however, be challenging.

When the association of cohesin's head domains was probed with FRET in live cells of budding yeast[87], a high FRET value was observed throughout the cell cycle, indicating that the ATPase heads are in proximity of each other at most times. No interactions between the hinge and the heads were detected; thus, if this interaction occurs *in vivo*, it is very transient. Likewise, no association among different cohesin complexes could be detected in this *in vivo* assay. The proximity of the coiled coils of both MukB and BsSMC was also probed *in vitro* with FRET[54]. A truncated form of BsSMC showed a high FRET efficiency, whereas a MukB fragment showed low FRET, consistent with an I shape and V shape, respectively.

PERSPECTIVE

The molecular mechanism of SMC complexes and their function in directing chromosomal architecture are among the most actively investigated topics in cell biology today, and biophysical techniques are key to answering fundamental questions that are essential for their elucidation. Although crystallography will continue to provide insights into protein structures, the flexible and open conformations of the full complexes evade capture by this approach. Single-molecule AFM and EM imaging, which both circumvent this limitation, have already begun to illuminate SMC complex structure, and we expect many more results to emerge from improved imaging techniques such as high-speed AFM and cryo-EM in the coming years. Visualizing SMC dynamics with high-speed AFM and FRET enables resolution of the large conformational changes that are believed to underlie their function.

It remains important to consider how the results of *in vitro* single-molecule experiments can be extrapolated to the *in vivo* environment of the cell. *In vitro* studies of partial complexes in the absence of ATP are tricky to interpret, as partial and ATPase-deficient complexes are not often viable *in vivo*. *In vivo*, SMC complexes are regulated by many cofactors and modifications, depending on the stage in the cell cycle. These additional components will become amenable to single-molecule analysis as the field continues to make progress in purifying proteins and cofactors of increasing quality[85]. Alternatively, one can perform single-molecule experiments on proteins derived from cell extracts, which may retain their modifications and cofactors. Single-molecule experiments of increased complexity may permit examination of minimal forms of chromatin instead of naked DNA; such assays appear well within reach given that reconstitution of chromosomes requires a surprisingly low amount of factors[88].

It will also be of interest to consider whether prokaryotic and eukaryoti c SMC complexes might employ different mechanisms. For example, the prokaryotic BsSMC was reported to require recruitment factors to become active[89,90]. Such factors have not been reported for eukaryotic complexes; indeed, all *in vitro* single-molecule studies on eukaryotic condensin so far have reported DNA compaction activity in the absence of a loading factor. This apparent difference between eukaryotic and prokaryotic condensin is unexpected, as, from an evolutionary perspective, one would expect the eukaryotic SMC to exhibit a higher complexity with additional cofactors.

The differences and similarities among various eukaryotic SMC complexes remain largely unresolved. For example, motor activity has only been established for eukaryotic condensin and not for bacterial SMC or for cohesin. It will be interesting to learn whether this reflects an intrinsic difference among the factors or is related to purification methods or cofactor function. A very recent study that combined Hi-C and computer simulations unexpectedly found that cohesin, and not condensin, was responsible for chromosome compaction in budding yeast[91]. It may be the case that cohesin and condensin share very similar mechanisms. Alternatively, the same homologous complex, say condensin, may function differently in different organisms. Critically evaluating differences between species and different SMC complexes with classical assays such as magnetic tweezers and DNA flow stretching is therefore of continued interest. Conducting these biophysical assays in the context of crowded environments, involving different cofactors known to interact with SMC proteins, will also more faithfully mimic *in vivo* conditions.

Progress can also be expected from the use of hybrid techniques that combine multiple single-molecule methods. Using magnetic tweezers in conjunction with fluorescence imaging would enable changes in DNA length or linking number and the action of fluorescently labeled SMC proteins to be monitored simultaneously. Similarly, the combination of FRET measurements on flow-stretched DNA could provide information on the local conformational changes within molecules while they perform their function on DNA. New developments in imaging and single-molecule techniques can thus be expected to significantly advance our understanding of the essential genome-organizing functions of SMC proteins in all organisms in the coming years.

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The authors declare no competing financial interests.

1. Uhlmann, F. SMC complexes: from DNA to chromosomes. *Nat. Rev. Mol. Cell Biol.* **17**, 399–412 (2016).
Excellent recent review of the current understanding of the mechanism of SMC complexes.
2. Nasmyth, K. & Haering, C.H. Cohesin: its roles and mechanisms. *Annu. Rev. Genet.* **43**, 525–558 (2009).
3. Hirano, T. Condensins: universal organizers of chromosomes with diverse functions. *Genes Dev.* **26**, 1659–1678 (2012).
4. Haering, C.H. & Gruber, S. SnapShot: SMC protein complexes part I. *Cell* **164**, 326–326.e1 (2016).
5. Nolivos, S. & Sherratt, D. The bacterial chromosome: architecture and action of bacterial SMC and SMC-like complexes. *FEMS Microbiol. Rev.* **38**, 380–392 (2014).
6. Britton, R.A., Lin, D.C. & Grossman, A.D. Characterization of a prokaryotic SMC protein involved in chromosome partitioning. *Genes Dev.* **12**, 1254–1259 (1998).
7. Wang, Q., Mordukhova, E.A., Edwards, A.L. & Rybenkov, V.V. Chromosome condensation in the absence of the non-SMC subunits of MukBEF. *J. Bacteriol.* **188**, 4431–4441 (2006).
8. Gruber, S. *et al.* Interlinked sister chromosomes arise in the absence of condensin during fast replication in B. subtilis. *Curr. Biol.* **24**, 293–298 (2014).
9. Danilova, O., Reyes-Lamothe, R., Pinskaya, M., Sherratt, D. & Possoz, C. MukB colocalizes with the oriC region and is required for organization of the two *Escherichia coli* chromosome arms into separate cell halves. *Mol. Microbiol.* **65**, 1485–1492 (2007).
10. Uhlmann, F., Lottspeich, F. & Nasmyth, K. Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. *Nature* **400**, 37–42 (1999).
11. Waizenegger, I.C., Hauf, S., Meinke, A. & Peters, J.M. Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. *Cell* **103**, 399–410 (2000).
12. Merkenschlager, M. & Nora, E.P. CTCF and cohesin in genome folding and transcriptional gene regulation. *Annu. Rev. Genomics Hum. Genet.* **17**, 17–43 (2016).
13. Ono, T. *et al.* Differential contributions of condensin I and condensin II to mitotic chromosome architecture in vertebrate cells. *Cell* **115**, 109–121 (2003).
14. Frosi, Y. & Haering, C.H. Control of chromosome interactions by condensin complexes. *Curr. Opin. Cell Biol.* **34**, 94–100 (2015).
15. Meyer, B.J. Targeting X chromosomes for repression. *Curr. Opin. Genet. Dev.* **20**, 179–189 (2010).
16. Lehmann, A.R. *et al.* The rad18 gene of *Schizosaccharomyces pombe* defines a new subgroup of the SMC superfamily involved in DNA repair. *Mol. Cell. Biol.* **15**, 7067–7080 (1995).
17. De Piccoli, G. *et al.* Smc5-Smc6 mediate DNA double-strand-break repair by promoting sister-chromatid recombination. *Nat. Cell Biol.* **8**, 1032–1034 (2006).
18. Lindroos, H.B. *et al.* Chromosomal association of the Smc5/6 complex reveals that it functions in differently regulated pathways. *Mol. Cell* **22**, 755–767 (2006).
19. Jeppsson, K. *et al.* The chromosomal association of the Smc5/6 complex depends on cohesion and predicts the level of sister chromatid entanglement. *PLoS Genet.* **10**, e1004680 (2014).
20. Dekker, J. & Mirny, L. The 3D genome as moderator of chromosomal communication. *Cell* **164**, 1110–1121 (2016).
21. Haering, C.H., Farcas, A.-M., Arumugam, P., Metson, J. & Nasmyth, K. The cohesin ring concatenates sister DNA molecules. *Nature* **454**, 297–301 (2008).
22. Cuylen, S., Metz, J. & Haering, C.H. Condensin structures chromosomal DNA through topological links. *Nat. Struct. Mol. Biol.* **18**, 894–901 (2011).
23. Kanno, T., Berta, D.G. & Sjögren, C. The Smc5/6 complex is an ATP-dependent intermolecular DNA linker. *Cell Rep.* **12**, 1471–1482 (2015).
24. Wilhelm, L. *et al.* SMC condensin entraps chromosomal DNA by an ATP hydrolysis dependent loading mechanism in *Bacillus subtilis*. *eLife* **4**, 11202–11212 (2015).
25. Cuylen, S., Metz, J., Hruby, A. & Haering, C.H. Entrapment of chromosomes by condensin rings prevents their breakage during cytokinesis. *Dev. Cell* **27**, 469–478 (2013).
26. Thadani, R., Uhlmann, F. & Heeger, S. Condensin, chromatin crossbarring and chromosome condensation. *Curr. Biol.* **22**, R1012–R1021 (2012).
27. Cheng, T.M.K. *et al.* A simple biophysical model emulates budding yeast chromosome condensation. *eLife* **4**, e05565 (2015).
28. Nasmyth, K. Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis. *Annu. Rev. Genet.* **35**, 673–745 (2001).
29. Alipour, E. & Marko, J.F. Self-organization of domain structures by DNA-loop-extruding enzymes. *Nucleic Acids Res.* **40**, 11202–11212 (2012).
30. Dolgin, E. DNA's secret weapon against knots and tangles. *Nature* **544**, 284–286 (2017).
31. Fudenberg, G. *et al.* Formation of chromosomal domains by loop extrusion. *Cell Rep.* **15**, 2038–2049 (2016).
32. Wang, X., Brandão, H.B., Le, T.B.K., Laub, M.T. & Rudner, D.Z. Bacillus subtilis SMC complexes juxtapose chromosome arms as they travel from origin to terminus. *Science* **355**, 524–527 (2017).
33. Sanborn, A.L. *et al.* Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes. *Proc. Natl. Acad. Sci. USA* **112**, E6456–E6465 (2015).
34. Goloborodko, A., Marko, J.F. & Mirny, L.A. Chromosome compaction by active loop extrusion. *Biophys. J.* **110**, 2162–2168 (2016).
35. Goloborodko, A., Imakaev, M.V., Marko, J.F. & Mirny, L. Compaction and segregation of sister chromatids via active loop extrusion. *eLife* **5**, e14864 (2016).
36. Diebold-Durand, M.-L. *et al.* Structure of full-length SMC and rearrangements required for chromosome organization. *Mol. Cell* **67**, 334–347.e5 (2017).
37. Kimura, K. & Hirano, T. ATP-dependent positive supercoiling of DNA by 13S condensin: a biochemical implication for chromosome condensation. *Cell* **90**, 625–634 (1997).
38. Kimura, K., Rybenkov, V.V., Crisona, N.J., Hirano, T. & Cozzarelli, N.R. 13S condensin actively reconfigures DNA by introducing global positive writhe: implications for chromosome condensation. *Cell* **98**, 239–248 (1999).
39. Bazett-Jones, D.P., Kimura, K. & Hirano, T. Efficient supercoiling of DNA by a single condensin complex as revealed by electron spectroscopic imaging. *Mol. Cell* **9**, 1183–1190 (2002).
40. Kim, H. & Loparo, J.J. Multistep assembly of DNA condensation clusters by SMC. *Nat. Commun.* **7**, 10200 (2016).
41. Ha, T. Single-molecule methods leap ahead. *Nat. Methods* **11**, 1015–1018 (2014).
42. Murayama, Y. & Uhlmann, F. Biochemical reconstitution of topological DNA binding by the cohesin ring. *Nature* **505**, 367–371 (2014).
43. Tanaka, T., Fuchs, J., Loidl, J. & Nasmyth, K. Cohesin ensures bipolar attachment of microtubules to sister centromeres and resists their precocious separation. *Nat. Cell Biol.* **2**, 492–499 (2000).
44. Ganji, M., Kim, S.H., van der Torre, J., Abbondanzieri, E. & Dekker, C. Intercalation-based single-molecule fluorescence assay to study DNA supercoil dynamics. *Nano Lett.* **16**, 4699–4707 (2016).
45. Greene, E.C., Wind, S., Fazio, T., Gorman, J. & Visnapuu, M.L. DNA curtains for high-throughput single-molecule optical imaging. *Methods Enzymol.* **472**, 293–315 (2010).
46. Juette, M.F. *et al.* The bright future of single-molecule fluorescence imaging. *Curr. Opin. Chem. Biol.* **20**, 103–111 (2014).
47. Gligoris, T. & Löwe, J. Structural insights into ring formation of cohesin and related Smc complexes. *Trends Cell Biol.* **26**, 680–693 (2016).
48. Bai, X.C., McMullan, G. & Scheres, S.H. How cryo-EM is revolutionizing structural biology. *Trends Biochem. Sci.* **40**, 49–57 (2015).
49. Ando, T. *et al.* A high-speed atomic force microscope for studying biological macromolecules. *Proc. Natl. Acad. Sci. USA* **98**, 12468–12472 (2001).
50. Katan, A.J. & Dekker, C. High-speed AFM reveals the dynamics of single biomolecules at the nanometer scale. *Cell* **147**, 979–982 (2011).
51. Niki, H. *et al.* E.coli MukB protein involved in chromosome partition forms a homodimer with a rod-and-hinge structure having DNA binding and ATP/GTP binding activities. *EMBO J.* **11**, 5101–5109 (1992).
The first electron micrographs of SMC proteins (MukB) showing the globular ATPase domains and coiled coils.
52. Melby, T.E., Ciampaglio, C.N., Briscoe, G. & Erickson, H.P. The symmetrical structure of structural maintenance of chromosomes (SMC) and MukB proteins: long, antiparallel coiled coils, folded at a flexible hinge. *J. Cell Biol.* **142**, 1595–1604 (1998).
53. Matoba, K., Yamazoe, M., Mayanagi, K., Morikawa, K. & Hiraga, S. Comparison of MukB homodimer versus MukBEF complex molecular architectures by electron microscopy reveals a higher-order multimerization. *Biochem. Biophys. Res. Commun.* **333**, 694–702 (2005).

54. Soh, Y.M. *et al.* Molecular basis for SMC rod formation and its dissolution upon DNA binding. *Mol. Cell* **57**, 290–303 (2015).
55. Mascarenhas, J. *et al.* Dynamic assembly, localization and proteolysis of the *Bacillus subtilis* SMC complex. *BMC Cell Biol.* **6**, 28 (2005).
56. Hirano, M., Anderson, D.E., Erickson, H.P. & Hirano, T. Bimodal activation of SMC ATPase by intra- and inter-molecular interactions. *EMBO J.* **20**, 3238–3250 (2001).
57. Fuentes-Perez, M.E., Gwynn, E.J., Dillingham, M.S. & Moreno-Herrero, F. Using DNA as a fiducial marker to study SMC complex interactions with the atomic force microscope. *Biophys. J.* **102**, 839–848 (2012).
58. Kamada, K., Miyata, M. & Hirano, T. Molecular basis of SMC ATPase activation: role of internal structural changes of the regulatory subcomplex ScpAB. *Structure* **21**, 581–594 (2013).
59. Kamada, K., Su'etsugu, M., Takada, H., Miyata, M. & Hirano, T. Overall shapes of the SMC-ScpAB complex are determined by balance between constraint and relaxation of its structural parts. *Structure* **25**, 603–616 e4 (2017).
60. Bahng, S., Hayama, R. & Marians, K.J. MukB-mediated catenation of DNA is ATP and MukEF independent. *J. Biol. Chem.* **291**, 23999–24008 (2016).
61. Badrinarayanan, A., Reyes-Lamothe, R., Uphoff, S., Leake, M.C. & Sherratt, D. J. In vivo architecture and action of bacterial structural maintenance of chromosome proteins. *Science* **338**, 528–531(2012).
62. Anderson, D.E., Losada, A., Erickson, H.P. & Hirano, T. Condensin and cohesin display different arm conformations with characteristic hinge angles. *J. Cell Biol.* **156**, 419–424 (2002).
63. Haering, C.H., Löwe, J., Hochwagen, A. & Nasmyth, K. Molecular architecture of SMC proteins and the yeast cohesin complex. *Mol. Cell* **9**, 773–788 (2002).
64. Elbatsh, A.M.O. *et al.* Cohesin releases DNA through asymmetric ATPase-driven ring opening. *Mol. Cell* **61**, 575–588 (2016).
65. Huis in 't Veld, P.J. *et al.* Characterization of a DNA exit gate in the human cohesin ring. *Science* **346**, 968–972 (2014).
66. Kulemzina, I. *et al.* A reversible association between Smc coiled coils is regulated by lysine acetylation and is required for cohesin association with the DNA. *Mol. Cell* **63**, 1044–1054 (2016).
67. Sun, M., Nishino, T. & Marko, J.F. The SMC1-SMC3 cohesin heterodimer structures DNA through supercoiling-dependent loop formation. *Nucleic Acids Res.* **41**, 6149–6160 (2013).
68. Bazett-Jones, D.P. & Hendzel, M.J. Electron spectroscopic imaging of chromatin. *Methods* **17**, 188–200 (1999).
69. Eeftens, J.M. *et al.* Condensin Smc2-Smc4 dimers are flexible and dynamic. *Cell Rep.* **14**, 1813–1818 (2016).
High-speed AFM study showing that condensin dimers dynamically adopt different conformations.
70. Gruber, S. *et al.* Evidence that loading of cohesin onto chromosomes involves opening of its SMC hinge. *Cell* **127**, 523–537 (2006).
71. Nasmyth, K. Cohesin: a catenase with separate entry and exit gates? *Nat. Cell Biol.* **13**, 1170–1177 (2011).
72. De Vlaminck, I. & Dekker, C. Recent advances in magnetic tweezers. *Annu. Rev. Biophys.* **41**, 453–472 (2012).
73. Strick, T.R., Kawaguchi, T. & Hirano, T. Real-time detection of single-molecule DNA compaction by condensin I. *Curr. Biol.* **14**, 874–880 (2004.)
Pioneering magnetic-tweezers study showing that condensin can compact DNA.
74. Eeftens, J.M., Bisht, S., Kerssemakers, J., Haering, C. & Dekker, C. Real-time detection of condensin-driven DNA compaction reveals a multistep binding mechanism. *bioRxiv* http://www.doi.org/10.1101/149138 (2017).
75. Cui, Y., Petrushenko, Z.M. & Rybenkov, V.V. MukB acts as a macromolecular clamp in DNA condensation. *Nat. Struct. Mol. Biol.* **15**, 411–418 (2008).
76. Petrushenko, Z.M., Cui, Y., She, W. & Rybenkov, V.V. Mechanics of DNA bridging by bacterial condensin MukBEF in vitro and in singulo. *EMBO J.* **29**, 1126–1135 (2010).
77. Pyle, A.M. Translocation and unwinding mechanisms of RNA and DNA helicases. *Annu. Rev. Biophys.* **37**, 317–336 (2008).
78. Singleton, M.R., Dillingham, M.S. & Wigley, D.B. Structure and mechanism of helicases and nucleic acid translocases. *Annu. Rev. Biochem.* **76**, 23–50 (2007).
79. Yang, W. Lessons learned from UvrD helicase: mechanism for directional movement. *Annu. Rev. Biophys.* **39**, 367–385 (2010).
80. Seidel, R., Bloom, J.G., Dekker, C. & Szczelkun, M.D. Motor step size and ATP coupling efficiency of the dsDNA translocase EcoR124I. *EMBO J.* **27**, 1388–1398 (2008).
81. Stigler, J., Çamdere, G.Ö., Koshland, D.E. & Greene, E.C. Single-molecule imaging reveals a collapsed conformational state for DNA-bound cohesin. *Cell Rep.* **15**, 988–998 (2016).
One of three studies demonstrating diffusion of cohesin on stretched DNA and their ability to move across roadblocks.
82. Davidson, I.F. *et al.* Rapid movement and transcriptional re-localization of human cohesin on DNA. *EMBO J.* **35**, 2671–2685 (2016).
83. Ladurner, R. *et al.* Cohesin's ATPase activity couples cohesin loading onto DNA with Smc3 acetylation. *Curr. Biol.* **24**, 2228–2237 (2014).
84. Murayama, Y. & Uhlmann, F. Chromosome segregation: how to open cohesin without cutting the ring? *EMBO J.* **32**, 614–616 (2013).
85. Kanke, M., Tahara, E., Huis In't Veld, P.J. & Nishiyama, T. Cohesin acetylation and Wapl-Pds5 oppositely regulate translocation of cohesin along DNA. *EMBO J.* **35**, 2686–2698 (2016).
86. Terakawa, T. *et al.* The condensin complex is a mechanochemical motor that translocates along DNA. *Science* http://dx.doi.org/10.1126/eaan6516 (2017).
87. Mc Intyre, J. *et al. In vivo* analysis of cohesin architecture using FRET in the budding yeast *Saccharomyces cerevisiae*. *EMBO J.* **26**, 3783–3793 (2007).
88. Shintomi, K., Takahashi, T.S. & Hirano, T. Reconstitution of mitotic chromatids with a minimum set of purified factors. *Nat. Cell Biol.* **17**, 1014–1023 (2015).
89. Gruber, S. & Errington, J. Recruitment of condensin to replication origin regions by ParB/SpoOJ promotes chromosome segregation in B. subtilis. *Cell* **137**, 685–696 (2009).
90. Sullivan, N.L., Marquis, K.A. & Rudner, D.Z. Recruitment of SMC by ParB-parS organizes the origin region and promotes efficient chromosome segregation. *Cell* **137**, 697–707 (2009).
91. Schalbetter, S.A. *et al.* SMC complexes differentially compact mitotic chromosomes according to genomic context. *Nat. Cell Biol.* **19**, 1071–1080 (2017).