Structure-function relationships of SMC protein complexes for DNA loop extrusion

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Structural Maintenance of Chromosome (SMC) complexes are vital for chromosome organization. They extrude DNA loops to compact 2 meters of DNA into a micrometer-sized chromosome structure. The DNA loop extrusion process is believed to be a universal mechanism of SMC complexes for spatiotemporal chromosome organization conserved in almost all species from prokaryotes to eukaryotes. However, the molecular mechanism of DNA loop extrusion by SMC complexes is under debate; various tentative mechanistic models have been suggested, but there is no clear consensus. Here, we review the structural studies of various SMC complexes from prokaryotes to eukaryotes to understand the structure-function relationships of SMC complexes involved in DNA loop extrusion. We introduce controversial observations of the conformations of SMC complexes based on previous reports and discuss various proposed mechanisms of DNA loop extrusion suggested by experimental observations of the conformations of diverse SMC complexes.

INTRODUCTION

The key proteins for chromosome organization, named Structural Maintenance of Chromosomes (SMC) complexes – including condensin, cohesin, and the SMC5/6 complex – are essential and conserved through all kingdoms of life from prokaryotes to eukaryotes (Nasmyth and Haering, 2005; Aragon et al., 2013; Jeppsson et al., 2014; Hirano, 2016; Uhlmann, 2016; Rowley and Corces, 2018; van Ruiten and Rowland, 2018). SMC complexes are vital for the spatiotemporal organization of chromosomes, playing key roles in sister chromatid cohesion, chromosome resolution, chromosome segregation, DNA replication, and DNA repair (Nasmyth and Haering, 2005; Haering and Gruber, 2016). Moreover, many mutations in the SMC complexes are related to human diseases such as Cornelia de Lange syndrome (CdLS) and many cancers (Romero-Pérez et al., 2019; Waldman, 2020). Therefore, elucidating the molecular mechanisms of SMC complexes is crucial to understanding the most fundamental building blocks of chromosome organization and determinants of chromosome-related diseases.

SMC complexes are believed to be key universal mechanochemical motor proteins that extrude DNA loops processively using ATP hydrolysis, contributing to macroscale chromosome organization. Because this “DNA loop extrusion hypothesis” can explain the fundamental principles of how the chromosome is precisely organized, a recent article in *Nature* described the phenomenon as “a complete revolution in DNA enzymology” and “kind of the Higgs boson” of the chromosomal biology field (Dolgin, 2017). Moreover, emerging evidence has supported the DNA loop extrusion hypothesis using chromosome capture methods (such as High-throughput sequencing experiment (Hi-C)), 3D polymer simulations, and *in vitro* single-molecule

fluorescence experiments. In particular, recent *in vitro* single-molecule fluorescence (Terakawa et al., 2017; Ganji et al., 2018; Davidson et al., 2019; Kim et al., 2019; Golfier et al., 2020; Kong et al., 2020) and Atomic Force Microscopy (AFM) (Ryu et al., 2020a) studies directly observed that SMC complexes are mechanochemical motor proteins that extrude DNA into large loops using ATP hydrolysis. These studies showed very high speeds of DNA loop extrusion (600 bp/s) despite low ATPase activity (~2 ATP/s) (Ganji et al., 2018). This indicates that SMC complexes generate large step sizes (~50 nm) for DNA loop extrusion, suggesting they fall within an entirely different class of motor proteins from other DNA-related motor enzymes such as translocases, polymerases, and helicases, which generate small steps (~1 bp/ATP) (Seidel et al., 2004; Pease et al., 2005; Graham et al., 2010; Sirinakis et al., 2011; Liu et al., 2014; Hsieh et al., 2015). Although uncovering special characteristics like the large step size is crucial to understanding chromosomal structure, the molecular mechanism of DNA loop extrusion driven by SMC complexes is still largely under debate due to many controversial observations.

This review focuses on recent structural studies that suggest mechanisms for DNA loop extrusion by SMC complexes. We introduce conserved SMC architectures and the emerging evidence for DNA loop extrusion. In addition, we introduce several controversial studies on the structures of different SMC complexes and discuss contradictory working models.

CONSERVED ARCHITECTURES OF SMC COMPLEXES

The protein complexes classified as the SMC protein family are diverse: cohesin, condensin, and SMC5/6 complexes in eukaryotes; Smc-ScpAB, MukBEF, and MskBEF in prokaryotes

(Figure 1A and Table 1). The key feature of this family is a unique tripartite ring structure, which is formed by the connection between the ends of two SMC subunits and one kleisin subunit. Each SMC complex includes peripheral subunits classified as HAWK or KITE proteins, which interact with the kleisin subunit (Figure 1A and Table 1) (Palecek and Gruber, 2015; Wells et al., 2017).

SMC subunits are the main components of the SMC complexes, of which amino acid sequences and architectures are conserved through species (Bürmann et al., 2017; Haering et al., 2002). Every SMC subunit is a long polypeptide which consists of 1,000-1,500 amino acids and contains a globular domain at

its middle, which is called a “hinge” (Haering et al., 2002). Each SMC polypeptide is folded at its central hinge domain, forming an anti-parallel coiled-coil (Jeppsson et al., 2014) (Figure 1B). The N-terminal Walker A motif and the C-terminal Walker B motif of an SMC subunit are combined and form an ATPase “head” domain which is classified as an ATP-binding cassette (ABC) ATPase (Löwe et al., 2001). Two SMC subunits form a homodimer in prokaryotes and a heterodimer in eukaryotes through the interaction interfaces in their hinge domain (Figure 1B, C) (Haering et al., 2002; Haering and Gruber, 2016). A pair of head domains also forms a transient dimer by sandwiching two ATP molecules between their Walker A/B motifs (Figure

FIGURE 1 | Structural features of SMC complexes. (A) Overall architectures of representative SMC family proteins. Eukaryotic cohesin, condensin, Smc5/6, prokaryotic Smc-ScpAB and MukBEF are displayed (left to right). Names of each subunit are listed in Table 1. Various colorations are used to identify each subunit. Nomenclatures of the subunits of eukaryotic proteins follow the names of homologous proteins of *S. cerevisiae* species. The names of HAWK and KITE subunits of each SMC complex are depicted in the bottom and listed in Table 1 with red and blue colors, respectively. (B) Schematic of the folding of an SMC dimer molecule and the binding sites for its kleisin partner. κ-SMC (cyan) designates the SMC subunit binding to the C-terminal winged-helix domain of kleisin (green) while v-SMC (blue) designates the SMC subunit binding to the N-terminal helical bundle of kleisin. The binding interfaces of kleisin are denoted by dashed green lines. (C) Representative crystal structures of SMC hinge domains. Homodimer structure of the hinge domain from *P. furiosus* Smc (PDB ID: 4RSJ, left) and heterodimer structure of the hinge domain from *S. cerevisiae* Smc2/4 (4RSI, right). (D) Representative crystal structures of SMC head domains. ATP-engaged Smc head dimer from *P. furiosus* (1XEX, left), Smc1 head binding the C-terminal WH domain of Scc1 from *S. cerevisiae* (1W1W, middle), and Smc3 head with proximal coiled-coil binding the N-terminal helices of Scc1 from *S. cerevisiae* (4UX3). Colorization follows that of Figure 1A. (E) Experimentally determined structures of HAWK proteins. Pds5 (5F0O), Scc3 (4UVK), Scc2 (5T8V), Ycs4 (6QJ3), and Ycg1 (5OQR) (left to right). Nomenclatures of the subunits of eukaryotic proteins follow the names of homologous proteins from *S. cerevisiae*. (F) Structural alignment of KITE proteins. *B. Subtilis* ScpB (4I98) and *H. Sapiens* Nse1/3 (5WY5) were superimposed based on their secondary structures.

TABLE 1 | Subunit composition of diverse and conserved SMC complexes

Protein name	Cohesin			Condensin		SMC5/6		Prokaryotic SMC complex		
	Species	<i>S. cerevisiae</i>	<i>H. sapiens</i>	<i>S. cerevisiae</i>	<i>H. sapiens</i>	<i>S. cerevisiae</i>	<i>H. sapiens</i>	Smc-ScpAB	MukBEF	MksBEF
				condensin I	condensin II			Most Prokaryotes	Enterobacteria	Distributed among bacteria
κ -SMC	Smc1	SMC1	Smc4	SMC4	SMC4	Smc5	SMC5	Smc	MukB	MksB
ν -SMC	Smc3	SMC3	Smc2	SMC2	SMC2	Smc6	SMC3	Smc	MukB	MksB
Kleisin	Sccl	SCC, RAD21L	Brn1	CAP-H	CAP-H2	Nse4	NSE4A, NSE4B	ScpA	MukF	MksF
HAWK _A	Pds5	PDS5A, PDS5B	Ycs4	CAP-D2	CAP-D3	Nse1	NSE1	-	-	-
HAWK _B	Sccl	SA1, SA2	Ycg1	CAP-G	CAP-G2	Nse3	NSE3	-	-	-
KITE _A	-	-	-	-	-	Nse5	NSE5	ScpB	MukE	MksE
KITE _B	-	-	-	-	-	Nse6	NSE6	-	-	-

Components of eukaryotic cohesin, condensin, SMC5/6, and prokaryotic condensin are listed from left to right, respectively. For eukaryotic SMC complexes, subunit names of *S. cerevisiae* and *H. sapiens* were listed as representative species. HAWK and KITE subunits are highlighted with red and blue colors.

1D) (Lammens et al., 2004). Each SMC subunit interacts with the kleisin subunit through the two binding interfaces of kleisin: the proximal coiled-coils of its head (called the “neck”) and the bottom part of its head (“cap”). The SMC subunit that binds the neck of the kleisin subunit is called “ ν -SMC” (ν from “neck”) while the other SMC subunit binding the cap of the kleisin subunit is called “ κ -SMC” (κ from “cap”) (Figure 1B, D and Table 1).

Each SMC complex interacts with only one kleisin subunit: an Sccl homolog, a Brn1 homolog, Nse4, ScpA, MukF, and MksF are kleisin subunits for eukaryotic cohesin, eukaryotic condensin, SMC5/6 complex, Smc-ScpAB, MukBEF, and MksBEF, respectively. The kleisin subunits differ in their amino acid sequences, but share similar structural features in their N- and C-termini: an N-terminal helical bundle binds to the neck of ν -SMC, and a C-terminal winged-helix (WH) domain binds to the cap of κ -SMC (Figure 1B, D). A crystal structure of MukF revealed that the N-terminal domain provides the binding interface for a kleisin homodimer (Woo et al., 2009) (Figure 1A, right-most panel). This binding characteristic of MukF and subsequent reports suggest that two MukBEF complexes act as a functional unit (Fennell-Fezzie et al., 2005; Badrinarayanan et al., 2012; Sherratt, 2020). The middle region of the kleisin subunits seems largely unstructured based on their predicted secondary structures and experimentally determined partial structures (Woo et al., 2009; Bürmann et al., 2013; Kamada et al., 2013).

The peripheral subunits interacting with the kleisin subunits can be separated into two superfamilies: HAWK (HEAT proteins Associated With Kleisin) with eukaryotic cohesin, condensin, and SMC5/6; and KITE (Kleisin Interacting Tandem winged helical Elements of SMC complexes) with eukaryotic SMC5/6 and prokaryotic SMC complexes (Figure 1A, bottom; Table 1)

(Wells et al., 2017). HAWK proteins consist of several HEAT (Huntingtin/EF3/PP2A/Tor1) repeat domains, and the HEAT repeat domain contains partial alpha-solenoids which consist of the repeats of two alpha-helices. In addition, every eukaryotic SMC complex adopts distinct HAWK components: Sccl, Pds5, and Sccl2 homologs for cohesin; Ycg1 and Ycs4 homologs for condensin; and Nse5/6 for SMC5/6 complex in *S. cerevisiae* (Table 1). Although the HAWK proteins show a low amino-acid sequence similarity and differ in three-dimensional structures, their structures show a common hook-shaped feature, especially when they form a subcomplex with kleisin (Figure 1E) (Kschonsak et al., 2017; Kong et al., 2020; Lee et al., 2020; Shi et al., 2020). In the case of cohesin, while Nimbl^{Sccl2}, the human homolog of Sccl2, is directly involved in the DNA loop extrusion process by stimulating the ATPase activity of the cohesin complex, Pds5 is not necessary for DNA loop extrusion (Davidson et al., 2019). However, Pds5, the unloading factor of cohesin, competes against a cohesin loader, Nimbl^{Sccl2}, for the same binding site on the kleisin protein of the cohesin complex (Murayama and Uhlmann, 2015) (Figure 1A, left-most panel). In the case of condensin, Ycg1 seems to anchor condensin onto DNA using a safety-belt mechanism with Brn1 (Kschonsak et al., 2017). In contrast, KITE proteins consist of tandem WH domains; two WH domains are connected by a flexible linker to form a homo- or heterodimer in prokaryotic SMC complexes and the eukaryotic SMC5/6 complex (Woo et al., 2009; Bürmann et al., 2013; Kamada et al., 2013; Palecek and Gruber, 2015). Moreover, alignment of WH domains based on secondary structure shows high structural similarity (Figure 1F). Although the structural stabilization of the kleisin-KITE subcomplex is certainly important to control aspects of the SMC complex such as ATPase activity (Kamada et al., 2013), the functional role of the KITE proteins is still unknown.

DNA LOOP EXTRUSION: FUNDAMENTAL PRINCIPLES OF SMC-MEDIATED CHROMOSOME ORGANIZATION

The loop extrusion hypothesis proposed by Arthur Riggs was that an unspecified enzyme reels DNA in to induce 50 – 100 kbp DNA loops for chromosome organization (Riggs, 1990). Over two decades ago, cohesin was suggested to be a new class of mechanochemical motor that forms chromosome loops (Guacci et al., 1993; Strunnikov et al., 1993); condensin was also suggested to extrude DNA in a processive manner to induce loops (Hirano and Mitchison, 1994; Kimura et al., 1999; Nasmyth, 2001). Furthermore, emerging evidence supports the long-standing hypothesis that every SMC protein uses a DNA loop extrusion mechanism for chromosome organization universally from prokaryotes to eukaryotes, although the exact function of each SMC protein complex seems to differ.

Recent progress using chromosome conformation capture methods such as Hi-C and micro-C has provided evidence of DNA loop extrusion by SMC complexes (Sofueva et al., 2013; Rao et al., 2014; Gibcus et al., 2018; Costantino et al., 2020; Krietenstein et al., 2020). To obtain contact probability maps of the chromosome within a nucleus, the chromosome is cross-linked and digested into short DNA pieces, and these pieces

are sequenced and displayed in a matrix of contact frequencies as a heat map. In a Hi-C map, it was shown that the cohesin complex extrudes DNA loops to fold chromatin into Topologically Associating Domains (TADs), 100 kb – 1 Mb regions of high self-interaction frequency present in interphase in vertebrate cells (Dixon et al., 2012; Sofueva et al., 2013; Rao et al., 2014; Gassler et al., 2017; Schwarzer et al., 2017; Wutz et al., 2017). Figure 2A shows a typical Hi-C map of TADs, organized by cohesin complex. Each TAD is separated by distinct boundary regions which are normally defined by the orientation of CCCTC-binding factor (CTCF)-binding sites (Dixon et al., 2012; Sofueva et al., 2013; de Wit et al., 2015; Guo et al., 2015; Holzmam et al., 2019; Busslinger et al., 2017; Wutz et al., 2017), and stable corner peaks at some TADs are observed at convergent CTCF sites, suggesting the cohesin complex is an active DNA loop extruder that translocates along chromatin until it encounters a CTCF site in a proper orientation (Sanborn et al., 2015; Fudenberg et al., 2016). This process is strongly supported by Hi-C control experiments. Firstly, a cohesin depletion experiment showed the disruption of both TADs and the peaks located at CTCF convergent sites. In addition, the depletion strengthened compartmentalization patterns (Rao et al., 2017). Secondly,

FIGURE 2 | Experimental basis of the DNA loop extrusion hypothesis. (A) Cartoon representation of the interaction signals underlying a Hi-C map (top) (Dixon et al., 2012; Sexton et al., 2012; Lajoie et al., 2015; Rowley and Corces, 2018) and a schematic model of the structure of a TAD in a metazoan cell (bottom). Both dark and light magenta areas represent Hi-C signals (top). Peak ChIP-seq signals (black wedges) imply the binding of the boundary protein, CTCF, to DNA (middle). Smaller subTADs characterized by darker magenta are shown in a TAD (top), and CTCF loops shown as dots at the apexes of each TAD domain correspond to DNA loops (middle) formed by cohesin complexes between CTCF sites (bottom). The coloration of cohesin follows that of Figure 1A. The direction of each CTCF site is depicted by the orientations of the yellow arrowheads in the schematic model. (B) Cartoon representation of a Hi-C map of the *B. subtilis* strain BWX3352 contains a single ParS site close to 0° (top) (Wang et al., 2017). Maps display contact frequencies during the propagation of the juxtaposition of chromosome of *B. subtilis*. The extension of off-diagonal (dark magenta, top) is highly correlated with the localizations of Smc-ScpAB observed by a ChIP-seq experiment (red lines; middle), implying that loading and translocation of SMC proteins (cyan) form juxtaposed chromosome arms from a site adjacent to the replication origin of DNA to the opposite site (bottom). (C) Cartoon representation of the simulated TAD organization mediated by cohesin complex and CTCF (Fudenberg et al., 2016). Directionalities (green and red arrows) of boundary elements (CTCF proteins: yellow arrowheads) determine the direction of loop-extruding factors such as cohesin complex (cyan rings) during interphase loop extrusion (left). The Hi-C simulation results correspond to the topological features of DNA tethered by boundary elements (right). (D) Cartoon representation of an *in vitro* single-molecule DNA loop extrusion experiment (Ganji et al., 2018). Single DNA loop, extruded by a loop extruding factor (cyan ring), is visualized during orthogonal fluid flow (blue arrow).

depletion of Nipbl, a human cohesin loader, disrupted TADs and peaks at CTCF binding sites (Busslinger et al., 2017), while depletion of Wapl, the unloading factor of the cohesin complex, induced more TADs and longer loops and formed a vermicelli shape of the chromosome structure (Haarhuis et al., 2017). Lastly, an ATP depletion experiment using oligomycin showed a dramatic change of TAD distribution across the genome (Vian et al., 2018). These results strongly support that the cohesin complex is a motor protein that has ATPase activity, and that CTCF functions as a roadblock to stall the DNA loop extrusion process driven by the cohesin complex (Vian et al., 2018).

Hi-C experiments investigating the prokaryotic SMC complex also support a DNA loop extrusion mechanism. For example, the Hi-C pattern of Smc-ScpAB showed close contacts between the juxtaposed chromosome arms of the circular chromosome in *B. subtilis* (Figure 2B) (Le et al., 2013; Marbouty et al., 2015; Wang et al., 2015). The Hi-C results suggest that the Smc-ScpAB complexes were loaded at *parS* sites close to the replication origin (close to 0°) via interaction with ParB (Le et al., 2013; Wilhelm et al., 2015), and the ATPase activity of Smc-ScpAB seems to be used for translocation from *parS* sites to the replication terminus (Wang et al., 2018). After that, Smc-ScpAB is likely unloaded by XerD at *ter* region (Karaboja et al., 2021). The rapid processive translocation speed of Smc-ScpAB along the juxtaposition of the opposite chromosome arms (up to 800 bp/s) strongly indicates the existence of the DNA loop extrusion process in bacteria.

Meanwhile, *in silico* polymer simulations strongly support the DNA loop extrusion model suggested from Hi-C experiments. A simulated Hi-C map successfully reproduced the TAD patterns shown in Hi-C experiments by assuming that a cohesin complex translocates along DNA to extrude a *cis* DNA loop until it meets a boundary element (Figure 2C) (Fudenberg et al., 2016). Also, this simulation predicts that the exceedingly strong corner peaks of TADs indicate the activity of cohesin in the region between two boundary elements – that is, CTCFs.

Additionally, single-molecule double-tethered DNA assays have directly visualized the DNA loop extrusion process driven by *S. cerevisiae* condensin, human cohesin and condensin I/II, and *Xenopus* cohesin and condensin (Ganji et al., 2018; Davidson et al., 2019; Kim et al., 2019; Golfier et al., 2020; Kong et al., 2020; Ryu et al., 2020a). To reconstitute the DNA loop extrusion process, SMC complexes and ATP are added to an immobilized λ DNA, and a flow perpendicular to the DNA strand is applied (Figure 2D). A clear DNA loop structure can be observed, indicating direct visualization of loop extrusion mediated by the SMC complex. This *in vitro* assay showed that DNA loop extrusion by a single yeast condensin complex is asymmetric because condensin anchors DNA using the safety-belt mechanism of Ycg1-Brn1 and reels it in from only one side (Kschonsak et al., 2017; Ganji et al., 2018). On the contrary, the human cohesin complex extrudes a DNA loop symmetrically, suggesting that the anchoring mechanism of the cohesin

complex differs from that of the condensin complex (Davidson et al., 2019; Kim et al., 2019). One possible explanation for the discrepancy in symmetry is that, in the case of cohesin, CTCF might function as an anchor when bound to one of the HAWK subunits (Li et al., 2020). The *in vitro* experiments showed that SMC complexes reel in DNA up to 1.5 kbp/s at low forces despite low ATPase activity (~2 ATP/s), suggesting a large step size (~50 nm) per hydrolyzed ATP molecule. How the interaction between SMC complexes and DNA induces the large step is still under debate, although numerous studies have been done to elucidate the DNA binding sites and conformational changes of SMC complexes.

INTERACTIONS BETWEEN DNA AND SMC COMPLEXES

To reel in DNA for loop extrusion, SMC complexes must have at least two DNA binding sites. Recent studies report that SMC subunits bind DNA mainly through two interfaces: the hinge domain with its adjacent coiled-coil, and the ATP-bound head dimer. In addition, HAWK subunits are also known to provide additional interaction sites with DNA through their multi-conformational HEAT-repeats.

Various studies report DNA-binding properties of the dimerized hinge at the inner part of the ring. Since the inner region of the hinge contains a positively-charged patch, it is plausible that DNA inside the ring can bind to the hinge domain (Griese et al., 2010; Soh et al., 2015; Diebold-Durand et al., 2017). Indeed, gel-shift assays and fluorescence anisotropy experiments showed DNA binding affinity to the hinge domain of mouse condensin (Griese et al., 2010), yeast condensin (Piazza et al., 2014), bovine cohesin (Chiu et al., 2004), and prokaryotic condensin (Hirano and Hirano, 2006). Previous AFM studies also showed DNA binding by the hinge domain of *E. coli* MukB (Kumar et al., 2017), *S. cerevisiae* condensin (Ryu et al., 2020a), and *S. pombe* condensin (Yoshimura et al., 2002), suggesting a conserved DNA-binding capability of the hinge domain. DNA binding affinity measurements of hinge domains with varied coiled-coil lengths imply that the juxtaposed hinge-proximal coiled-coil should be opened to provide space for DNA binding (Griese et al., 2010). The hinge and its adjacent coiled-coil seem to undergo a conformational change upon binding of DNA to the hinge and ATP to the head (Soh et al., 2015; Minnen et al., 2016; Ryu et al., 2020a). The ATPase activity of the head domain seems to control DNA binding to the distant hinge domain (Hirano and Hirano, 2006; Soh et al., 2015; Diebold-Durand et al., 2017). Recent HS-AFM studies showed that the hinge angle of the O-shaped condensin changes dynamically with ATP hydrolysis, indicating that the hinge domain is very flexible (Ryu et al., 2020a). This is consistent with previous crystal structures of hinge domains that showed two different states of the fully associated and half-dissociated conformations (Haering et al., 2002; Griese et al., 2010; Ku et al., 2010; Li et al., 2010; Niki, 2017).

SMC head monomers show low binding affinity to DNA; however, the binding affinity largely increases when they

are dimerized upon ATP binding. A crystal structure of a dimerized *E. coli* MukB head domain shows a positive patch possibly capable of binding a DNA strand (Woo et al., 2009). A dimerized Rad50 head domain shows stable DNA binding based on X-ray crystallography (Seifert et al., 2016). Recent Cryo-EM structures of the cohesin complex also showed that DNA binds the ATP-bound head dimer (Collier et al., 2020; Higashi et al., 2020; Shi et al., 2020).

In addition, HAWK subunits were observed to have DNA binding affinity. Based on the crystal structure of the Ycg1-Brn1 complex, Ycg1 contains a positive patch inside its groove, providing a strong DNA binding site for condensin. The Ycg1-binding DNA is embraced by a kleisin loop, and this loop is considered to be a safety belt during the sliding movement of condensin complex along DNA (Kschonsak et al., 2017). The partial positive patch in the inner groove responsible for DNA binding is also conserved in Scc3 of the cohesin complex (Li et al., 2018). Recent Cryo-EM structures also report that the parts of HEAT-repeats of the Scc2/4 cohesin loader complex also participate in DNA binding (Collier et al., 2020; Higashi et al., 2020; Shi et al., 2020). In contrast of HAWK subunits, the DNA-binding ability of KITE subunits has rarely been reported. ScpB was regarded as a DNA-binding protein because it contains a WH domain, which is one of the representative DNA-binding motifs, but its DNA-binding affinity seems very low and not necessary for the function of Smc-ScpAB complexes in prokaryotes (Jeon et al., 2020; Kim et al., 2006). A DNA-binding capability of MukE has also not been reported.

CONTROVERSIAL STRUCTURAL STUDIES OF SMC PROTEINS

Comparison of ATP hydrolysis rates in bulk and the measurements of DNA loop extrusion speeds suggest that condensin reels in DNA with large step sizes on the scale of the SMC complex (~50 nm) in a single ATP cycle. This indicates that an extensive conformational change is necessary to take a

large step along DNA. Recently, quite enormous structural studies on SMC proteins using various techniques such as X-ray crystallography, negative stain EM, rotary shadow EM, cryo-EM, and AFM have been performed and many different configurations, such as I, O, V, Y, B, P, and a folded state have been observed (Figure 3A) (Diebold-Durand et al., 2017; Eeftens and Dekker, 2017; Bürmann et al., 2019; Ryu et al., 2020a). However, it is still not clear which of the different conformations the SMC proteins use to extrude DNA loops (Table 2).

We can categorize the controversial studies of the conformational changes of SMC complexes according to the shape transitions that they report: (i) I to O – from a rigid, extended I-shape (the apo state) to an O-shape (the ATP-bound state) (Figure 3B), (ii) I to folded state (Figure 3C), and (iii) O to B (Figure 3D) – from the apo state to the ATP-bound state. The I-to-O shape transition is supported by the crystal structure of bacterial SMC proteins and negative stain EM (Diebold-Durand et al., 2017; Kamada et al., 2017). The structure of the full length of the SMC arms of Smc-ScpAB showed the closely juxtaposed SMC coiled-coils (I-shape) and ATP γ S-bound dimerized heads with open SMC arms (O-shape), suggesting the lever arm movement of the head domains induces the O-shape via ATP binding. This dimerization process seems to tilt the proximal coiled-coils of the head domains and generate a large tension on the coiled-coils to open the closed ring. On the other hand, the I-to-folded-state transition is supported by recent Cryo-EM studies of MukBEF, yeast cohesin, yeast condensin (Bürmann et al., 2019), and an early AFM study on *S. pombe* condensin (Yoshimura et al., 2002). However, it is not clear how ATP binding changes the structure of the SMC arms in these studies. On the contrary, recent liquid-phase HS-AFM imaging on yeast condensin complex shows a predominantly extended O-shape to collapsed B-shape (Ryu et al., 2020a). Interestingly, real-time imaging using the EQ mutant, which blocks the ATP hydrolysis of SMC proteins, showed the O-to-B shape transition, suggesting that the B-shape is induced by ATP binding to the head domains.

FIGURE 3 | Various configurations of SMC complexes. (A) Various shapes of SMC proteins according to alphabet letters. (B) Conformational change of SMC from the I-shape to the ATP-bound O-shape. The binding of two ATP molecules (red dots) promotes head-head engagement and opening of rigidly juxtaposed coiled-coil pairs. (C) Folding of the I-shape. Kinks in the middle of the coiled-coil (termed the elbow) seem to enable bending and folding of rigid SMC proteins. (D) Transition of SMC states from O- to B-shape upon ATP binding. Some experimental results imply dynamic flexibility or bending of SMC which enables its B-shaped state.

TABLE 2 | List of reported experiments implying the conformational changes of SMC complexes

Conformation	Species	Protein	Method	Note	Reference
I and O	<i>B. subtilis</i>	Smc coiled-coil	X-ray crystallography	Four segments of the SMC arms were used to reconstruct the full length of SMC arms. I shape : apo state O shape : ATP-bound state	(Diebold-Durand et al., 2017)
	<i>S. pombe</i>	condensin holocomplex	Dry AFM	apo state	(Yoshimura et al., 2002)
Folded state	<i>E. coli</i>	MukBEF holocomplex	X-ray crystallography, negative stained EM, and Cryo-EM	MukB coiled-coil elbow was used for crystallography apo state	(Bürmann et al., 2019)
	<i>S. cerevisiae</i>	cohesin holocomplex	Negative staining EM	apo state	
	<i>S. cerevisiae</i>	Smc2/4-Brn1-Ycs4	Cryo-EM	apo state and ATP-bound state	(Lee et al., 2020)
	<i>S. cerevisiae</i>	condensin holocomplex	Dry AFM and liquid-phase HS AFM	O shape: apo state B shape: ATP-bound state	(Ryu et al., 2020a)
O and B	<i>H. sapiens</i>	cohesin holocomplex and Nipbl	Cryo-EM	With DNA B shape: ATP-bound state	(Shi et al., 2020)
	<i>S. cerevisiae</i>	Smc1/3-Scc1-Scc2	Cryo-EM	with DNA B shape: ATP-bound state	(Collier et al., 2020)
	<i>S. pombe</i>	cohesin holocomplex with Mis4-Ssl3	Cryo-EM	with DNA B shape: ATP-bound state	(Higashi et al., 2020)

Structural studies which support the transition from I-shape (the apo state) to O-shape (the ATP state) or folded state (the apo state), and O-shape (the apo state) to B-shape (the ATP-bound state).

This result is also supported by the recent Cryo-EM studies of human cohesin and yeast cohesin complexes showing that the hinge domain is engaged with the heads and HAWK (Collier et al., 2020; Higashi et al., 2020; Takaki et al., 2021).

CONTROVERSIAL MECHANISMS FOR DNA LOOP EXTRUSION

The discrepancy in the observed conformations of SMC complexes may suggest different mechanisms. In a tethered inchworm model (Nichols and Corces, 2018), the hinge domain of the SMC complex anchors DNA, and two head domains – each head domain binds a HAWK or a KITE domain – are tethered by a kleisin subunit (Figure 4A). The SMC protein moves along DNA using head-head motions through the transition from the V-shape to the O-shape. In the V-shape the two heads are separated, and ATP binding induces the transition to the O-shape via dimerization of the two head domains. The dimerization process of the heads can generate a DNA loop-extrusion step by pulling the DNA region bound to another head domain, while ATP hydrolysis releases the head domain to find a new target DNA region to repeat the cycle. In addition, the kleisin subunit would be stretched and relaxed because the distance between two head domains is restricted by the length of the kleisin subunit. These conformational changes of the kleisin subunit may also be involved in pulling the DNA strand. However, this contradicts the previous observation that Ycg1-Brn1 of yeast condensin provides a DNA anchoring site, because this model assumes that the hinge domain works as a DNA anchor. In addition, both eukaryotic and prokaryotic SMC proteins have weak binding affinity to dsDNA in the hinge region,

which also contradicts the observation that the hinge can be an anchor (Chiu et al., 2004; Griesse et al., 2010; Piazza et al., 2014; Soh et al., 2015; Diebold-Durand et al., 2017). Furthermore, with this model, it is difficult to explain how the hinge replacement of Smc to a structurally distinct zinc-hook domain of Rad50 did not eliminate the functional activity of the SMC complex during fast cell division in *B. subtilis* (Bürmann et al., 2017). Moreover, this model seems to contradict the recent Cryo-EM and HS-AFM results that showed the collapsed state of the SMC complex where the hinge is in close proximity with heads and HAWK subunits (Bürmann et al., 2019; Higashi et al., 2020; Lee et al., 2020; Ryu et al., 2020a; Shi et al., 2020).

On the other hand, the structural studies on the Smc-ScpAB complex in prokaryotes suggested a ‘DNA pumping model’ that involves a transition between opening and closing of the SMC arms (Figure 4B) (Diebold-Durand et al., 2017; Marko et al., 2019). Structural and biochemical studies revealed that Smc-ScpAB takes an I-shape in the absence of ATP, and the binding of non-hydrolyzable ATP analogs induced the O-shape by the dimerization of heads and the opening of the two SMC arms from the head domains. Because full-length SMC arms are difficult to crystallize, crystal structures of the four different segments of the SMC arms were obtained and co-aligned to reconstruct the structure of full-length SMC arms. In the DNA pumping model (Diebold-Durand et al., 2017; Marko et al., 2019), a zipping of the two SMC arms pushes pseudo-topologically entrapped DNA from the hinge domain to the head domains, while an ATP-induced dimerization of the head domains changes the SMC protein conformation from an I-shape to an O-shape to target new binding of DNA for subsequent ATP hydrolysis cycles.

FIGURE 4 | Possible models of loop extrusion mechanism driven by active SMC complexes. (A) Tethered inchworm model. (B) DNA pumping model. (C) Extended scrunching model. The coloration of the SMC complex follows that of the condensin complex in Figure 1A. Black anchor symbols denote the anchored positions of the hinge or head on DNA. Hinge-proximal (magenta) and head-proximal sites (cyan) of DNA are marked to indicate the positional movement of DNA. Details are described in the main text.

Moreover, this is strongly supported by electron paramagnetic resonance (EPR-DEER) which measures the heterogeneous distance between two different regions (1.5 ~ 10 nm) of a protein by measuring the coupling of electron spins (Nunez et al., 2021). However, this seems to contradict recent eukaryotic SMC studies with Cryo-EM and AFM imaging that showed folded states or B-shapes where the hinge region is close to the heads and HAWK subunits. Thus, the DNA pumping model seems to work for SMC-ScpAB, but not for eukaryotic condensin or cohesin, and future studies need to show whether the different results were due to the differences of methods or intrinsic properties of SMC-ScpAB. Because SMC-ScpAB shows a different Hi-C pattern from that of eukaryotic cohesin or condensin, the intrinsic behavior of SMC complexes may vary (Rao et al., 2017; Wang et al., 2017).

In a 'scrunching model', a HAWK subunit anchors DNA, and the hinge domain cyclically binds another region of the DNA and transfers it to the heads and non-SMC subunits using an extension and retraction of the flexible SMC arms connected to

the hinge domain (Figure 4C). When the DNA binds to the head and non-SMC subunits, the hinge releases it to subsequently grab a new region of DNA to reel in (Hassler et al., 2019; Ryu et al., 2020a; Terakawa et al., 2017). This model is supported by recent Cryo-EM and AFM experiments that showed the folded state or B-shape when the hinge domain contacts the heads and HAWKs. Moreover, recent *in vivo* studies of cohesin support that the hinge can be in close proximity with the head domain. Furthermore, the force-dependent step sizes observed by magnetic tweezers fits a simulation based on the scrunching model (Ryu et al., 2020b; Takaki et al., 2021). A recent study suggested a Brownian ratchet model in which the conformational changes between the O- and B-shapes are induced by thermal fluctuations due to the flexible SMC arms (Hwang and Karplus, 2019). Further reports indicate that transitions between the two states are determined by the interaction between the hinge domain and the globular domain via ATP binding (Higashi et al., 2020; Ryu et al., 2020a; Shi et al., 2020). ATP binding induces the dimerization of the head domains and engagement of the hinge domain with the globular head and non-SMC subunits to form a B-shape while ATP hydrolysis or release induces the dissociation of the hinge from the head and non-SMC subunits to form an O-shape. For eukaryotic cohesin and condensin, which have HAWK subunits, the scrunching model seems to be the most relevant.

DISCUSSION

In this review, we introduced the current understanding of the molecular mechanism of DNA loop extrusion, and we explored the structure-function relationships of SMC proteins and DNA loop extrusion. Different mechanisms for DNA loop extrusion were discussed based on controversial structural results. Below, we discuss the limitations of current studies, which might be able to explain the controversial observations.

POSSIBLE REASONS FOR THE CONTROVERSIAL OBSERVATIONS

The reason for the differences between previous structural studies of SMC proteins may result from different organisms, the protein purification process, and sample preparation methods. First, SMC proteins from different organisms show different shapes. For example, *S. cerevisiae* cohesin, human cohesin, and yeast condensin were found to adopt a folded conformation or B-shape (Bürmann et al., 2019; Higashi et al., 2020; Ryu et al., 2020a; Shi et al., 2020), but *B. subtilis* SMC proteins showed an I-shape in the apo state and an open shape in the ATP-bound state (Soh et al., 2015; Diebold-Durand et al., 2017; Bürmann et al., 2019). Second, the purification process may induce certain configurations because SMC complexes are macrocomplexes; purifying intact functional complexes is quite challenging. Before performing structural studies, purified proteins should be assayed for their ability to extrude DNA loops to ensure functional complexes are used. To date, purified yeast condensin, human

condensin I/II, and human cohesin have shown clear DNA loop extrusion activity (Ganji et al., 2018; Davidson et al., 2019; Kim et al., 2019; Kong et al., 2020). Moreover, during purification, different post-translational modifications may also alter the conformational state. For example, cohesin contains numerous post-translational modification sites, such as for acetylation or phosphorylation, and some modifications were suggested to be involved in the interaction between the two SMC arms, inducing the juxtaposed structure (Kulemzina et al., 2016). Still, it is largely unclear how the post-translational modifications are exactly involved in the structural transitions of SMC proteins. Third, different sample preparation methods can induce or bias the observation of certain configurations. During sample preparation for structural studies, experimental factors such as surface adhesion, metal staining, crystallization, and air exposure during sample preparation can induce structural changes in proteins.

LIMITATIONS OF THE METHODS FOR SMC PROTEIN STUDIES

Recently, the development of state-of-the-art techniques such as high-resolution Cryo-EM, and liquid-phase HS-AFM have led to improved structural studies of SMC proteins and brought greater insight into their mechanistic functions. However, there is still no perfect technique to clarify the structure-function relationships of SMC proteins. Crystallography has been largely used to obtain atomic-resolution structures of subunits or parts of the SMC arms. There remains no crystal structure of the full-length SMC arms, although four partial crystal structures of truncated arms of bacterial SMC-ScpAB were determined to display the structure of the full length of the SMC arms. The full-length SMC arms might be difficult to crystallize due to their flexibility, as observed by liquid-phase HS-AFM (Eeftens et al., 2016; Ryu et al., 2020a). In the case of Cryo-EM, although the recent revolution in resolution has enabled atomic-resolution structures of SMC proteins, single-particle analysis can bias the structure of the flexible SMC arms because of an ever-luring effect (Lyumkis, 2019). Moreover, as a macromolecule, the SMC complex can be easily exposed to an air-water interface due to its large structure (~50 nm) because protein samples are trapped in a thin ice layer during sample preparation (thickness ~50 nm). This air exposure can cause a preferred orientation (Noble et al., 2018) or denaturation (D'Imprima et al., 2019) of proteins. Moreover, with crystallography and Cryo-EM, it is not possible to obtain the dynamics of structural transitions, but liquid-phase HS-AFM imaging is a unique tool that can show the dynamics of the structural changes of a protein in aqueous solution without any staining or labeling. One limitation of this technique is that it cannot avoid non-specific binding of proteins to the surface. Furthermore, many structural studies have complemented crosslinking experiments to support their results, but the possibility of crosslinking artifacts cannot be excluded. Therefore, more studies on the architecture of SMC complexes are necessary to form a clear picture of their mechanisms.

To understand the reasons for these contradictory results, systematic approaches are needed to compare the experiments from various SMC proteins, methods, and conditions. Firstly, we need to understand whether every SMC complex can extrude a DNA loop and characterize biophysical properties the DNA loop extrusion by each SMC complex. For example, it is clear that yeast condensin forms a single SMC complex to extrude a single loop, but with human cohesin it is not clear whether a single or double complex is involved (Ganji et al., 2018; Davidson et al., 2019; Kim et al., 2019). On the other hand, the MukBEF complexes are dimerized via the N-terminal helical bundle of MukF, suggesting two MukBEF complexes can act as a single functional unit for the DNA loop extrusion process (Fennell-Fezzie et al., 2005; Woo et al., 2009; Badrinarayanan et al., 2012). However, DNA loop extrusion mediated by MukBEF has not yet been observed. In addition, whether the different SMC proteins extrude DNA loops symmetrically or asymmetrically should be also tested, as yeast condensin clearly showed asymmetric DNA loop extrusion while the process was symmetric for human cohesin (Ganji et al., 2018; Davidson et al., 2019). Therefore, there is a possibility that the conformational changes could vary with each SMC protein. Secondly, each SMC complex needs to be tested using both liquid-phase HS-AFM and Cryo-EM in the presence of all subunits and DNA via controlled conditions of ATP addition. These two techniques are complementary because HS-AFM provides fast and dynamic information while Cryo-EM can provide static atomic-resolution structural information. Thirdly, simultaneous observation of the conformation of SMC complexes during DNA loop extrusion would provide the most direct evidence to explain the mechanism. We expect that future studies will soon clearly show the molecular mechanism of the DNA loop extrusion process, and answer specific questions such as the causes of one-sided or two-sided DNA loop extrusion and the reason for its unidirectional motion.

CONCLUSION AND PERSPECTIVES

Summing up, we have discussed three different mechanistic models of DNA loop extrusion by SMC proteins. Although DNA loop extrusion seems to be a universal function of the entire SMC protein family, the mechanism has remained mysterious. Further studies of the conformational changes of SMC proteins will likely unravel their modes of action. Although various conformations of SMC complexes were observed in precise experiments, the results can be affected by buffer composition, species of the SMC protein, or detection methods. Therefore, systematic studies to compare many different SMC proteins using different methods will be necessary to clearly describe the mechanism of DNA loop extrusion by SMC proteins.

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