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REVIEW

Cohesin Complex: Structure and Principles of Interaction with DNA

Arkadiy K. Golov[1,2,a]* and Alexey A. Gavrilov[1,b]*

[1]*Institute of Gene Biology, Russian Academy of Sciences, 119334 Moscow, Russia*
[2]*Technion – Israel Institute of Technology, 3525433 Haifa, Israel*
[a]*e-mail: golovstein@gmail.com* [b]*e-mail: aleksey.a.gavrilov@gmail.com*

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Abstract—Accurate duplication and separation of long linear genomic DNA molecules is associated with a number of purely mechanical problems. SMC complexes are key components of the cellular machinery that ensures decatenation of sister chromosomes and compaction of genomic DNA during division. Cohesin, one of the essential eukaryotic SMC complexes, has a typical ring structure with intersubunit pore through which DNA molecules can be threaded. Capacity of cohesin for such topological entrapment of DNA is crucial for the phenomenon of post-replicative association of sister chromatids better known as cohesion. Recently, it became apparent that cohesin and other SMC complexes are, in fact, motor proteins with a very peculiar movement pattern leading to formation of DNA loops. This specific process has been called loop extrusion. Extrusion underlies multiple functions of cohesin beyond cohesion, but molecular mechanism of the process remains a mystery. In this review, we summarized the data on molecular architecture of cohesin, effect of ATP hydrolysis cycle on this architecture, and known modes of cohesin–DNA interactions. Many of the seemingly disparate facts presented here will probably be incorporated in a unified mechanistic model of loop extrusion in the not-so-distant future.

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INTRODUCTION

Cohesin is a paneukaryotic protein complex belonging to the group of structural maintenance of chromosomes proteins (SMC) participating in the processes of individualization of sister chromatids, chromosome decatenation, mitotic and meiotic cohesion, establishment and maintenance of specific DNA folding in cells, repair of double-strand breaks, and stabilization of stalled replication forks [1, 2]. Similar to other SMC complexes, cohesin, on the one hand, comprises a multisubunit ABC-ATPase, and, on the other hand, – a non-sequence-specific DNA-binding complex. Binding and hydrolysis of ATP are coupled with conformational changes in cohesin, as well as with changes in affinity of different components of the complex to DNA. The repeating binding/ATP hydrolysis cycles result in the directional movements of the complex along the DNA thread; hence, cohesin is a motor protein – DNA translocase.

Cohesin performs two different functions in the eukaryotic cells: mediating sister chromatid cohesion and so-called chromatin loop extrusion (Fig. 1a). The process of extrusion starts with the capture of a small DNA loop followed by its processive enlargement by pulling DNA flanking regions inside [3-6]. An active SMC complex is located at the basis of growing loops, and their size could reach up to hundreds of thousands of base pairs (Fig. 1a (*2*)). Extrusion is coupled with ATP hydrolysis and represents a universal biochemical activity of all SMC complexes [1]. Most likely, extrusion appeared at the dawn of cellular evolution as a way of post-replicative separation of the massive molecules of genomic DNA (Fig. 1b); in this process the

Abbreviations: HAWK, HEAT protein associated with Kleisin; SMC, structural maintenance of chromosomes proteins.

* To whom correspondence should be addressed.

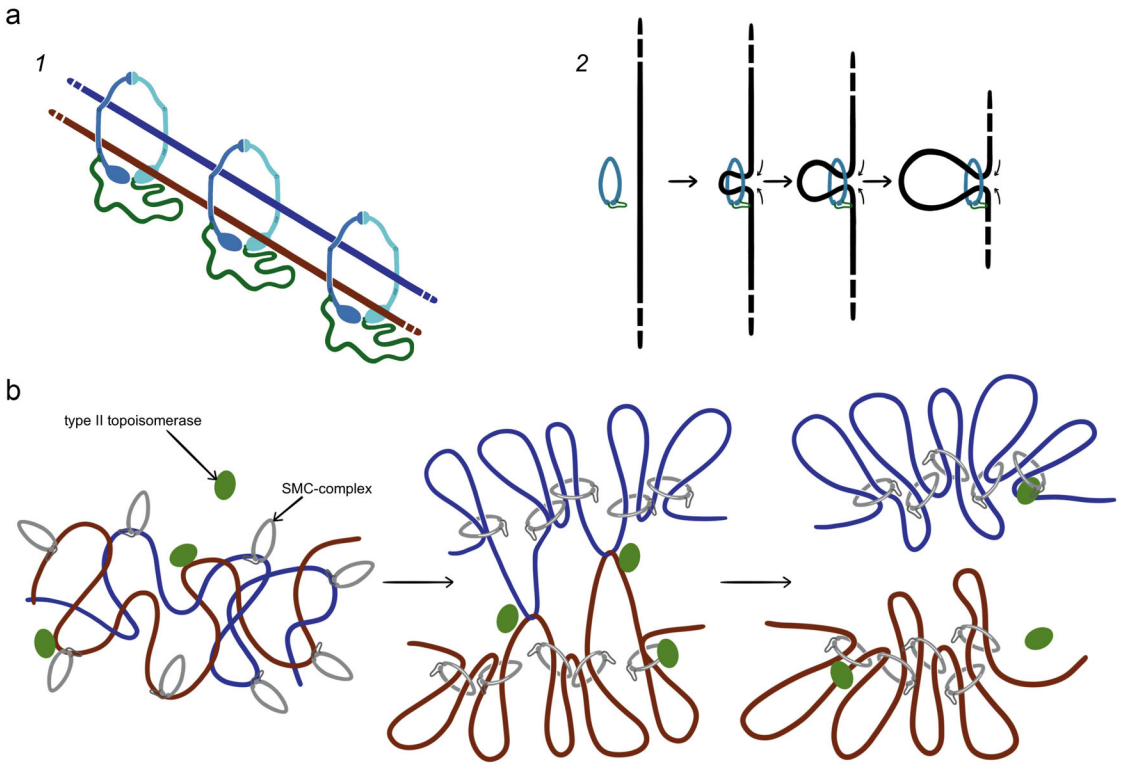

Fig. 1. Sister chromatid cohesion and DNA loop extrusion is mediated by SMC complexes. a) Two basic activities of cohesin: cohesion (*1*) and extrusion (*2*). b) Extrusion mediated by SMC complexes, and activity of type II DNA topoisomerases ensure post-replicative individualization of sister genomes in all cells, prokaryotic and eukaryotic.

SMC complexes cooperate with the type II topoisomerases. Cohesion of sister chromatids mediated by cohesin rings (Fig. 1a (*1*)), most likely, does not depend on the extrusion activity of the complex [7, 8], although ATP hydrolysis is also required for the loading of cohesin rings onto DNA [7, 9].

Information on the protein structure is important for understanding their activity and even more important for understanding activity of motor proteins. In this review the data on cohesin structure as well as current understanding of its interactions with DNA, conformational changes, and how interactions with DNA and binding/ hydrolysis of ATP control these conformational changes are presented. Detailed data on the biological role of the cohesin-dependent extrusion, as well as molecular mechanism of this process are presented in the second part of this review published in the same issue of this journal [10].

ANNULAR CORE COHESIN TRIMER COMPLEX

All eukaryotic genomes encode at least four SMC complexes from three different classes: two variants of cohesin (mitotic and meiotic), condensin I, and SMC5/6 complex [3, 4] (table). Trimer composed of two SMC subunits and one kleisin subunit forms an invariant basis of SMC complexes [2, 3, 11]. SMC proteins [12] and kleisins [13] represent two unrelated protein families. Different classes of SMC complexes are separated by hundreds of millions of years of evolution and lost their homology in the larger part of their amino acid sequences, while similarity within the short functionally important motifs was retained, as well as their common basic structure [14]. Nevertheless, a significant degree of conservation has been observed within the six main subfamilies of eukaryotic SMC proteins (SMC1-6) and three subfamilies of eukaryotic kleisins (kleisins α-γ). The especially high degree of conservation is observed within the SMC1- and SMC3-subfamilies. For example, the human and mouse SMC3 are practically identical.

In the case of cohesin (here and further we consider the better described mitotic variant of the complex) the pair of SMC proteins is represented by the SMC1 (Smc1)–SMC3 (Smc3)[1] heterodimer and the kleisin subunit – by the RAD21 protein (Scc1). SMC proteins are rod-like molecules with length of about 50 nm with two globular domains at the ends connected via relatively labile fibrillar structure (Fig. 2a) [16-18]. Within the rod-like structure of the SMC protein 1200-1300 aa long polypeptide chain is folded back on itself so that

[1] Names of human proteins are used in the main text and names of homologous *Saccharomyces cerevisiae* proteins are given in parenthesis (at first mention).

Subunit composition of paneukaryotic SMC complexes

Complex → / ↓ Subunit	Meiotic cohesin	Mitotic cohesin	Condensin I	SMC5/6 complex
ν-SMC	SMC3 (Smc3)	SMC3 (Smc3)	SMC2 (Smc2)	SMC6 (Smc6)
κ-SMC	SMC1 (Smc1)	SMC1α (Smc1)	SMC4 (Smc4)	SMC5 (Smc5)
Kleisin	REC8 (Rec8)	RAD21 (Scc1)	CAP-H (Brn1)	NSE4A/B (Nse4)
$HAWK_A$	PDS5A/B (Pds5)	NIPBL (Scc2) and PDS5A/B (Pds5)	CAP-D2 (Ycs4)	–
$HAWK_B$	STAG3 (Scc3)	STAG1/2 (Scc3)	CAP-G (Ycg1)	–
$KITE_A$	–	–	–	NSE5 (Nse5)
$KITE_B$	–	–	–	NSE6 (Nse6)

Note. HAWK-subunits are present only in cohesins and condensins, while prokaryotic SMC complexes and SMC5/6 complex have auxiliary subunits in their composition belonging to the group of KITE-proteins [15]. Names of the human subunits are presented as well as names of homologous subunits of *Saccharomyces cerevisiae* (in parenthesis).

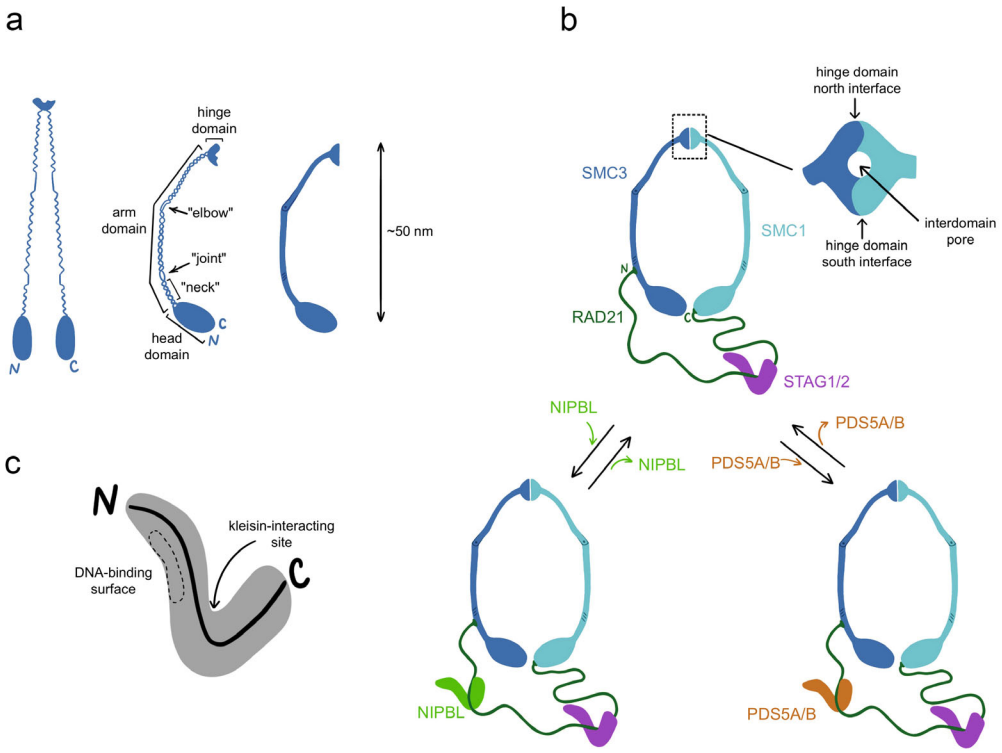

Fig. 2. Subunit structure of cohesin complex. a) Folding and main structural features of SMC proteins. b) General structure of three-part cohesin ring and interaction of HAWK-subunits with it. c) General structure of hook-shaped HAWK-subunits in SMC complexes.

one of the terminal globular domains is formed as a result of interactions of N- and C-ends, and another is formed by the uninterrupted sequence located approximately in the middle of the linear sequence [17, 19]. The first of the globular domains was termed head domain, and the second – hinge domain; polypeptide chain of the SMC protein makes a 180° turn within the latter. Fibrillar structure connecting two globular domains comprises an intramolecular coiled-coil structure termed arm domain.

Stable dimerization of the SMC proteins is achieved via homotypic interactions between the hinge domains [17, 18]. Coiled-coil arm domains are directed to the same direction away from the interacting hinge domains; hence, the SMC1–SMC3 dimer in the absence of ATP comprises a V-shaped structure with dimerized

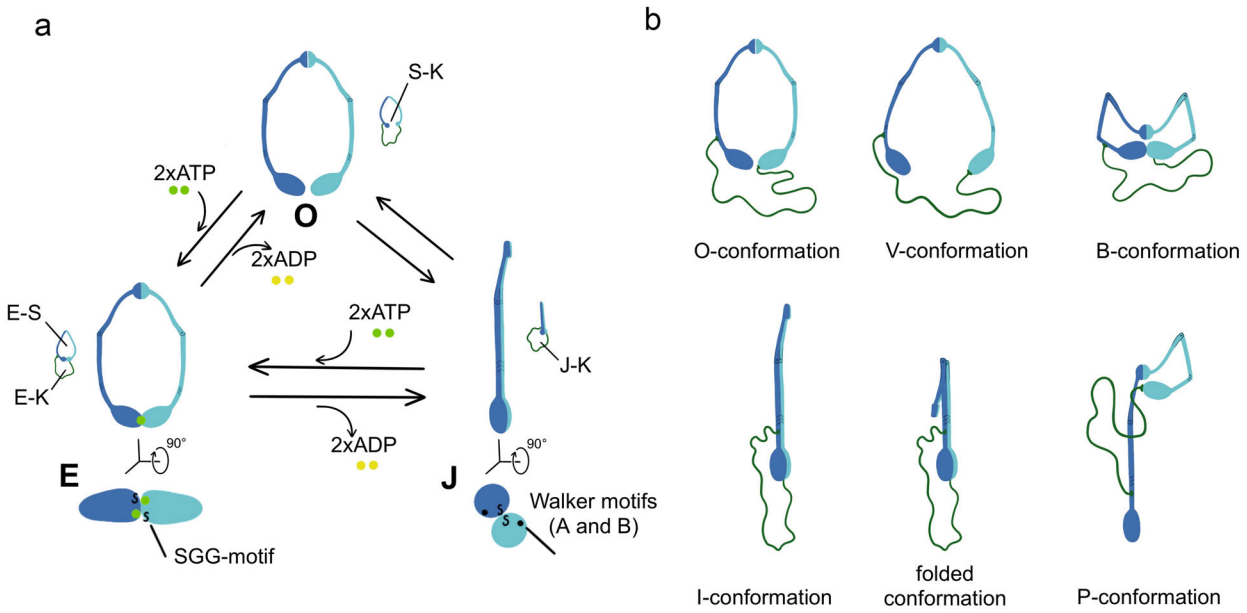

Fig. 3. Diversity of cohesin conformational states. a) O-, E-, and J-configurations of cohesin head domains and transitions between them, RAD21-subunit is not shown for better clarity. S-K-ring and subcompartments (E-S, E-K, and J-K) formed as a result of head domains engagement are shown in pictograms. b) Major conformational states of cohesin detected using microscopy.

hinge domains at one pole and pair of separated head domains at another pole [17, 20].

The RAD21-kleisin subunit has length of approximately 500-700 aa with two structured domains at N- and C-ends, and extended mostly disordered region between them [13]. The N-terminal domain of kleisin interacts with the region connecting the head and arm domains of SMC3 subunit, while the C-terminal domain – with the head domain of SMC1 (Fig. 2b) [21].

Molecules of the three core subunits of cohesin form a closed annular structure through stable interactions between the termini [17, 22]. Elongated shape of each of the core subunits results in formation of cohesin conformations in which it assumes an expanded topologically closed intersubunit compartment called S-K-ring (Fig. 3a). Opening of the S-K-ring, also called S-K-compartment, allows passage of globular particles with diameter of up to around 10 nm through it [23, 24]. It was shown that one or two DNA threads can be entrapped within the S-K-compartment, thus the ring-shaped complex can be put on the DNA as a bead on a string. In the G2-phase of cell cycle such topological interactions of cohesin molecules with the pairs of sister chromatids mediate cohesion [7].

Hinge domains of the SMC proteins comprise compact structures consisting of two (N- and C-terminal) subdomains of α/β-class; each of the subdomains contains a small β-sheet [17, 18]. The N-terminal subdomain interacts with the C-terminal subdomain of its dimerization partner with merger of their β-sheets. Hence, two separate surfaces are involved in the interaction between two hinge domains of the dimer. Due to the fact that the two hinge subdomains are separated by a small groove, the dimerized hinge domains look like a toroidal structure with the 2-fold rotational pseudosymmetry (Fig. 2b). Small channel inside the hinge domain dimer contains functionally important positively charged amino acids, which are assumed to be able to participate in electrostatic interactions of the complex with DNA. Asymmetry in the heterodimer of cohesin SMC subunits allows distinguishing two interaction surfaces of the hinge domains. One of them, which is at a larger distance from the head domains is commonly known as a northern one, and another – as a southern (Fig. 2b).

The arm domain of each SMC protein comprises a pair of antiparallel coiled-coils each consisting of 300-400 aa. Coiled-coils of the arm domains have a number of defects in which one or both antiparallel chains lose their regular α-helical structure [19]. Two such breaks in the coiled-coil of the arm domain have high degree of evolutionary conservation and, most likely, play an important role in the activity of SMC complexes. The first one, located around the middle of the arm domain a bit closer to the hinge domain has been called ‘elbow’ (Fig. 2a). This region ensures mechanical flexibility of the arm domains: simultaneous bending of the two ‘elbow’ regions could bring the hinge domains into the close proximity of the head domains (Fig. 3b). The second conserved break of the coiled-coil is located close to the head domain at the distance of approximately 50 aa; it is commonly called ‘joint’ (Fig. 2a). ‘Joints’ in the SMC subunits are important hubs of interactions with the auxiliary subunits

of the complex [25], they also participate in the movements of the head domains relative each other [26].

Head domains of the SMC proteins comprise ABC-type ATPases [16, 27]. Similarly to other ABC-proteins, cohesin is capable of hydrolyzing ATP only during physical interaction between ATPase domains of two SMC subunits (Fig. 3a) [28, 29]. Head domain of one of the subunit of the dimer binds an ATP molecule, and the head domain of the other is required for hydrolysis of the bound molecule. Each cycle of the cohesin-dependent ATP hydrolysis involves binding of ATP by each of the head domains, and as a result two ATP molecules are hydrolysed. Engagement of head domains is terminated after nucleoside triphosphate hydrolysis and is established again after binding of a new pair of ATP molecules. Hence, in contrast to interaction of the hinge domains with each other, dimerization of the head domains depends on the presence of the substrate and has dynamic nature (Fig. 3a).

From the structural point of view the ABC-ATPase of the head domain is a globule with the core consisting of an open β-cylinder with β-strands belonging to both N- and C-terminal parts of the SMC protein [9, 16, 28]. At one of the poles of the β-cylinder there are two conserved catalytically significant sequences: Walker A motif (also known as a P-loop or phosphate-binding loop) and Walker B motif. Both these motifs are typical not only for all representatives of the ABC-ATPase superfamily, but also for the wider monophyletic protein group called P-loop NTPases [27]. The Walker A motif, located in the N-terminal part, is responsible for binding β- and γ-phosphate groups of ATP. The Walker B motif is located in the C-terminal part and is responsible for coordination of Mg^{2+} in the active center. Unlike in the case of other P-loop NTPases, effective hydrolysis of the phosphate bond by the ABC-ATPases requires spatial interaction between nucleotide triphosphate bound by the Walker A and B motifs and the serine residue within the so-called SGG-motif. The SGG-motif is also located in the head domain, but at the relatively large distance from the ATP-binding β-cylinder, hence, such interaction is impossible within the head of SMC monomer, it could be realized only between the ATP-binding site of one head domain and SGG-motif of another one during their dimerization (Fig. 3a). That is why ATPase activity of cohesin can be realized only during physical engagement of the head domains of two SMC subunits of the complex.

The structured N- and C-terminal domains of RAD21 interact with two head domains of two SMC proteins asymmetrically (Fig. 2b) [30]. The N-terminal domain of RAD21 binds to the site on the arm coiled-coil coming out of the head domain of SMC3 Part of the arm domain that binds N-terminal domain of kleisin is located between the head domain and 'joint' and is called 'neck'. Fragment of the neck site of the SMC3 coiled-coil domain interacts with two N-terminal α-helices of RAD21 with formation of four-α-helix bundle [30, 31]. The C-terminal globular domain of RAD21 has a winged-helix fold and interacts with the top of the SMC1 head domain [32].

INTERACTIONS OF THE REGULATORY HAWK-PROTEINS WITH THE KLEISIN

The core cohesin trimer interacts with three auxiliary subunits: STAG1/2[2] (Scc3), PDS5A/B (Pds5), and NIPBL (Scc2). The auxiliary subunits participate in the complex binding to DNA and regulation of its activity [1-3]. The disordered central part of RAD21 represents the major binding site for the auxiliary subunits on the core trimer [33-35].

All three auxiliary subunits of cohesin belong to the family of HAWK-proteins (HEAT proteins associated with kleisin) [15]. These α-helical hook-shaped proteins (Fig. 2c) [33, 36] are composed of around 20 tandem HEAT-repeats [37]. Each repeat consists of 30-40 aa organized as a pair of interacting antiparallel amphipathic α-helices. α-Helices of each HEAT-repeat are arranged perpendicularly to the axis of the HAWK-subunit hook. Inner concave surface of the HAWK hook interacts with kleisin-subunit.

STAG1/2 binds to kleisin stably and, hence, is a constitutive component of the complex [38]. NIPBL and PDS5A/B interact with kleisin transiently; moreover, these two subunits compete with each other for the same binding site [35]. Hence, at each time point the complex could contain one (STAG1/2) or two (STAG1/2 + NIPBL or STAG1/2 + PDS5A/B) HAWK-subunits (Fig. 2b). Binding of NIPBL or PDS5A/B dramatically changes activity of the cohesin complex [35]. NIPBL increases substantially ATPase activity of the complex [39, 40]. This subunit is essential for the cohesin-dependent loop extrusion [40, 41]. PDS5A/B-subunit supresses ATPase activity, and in its presence the WAPL protein could be recruited to the cohesin ring, which stimulates dissociation of the N-terminal part of RAD21 from the SMC3 subunits [42, 43]. Acetylation of the SMC3 subunit, as well as interaction of the complex with other chromatin components such as insulator protein CTCF, regulates binding of NIPBL and PDS5A/B to the cohesin ring [25, 44-46].

[2] Vertebrate genomes generally encode a pair of somatically expressed paralogs for both Scc3 and Pds5 HAWK-proteins: STAG1/STAG2 and PDS5A/PDS5B. These paralogs in the majority of cases are structurally and functionally equivalent, hence, here and further in the text the designations STAG1/2 and PDS5A/B are used.

CONFORMATIONAL POLYMORPHISM OF COHESIN COMPLEX

Activity of all proteins one way or another is associated with ligand-dependent conformational changes. SMC complexes demonstrate ability of radical conformational rearrangements, which mediate their motor activity.

Various microscopic techniques, X-ray crystallography, as well as indirect approaches for elucidation of structure such as Förster Resonance Energy Transfer (FRET), chemical cross-linking, and analysis of stability of DNA–protein complexes in various physicochemical conditions facilitated discovery of a wide spectrum of cohesin conformational states (Fig. 3b) [3, 4].

Entire variety of the known cohesin conformations could be described to a large degree by three parameters: (1) engagement of head domains of the two SMC subunits, (2) distance between the hinge and head domains controlled by the degree of the elbow bending, (3) distance between the arm coiled-coils of two SMC subunit. For example, in both I-conformation and folded conformation head domains are engaged, and arm's coiled coils interact along the entire length; difference is in the fact that in the first conformation arm domains are in the straightened state, while in the second conformation – in the fully bended state. O- and B-conformations differ from the previous pair by the fact that interactions between the arm domains are absent (Fig. 3b).

Part of conformational rearrangements of cohesin is strictly coupled with ATP binding or hydrolysis, while others occur spontaneously. It is likely though that the direction of some spontaneous cohesin rearrangements could be controlled by the irreversible processes of ATP binding and hydrolysis.

Dimerization of head domains and ATP hydrolysis. ATP binding induces tight interaction between the two cohesin head domains, the so-called E-state (engaged state) [9, 28, 47]. This short-lived structure breaks down after ATP hydrolysis (Fig. 3a). ATP hydrolysis is followed by the release of the products of hydrolysis and change in positioning of the head domain relative to each. It was originally assumed that ATP hydrolysis inevitably leads to a complete separation of head domains in a structure known as an O-state (open state). However, it has been later discovered that even in the absence of ATP the structure called J-state (juxtaposed state) can be formed in which two head domains interact in such a way that the SGG-motifs of the pair of SMC proteins are brought close to each other [26, 48] (Fig. 3a). Considering that this interaction is realized through the surfaces located at a relatively large distance in the E-state, transitions from E-state to J-state and back involve significant rotation of the head domains relative to each other.

Additional conformational changes coupled with binding and hydrolysis of ATP. ATP binding and engagement of head domains in E-state is coupled with several important conformational rearrangements: local separation of arm domains, temporal dissociation of kleisin from SMC3, establishing of an additional bridge between the two head domains formed by NIPBL [25, 44, 49].

Coiled-coils of the arm domains that are adjacent to the head domains are in fixed orientation relative to each other in the J- and E-states. Despite the relative flexibility of the arm domains, comparatively long regions of the coiled coils adjacent to the head domains are constitutively separated in the E-state [25, 44, 49]. At the same time, position of the head domains in the J-state ensures close proximity of the coiled-coil regions of SMC1 and SMC3 [26, 48]. The available structural data indicate that the arm domains in the J-state are in contact with each other over their entire length (Fig. 3a). Hence, while in the absence of ATP (in the so-called apo-form) the arm domains could be either separated, or interact with each other to a certain degree, binding of ATP results in separation of the coiled coils at least in the regions directly adjacent to the head domains.

During interaction of the head domains with each other in the E- and J-state the united S-K-compartment is divided into two compartments (S and K) confined by, respectively, SMC dimer and kleisin subunit (Fig. 3a). Depending on the nature of the interaction between the head domains, the following subcompartments can be distinguished: E-K, E-S, J-K, and J-S; due to the tight contact between the coiled coils of the arm domain, the latter does not have any opening.

Another change of cohesin conformation associated with ATP binding in the active center is short-term opening of the so-called N-kleisin gates of the complex. At the moment of formation of the E-state, the N-terminal domain of RAD21 dissociates from its binding site at the SMC3 subunit [47, 49], which results in temporal disruption of integrity of the E-K compartment.

ATP binding also causes changes in the pattern of NIPBL interaction with other subunits. In addition to its main binding site in the central part of kleisin, NIPBL has additional ATP-regulated sites of interaction with the SMC subunits. In the absence of ATP NIPBL interacts with the dimer of hinge domains; ATP binding results in dissociation of NIPBL from the hinge domains and attachment to the head domain of SMC3 [50]. This jump of NIPBL, likely, stimulates formation of the E-state, in which NIPBL forms additional contacts with the head domain of SMC1 [25, 44, 49]. A pair of conserved lysine residues in SMC3, K105/K106 (K112/K113 – in Smc3 *S. cerevisiae*), plays an important role in the interaction of NIPBL with the SMC3 head domain, acetylation of these residues by acetyl trans-

ferase ESCO2 (Eco1) is a key factor of stabilization of the cohesive binding. Following ATP hydrolysis NIPBL loses its interaction with the head domains and restores interaction with the hinge domain site [50].

Spontaneous changes of cohesin conformation. Microscopic observations, as well as the FRET data indicate that the arm domains of cohesin and other SMC complexes are rather labile structures mostly due to the possibility of bending at elbows [17, 19, 49-51]. Amplitude of this bending reaches up to 180° which makes possible direct physical interaction of the head and hinge domains in the completely bent state (Fig. 3b).

Discovery of the J-state and recognition of the fact that transitions between the E- and J-states should be accompanied by the significant changes in the mutual position of the arm domains led to the suggestion of a hypothesis according to which bending and straightening of the elbow domains should be strictly controlled by the ATP hydrolysis cycle [19]. However, experimental data obtained later indicate that bending and straightening of the elbows occur spontaneously [50].

The most important consequence of bending and straightening of the elbows is the engagement of the hinge domains with the head domains in the completely bent state. The possibility of temporal interaction between the head and hinge domains is a crucial premise of many mechanistic models of SMC-dependent loop extrusion [19, 50, 52, 53]. Despite the fact that bending of the arm domains occurs spontaneously, there are indication that there could be a ratchet mechanism coupling bending and ATP hydrolysis. In particular, it has been suggested that the complete bending of the elbow domain could be required for the formation of the E-state [25, 44, 49, 50].

Another known spontaneous change of the cohesin architecture is reversible interaction of the coiled-coils adjacent to the hinge domains. Such interaction spreads from the hinge domain towards the elbows and, likely, is associated with winding of the two coiled-coil domains of the arm around each other [50]. It is hypothesized that this winding can be a necessary prerequisite for the bending of the elbows.

INTERACTIONS BETWEEN DNA AND COHESIN

Binding to DNA is a key aspect of activity of SMC complexes. There are two characteristic features of interactions between SMC complexes and DNA: (1) association between changes in affinity of different sites to DNA and changes in complex conformation and (2) ability of the complexes to entrap DNA topologically.

Unlike the classic electrostatic interactions typical for the majority of DNA-binding proteins, topological binding represents a qualitatively different mode of non-covalent binding for which there is no need in direct physical contact between the DNA and the protein [1, 44]. Topologically entrapped DNA is threaded through the closed S-K-ring of the complex like a string threaded through a bead (Fig. 4a). In this scenario complex can be stably associated with DNA even in the absence of electrostatic interactions.

Cohesin can also interact with DNA electrostatically: several positively charged sites on the complex surface mediate such interactions (Fig. 5) [3, 50]. An important feature of the electrostatic interactions of cohesin with DNA is cooperativity: small individual sites dispersed over the different subunits are not generally capable to form stable contacts with DNA on their own, sufficient affinity is achieved only by the composite surfaces formed by several DNA-binding sites from different subunits coming together. Such composite surfaces are assembled in some cohesin conformations and disassembled in others. The dynamically assembled DNA-binding sites are crucial elements of the machinery that couples the processes of ATP hydrolysis, structural changes of the complex, and its movement along the DNA thread during loop extrusion.

Topological entrapment of DNA within cohesin. Cohesin, same as almost all other SMC complexes, is capable of binding DNA topologically[3]. Three observations underlie the ring hypothesis according to which cohesin could entrap DNA topologically inside the three-part S-K-ring [22, 54]: (1) complex structure suggesting existence of the extended topologically closed compartment [17, 55]; (2) capability of the complex to establish extremely stable interactions with chromatin during the G2-phase [56, 57], and (3) immediate destabilization of these interactions upon proteolysis of the kleisin subunit in anaphase [21, 58]. After more than a decade of accumulation of indirect clues, this hypothesis was finally confirmed with the experiments including covalent cross-linking of the cohesin rings *in vivo* [7, 30, 48].

A number of experimental approaches have been suggested for confirming topological nature of protein and protein complexes binding to DNA. Topological nature of interaction may be indicated by the following experimental observations: (i) stability of binding in the high ionic strength buffers [39, 56] (Fig. 4b (*1*)); (ii) sensitivity of the interaction to proteolytic cleavage

[3] In certain conformations cohesin can form, in addition to S-K ring, other non-covalently closed compartments in which DNA strand can be entrapped. Such structures include NIPBL-SMC3-subcompartment, E-S-, E-K-, and J-K-subcompartments. Integrity of such subcompartments, unlike integrity of the S-K-ring, is usually very quickly disrupted. In the text term 'topological binding' refers to the DNA entrapment within the stable S-K-ring.

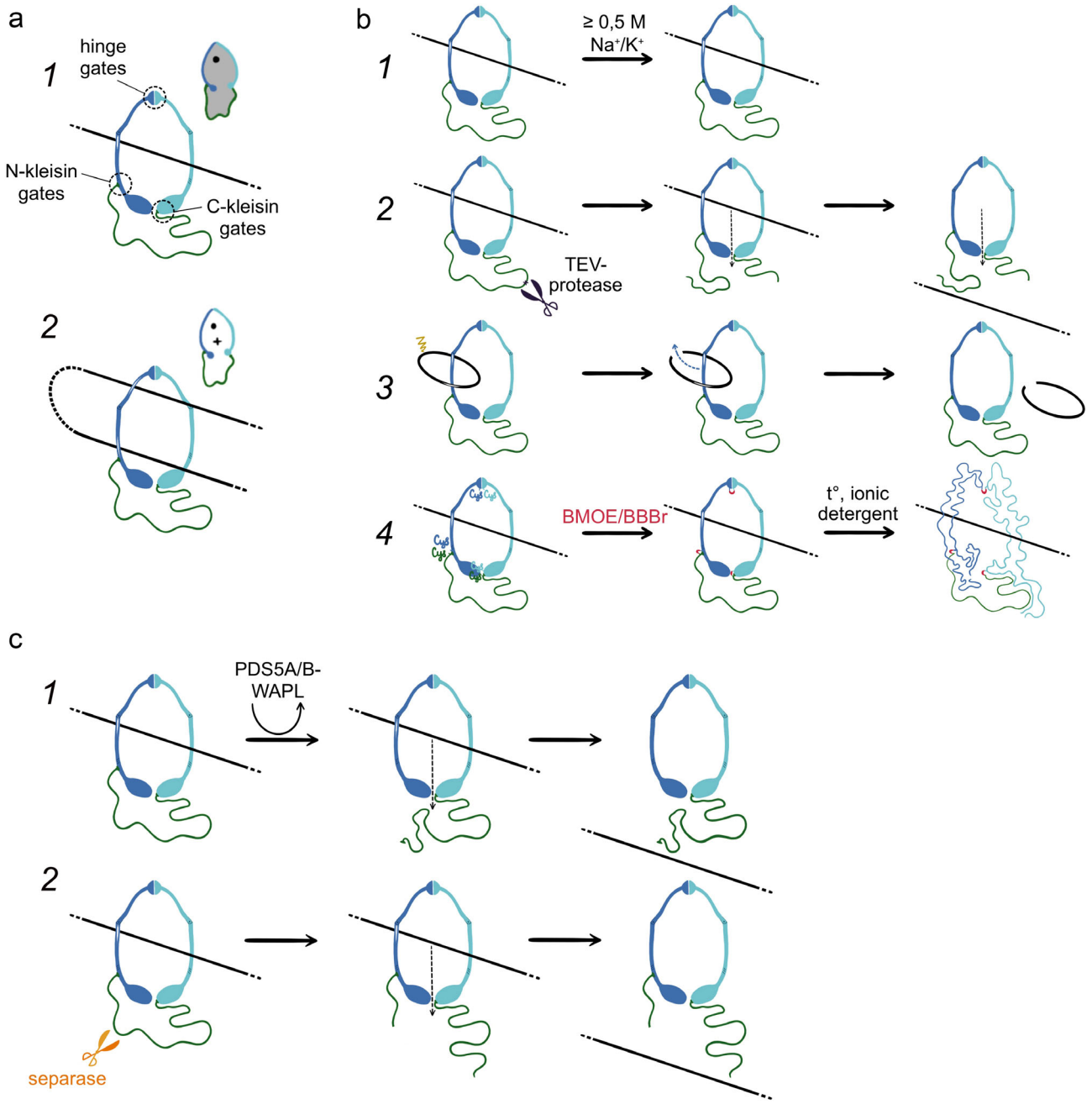

Fig. 4. Topological interaction of the cohesin ring with DNA. a) Topological (*1*) and pseudo-topological (*2*) interaction of cohesin with DNA. In the pictograms direction of DNA-thread relative to the figure plane is shown with • and + symbols. b) Methods used to establish topological nature of interaction between the protein complex and DNA: analysis of stability of the binding in the high ionic strength buffers (*1*); analysis of sensitivity of interaction to proteolytic cleavage of one of the subunits (*2*); analysis of sensitivity of interaction to the break in the DNA molecule (*3*); analysis of the interaction stability under denaturing conditions after covalent cross-linking of the protein ring with the help of cysteine specific cross-linking agents (BMOE, bBBr) (*4*). c) Two pathways for removal of topologically bound cohesin rings from DNA realized in eukaryotic cells: WAPL-dependent (*1*) and proteolytic (*2*).

of one of the subunits of the complex [22, 39] (Fig. 4b (*2*)); (iii) sensitivity to double-strand DNA break (if circular DNA molecule participates in the interaction) [39, 59] (Fig. 4b (*3*)); (iv) resistance of the interactions to denaturing conditions after covalent cross-linking of the protein ring [30, 60] (Fig. 4b (*4*)). Conclusions made based on the results of experiments using first three of the mentioned approaches are mostly of preliminary nature, while the experiments involving cross-linking of the protein ring allow concluding with confidence on the mode of the complex binding to DNA.

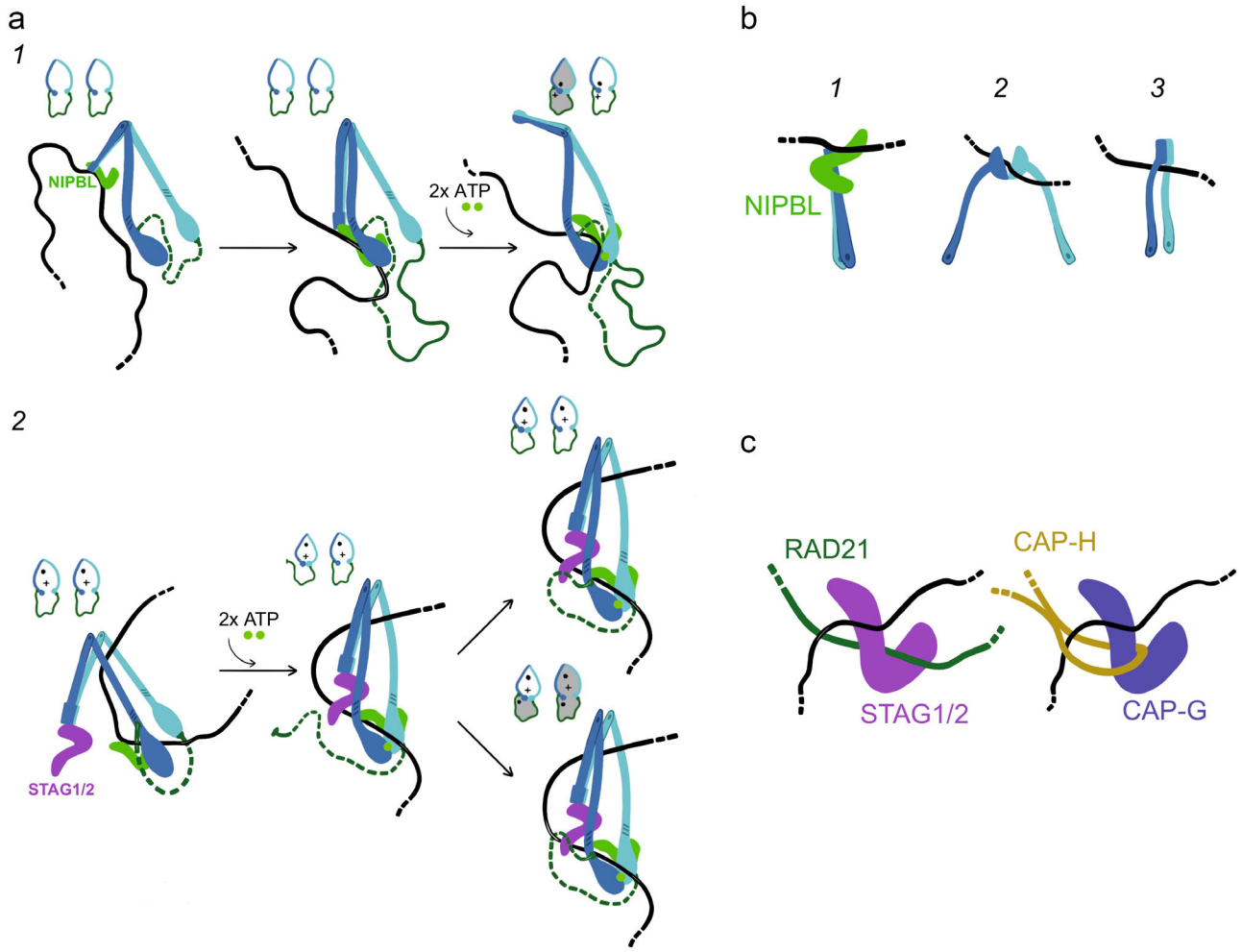

Fig. 5. Electrostatic interactions between cohesin and DNA. a) Two hypothetical pathways of the formation of 'DNA gripping' structure suggested based on observations of *S. cerevisiae* (*1*) and *Schizosaccharomyces pombe* (*2*) cohesins. In the pictograms direction of DNA-thread relative to the figure plane is shown with • and + symbols, gray fill indicates topological binding. In the pairs of pictograms, the left one reflects the formation of E-S- and E-K-subcompartments and position of DNA threads relative to them; the right one – position of DNA threads relative to the S-K-ring. Dashed parts of the RAD21-subunit correspond to the regions in which path of the protein chain is shown arbitrary for clarity (in reality HAWK-subunits remain bound to RAD21 at all presented stages). b) Electrostatic interactions of the cohesin hinge domains with DNA. Three (not mutually exclusive) scenarios are shown: NIPBL-subunit-mediated interaction (*1*), direct contact of the inner surface of the hinge domain pore with DNA (*2*), and direct contact of DNA with the southern pole of the hinge domains dimer (*3*). c) Interaction of HAWK-B subunits of cohesin (STAG1/2) and condensin (CAP-G) with DNA. Peptide loop, called 'safety belt', formed by the kleisin subunit of condensin (CAP-H), additionally stabilizes binding of the HAWK-B subunit (CAP-G) to DNA.

The most reliable proofs of the ability of SMC complexes to interact with DNA topologically were obtained during investigation of the covalently cross-linked S-K-rings [7, 60, 61]. Resolution of the atomic structure of three sites of interactions between the pairs of the subunits forming S-K-ring (dimer of the SMC1 and SMC3 hinge domains, N-terminal RAD21 domain interacting with the neck region of SMC3, and C-terminal domain of RAD21 interacting with the SMC1 head domain) allowed to genetically engineer cohesin complexes so that the S-K-compartments can be covalently crosslinked with the bifunctional thiol-specific reagents: bis-maleimidoethane (BMOE) or dibromobimane (bBBr). Capability of complex to be cross-linked is achieved by introduction of three pairs of cysteine residues at the sites of interaction of three core subunits in such a way that two cysteines in each pair are located opposite to each other on different subunits of the complex at a distance no larger than 1 nm (Fig. 4b (*4*)). Long linear molecules of genomic DNA and circular plasmids topologically interacting with the modified cohesin complexes cannot be separated after treatment with the cross-linking agents from the protein trimer even under the harsh denaturing conditions, which allows easy detection of such DNA molecules by the decrease in their electrophoretic mobility. Existence of cohesin complexes topologically interacting with DNA not only in artificial *in vitro* systems,

but also in living cells has been finally proved with the help of such approach [7, 30, 48]. Besides detection of the mere fact of topological interaction thiol-specific cross-linking of other pairs of artificially introduced cysteines potentially allows establishing the identity of specific subcompartment of the S-K-ring (E-S, E-K, J-S, or J-K) involved in topological interaction between cohesin and DNA (Fig. 3a) [44, 48, 62].

In order to understand physiological activity of cohesin it is important to investigate mechanisms of formation and termination of topological interactions of the S-K-ring with DNA. Theoretically such interaction could be established and terminated during temporal disengagement of one of the three non-covalent interfaces forming the S-K-ring: interface between SMC3 and SMC1 hinge domains (hinge gates), interface between kleisin and SMC3 subunit (N-kleisin gates), or interface between kleisin and SMC1 (C-kleisin gates) (Fig. 4a (*1*)). In addition, topological interaction of cohesin with DNA could be disrupted due to proteolysis of one of the core subunits of the complex.

Mechanisms of termination of the topological binding of cohesin are relatively well understood. It has been established that non-proteolytic removal of the complex from DNA (Fig. 4c (*1*)) is catalyzed by the conserved nuclear protein WAPL (Wpl1) (winged-apart like) and is caused by the opening of N-kleisin gates [42, 63, 64]. The structured C-terminal domain of WAPL consists of eight HEAT-repeats and it can interact with the engaged SMC head domains in the E-state. The non-structured N-terminal domain contains conserved YSR- and FGF-motifs participating in the interaction with the HAWK-subunits: PDS5A/B and STAG1/2 [43]. WAPL is not a constitutive component of the cohesin complex, but can transiently interact with the PDS5A/B-containing rings [42, 65]. It is assumed that the WAPL binding and DNA release occur when the complex is in the E-state. Formation of the E-state always results in the short-term opening of the N-kleisin gates, however, WAPL stabilizes the complex in such open state, which increases the probability of DNA leaving the ring [43, 66]. According to this mechanism in order for the DNA to escape the ring it should be first transferred into the E-K-subcompartment. After the DNA release from the complex, dissociation of WAPL from the complex and closing of exit gates occur.

Topological interaction of cohesin with DNA is disrupted also due to proteolysis of the kleisin subunit in anaphase [21, 58, 67]. After the passage of the anaphase checkpoint the serine protease separase cleaves the central non-structured part of the RAD21-subunit and, thus, releases the sister chromatids from the topological entrapment within the cohesin ring (Fig. 4c (*2*)).

Much less is known about how topological binding of cohesin to DNA is established. Ability of cohesin to hydrolyse ATP as well as presence of NIPBL and STAG1/2 are required for topological loading of the complex in cells and in *in vitro* systems [7, 44]. At the same time, other data indicate that at least *in vitro* the PDS5A/B–WAPL complex could catalyze reaction of cohesin loading onto DNA in the absence of the NIPBL-subunit [39, 68].

Not only the details of the loading mechanism are still unknown, but also what particular gates of the SMC complex let DNA inside the ring. On the one hand, it has been assumed that formation of the E-state is accompanied by the transient opening of the N-kleisin gates through which the DNA thread could enter the S-K-compartment [49]. On the other hand, it was shown that the complexes with covalently closed N- or C-kleisin gates despite all that can be topologically loaded onto DNA [7]. Moreover, it has been shown that the positively charged amino acids located inside the pore between the hinge domains are required for establishing topological interactions between the cohesin and DNA, which indicates possible role of the hinge gates in this process [7, 8].

From the early days of the hypothesis suggesting topological mode of cohesin–DNA interactions it was assumed that the S-K-ring can accommodate two DNA strands simultaneously ensuring cohesion of sister chromatids in the G2-phase of cell cycle [22, 54]. Later this mechanism of cohesion was confirmed experimentally [7, 48]. Some of the currently discussed models of cohesin-dependent loop extrusion suggest that the base of growing loop consists of two DNA strands entrapped within a single S-K-ring [26, 53, 62]. Such loop can be formed while the S-K-compartment remains constantly closed, it does not contain true topological link between the SMC complex and DNA, and therefore it represents an example of the so-called pseudo-topological interactions (Fig. 4a (*2*)). Unlike the topological entrapment of DNA within cohesin, pseudo-topological interaction is still considered to be a speculative model rather than the observed phenomenon.

The topologically engaged cohesin complexes are capable of passive one-dimensional diffusion along the DNA thread [23, 24]. *In vitro* studies showed that the size of cohesin pore allows this complex to bypass small DNA-bound proteins during such diffusion, however, nucleosome particles with diameter of 10 nm represent a significant obstacle, while particles with diameter 20 nm are impassable barriers for such motion [23]. It has been suggested that the pseudo-topologically loaded cohesin rings are also capable of diffusion along the DNA threaded through the complex pore [40, 53].

DNA gripping: transient ATP-dependent binding of head domains and NIPBL-subunit to DNA. The most important finding in structural biology of cohesin was the discovery of coupling between the formation of E-state in the presence of ATP and cohesin

binding to DNA. It was observed that the cohesin complexes with EQ-mutations in the Walker B motif (suppressing ATPase activity) in the presence of ATP, as well as wild type cohesin complex in the presence of nonhydrolyzable ATP analogues form stable complexes with DNA called gripping state/DNA clamping [25, 44, 49]. Presence of NIPBL, which is not a constant component of cohesin, is also required for formation of such complex. During the gripping double helix interacts with the dimerized head domains (Fig. 5a), moreover, DNA is found pinned to the SMC3 head domain with the help of NIPBL, which in the presence of ATP binds to the joint region of the SMC3 arm domain and head domains of both SMC subunit.

In the gripping configuration the DNA sugar-phosphate backbone forms a series of electrostatic interactions with different cohesin sites: upper surface of the dimerized SMC head domains, DNA-binding surface of NIPBL and N-terminal domain of RAD21. All DNA binding sites participating in DNA gripping come close to each other and form a small channel complementary to the bound DNA double helix only for a short time, while the complex is in the gripping state. After ATP hydrolysis, the E-state is disassembled, and at the same time the composite DNA-binding surface is taken apart. Hence, in the wild type complexes, gripping is a short-lived state, formation and disruption of which is coupled with ATP binding and hydrolysis.

In the presence of ATP, DNA, NIPBL, and wild type cohesin the head SMC domains and NIPBL-subunits periodically interact with each other; replacement of ATP with nonhydrolyzable analogue or introduction of EQ-mutations renders this interaction constitutive [49, 50]. Remarkably, on binding of ATP, the DNA first interacts with the SMC3 head domain and NIPBL-subunit in the so-called pre-engaged clamp, and only after this engagement of the head domains occur with formation of the full-fledged gripping configuration (Fig. 5a (*1*)) [50].

In the absence of DNA, the head SMC domains are capable of dynamic interactions with fast formation and disruption of the E-state [49, 50]; addition of DNA to the system does not increase frequency of such interactions [49, 50], however, it does stimulate significantly ATPase activity of cohesin [39, 40]. Most likely, DNA increases productivity of the interactions between the head domains due to allosteric effect on the active center which makes ATP hydrolysis a necessary condition for disengagement of the head domains [50]. This explains why catalytically inactive (containing EQ-mutations) SMC complexes in the presence of DNA and ATP remain frozen in the gripping configuration [44, 49].

Two alternative pathways were suggested for the formation of gripping state. The data obtained with the help of thiol-specific cross-linking of *S. cerevisiae* cohesin show that during formation of gripping state DNA thread is trapped simultaneously in both E-K- and E-S-subcompartments. However, DNA does not enter the S-K-compartment; hence, it is assumed that in this case the S-K-ring harbors a small DNA loop in a pseudo-topological manner (Fig. 5a (*1*)) [44]. It has been suggested that formation of such complex occurs due to the approaching of DNA to cohesin from the side of disengaged head domains, and passing through them prior to formation of the E-state makes DNA trapped simultaneously in the E-K- and E-S-subcompartments (Fig. 5a (*1*)). As a result, formation of the gripping state according to this mechanism is not accompanied by DNA topological entrapment within the S-K-ring. The data on the kinetics of DNA entrance into the E-K- and E-S-subcompartments are in agreement with the proposed mechanism: short incubation of DNA with cohesin is sufficient for realization of these reactions, moreover both reactions follow the same kinetics.

The results obtained during the study of DNA-gripping state formation by the *Schizosaccharomyces pombe* cohesin contradict the scenario described above [49]. These data demonstrate that first small DNA loop is threaded through the S-K-ring, this is followed by the formation of the gripped state with the lower part of the loop located closer to the head domains being clamped (Fig. 5a (*2*)). It is suggested that while the head domains establish E-state interaction the N-kleisin gates open temporarily and could occasionally let DNA within the S-K-ring. Thus, according to this model the DNA gripping state can be one of the intermediates of the process of topological loading. However, in the framework of this model the DNA thread usually does not enter the N-kleisin gates during formation of the gripping state, and thus the majority of the ATP hydrolysis cycles do not result in the topological loading of the complex.

Deciphering process of DNA gripping state formation is crucial for understanding of the mechanisms of cohesin-dependent loop extrusion and of the relationships between loop extrusion and topological entrapment of DNA within the S-K-rings [4, 5, 53].

Electrostatic interactions of hinge domains with DNA. The dimerized hinge domains of the cohesin SMC subunits interact with double-stranded DNA electrostatically [50, 69, 70]. In the absence of ATP (in the apo-form of the complex), hinge domains interact with NIPBL [50], which results in formation of a stronger binding site combining DNA-binding surfaces of the hinge domains and of the HAWK-subunit (Fig. 5b (*1*)). Binding of ATP by the head domains results in disruption of this composite site and decrease in affinity of the hinge domains to DNA.

There is no reliable information on particular amino acids in the hinge domains that participate in

the DNA binding (Fig. 5b (*2* and *3*)). Despite the fact that the positively charged amino acids lining the small pore of the dimer of hinge domains are required for the DNA entrapment within the S-K-compartment [7, 8], their physical interaction with DNA has not been demonstrated yet. The pore is too small to trap the double-stranded DNA within it; nevertheless, some authors suggest that the loss of contacts between the hinge domains at one pole of the dimer could expose inner surface of the pore for DNA binding [7, 71] (Fig. 5b (*2*)). Interestingly enough, one of the published structures of the cohesin complex in the gripping configuration contains hinge domains with disengaged northern dimerization surface [25].

According to other data, the sites on the hinge domains located at the southern pole of the dimer looking inside the S-K-ring as well as adjacent regions of the coiled coils are responsible for DNA binding [50, 70] (Fig. 5b (*3*)).

Participation of STAG1/2 in cohesin–DNA interaction. Unlike NIPBL, which, most likely, contacts DNA exclusively as a constituent of the composite DNA-binding sites in cooperation with the head or hinge domains, STAG1/2 is more or less autonomous DNA-binding module (which, however, does not rule out its participation in cooperative binding; in particular, there are indications that STAG1/2 interacts with DNA in the gripping state in close proximity of the site of DNA interaction with the NIPBL-subunit) [25]. Amino acid residues responsible for interaction of STAG1/2 with DNA are localized in the regions homologous to the corresponding DNA-binding patches on the NIPBL surface (Fig. 5c). One of the main such regions is the positively charged groove at the side surface of the long N-terminal subdomain of the hook-shaped molecule [50, 72]. Interestingly enough, electrostatic interaction of the CAP-G/CAP-G2-subunit of condensin – STAG1/2 homolog – with DNA is additionally stabilized due to the fact that the kleisin subunit CAP-H/CAP-H2 forms a non-covalent peptide loop around the DNA thread interacting with CAP-G/CAP-G2 [62, 73, 74] (Fig. 5c). This loop with length of around 100 aa is called ‘safety belt’ and is formed as a result of interaction between the two short hydrophobic fragments in the middle of the non-globular part of the kleisin. The kleisin cohesin subunit, RAD21, most likely, does not have a structure similar to the condensin’s ‘safety belt’.

CONCLUSIONS

Some structural features of the SMC complexes such as ring architecture and their ability to topological entrap DNA were described long before the discovery of the loop extrusion process. Exploration of cohesin ability to topologically entrap DNA was coupled with elucidation of molecular mechanisms of establishing, maintenance, and termination of cohesion. However, cohesion, which is not directly associated with extrusion activity, is an exception rather than the rule: vast majority of physiological activities of cohesin and other SMC complexes depend on their ability to actively create DNA loops. The fact that these complexes were found to be motor proteins spurred further interest in their structure.

Aspiration to decipher molecular mechanism of extrusion motivated researchers to investigate structure of cohesin in recent years. Breakthroughs in this area are at large associated with the development of experimental technologies that enable exploration of dynamic and, to a certain degree, polymorphic structure of massive multisubunit SMC complexes. Technologies that substantially contributed to the current understanding of the cohesin structure include cryogenic electron microscopy [25, 44, 49], atomic force microscopy [50], FRET [49, 50], and protocols for real-time imaging of loop extrusion in reconstituted *in vitro* systems [8, 40]. These methods unraveled many disparate molecular details of the extrusion process. One of the most important findings in this respect was the discovery of the periodical formation of cohesin DNA-gripping state during the loop extrusion process, as well as establishing of the central role of NIPBL in the assembly of this structure.

Unfortunately, disparate structural, biochemical, and genetic observations still do not provide a comprehensive model describing the process of cohesin-dependent loop extrusion (hypothetical mechanistic models of this process based on the available data on the structure and activity of SMC complexes are presented in the second part of this review [10]). Nevertheless, there is every reason to believe that accumulation of empirical, primarily structural data could facilitate elucidation of the molecular mechanism of SMC-dependent loop extrusion in the nearest future.

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