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REVIEW

Cohesin-Dependent Loop Extrusion: Molecular Mechanics and Role in Cell Physiology

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Abstract—The most prominent representatives of multisubunit SMC complexes, cohesin and condensin, are best known as structural components of mitotic chromosomes. It turned out that these complexes, as well as their bacterial homologues, are molecular motors, the ATP-dependent movement of these complexes along DNA threads leads to the formation of DNA loops. In recent years, we have witnessed an avalanche-like accumulation of data on the process of SMC dependent DNA looping, also known as loop extrusion. This review briefly summarizes the current understanding of the place and role of cohesin-dependent extrusion in cell physiology and presents a number of models describing the potential molecular mechanism of extrusion in a most compelling way. We conclude the review with a discussion of how the capacity of cohesin to extrude DNA loops may be mechanistically linked to its involvement in sister chromatid cohesion.

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Keywords: cohesin, SMC complexes, loop extrusion, cohesion, DNA gripping state

INTRODUCTION

Cohesin is a protein complex, which is absolutely essential for reproduction of eukaryotic cells [1, 2]. First cohesin subunits were discovered more than 25 years ago as factors participating in pairing of sister chromatids in mitosis [3-5]. It has been found out later that this phenomenon termed ‘cohesion’ is based on the fact that the pairs of sister chromosomes after replication end up being threaded through multiple cohesion complexes (each of which having ring-shaped structure with a relatively large intersubunit pore), similar to two threads passing through a series of beads (Fig. 1a) [6-8]. Maintenance of cohesion during the G2-phase of cell cycle and its controlled release in anaphase occurring due to proteolysis of the RAD21 subunit of cohesin ensures correct attachment of spindle microtubules to kinetochores and subsequent equal distribution of genetic material between the two daughter cells [9].

No less important activity of cohesin besides cohesion is its ability to form DNA loops via the mechanism called extrusion [2, 10, 11]. Extrusion begins with cohesin binding to small DNA fragment followed by the ATP-dependent movement of the complex along the DNA resulting in processive pulling of the flanking DNA inside the growing loop, the length of which could eventually reach hundreds of thousands of base pairs (kbp). Theoretically flanking DNA can be continuously pulled inside from one side of the loop held by the complex (in the case of unidirectional extrusion) or from both sides (in the case of bidirectional extrusion). Extrusion is typical not only for cohesin, but for an entire group of protein complexes, known as SMC complexes (structural maintenance of chromosomes proteins), with cohesin being one of the representatives of this group [12-14]. Synergistic activity of the type II DNA topoisomerases and SMC-dependent extrusion is required for post-replicative individualization of sister genomes in all cells (prokaryotic and eukaryotic).

Abbreviations: CAR, cohesin associated region; E-P, enhancer-promoter (interactions); FRET, Förster resonance energy transfer; HAWK, HEAT protein associated with Kleisin; SMC, structural maintenance of chromosomes.

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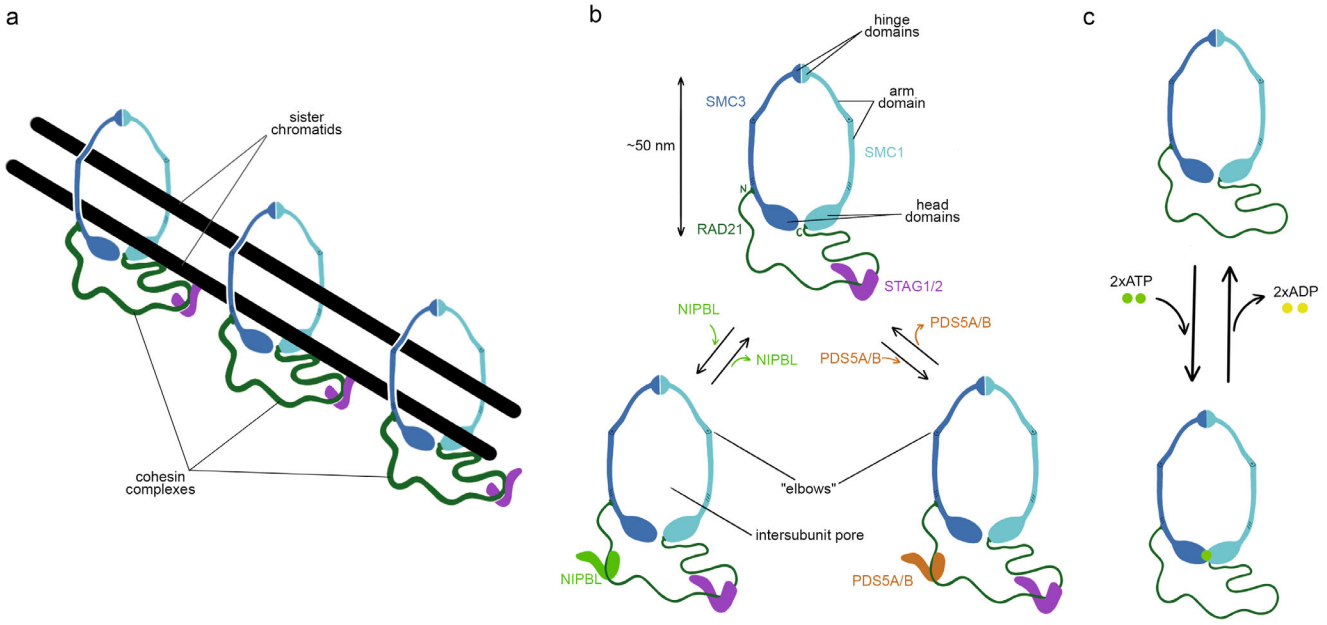

Fig. 1. Cohesin structure and its participation in sister chromatid cohesion. a) Topological loading of cohesin rings onto sister chromatids during cohesion. b) Schematic representation of core trimer forming the cohesin ring; recruitment of auxiliary HAWK subunits to the core trimer. c) Dimerization of head domains of SMC subunits required for ATP hydrolysis.

Extrusion can also facilitate orderly compaction of DNA; formation of mitotic chromatids in prophase/metaphase in the vertebrate cells is the best example of such compaction. Chromatids are elongated structures consisting of the densely packed chromatin loops anchored on the proteinaceous axial core. The main structural component of this axis is condensin – SMC complex responsible for mitotic loop extrusion, which eventually results in formation of chromatids. The cohesin-dependent loop extrusion contributes to individualization of eukaryotic chromosomes, in many cases is responsible for chromatin compaction and also has a number of secondary functions.

Cohesion, unlike extrusion, is typical exclusively for cohesin, and not for any other representatives of SMC complexes [1, 2]. Cohesive activity likely emerged in the primordial cohesin during early eukaryogenesis [15], this acquisition did not result, however, in the loss of extrusive activity of the complex [16-18]. This commonly accepted scenario raises a number of interesting questions on mechanistic, functional, and evolutionary interrelationships between the cohesion phenomenon and the process of loop extrusion.

In this review we summarize current understanding of cohesin-dependent loop extrusion, its role in cellular processes, and its molecular mechanisms. We also briefly discuss disparate data indicating possible mechanistic relationship between extrusion and cohesion. Detailed description of the cohesin complex structure and principles of its interactions with chromatin can be found in the first part of the review published in the same issue of the journal [19].

STRUCTURE OF COHESIN COMPLEX

Cohesin is a protein complex with a ring-shaped structure, which is based on the trimer of core proteins: SMC1 (Smc1)[1], SMC3 (Smc3), and RAD21 (Scc1). All three proteins have elongated shape and interact with each other through terminal globular domains (Fig. 1b). Such organization results in formation of the extensive intersubunit pore, which is able to let through globular particles with diameter up to around 10 nm [20, 21]. Presence of the intersubunit pore makes possible topological entrapment of DNA within the complex with DNA being threaded through the protein ring [6-8].

SMC1 and SMC3 subunits are paralogs belonging to the family of ATPases called SMC proteins [22]. SMC1 and SMC3 form a stable V-shaped heterodimer via homotypic interaction between the hinge domains of two subunits [20, 23]. Head domains, which are located at the opposite end of the rod-shaped molecule from the hinge domains, are responsible for ATPase activity. In the presence of ATP intermittent engagement of the head domains of two SMC subunits occurs, such dimerization is required for hydrolysis of bound

[1] In the paper names of human proteins are presented in the main text; names of *Saccharomyces cerevisiae* homologs are shown in parenthesis (at first mention).

ATP molecules (Fig. 1c). The hinge and head domains of each of the SMC subunits are separated by the long and relatively flexible coiled-coil arm domain. Flexibility of the arm domain is to a large degree associated with the presence of evolutionary conserved defect in the regular coiled-coil structure, the so-called elbow region (Fig. 1b). Bending of 'elbows' has rather large amplitude; and simultaneous bending of elbow sites in both SMC subunits could facilitate direct physical interaction of the hinge and head domains of the complex. The RAD21 protein, also called kleisin subunit, forms a constant bridge between the head domains of the two SMC subunits, thus closing the ring structure.

Auxiliary subunits belonging to the family of HAWK proteins (HEAT protein associated with Kleisin) including STAG1/2[2] (Scc3), NIPBL (Scc2), and PDS5A/B (Pds5) bind to the core trimer [16, 20, 23]. The primary site of interaction of the HAWK subunits with the core trimer is the kleisin subunit, however, only STAG1/2 forms a stable contact with RAD21 and, hence, is a constitutive component of the complex. NIPBL and PDS5A/B compete for the shared binding site at the kleisin subunit, and both these proteins interact transiently with the stable cohesin tetramer (SMC3–SMC1–RAD21–STAG1/2) [24-26]. Therefore, at each particular moment the complex could contain one (STAG1/2) or two (STAG1/2 + NIPBL or STAG1/2 + + PDS5A/B) HAWK subunits (Fig. 1b). Binding of NIPBL to the core cohesin complex (and presence of DNA) is required for effective hydrolysis of ATP, replacement of NIPBL with PDS5A/B dramatically changes activity of the complex [26].

CELLULAR CONTEXT OF COHESIN-DEPENDENT LOOP EXTRUSION

Cohesin-dependent loop extrusion throughout the cell cycle. Cohesin-dependent extrusion is realized in eukaryotic cells throughout interphase and mitosis (Fig. 2a). Various estimates indicate that in the G1-phase vertebrate cells there are around 100,000 cohesin rings, and this number doubles in the G2-phase [27, 28]. Nucleoplasm of the cells in G1-phase contains two coexisting subpopulations of cohesin complexes of approximately the same size, which are in dynamic equilibrium: (1) freely diffusing cohesin and (2) cohesin involved in extrusion (with chromatin residence time of about 10-30 min in vertebrate cells and around 1 min in yeast cells) [28-31]. When cohesion is established in the S-phase a third subpopulation emerges: stably bound cohesive rings excluded from extrusion initiation/termination cycle [28, 29, 32]. Complete arrest of cohesin-dependent extrusion occurs during mitosis. In vertebrates phosphorylation of the HAWK subunits in prophase induces termination of cohesion in chromosome arms; most likely this phosphorylation also leads to dissociation of extruding cohesin from DNA [29, 33]. Resumption of cohesin-dependent extrusion occurs at the end of telophase [34, 35]. In the budding yeasts the cohesin-dependent extrusion is realized in mitotic cells up to the start of anaphase [36], when the separase-dependent proteolysis of the kleisin subunits results in degradation of the whole cellular pool of cohesin complexes and is resumed only at the end of G1-phase with restoration of the population of intact cohesin rings [4, 36].

Genomic distribution of initiation sites. Initiation of the cohesin-dependent loop extrusion is not strictly localized to particular genomic sites, however, there are some preferences in extrusion complex binding to DNA. Centromeres and regions adjacent to them are examples of such sites [8, 26]. Initiation of extrusion, however, also occurs constantly outside of the centromere regions. It has been assumed up until now that initiation of the cohesin-dependent extrusion in the chromosome arms is mainly associated with open chromatin [37-39]. However, this model is questioned now. The recently reported experimental data on the genomic distribution of cohesin binding, as well as the results of computer modeling indicate that probability of extrusion initiation is distributed evenly along the genome outside of the centromeres [40].

WAPL and PDS5A/B are negative regulators of processivity, NIPBL – positive. The final size of the loops formed in the process of extrusion is determined by processivity of the cohesin complexes and genomic distribution of the extrusion pause sites [36, 41-45], it varies from several dozens of kb in yeasts [44, 46] to hundreds of kb in vertebrates [41, 42].

Processivity of the cohesin-dependent extrusion is suppressed by the HAWK subunit PDS5A/B, as well as by the WAPL protein recruited by this subunit. Depletion of PDS5A/B and WAPL (together or individually) significantly increases chromatin residence time of the extruding cohesin, as well as length of the forming DNA loops [36, 41, 42, 44, 45]. Interestingly enough, both these proteins also participate in non-proteolytic termination of cohesion as a part of the so-called prophase cascade: recruitment of the WAPL protein to cohesin complexes containing PDS5A/B subunit results in non-proteolytic opening of the protein rings in the prophase cells of vertebrates and their removal from the chromosomal arms [47, 48]. This dual activity

[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.

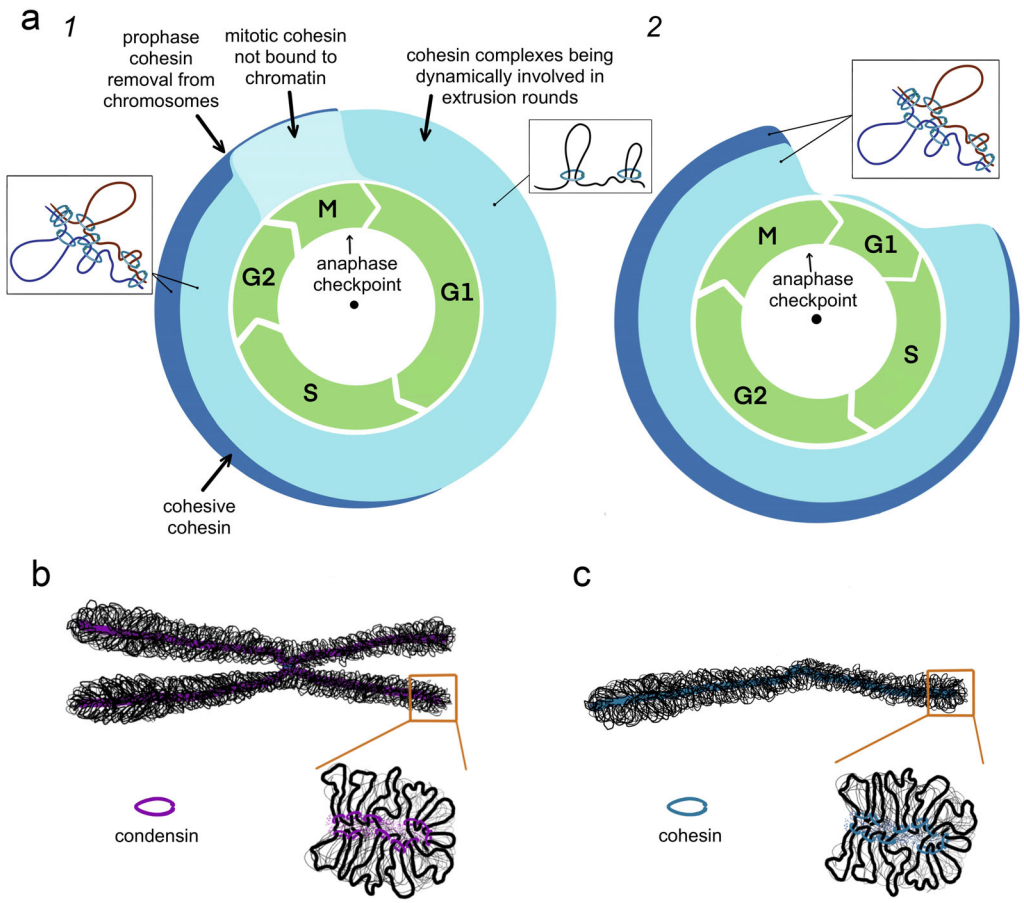

Fig. 2. Cohesin activity throughout the cell cycle and chromatin compaction due to SMC-dependent loop extrusion. a) Intact cohesin complex quantity and their activity throughout mitotic cycle in vertebrate (*1*) and *S. cerevisiae* (*2*) cells. b) Metaphase chromosomes of vertebrates are formed due to extrusion activity of condensin complexes eventually accumulated in axial structures. Typical X-shaped structure is maintained due to residual cohesion in centromeres of two metacentric sister chromosomes. c) Compact chromatid-like "vermicelli" structures formed due to cohesin-dependent loop extrusion in interphase vertebrate cells with suppressed WAPL activity. Cohesin is the primary structural component of the axial structures in such chromosomes.

of PDS5A/B and WAPL implies existence of mechanistic relatedness between the process of loop extrusion and the phenomenon of cohesion.

Another HAWK subunit, NIPBL, on the contrary, is a positive regulator of the extrusion processivity. NIPBL is commonly considered as a cohesin loader; however, this point of view probably requires reconsideration as the new data have been reported demonstrating that NIPBL could be dispensable for the primary loading of cohesin onto chromatin [49]. It has been firmly established that recruitment of NIPBL to the complex is necessary for active extrusion: functional depletion of NIPBL results in suppression of cohesin-dependent loop interactions in vertebrates and inhibition of the cohesin translocation from the primary loading sites in yeasts [26, 42, 50]. During each round of extrusion initiation/termination cycle NIPBL is repeatedly recruited to and released from the complex: chromatin residence time of NIPBL subunit in the vertebrate G1-cells is around 1 min, which is an order of magnitude less than average duration of each round of extrusion [25, 31]. During NIPBL absence its binding site can be occupied by the PDS5A/B subunit recruiting WAPL, which, likely, leads to extrusion termination by not yet elucidated mechanism [2].

Cohesin-dependent interphase extrusion, unlike mitotic condensin-dependent extrusion, usually does not result in formation of condensed chromatid-like structures with proteinaceous axial cores to which DNA loops are anchored. This can be a consequence of low processivity of the cohesin-dependent extrusion. Suppression of activity of PDS5A/B and WAPL in the vertebrate cells results in interphase condensation of chromatin accompanied by the formation of microscopically visible elongated structures with axial cores containing cohesin [41, 42, 51]. Such compact structures with characteristic shape resembling metaphase chromatids (Fig. 2b) have been called 'vermicelli' (Fig. 2c).

Interphase 'vermicelli' in a structural sense is similar to chromatids formed in the meiosis I prophase [52]. Compaction of meiotic chromosomes is achieved by the extruding activity of cohesin that eventually accumu-

lates in the axial structures, which later become an important component of synaptonemal complex. Discovery of cohesin-mediated formation of the condensed structures similar to metaphase chromatids in the meiosis I prophase became one of the early indications that extrusion of DNA loops might be a universal activity of all SMC complexes [51, 53].

Site-specific arrest of loop extrusion. Cohesin-dependent extrusion differs from extrusion mediated by other SMC complexes by the ability for a regulated arrest at the specific genome loci. It is not known exactly whether such arrest is a temporary pause or final termination of extrusion. In any case, stability of the formed DNA loop is maintained by the extrusion complex for a certain time after arrest until dissociation of the complex from DNA [31, 54]. At least two mechanisms of site-specific arrest of extrusion cohesin complexes have been described: CAR-dependent (Cohesin Associated Region) and CTCF-dependent. The first one, which is evolutionary more ancient and typical for the cells of lower eukaryotes, is associated with extended genome regions of cohesin accumulation, CAR-regions [44, 46, 55]. CAR-regions are preferably immunoprecipitated by the antibodies against cohesin subunits and, as a rule, are located at the 3′-ends of the convergently transcribed genes [56, 57]. The second mechanism of extrusion arrest associated with activity of the insulator protein CTCF is realized in the vertebrate cells [58, 59]. Blocking of extrusion complexes at the CTCF-sites results in colocalization of the vast majority of strong cohesin binding sites with the CTCF binding sites in the vertebrate cells.

CAR-dependent and CTCF-dependent mechanisms of extrusion arrest have much in common both with each other and with the processes of stabilization of cohesive ring binding to chromatin (Fig. 3a). In both cases arrest is realized due to the fact that the blocking sites favor replacement of NIPBL with PDS5A/B [44, 58]. This replacement is facilitated by the ESCO1 (Eco1)-dependent acetylation of the SMC3 protein occurring at the arrest sites [43, 45]. Acetylation of the SMC3 subunit at the conserved lysine residues K105/K106 (K112 and K113 – in yeasts) suppresses extrusion activity of cohesin likely due to decrease in affinity of the complex to NIPBL [60, 61]. Additionally, acetylation inhibits activity of the WAPL protein [62], which, as has been mentioned above, removes from chromatin not only the topologically engaged cohesin complexes, but also complexes participating in extrusion [41, 42, 51]. Interestingly enough, stabilization of the cohesive rings on chromatin in the G2-phase occurs due to acetylation of SMC3 at the same amino acid residues (in many species an auxiliary protein sororin also participates in stabilization). Acetylation facilitating stabilization of cohesion is established in the S-phase co-replicatively due to activity of the ESCO1 paralog, acetyltransferase ESCO2 (Eco1), and it also suppresses activity of WAPL towards the acetylated complexes.

Existence of the genomic elements blocking extrusion results in accumulation of cohesin complexes interacting with CAR-elements and CTCF-sites, as well as in formation of interphase chromatin typical folding patterns: structural chromatic loops anchored in the mentioned genomic elements and topologically-associated domains (TADs) (Fig. 3, b and c) [44, 46, 58, 59, 63]. In vertebrates the N-terminal fragment of CTCF is responsible for the arrest of extruding cohesin complexes, as well as for inhibition of the activity of WAPL subunit [58, 59, 64]. Steric characteristics of CTCF molecule bound to the binding site allow effective interaction of its N-terminal fragment with cohesin only if the extrusion complex approaches the CTCF-motif from the side of the 3′-end (N-terminal zinc-fingers of CTCF bind there to DNA); this interaction blocks extrusion and stabilizes cohesin at the CTCF-bound site (Fig. 3a). At the same time when extrusion complex approaches CTCF-motif from the side of the 5′-end it does not cause prolonged stalling of cohesin. These features of protein–protein interactions result in the peculiar regularity in orientation of the CTCF-motifs located at the bases of structural loops and at the boundaries of topological domains in vertebrates: pairs of CTCF-motifs located at the bases of structural loops are generally oriented in such a way that their 3′-ends face each other (convergent orientation); at the same time several CTCF-motifs (at least a pair) that are typically present within each TAD boundary face interior of the closest topological domain with their 3′-ends (divergent orientation) (Fig. 3, b and c) [63, 65-67].

Interplay with transcriptional apparatus of the cell. Why CAR-sites that stop extrusion in the yeast cells are located at 3′-ends of the convergently transcribed genes? Theoretically this could be a consequence of the fact that collision of the transcribing polymerase with extruding cohesin complexes moving in the opposite direction results in translocation of the latter to the end of the transcriptional units. This model is in agreement with the fact that turning off transcription leads to the removal of cohesin from the CAR-sites [56].

The mechanisms underlying transcription-dependent accumulation of cohesin rings at the 3′-end of the genes are operating not only in yeast cells, they are universal. For example, despite the fact that the vertebrate cells do not have classic CAR-sites, simultaneous depletion of the CTCF and WAPL results in relocation of cohesin complexes from the CTCF-sites to the 3′-ends of actively transcribing genes [38, 40]. These new cohesin-bound sites differ significantly from the original genomic peaks colocalized with CTCF; they comprise extended regions several kbp in length appropriately

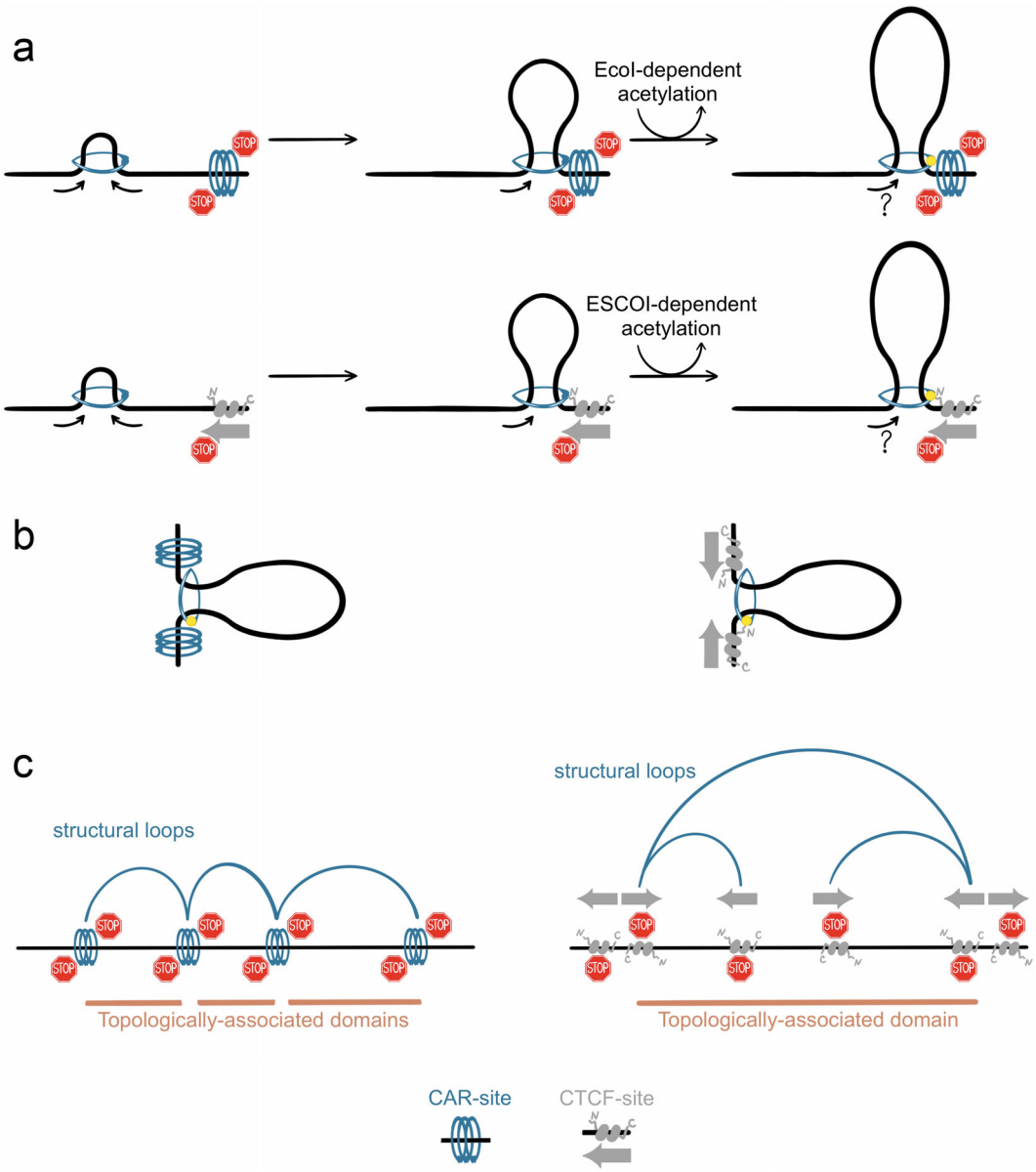

Fig. 3. Mechanisms of cohesin-dependent extrusion arrest and chromatin folding patterns emerging as a result of extrusion block and subsequent stabilization of extruding complexes. a) CAR-dependent and CTCF-dependent arrest of loop extrusion. Collision of cohesin with an individual site likely converts bi-directional cohesin-dependent extrusion into unidirectional. ESCO1 (Eco1)-dependent acetylation of SMC3 subunit (acetylation of SMC3 is shown as a yellow dot) plays an important role in arrest of extrusion and in protection of the stalled complex from WAPL. b) Structural loops with CAR- or CTCF-sites located at their bases are formed due to complete (two-sided) arrest of extrusion. c) Distribution of CAR- and CTCF-sites along the genome predetermines formation of typical supranucleosomal chromatin folding patterns: structural loops and topologically associated domains.

named 'cohesin islands'. The islands, similarly to the yeast CAR-sites are predominantly located at the 3′-ends of convergently transcribed genes; their formation can also be prevented by inhibition of transcription [38]. Emergence of cohesin islands is coupled with formation of cohesin-dependent chromatin loops between the pairs of neighbouring islands, which makes their homology with CAR-sites even more apparent [40].

A number of observations contradict the simplistic notions according to which RNA polymerase directly interacts with extrusion complexes. One of the alternative hypotheses suggests that RNA-polymerase relocates the topologically loaded cohesin rings not participating in extrusion to the 3′-ends of the genes, and those, in turn, block the extrusion process [44, 68]. The following facts speak in favor of such mechanism: (i) preferable localization of CAR-sites at the 3′-ends of only convergently oriented genes (not at 3′-ends of each and every active gene), (ii) kinetics of accumulation of cohesin complexes at the CAR-sites during

cell cycle and temporarily delayed kinetics of loop formation between the CAR-sites [44], and (iii) ability of extruding SMC complexes to bypass large chromatin-bound protein obstacles, including transcribing RNA polymerase, demonstrated *in vitro* [69].

It is likely that the transcribing RNA polymerase on its own does not represent a significant barrier for the extruding cohesin complexes, however, active promoters, which, in addition to RNA polymerase, recruit a large number of auxiliary proteins involved in initiation, may constitute such barriers. The known phenomenon of the promoter-associated topological insulation supports the notion that promoters hinder movement of extruding cohesin complexes approaching from both directions [40, 70, 71]. Promoters are less effective extrusion barriers than CAR-regions and CTCF-sites. Moreover, the cohesin complexes stopped at promoters are not stabilized via the ESCO1/2-dependent acetylation; that is why promoters are generally not localized at the bases of metastable DNA loops held by cohesin.

ROLE OF COHESIN-DEPENDENT LOOP EXTRUSION IN CELL PHYSIOLOGY

Post-replicative individualization of sister chromosomes and condensation of mitotic chromosomes. Individualization of sister chromosomes is a primordial function of SMC complexes [1, 2]. Mechanics of the process of replication of double-helical DNA molecule in the cells inevitably leads to the certain degree of topological linkage between two newly synthesized sister DNA threads [72, 73]. Post-replicative individualization relies on SMC-dependent extrusion, which directs activity of the type II topoisomerases towards decatenation of the topologically linked newly replicated genomic DNA molecules and facilitates spatial separation of the unlinked DNA threads [1, 2, 74]. In the vast majority of cases, bacterial and archaeal genomes encode a single SMC complex (belonging to one of the two classes: Smc–ScpAB or MukBEF), main function of which is exactly post-replicative individualization of sister chromosomes [1, 2, 75]. In eukaryotic cells this process is realized through the complementary activities of two SMC complexes: cohesin and condensin. In addition to individualization, these complexes also participate in compaction (condensation) of mitotic chromosomes, which enables relocation of chromosomes to the spindle poles in anaphase.

Extrusion activity of condensin is suppressed in interphase [76-79], at the same time, throughout G2-phase cohesin not only maintains cohesion, but also participates in extrusion, which ensures individualization of sister chromosomes (to a large extent) even before the beginning of mitosis [17, 80, 81]. At the moment when the bulk of cohesin rings (in prophase – in vertebrates and many other eukaryotes, and in anaphase in *S. cerevisiae*) is removed from the chromatin, sister chromosomes are already separated from each other to a large extent and topological links are likely preserved predominantly at the sites of cohesion: centromeres, ribosomal repeats, CAR-regions, and CTCF-sites [17, 80, 81]. Removal of the residual topological links and ultimate individualization of sister chromosomes rely on mitosis-specific condensin-dependent extrusion [17, 82, 83].

Extrusion performed by condensin and cohesin also ensures mitotic (and meiotic) chromatin compaction (Fig. 2, b and c); however, contribution of each of the complexes differs in the cells of different organisms [1, 2]. In particular, cohesin contributes substantially to condensation in the budding yeasts, where the cohesin-dependent extrusion continues up to the start of anaphase [44, 84, 85]; at the same time, in vertebrate cells in which cohesin binding in chromosomal arms is already terminated by the end of prophase, formation of compact mitotic chromosomes depends primarily on condensin [76, 86]. Highly processive activity of condensin in mitotic cells of vertebrates results in formation of chromatids – compact elongated bodies with chromatin loops anchored on the axial proteinaceous core with condensins (vertebrate genomes encode two types of condensin complexes) and topoisomerase II being the main constituent of it [87].

It is noteworthy that the processive extrusion activity of cohesin can result in formation of chromatid-like structures outside of mitosis. In particular, as has been mentioned above, cohesin-dependent formation of the chromatid-like “vermichelli” structures occurs in the interphase cells with suppressed activity of the WAPL, and axial core of such structures consists of cohesin rings [41, 42, 51] (Fig. 2c). In the same way, meiotic chromosomes including transcriptionally active lampbrush chromosomes comprise elongated structures with chromatin loops originating from axial cores that have cohesin meiotic variants and topoisomerase II in their composition in addition to other proteins [52].

Maintenance of decatenated state of the genome. Interphase cohesin-dependent extrusion also ensures specific non-equilibrium folding of chromosomal DNA. Firstly, the constantly ongoing rounds of extrusion increase frequency of local *cis*-interactions and to a certain extent suppress long-range *cis*- and *trans*-interactions [41, 46, 88]. Suppression of *trans*-interactions, maintained to some extent by cohesin-dependent extrusion, manifests itself in the formation of more or less defined chromosome territories in the interphase eukaryotic cells [89]. Actively maintained bias towards local DNA contacts in the interphase genome, as will be discussed later in the review, facilitates accuracy of DNA repair processes.

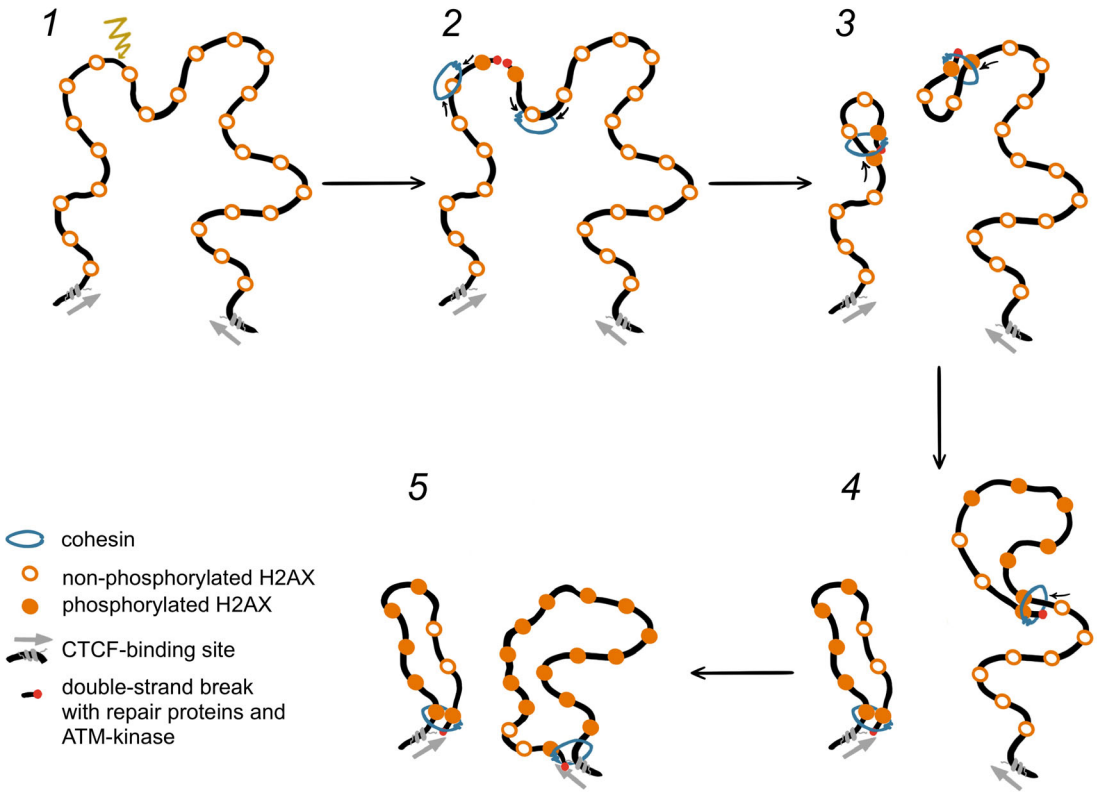

Fig. 4. Cohesin-dependent loop extrusion in the vicinity of double-strand breaks (according to the data reported by Arnould et al. [102]). Emergence of double-strand breaks (*1*, *2*) leads to the formation of new sites of cohesin-dependent extrusion arrest at the break site (*3*), to the spread of the γH2AX-signal from the break site along the topological domain due to unidirectional extrusion (*3*-*5*), and to the repositioning of the break site to the interior part of the chromosome territory (*5*).

The second global consequence of the interphase cohesin-dependent extrusion is maintenance of genomic DNA in decatenated state [90-92]. Similar to decatenation of sister chromosomes, cohesin directs activity of topoisomerase II towards resolving, but not establishing of the interchromosome links. Decatenated chromatin state facilitates realization of the DNA template dependent reactions in interphase [93] and individualization of non-sister chromosomes in mitotic prophase [85].

Double-strand break repair. Cohesin activity enables accurate DNA damage repair, primarily repair of double-strand breaks [94-96]. In addition to cohesion, which facilitates search of homologous recombination partner in G2-phase of the cell cycle [94, 97], loop extrusion is also an important component of the reparative activity of cohesin.

Firstly, the interphase cohesin-dependent extrusion creates and actively maintains specific non-equilibrium folding of eukaryotic chromatin characterized by suppression of *trans* DNA contacts and long-range *cis* DNA contacts [41, 46, 88]. Such bias towards local DNA interactions enables fast and errorless repair of double-strand breaks. This bias facilitates search for partners during non-homologous end joining (NHEJ) DNA repair [96], and also decreases the probability of ectopic recombination during the recombinational repair [98, 99].

Moreover, cohesin is selectively recruited to the double-strand breaks and participates in their repair presumably by limiting diffusion of the break ends and by blocking their interaction with other chromosomes [96, 98, 100, 101]. A number of recent studies showed a clear connection between this function of cohesin and its extrusion activity [99]. It has been suggested that a double-strand DNA break can block movement of a cohesin extrusion complex in a manner similar to CAR-regions and CTCF-sites. Bidirectional extrusion is converted to unidirectional extrusion upon collision with a double-strand break site, thus a loop held by an actively extruding cohesin complex is formed on each side of the break with a break end being one of the bases of this loop (Fig. 4). Such configuration explains how exactly cohesin limits break end diffusion and recruits them to the inner part of the chromosome territory [99]. This process can be likened to the formation of axial structures in metaphase chromosomes via the condensin-dependent loop extrusion. Despite the fact that interphase extrusion does not lead to the formation of chromatid-like structures in the wild type cells, the bases of the cohesin-associated loops still tend to be located in the inner part of the chromosome

territory somewhat similar to the bases of condensin-formed loops of the mitotic chromosomes. It is likely that extrusion exerts similar effect on the ends of double-strand breaks, which become bases of the cohesin-dependent loops. Restriction of break end diffusion and their *trans*-interactions can facilitate accurate restoration of the DNA thread integrity in both NHEJ repair pathway and reparative recombination pathway.

Finally, extrusion is also important for the signaling cascades associated with double-strand breaks, in particular for the spread of γH2AX mark (phosphorylated variant form of H2AX histone) involved in recruitment of repair factors to the genomic region surrounding the break site (Fig. 4) [102-104]. Unidirectional extrusion ensures systematic recruitment of the break ends to the surrounding genomic fragments residing within the same topological domain, which, in turn, enables the spread of γH2AX-signal from the break site. The thing is that activity of the enzymes responsible for the phosphorylation of the H2AX histone (primarily of ATM kinase) is localized strictly to the site of DNA damage itself, and in order for this modification to spread several kbp away in both directions physical recruitment of these loci to the break site is necessary. Such recruitment is enabled by the cohesin-dependent extrusion.

Regulation of transcription in vertebrates. Interphase cohesin-dependent loop extrusion is also important for fine tuning of transcriptional activity in vertebrates. Cause-and-effect relationships between the supranucleosomal DNA folding and transcriptional activity are established in vertebrates due to the existence of activating remote regulatory elements, enhancers, in their genomes[3] [105, 106]. The generally accepted model of enhancer activity assumes that the enhancer-activated initiation of transcription is coupled with the physical interaction between the enhancer and promoter of its target gene. Prevalence of physical interactions between enhancers and promoter of their target genes, enhancer–promoter (E–P) loops, in vertebrate cells have been experimentally shown at the genome-wide scale in recent years [107-109].

Up until recently the notion of the key role of cohesin-dependent extrusion in the formation of E–P loops prevailed [110, 111]. New experimental data, however, contradict this notion: depletion of cohesin and suppression of interphase extrusion do not result in a breakdown of the majority of E–P loops; formation of these structures, most likely, does not depend on extrusion [108, 112, 113]. It cannot be ruled out that cohesin-dependent extrusion is, nevertheless, important for activity of at least some specific types of enhancers – primarily a small group of enhancers containing CTCF binding sites within them [112, 114, 115].

Despite the fact that suppression of the cohesin-dependent extrusion in interphase vertebrate cells does not perturb E–P loop landscape in general, it causes reproducible transcriptional changes. Although the observed effects are mostly marginal, hundreds of genes change the expression level [88, 108]. The observed changes in transcription can be explained by disturbance of activity of CTCF-associated enhancers in only a few cases; at least two alternative mechanisms could explain bulk of the observed changes: (1) insulation effect of TAD boundaries and (2) cohesin-dependent suppression of promiscuous interactions between the active genomic regions.

The majority of topological domains and their boundaries in vertebrates represent an epiphenomenon of cohesin-dependent loop extrusion. Hence, suppression of extrusion could increase frequency of E–P interactions between the regulatory elements, which under normal conditions are separated by the insulating TAD boundaries. Increased intensity of such ectopic E–P contacts likely results in the changes of transcriptional output for some genes [88, 112]. Importance of the regulatory insulation provided by the TAD boundaries is confirmed by the genetic observations: genomic rearrangements causing the disruption of topological insulation have been shown to be associated with the developmental disorders and oncogenesis. For some model systems it has been even demonstrated that specifically ectopic activation of transcription mediates pathological changes caused by the disturbances of TAD landscape [116-118].

The last potential pathway connecting loop extrusion to transcription is related to the fact that cohesin-dependent extrusion suppresses long-range interactions between the active genomic regions. The not-fully understood from biochemical point of view mechanism facilitates clustering of active chromatin within the nucleus, and such clustering results in the formation of active nuclear compartment or A-compartment at the level of cell population [65, 119]. Active chromosomal regions separated by tens of millions of bp, as well as active sites from different chromosomes can interact within the A-compartment. Shutdown of the cohesin-dependent extrusion leads to the uncontrolled increase of interactions between the active genomic regions [42, 50, 88]; it has been suggested that this may result in decreased transcription of some genes and increased transcription of the others potentially due to establishment of super long-range E–P contacts within the A-compartment [88].

[3] In addition to enhancers activating transcription of their target genes, there are silencers that suppress transcription in vertebrate cells; in this review only enhancers will be discussed, as information on silencers is currently scarce, nevertheless, all the described regularities most likely can be extrapolated to silencers.

Hence, loop extrusion in vertebrate cells affects transcription regulation via three separate pathways: via participation in topological insulation, via suppression of excessive genomic compartmentalization, and, to a lesser degree, via direct involvement in the formation of E–P loops.

CYCLE OF CONFORMATIONAL CHANGES OF COHESIN COMPLEX DURING LOOP EXTRUSION

Hypothesis according to which compaction and individualization of mitotic chromosomes are associated with the extrusion of DNA loops has been suggested more than three decades ago [120]. Slightly later Nasmyth [53] suggested that SMC complexes might be the key components of the cellular machinery of extrusion. Over the years this speculative concept of SMC-dependent extrusion has found support in the data on interphase chromatin structure [65, 66, 88], as well as in the results of computer modeling of mitotic and interphase chromosomes [111, 121]. Eventually the ability of cohesin, condensin, and SMC5/6 complex to perform extrusion of DNA loops has been recently demonstrated directly in the reconstituted *in vitro* systems [12, 13, 122, 123].

The accumulated data enabled determination of many specific characteristics of SMC complex-dependent extrusion, particularly cohesin-dependent. It was found out that extrusion can be realized exclusively by the NIPBL containing cohesin complex [122, 123]. The speed of cohesin-dependent extrusion *in vivo* and *in vitro* is ~1 kb/s (estimates in different publications vary from 0.4 to 3 kb/s) [33, 88, 122-124]. Only one cycle of ATP binding/hydrolysis occurs each second during cohesin-dependent extrusion [123, 124]. High speed and the step size as large as ~1 kb (which is equivalent to tens of nanometers even for the nucleosome-packed DNA) distinguishes SMC complexes from other classes of DNA translocases (polymerases, helicases, etc.), speeds of which are lower by several orders of magnitude and typical step size is equal to 1 bp. Despite the impressive speed of cohesin-dependent extrusion, even relatively weak forces (<1 pN) applied to DNA thread can slow down or even completely block the process [122, 125].

Observations of extrusion *in vitro* additionally showed that monomeric cohesin ring performs bidirectional extrusion [Fig. 5a (*1*)] [33, 123, 125], while the closest cohesin homolog – condensin – performs unidirectional extrusion [Fig. 5a (*2*)] [12, 126]. Directionality is one of the most interesting characteristics of the extrusion process; it is intimately connected to the molecular mechanism of the process. Emerging DNA loop can theoretically grow due to the pulling of the DNA thread from one side of the extruding SMC complex, in this case extrusion is called unidirectional or asymmetric. In this situation two poles of the loop can be distinguished: fixed (stable) base, or anchor, and translocating (mobile) base. Another possible variant of extrusion is bidirectional or symmetric extrusion in which the loop grows due to the pulling of DNA inside the loop from both sides of the active protein complex. In the case of cohesin-dependent extrusion the bidirectional variant of the process is realized. From the molecular point of view apparently bidirectional extrusion can be the result of asymmetric activity of SMC complex coupled with frequent switching of the movement direction between the individual cycles of ATP binding/hydrolysis [126-128]. In such a process of "switching" bidirectional extrusion, in each ATP hydrolysis cycle the growing loop has a fixed and a translocating base, but in each subsequent cycle the bases can switch the roles.

The covalently linked SMC rings can realize extrusion *in vitro* and form loops on par with the wild type complexes [69, 123]. Moreover, the mutant forms of cohesin incapable of maintaining cohesion and, most likely, incapable of topological engagement with DNA, can nevertheless be capable of loop extrusion in cells [8, 18]. Hence, opening of the SMC ring is probably not an essential step of the extrusion process: cohesin (and other SMC complexes) interact with DNA non-topologically during extrusion (not holding any of the two bases of the growing loop inside the intersubunit pore and not forming true topological links with DNA) (Fig. 5b).

Capability of the complex to traverse DNA-bound particles with sizes manifold larger than the linear sizes of the complex itself during the process of loop growth was an unexpected observation during the study of cohesin-dependent extrusion [69]. It was shown that during active extrusion cohesin can bypass DNA-associated particles with diameter of up to 200 nm. Interestingly such bypassing of the obstacles occurs with a minimal slowdown of the complex movement along the DNA. Traversing massive obstacles indirectly indicates that the process of extrusion has a non-topological nature [69, 129]. Non-topological nature of extrusion has been also indirectly supported by the following observation: binding of cohesin to DNA at the bases of chromatin loops formed during extrusion are disrupted in the permeabilized nuclei incubated in buffer with comparatively mildly increased ionic strength [130].

Despite the fact that many general characteristics of the cohesin-dependent loop extrusion have been documented in the recent years (especially substantial progress was achieved due to the development of *in vitro* reconstituted systems of extrusion), molecular mechanism of extrusion is still not completely elucidated.

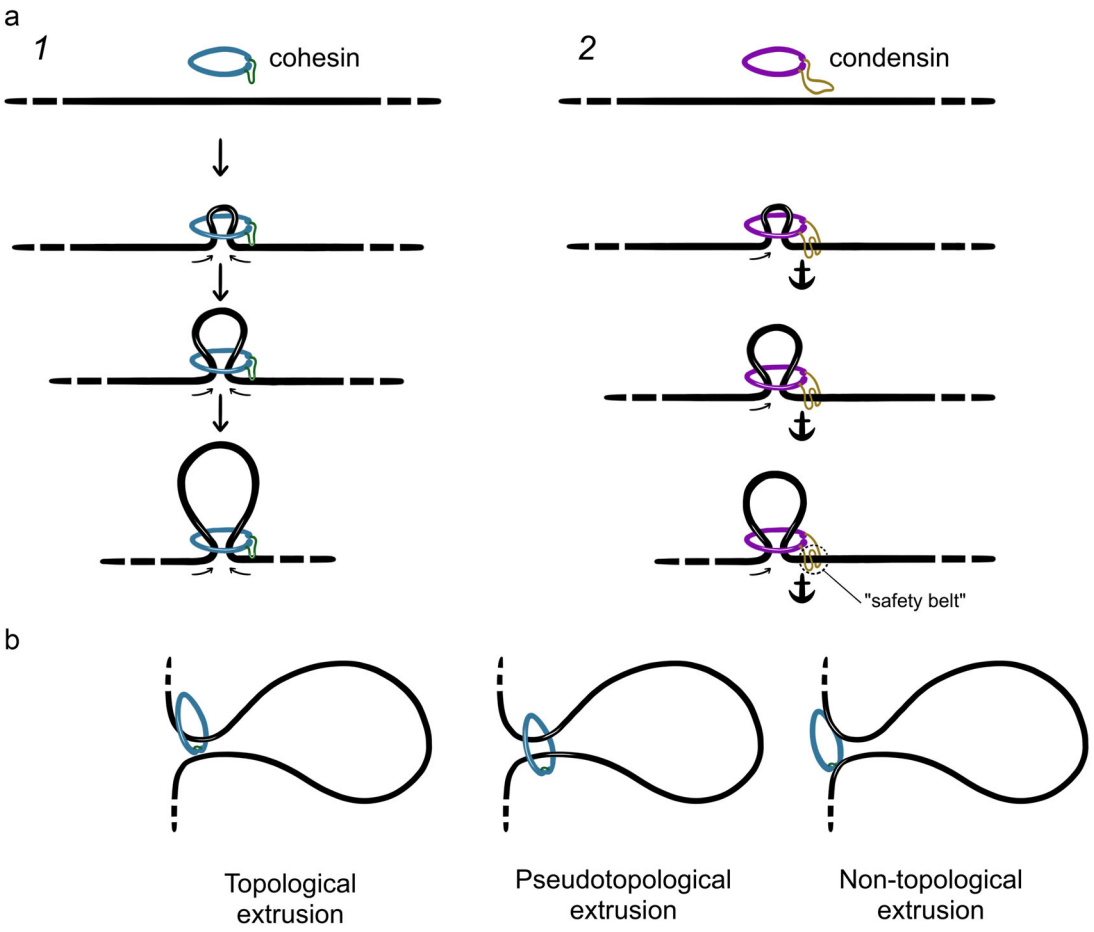

Fig. 5. Molecular details of SMC-dependent extrusion. a) Bidirectional extrusion realized by cohesin monomers (*1*), and unidirectional extrusion realized by the condensin complexes (*2*). 'Safety belt', likely, stabilizes binding of the complex to one of the bases of the growing loop during condensin-dependent extrusion. b) Hypothetical modes of SMC complex binding to DNA of the growing loop during extrusion.

We do not know how exactly the cycle of ATP binding/hydrolysis is coupled with conformational changes of the complex, and how these changes orchestrate the growth of DNA loops. Due to considerable interest to this problem and due to insufficient amount of experimental data, several competing models of the process have been suggested. Three such models, which are in greatest agreement with the available structural, genetic, and biochemical data, are: pumping/hold-and-feed model, Brownian ratchet model, and swing-and-clamp model. Below, brief description of each of these models is provided. It must be emphasized that despite the elegance and impressive explanatory power of each of the discussed model, none of them is in agreement with the whole entirety of the available experimental data.

Gripping state/DNA clamping. Despite the fact that the models of extrusion process described below differ in numerous significant parameters, one of the crucial intermediates of the extrusion cycle – the so-called DNA 'gripping state' – is common for all the models. DNA gripping has been described in the very recent years as a result of investigation of cohesin and condensin structures with the help of cryogenic electron microscopy [61, 126, 131]. It was discovered that in the presence of NIPBL, double-stranded DNA, and non-hydrolyzable ATP analogs (or alternatively if cohesin contains SMC subunits with mutations preventing ATP hydrolysis) cohesin forms specific type of stable complexes with DNA called 'DNA gripping state'. In the gripping state DNA electrostatically binds to the upper surface of the head domains engaged in the presence of ATP (Fig. 6a). NIPBL interacts with the arm region of the SMC3 subunit and simultaneously with the dimerized head domains thus forming a protein bridge pinning down the DNA thread to the head domains from above. NIPBL in the described structure also forms a series of electrostatic contacts with DNA. The data obtained using FRET (Förster Resonance Energy Transfer) indicate that in the presence of hydrolysable ATP the gripping state is rapidly formed and disassembled [124]. It is likely that this cycle of formation and disassembly is strictly coupled to the ATP hydrolysis cycle: ATP hydrolysis leads to the disruption

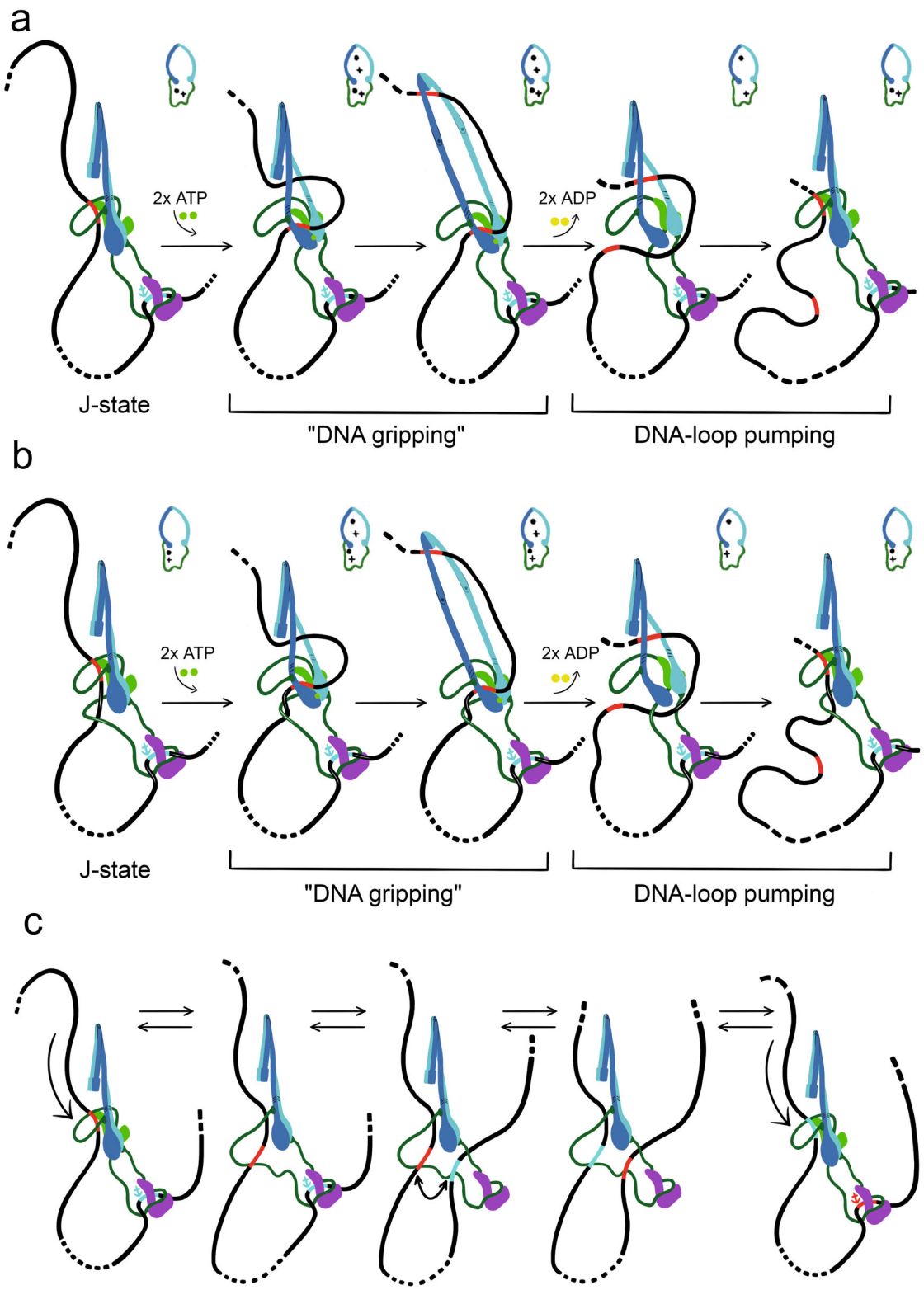

Fig. 6. Cohesin-dependent extrusion according to the pumping/hold-and-feed model. a) Pseudo-topological variant of the extrusion pumping/hold-and-feed mechanism (according to the data reported by the Hearing group [126]). b) Non-topological variant of the extrusion pumping/hold-and-feed mechanism (according to the data reported by Oldenkamp and Rowland [10]). c) Exchange of the two bases of the growing loop during pseudo-topological extrusion could enable frequent switch of the movement direction and be manifested as an apparent bidirectionality of the process. In all pictograms direction of DNA thread relative to the figure plane is shown with • and + symbols. DNA sites, which are or were at previous stages dynamic bases of the loop are shown in red, and stable bases – in blue. Dashed line fragment of DNA thread reflects potentially unlimited size of the growing loops.

of the head domain dimer, which contains an extended DNA-binding groove on its surface. This probably also leads to the dissociation of NIPBL and eventually to the release of the clamped DNA fragment. Binding of a new pair of ATP molecules by the head domains results in reassembly of the gripping state. The described coupling of ATP hydrolysis cycle and reversible DNA binding to cohesin in the gripping state clearly indicates that this structure may play an important role in the translocation of cohesin along the DNA during extrusion. However, the described static structure of DNA gripping state does not tell anything about the structural rearrangements leading to the directional movement of cohesin and binding of new fragments of the DNA thread in each next cycle in the gripping state formation.

Pumping/hold-and-feed model. One of the prominent structural characteristics of DNA gripping is disengagement of the arm domains of the two SMC subunits [61, 132]. It is known that after ATP hydrolysis the interaction between head domains of SMC subunits can be rearranged resulting in the formation of the so-called juxtaposed state (or J-state), which leads to the closing of intersubunit pore and establishment of tight interactions between the arm domains along their entire length [133, 134]. There are some indications that the closing of the intersubunit pore during transition to the J-state occurs processively from top to the bottom similarly to zipper closing [135, 136]. The pumping/hold-and-feed model suggests that the translocation of cohesin complex along DNA is mediated through repeated cycles of arm domain 'zipping' coupled with the cycles of ATP hydrolysis (Fig. 6a) [136, 137]. The most elaborate version of this model recently presented by the Haering group [126] is based on the cryogenic electron microscopy data, as well as data regarding entrapment of DNA threads within different subcompartments of the SMC ring obtained with the help of thiol-specific complex cross-linking.

This model suggests that one of the DNA-bases of the growing loop is anchored on STAG1/2 (Fig. 6a). DNA of the loop mobile base is held by NIPBL close to the SMC head domains. Translocation of the complex along DNA occurs due to the fact that formation of the gripping state is mechanically coupled with threading of the DNA micro loop through the intersubunit pore. ATP hydrolysis leads to coalescence of the captured micro loop with the main DNA loop held in the vicinity of the head domains of the complex. Coalescence results in substitution of the original mobile base of the loop with a new one located downstream. DNA site electrostatically bound to the inner surface of the dimerized hinge domains during initial capture of the micro loop assumes role of such new mobile base. This site is handed over to the NIPBL subunit in the course of 'zipping' of arm domains. 'Zipping' returns the complex to the initial J-state, while the loop gets longer than the original one due to the absorption of micro loop in the course of this process.

There are two principal variants of the pumping/hold-and-feed model: pseudo-topological variant in which each of the two bases of the growing loop are threaded through the cohesin pore while the complex is in the J-state (Fig. 6a), and non-topological variant in which the entire growing loop interacts with the complex in a non-topological manner (Fig. 6b).

The pseudo-topological variant of the model is in agreement with many experimental observations made in the reconstructed *in vitro* extrusion systems. Firstly, the possibility of efficient micro loop capture depends of the mechanical tension of the DNA molecule. This explains how even small mechanical forces (~ 1 pN) are capable of preventing micro loop capture and therefore of blocking extrusion [122, 125]. Secondly, in *in vitro* systems cohesin complexes perform bidirectional extrusion [123, 125]. Pseudotopological variant of pumping/hold-and-feed model suggests that dissociation of the NIPBL subunits from the complex (occurring periodically during extrusion) can result in formation of a single subcompartment holding both bases of the growing loop. It is assumed that interaction of the STAG1/2 subunit with the loop anchor also can be periodically disrupted (Fig. 6c). Restoration of binding of the loop bases to the HAWK subunits could result in the exchange of the two DNA threads, which is equivalent to the change in extrusion direction. Hence, the apparent symmetry of cohesin-dependent extrusion can be explained as a result of periodically occurring change in the direction of asymmetric process. The model also explains why the wild type condensin is a strictly unidirectional extruder [12, 126]. Presumably unidirectionality of condensin is ensured by the additional strength of the loop anchor binding to the complex; this strength is provided by the 'safety belt' – structural feature absent in the kleisin subunit of cohesin (Fig. 5a). Mutations destabilizing the 'safety belt' convert condensin into a bidirectional extruder similar to cohesin [126].

The pseudo-topological variant of the model, however, contradicts at least one crucial experimental observation: the ability of the complex to traverse massive DNA-binding particles in the course of *in vitro* extrusion [69]. In order to resolve this contradiction, a non-topological variant of the model was suggested [10]. It was found out that the vast majority of empirical observations, underlying initial pseudo-topological variant of the model, could be equally well explained under assumption that the growing DNA loop is not threaded through the complex (Fig. 6b). This variant of the model suggests that the mobile base of the loop itself is bound to the complex as a small pseudo-topological loop formed due to interaction of the

NIPBL subunit with the juxtaposed head domains. Upon ATP hydrolysis this small loop coalesces with the micro loop captured inside the intersubunit pore, next it is tightened to its initial size due to the 'zipping' of the arm domains. Such process, unlike the mechanism suggested in the pseudo-topological model, is in agreement with the observed phenomenon of the cohesin complex bypassing massive obstacles during extrusion.

Paradoxically, non-topological variant of the pumping/hold-and-feed model explaining the ability of cohesin to traverse obstacles, cannot explain bidirectional nature of the cohesin-dependent extrusion: hypothetical exchange of the DNA threads is only possible inside a single pseudo-topological compartment holding both bases of the growing loop. The majority of the attempts to describe molecular details of extrusion face this fundamental problem: impossibility to reconcile in one model the bidirectional nature of extrusion and the ability of the complex to bypass massive barriers. Pseudo-topological models usually cannot explain traversing of massive barriers, while the non-topological ones cannot be reconciled with the bidirectional nature of the process. Some authors, however, suggest that some variants of non-topological models may under certain assumptions include exchange of DNA threads, switch of the extrusion direction, and, eventually, bidirectional nature of the process [128].

Brownian ratchet model. Two other models described below belong to the class of extrusion models based on the bending movement of 'elbows' (scrunching models). These models assume that the 'elbow' bending and subsequent engagement of hinge domains with head domains is somehow coupled with the transfer of the mobile base of the loop from one pair of the domains to another [134, 138]. Unlike the pumping/hold-and-feed model in which the key conformational change coupled with the ATP binding/hydrolysis cycle is 'zipping' of the intersubunit pore, scrunching models of extrusion assume that the key conformational change enabling complex translocation is reversible bending of the 'elbows'.

This class of models rely on the ability of cohesin and other SMC complexes to assume conformations in which the 'elbows' are fully bent, and head and hinge domains are close to each other [134, 139]. The cryogenic electron microscopy data showed that such bending could be theoretically coupled with ATP binding and formation of DNA gripping state [60, 61, 131]. Additional structural data obtained with the help of atomic force microscopy, FRET, covalent cross-linking of the complexes, and observations of the ability of mutant complexes to form loops *in vitro* enabled the development of two most detailed scrunching extrusion models: Brownian ratchet model [127, 131] and swing-and-clamp model [124].

In the Brownian ratchet model "elbow" bending results in pulling of pseudo-topological DNA loop through the cohesin ring. The model was developed by the Uhlmann group [127, 131] as a result of analysis of one of the first cohesin gripping state structures. The authors noticed that in the gripping state structures resolved with the help of cryogenic electron microscopy STAG1/2 binds to DNA in vicinity to the clamped site, and that due to the elbow bending, dimer of the hinge domains is close to the STAG1/2 (Fig. 7a). Additional FRET-experiments demonstrated (these results were not however confirmed by the later studies) that proximity between the hinge domains and STAG1/2 in the complex has constitutive nature. Hence, Brownian ratchet model suggests that cohesin has two DNA-binding modules: head module associated with NIPBL, and hinge module associated with STAG1/2. Unlike the pumping/hold-and-feed model and the swing-and-clamp model, the Brownian ratchet model suggest that both HAWK subunits bind to the mobile base of the growing DNA loop, while retention of the loop anchor site within the complex occurs passively due to pseudo-topological interaction of the complex with the DNA loop.

The model assumes that disruption of DNA gripping caused by ATP hydrolysis results in straightening of the 'elbows' and destabilization of interaction of the STAG1/2-hinge module with DNA. This destabilization eventually leads to the loss of interaction, however, before the complete disruption of the bond between the STAG1/2-hinge module and DNA occurs, the 'elbows' have time to straighten up to at least some extent (Fig. 7a). Elbow straightening results in the growth of the captured pseudo-topological loop due to the movement of the complex along one of the DNA threads – unidirectional extrusion. Pseudo-topological interaction of the complex with DNA allows complex to hold the growing loop even after the complete loss of electrostatic interactions. The growing DNA loop is held pseudo-topologically inside the intersubunit pore up to the moment of ATP binding and assembly of the new DNA gripping state.

It is generally accepted that the elbow straightening is a reversible equilibrium process realized through the thermal motion; directional nature of extrusion is ensured in this model by the ratchet mechanism: power stroke movement always starts at the gripping state with elbows completely bend. These two features of cohesin movement in the Brownian ratchet mechanism are reflected in the name of this model.

Similar to the pseudo-topological variant of the pumping/hold-and-feed model, the Brownian ratchet mechanism explains bidirectional nature of the cohesin-dependent extrusion by exchange of the DNA threads of the growing loop, which potentially could occur in each cycle of ATP binding/hydrolysis at the

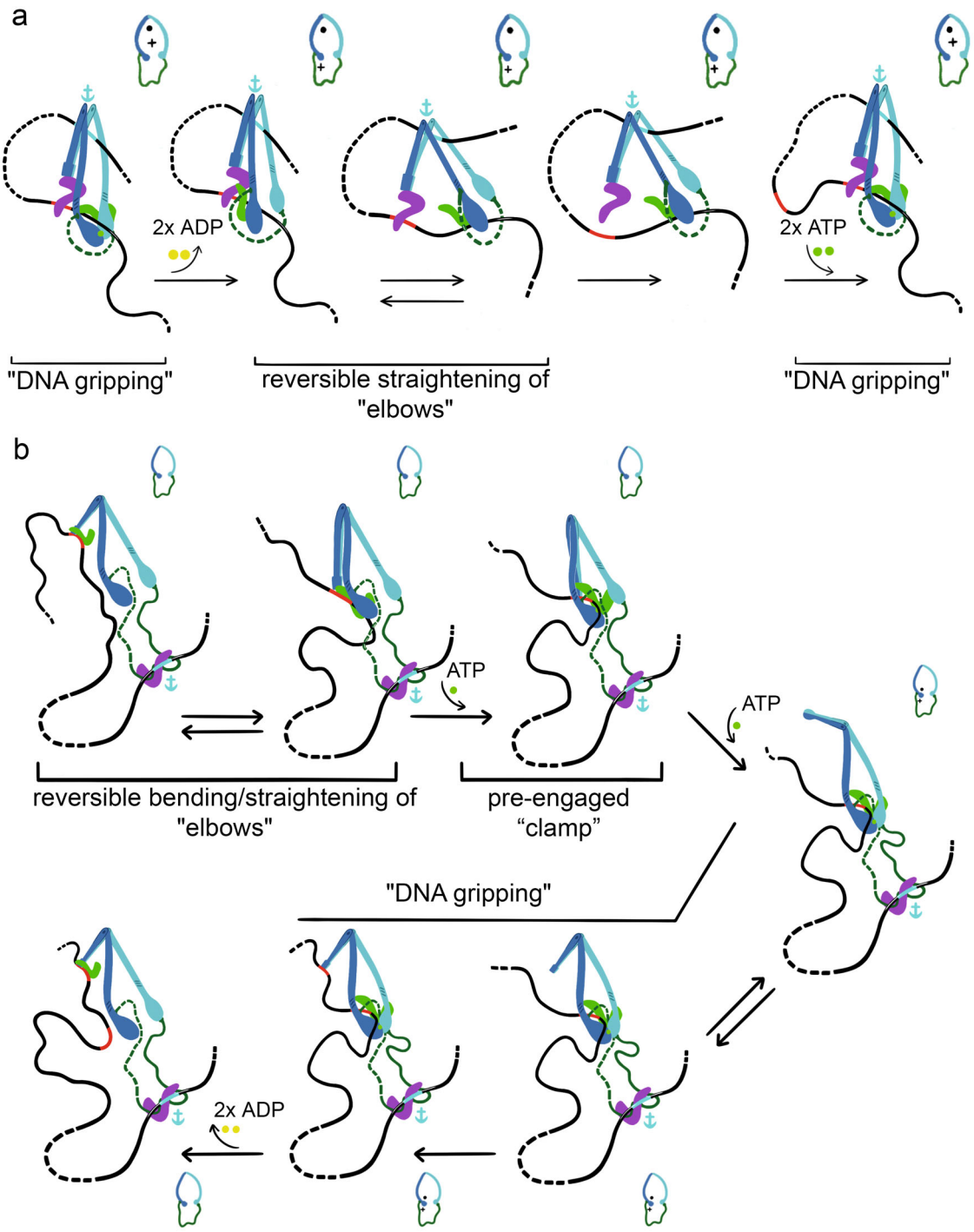

Fig. 7. Cohesin-dependent extrusion according to the 'scrunching' models. a) Extrusion according to the Brownian ratchet mechanism (according to the data reported by Higashi et al. [127]). b) Extrusion according to the swing-and-clamp mechanism (according to the data reported by Bauer et al. [124]). Fragments of RAD21 subunit depicted by dashed lines correspond to the regions in which path of the protein chain is shown arbitrarily to increase figure clarity (in reality HAWK subunits remain bound to RAD21 during all the presented stages). All other designations as in Fig. 6.

stage of passive trapping of the pseudo-topological loop inside the cohesin ring. Equilibrium character of the 'elbow' extension explains why even weak external forces completely block the SMC dependent extrusion *in vitro*.

There are however two significant drawbacks of the Brownian ratchet model: (1) impossibility to reconcile pseudo-topological extrusion with experimentally observed ability of cohesin to bypass massive barriers [69] and (2) absence of an independent validation for

the existence of the stable STAG1/2-hinge DNA-binding module.

Swing-and-clamp model. Another extrusion model based on 'elbow' bending proposed by the Peters group [123] suggests that binding of DNA by the hinge domains and bending of 'elbows' ('swing') precedes formation of gripping configuration, hence, this model was named 'swing-and-clamp' [124]. The model is based on two key observations obtained using FRET technique: 1) NIPBL protein constitutively interacting with RAD21 can also form temporary ATP-dependent contacts with other subunits of the complex and 2) despite the relative freedom of bending/straightening movements of arm domains, physical interaction between the hinge and head domains appears to be not compatible with the gripping; this means that 'elbows' during gripping are always in a more or less straightened state. This model suggests non-topological mode of extrusion with the stable base of the loop anchored at STAG1/2 (as in the pumping/hold-and-feed model). Move of the mobile base of the loop relative the SMC complex occurs due to transfer of DNA from the hinge domains to the head domains during formation of the DNA gripping state and due to the subsequent straightening of the 'elbows' (Fig. 7b). The DNA transfer occurs during the formation of DNA gripping state and is coupled with the switch in NIPBL–SMC interaction pattern. Prior to the formation of the gripping state NIPBL is bound to the hinge domains, and together they hold DNA of the loop mobile base. Assembly of the gripping state is associated with the transfer of both the loop mobile base and the NIPBL subunit holding it from the hinge domains to the head domains; in the process, hinge domains lose their interaction with NIPBL. Immediately afterwards straightening of the 'elbows' occurs due to allosteric effects of DNA gripping formation. It is assumed that prior to ATP hydrolysis and subsequent disassembly of the gripping state the hinge domains bind a downstream DNA site, which eventually becomes the new mobile base of the growing loop. Disassembly of the gripping state results in dissociation of NIPBL from the head domains and its reverse jump to the hinge domains thus beginning a new cycle of conformational changes. Hence, contrary to the Brownian ratchet model, in the swing and clamp model the mobile base of the loop is electrostatically bound to one or another DNA-binding surface of cohesin over the entire duration of the cycle. Such continuous contact is indispensable for non-topological maintenance of the growing DNA loop.

In addition to being in agreement with numerous structural data swing-and-clamp model also provides clear and specific explanation of how the processes of elbows bending/straightening are coordinated with the cycle of ATP binding/hydrolysis and with the transfer of the DNA thread from the hinge domains to the head domains. Bending of arm domains in this model is a prerequisite for the formation of DNA gripping state and ATP hydrolysis; DNA gripping state formation, in turn, is coupled with the transfer of the mobile loop base from one binding site to another. At least partial expansion of the elbows, which always precedes binding of the new mobile base by the hinge domains, ensures directional movement of the extrusion complex. It should be mentioned that in the swing-and-clamp model, like in the Brownian ratchet model, bending/straightening of the arm domains mediating movement of the cohesin complex along the DNA is an equilibrium process, which accounts for high sensitivity of the extrusion speed to external forces [122, 125]. Non-topological nature of extrusion postulated in this model is in agreement with the ability of extrusion complex to bypass massive DNA-bound particles [69]. At the same time, the swing-and-clamp model predicts exclusively unidirectional mode of extrusion, which contradicts the observations made in the reconstituted *in vitro* systems [123, 125].

COHESION AND COHESIN-DEPENDENT LOOP EXTRUSION MIGHT BE MECHANISTICALLY RELATED

From the time of its discovery and up until now cohesin is better known as a complex ensuring cohesion of sister chromatids through its ability for topological loading onto DNA. The fact that cohesin is also a DNA translocase capable of formation DNA loops via the extrusion mechanism is often considered as a minor detail, secondary addition to its primary cohesive function. Ever increasing number of cellular processes shown to be reliant on the cohesin-dependent loop extrusion unambiguously demonstrates the fallacy of such view: extrusion is along with cohesion one of the fundamental activities of cohesin. This point of view is in good agreement with the evolutionary primitiveness of extrusion which is known to be a feature of almost all SMC complexes [12-14, 123]. From phylogenetic point of view cohesive pairing of sister chromatids has likely emerged as an adaptation of the primordial cohesin to perform an additional function not typical for other related complexes [15-17]. Thus, one of the unresolved mysteries of cohesin biology, namely, whether cohesion and extrusion are parts of the single molecular pathway draws even more attention.

Many authors intuitively assume positive answer to this question [61, 127]. Nevertheless, even the most general scheme of such a pathway still remains a controversy. Development of the concepts regarding the nature of this pathway turned out to be closely intertwined with the ideas about the mode of cohesin binding to DNA during extrusion. The most general

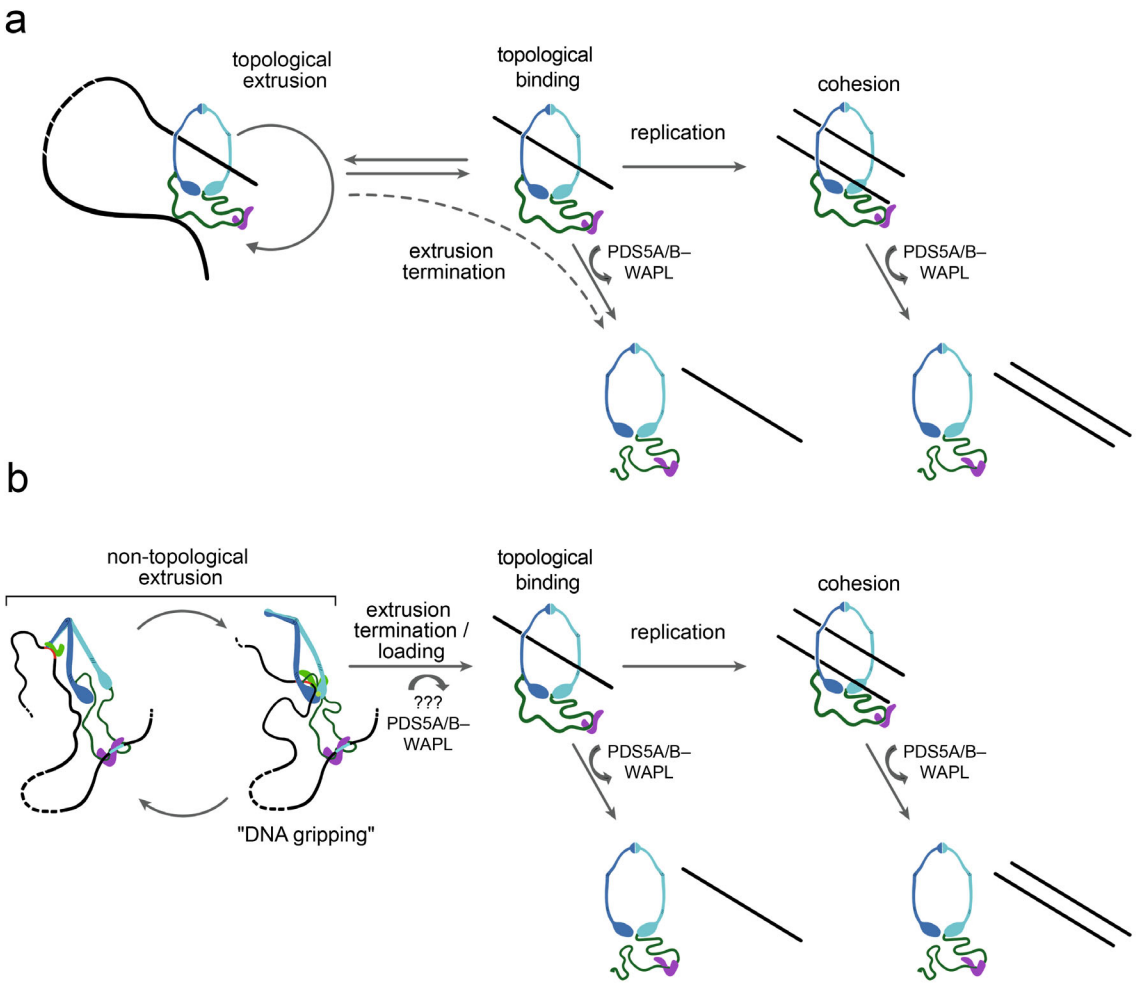

Fig. 8. Scheme of the hypothetical molecular pathway which includes both cohesin-dependent extrusion and cohesion. a) The scheme of originally proposed pathway of this kind, suggesting equivalence between topological cohesin loading and extrusion initiation. b) Scheme of the modified version of such a pathway taking into account the accumulated structural data on extrusion process: topological cohesin loading is suggested to be coupled with termination of extrusion. DNA gripping state plays here a key role in the switch between extrusion and topological engagement.

indication on the existence of mechanistic interplay between the extrusion and cohesion is the fact that the PDS5A/B subunit and WAPL protein participate in both removal of cohesive rings from DNA [47, 48], and in extrusion termination [41, 42, 44, 51]. Initially this observation prompted a hypothetical scheme in which extrusion is realized by the cohesin rings topologically loaded onto DNA, and these rings may be converted into cohesive complexes during replication (Fig. 8a).

However, accumulation of new data resulted in rejection of this naive model. We are primarily referring to the structural data indicating that extruding cohesin complexes are not topologically engaged with DNA [69, 123, 130]. These structural data have later received genetic validation: it was shown that certain mutations in SMC subunits can suppress ability of the complex for topological loading not affecting its capacity to extrude DNA loops [8, 18]. Today, when it can be assumed with sufficient certainty that extrusion follows either non-topological or pseudo-topological mechanism, another concept has come to prominence. According to this concept cohesin topological loading (and subsequent cohesion establishment) is just an alternative pathway for termination of extrusion-associated ATP hydrolysis cycle. Many authors suggest that DNA gripping as a key intermediate stage of the ATPase cycle where the choice between continuation of the extrusion and topological loading eventually resulting in extrusion termination is made (Fig. 8b) [61, 127]. The role of the PDS5A/B–WAPL tandem might be then in tipping the reaction towards termination of extrusion cycle and cohesin topological engagement with DNA [130]. This hypothetical scenario implies that the ability of the PDS5A/B–WAPL subcomplex to open cohesin ring is crucial for two different reactions taking place in the cell nucleus: cohesin topological loading/termination of extrusion and removal of topologically engaged cohesin rings from chromatin.

CONCLUSIONS

For many years DNA loop extrusion was more of a speculative concept; even then though some authors emphasized that only extrusion could explain a number of cellular processes such as decatenation of sister DNA molecules [53]. Last decade was the time of accumulation of first indirect and next direct experimental confirmations that this process indeed happens in cells, and SMC complexes plays the central role in this process [12, 66, 111, 123]. We have arguably witnessed an extremely rare event: validation of theoretical prediction in biology.

Structural studies of SMC complexes, analysis of molecular factors affecting SMC-dependent loop extrusion, and microscopic observation of extrusion in *in vitro* systems significantly clarified our understanding of the nature of the process. Let us mention the most significant observations regarding cohesin-dependent loop extrusion reported in recent years: 1) bidirectional nature of the process [122, 123]; 2) non-topological or pseudo-topological nature of binding of extrusion complex to DNA [8, 18, 69, 123, 130]; 3) high speed of loop growth reaching several kbp per second [33, 88, 122-124]; 4) key role of NIPBL subunit in the process of active extrusion [42, 122, 123]; 5) periodic ATP-dependent formation of DNA gripping state during the cycle of conformational changes associated with extrusion [60, 61, 131]; 6) participation of PDS5A/B and WAPL (recruited by the former) in termination of the process [41, 42, 44, 51]; 7) arrest of extrusion at the specific genomic regions, CTCF-sites and CAR-regions, resulting in formation of metastable loops anchored in these regions [44, 58, 59]. These results, despite their exceptional importance, are just a series of more or less disparate facts that do not add up to form a clear unified picture. The attempts to develop such a universal picture have been already made [140]. The most important component of such picture should be detailed description of molecular rearrangements of the complex during extrusion. In this review we among other things described the three most convincing molecular models of extrusion; each of the presented models being more or less speculative and in only partial agreement with the available experimental data. We also address the intriguing and poorly understood question of mechanistic relationships between cohesion and extrusion. Additional data that would be obtained in the near future hopefully can help to fill up the existing gaps in our understanding of loop extrusion mechanics and enable creation of a clear, unified, and experimentally grounded model of the process, such model seems to be essential for dispelling the mystery of cohesin, two-faced Janus of the eukaryotic chromosome biology.

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