Cohesin in Determining Chromosome Architecture

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Cells use ring-like structured protein complexes for various tasks in DNA dynamics. The tripartite cohesin ring is particularly suited to determine chromosome architecture, for it is large and dynamic, may acquire different forms, and is involved in several distinct nuclear processes. This review focuses on cohesin’s role in structuring chromosomes during mitotic and meiotic cell divisions and during interphase.

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SMC proteins complexes: universal chromosome organizers

Roughly one-fourth of all of our protein-encoding genes code for proteins involved in genome maintenance and cell division, which illustrates the enormous complexity and effort that eukaryotic cells have evolved to divide and faithfully transmit their genomes to the next generation. While many elaborate concepts describing these processes have been formulated and a wealth of information has been accumulated about many fundamental events of cell division, there are still huge gaps in our knowledge concerning, for example, chromosome architecture and dynamics.

Even though condensed metaphase chromosomes are known already to school-kids through their textbooks, the layers of structural organization that are required to assemble and partition these chromosomes remain poorly understood. Heterochromatin versus euchromatin, centromeric versus chromosomal arm organization, intergenic versus genic regions, repetitive versus non-repetitive elements, nucleolar or nuclear-envelope-associated regions and many other chromosomal features define chromosome architecture in space and time. It is therefore not surprising that cells have evolved a sophisticated molecular machinery to manage this complex level of organization.

Among the major chromosome organizers is a ubiquitous family of protein complexes based on structural maintenance of chromosomes (SMC) proteins, whose unique structural features make them particularly suited for handling an extensive polymer such as a chromosomal fiber. This was realized quickly after the first description of SMC proteins in 1993 [1], and SMC proteins were subsequently suggested to function as motor proteins, clamps, or crossties that centrally contribute to chromosome structure [2-5]. SMC proteins feature two globular domains at the ends of a ~45 nm long intra-molecular coiled coil that both serve for SMC protein dimerization (Fig. 1A). Specific pairs of SMC proteins form via a high-affinity interaction between the “hinge” domains at one end of the coil “arms”. At the same time, the ATPase “head” domains at the other end can dynamically associate and dissociate upon binding and hydrolysis of ATP, respectively [6]. A so-called kleisin protein further connects the two head domains to form a closed ring-like structure (Fig. 1A). This large ring architecture seems ideal to clasp chromosomes inter- or intra-molecularly between the SMC arms in order to tie them up.

The principle of entrapping DNA within a ring is certainly not unique to SMC complexes. A number of proteins that manipulate nucleic acids, including for example replicative helicases such as the MCM licensing factors, RNA helicases such as the Rho transcription terminator, or DNA replication processivity factors such as PCNA or the β–subunit of prokaryotic DNA polymerase III form rings with a six-fold symmetry and a central hole large enough encompass a double helix (Fig. 1A) [7]. Similarly, DNA mismatch repair proteins like MSH2 and MSH6 can form sliding clamps, i.e. rings that, once they hit a mismatched base-pair, may move further and recruit more clamps [8]. A ring architecture is in general well suited whenever protein complexes need to move along DNA, as rings can – in principle – rapidly slide along nucleic acid strands for long distances without falling off. What makes SMC complexes unique is their ring diameter, which is at least an order of magnitude larger than...
the rings of the above mentioned complexes, allowing SMCs to topologically encircle not only one DNA helix but two DNA helices, which may even be wrapped around nucleosomes. In this brief review, we discuss how a particular SMC complex named cohesin can exploit this mode of action to determine the architecture of chromosomes and draw parallels to the function of the related condensin, SMC5/6, and prokaryotic SMC complexes that are described in other articles of this special issue.

**Cohesin holds sister chromatids together**

The cohesin complex was first identified in genetic screens that aimed to identify proteins required for holding together sister chromatids [9, 10]. Biochemical and structural studies demonstrated that cohesin's kleisin subunit SCC1 (also named RAD21 or MCD1) simultaneously binds to both head domains of an SMC1/SMC3 heterodimer and to a fourth subunit that is predicted to be largely composed of HEAT-repeat motifs (named SCC3 in yeast and present in two isoforms named SA1 and SA2 in metazoan cells) (Fig. 1B) [11, 12]. In germ cells, some of these subunits can be replaced by meiosis-specific versions (see below).

Is there experimental proof that cohesin binds chromosomes by entrapping chromosomes within the ring-shaped structure formed by its SMC1, SMC3, and SCC1 subunits following the principle outlined in the introduction? The findings that (a) opening of the cohesin ring by site-specific proteolytic cleavage of SCC1 or SMC3 is sufficient to release cohesin from chromosomes and to destroy sister chromatid cohesion [13-16], (b) linearization of circular minichromosomes releases their association with cohesin in vitro [17], and (c) covalent connection of the interfaces between the three ring subunits renders minichromosome-cohesion resistant to protein denaturation [18, 19] are certainly compatible with the idea that cohesin rings encircle and slide along the chromatin fiber. In addition to entrapping chromosomal DNA topologically, cohesin may at least temporarily establish direct protein-chromatin contacts, for example during loading of cohesin onto chromosomes, or at specific chromosome loci [20]. This notion is consistent with the binding of isolated cohesin complexes or SMC proteins to DNA in vitro [21-23]. Other SMC protein complexes such as condensin, which also encircles chromosomal DNA within its ring structure [24], presumably make even more extensive high-affinity contacts with the chromatin fiber than cohesin [6].

If cohesin binds chromosomes topologically as the evidence suggests, cohesin rings could either hold sister chromatids together by entrapping both sisters within the same ring (Fig. 2A, top) or by the interaction of two or more cohesin rings that each entrap a single chromatid (Fig. 2A, bottom). While further work may be required to prove either alternative right [25], both models require that cohesin rings need to open transiently to allow entry and exit of the DNA helices. The observation that linkage of the SMC1–SMC3 hinge domains, but neither of the interfaces between SMC1 and SCC1 or SMC3 and SCC1, prevents cohesin loading onto chromosomes in yeast implies that the hinge domains form an entry gate for DNA to pass into the cohesin ring [26]. Cohesin’s loading onto chromosomes depends on SMC1–SMC3’s ATPase activity [27, 28] and a separate complex composed of SCC2 (also called MIS4 or NIPBL) and SCC4 proteins [29-32], which might control opening of the entry gate. Once DNA has been entrapped, ring re-opening may be prevented by cohesin sliding away from SCC2–SCC4 binding sites (reviewed in [33]). This model predicts that the population of cohesin that can be detected at SCC2–SCC4 sites [34-37] would be less stably bound to chromosomes than cohesin at loci away from SCC2–SCC4, which remains to be tested.

Any topological cohesin model faces the challenge that it needs to explain how cohesin rings specifically entrap sister

*Fig. 1 – Architecture of SMC proteins and the cohesin complex. (A) A ~45 nm long intra-molecular coiled coil separates an ATPase “head”, formed by the association of N- and C-terminal globular domains, from a central “hinge” that serves for SMC protein dimerization. Two SMC head domains can associate upon sandwiching two ATP molecules, resulting in a circular SMC architecture with a diameter of 30 nm or more, which is significantly larger than that of other ring-shaped DNA binding protein complexes shown to scale. (B) In cohesin complexes, the kleisin protein SCC1 connects the head domains of an SMC1–SMC3 dimer and recruits the HEAT-repeat containing subunit SCC3. Acetylation of SMC3’s head domain by the ECO1 acetyl-transferase counteracts the chromatin-releasing activities of the WAPL–PDS5 complex.*
chromatids and not just random chromosomes. The finding that, at least in an unperturbed cell cycle, establishment of cohesion is coupled to DNA replication may provide an elegant solution to this problem [38]. If cohesin rings were only able to connect two DNAs – be it by entrapment of both DNAs within the same ring or by connection of two rings that each bind to one DNA molecule – at active replication sites, then this would ensure that stable cohesive linkages form exclusively between the adjacent sister DNAs as they emerge from the replication fork. Since the turnover rate on chromosomes of a fraction of cohesin dramatically decreases post-replication [39], replication may promote the permanent closure only of cohesin rings that encircle sister chromatids. What could be the molecular basis for such a mechanism? Recent work suggests that acetylation of two conserved lysine side chains in the SMC3 head domain by the acetyl-transferase ECO1 counteracts destabilization of cohesin binding to chromosomes brought about by the action of a protein complex composed of PDS5 and WAPL proteins (Fig. 1B). SMC3 acetylation could therefore stabilize cohesin rings (reviewed in [40, 41]). ECO1’s activity may be spatially constrained to replication forks by its binding to PCNA [42] and temporally by SCF-dependent degradation following its phosphorylation through CDK1 after S phase [43]. Interestingly, preventing ECO1 degradation or de novo overexpression of ECO1 in G2/M allows cohesion establishment independent of DNA replication [43, 44]. Following cohesin’s release from chromosomes after anaphase (see below), SMC3 acetylation is reverted by the deacetylase HOS1 to allow cohesion establishment during S phase of the next cell cycle [45-47].

Finally, chromosomes need to find their way out of cohesin rings in order for sister chromatids to separate at the transition from metaphase to anaphase. This is achieved by the activation of a thiol-protease named separase after bi-orientation of all chromatid pairs. Separase cleaves distinct sites within cohesin’s kleisin subunit and thereby opens the cohesin ring [15, 48, 49]. In addition, there exists a cleavage-independent pathway of cohesin release mediated by the WAPL–PDS5 complex, which in mammalian cells is promoted by phosphorylation of cohesin’s SA1/SA2 subunits by polo-like kinase (PLK) during prophase [16, 50]. The molecular mechanism behind this alternative pathway is not yet understood, but it was recently suggested that WAPL–PDS5 may cause disengagement of the SMC3 head domain from SCC1 to open an exit gate for the escape of DNAs from their entrapment within cohesin rings [40].

**Cohesin regulates higher order chromosome structure during mitosis and meiosis**

Besides generating sister chromatid cohesion, ring-shaped complexes such as cohesin are well suited to contribute to other aspects of higher order chromosome structure. One may imagine inter-molecular links between two

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**Fig. 2** – Models for chromosome organization by cohesin complexes. (A) The ring model predicts that two sister chromatids are held together by their entrapment within the same cohesin ring. In alternative models, two cohesin rings, each encircling or holding on directly to a single sister chromatid, interact to provide sister chromatid cohesion. (B) At the centromeres of budding yeast chromosomes, a network of cohesin and condensin complexes may form intra-sister chromatid linkages to form a looped structure with spring-like properties that allows the balancing of spindle forces. (C) During meiotic prophase, cohesin organizes the structure of chromatin loops that emerge from the synaptonemal complex structure formed between paired homologous chromosomes.
chromosomes in G1 phase, intra-molecular connections between distinct regions on one chromosome, or even linkage of more than two double-stranded DNA molecules through multiple ring interactions. Indeed, there is evidence supporting at least the latter two modes.

In metaphase, mammalian chromosomes condense more than 10,000-fold, and several proteins and protein complexes are involved [51, 52]. Among them are topoisomerase II and condensin – their precise contribution, whether within multiple, redundant pathways or whether primarily contributing to stabilization of compacted chromosomes, is not yet entirely clear, as their individual elimination or reduction does not affect chromosome condensation as drastically as expected – and also cohesin. Besides perhaps having a structural scaffold function, which however was called into question, topoisomerase II is likely needed to remove entanglements between different chromosomes or even a high load of entanglements between sister chromatids. Such entanglements could disturb proper chromosome condensation and assembly on the metaphase plate. Topoisomerase II removes catenanes preferentially when spindle tension increases [53]. In its reverse reaction, however, topoisomerase II can also introduce catenation, and cohesin was recently shown to support this reaction or to retard decatenation [18] and may thus promote catenation of closely linked sister chromatids to support cohesion. This notion is consistent with the finding that inhibition of topoisomerase II rescues cohesion in absence of fully functional cohesin [54]. Cohesin appears to be one of the factors determining the reaction equilibrium of topoisomerase II decatenation and catenation activities and thereby contributes to higher order chromosome structure in mitosis.

Another function of SMC ring complexes is the organization of higher order chromosome structures at centromeres, where cohesion resists microtubule-generated forces and thereby generates tension in bi-oriented sister chromatids during metaphase. Cohesin and condensin localize to the pericentric region, which on each sister chromatid is thought to be organized in a large loop (Fig. 2B). While condensin aligns on each loop-forming chromatid in parallel to the spindle axis, cohesin localizes next to the pericentric loops and connects the sister chromatids [55]. Thus, condensin may stabilize the pericentric loop through linkage of the same DNA chain and cohesin may link pericentric sister chromatids inter-molecularly. Together, condensin and cohesin form a quartet of cylindrical axes at the pericentric region, which have been proposed to act to transform the centromeric region into a modular spring that could balance the tension forces [55]. The elastic properties of such a spring may be co-determined by the density of both SMC complexes and the flexibility of the complexes, which could be embedded within the HEAT-repeat domains of the non-SMC subunits. Whether interactions between cohesin and microtubules or associated proteins such as those proposed with the nuclear matrix protein NuMA [56] play a role in the pericentric balance of forces remains to be determined.

During meiosis I, four chromatids are paired within the synaptonemal complex, a meiosis-specific protein-DNA structure characterized by a core axis from which chromatin loops emanate. Two pairs of sister chromatids, each in cohesion, need to be brought together to pair. Cohesin may support this synopsis perhaps through inter-ring associations (Fig. 2C). Meiocytes from mice defective in certain cohesin subunits show high degrees of asynapsis, i.e. a failure to synapse (to pair) the two pairs of sister chromatids. The situation is more complex, however, since sister chromatid cohesion may also be deficient in those mice and thus asynapsis may be a consequence of loss of cohesion. In mice lacking the only meiosis-specific SMC protein, SMC1β, the remaining SMC1α complexes provide sufficient cohesion to keep sister chromatids together during prophase I, but still a high level of asynapsis is observed in Smc1β−/− meiocytes [57]. Loss of the meiosis-specific kleisin REC8 causes more dramatic loss of cohesion, and some chromosomes then show aberrant synopsis between individual sister chromatids instead of between pairs of sister chromatids [58, 59]. Meiocytes appear to attempt synopsis at whatever substrate there is.

In yet another mouse model with a deficiency in the second meiosis-specific kleisin RAD21L, defects in synopsis in spermatocytes and, to a lesser extend, in oocytes were observed. Localization studies suggested that each of the three kleisins follows distinct spatiotemporal patterns during entry in and execution of meiosis [60-63].

Since there exist four meiosis-specific cohesin proteins (SMC1β, REC8, RAD21L, SA3) in addition to the universal five cohesin proteins (SMC1α, SMC3, RAD21, SA1/SA2) in vertebrates and at least six if not more types of cohesin complexes with different subunit composition are formed in meiocytes, the contribution of each of those complexes to higher order chromosome structure remains to be determined [64]. It is clear, however, that not only synopsis but also the axis-loop architecture involves cohesin (Fig. 2C). In prophase I, SMC1β-deficient meiocytes show chromosome axes that are shortened by about 50 %, while the loops, i.e. the chromatin clouds that surround these axes, are extended by up to two-fold [57]. Probably fewer loop attachment sites on the axes are provided in a situation where only the SMC1α-type complexes remain. These loop attachment sites serve not only to anchor loops within the axis, but also to restrict the compaction force exerted by synaptonemal complex proteins such as SYCP3. This becomes apparent in oocytes of Smc1β−/− Sycp3−/− “double-knockout” mice, which show chromosome axes that are only slightly longer than in oocytes of wild-type mice [65], yet their loops are quite irregular with large
fluctuations. It should be fascinating to analyze mice with meiocyte-specific deficiency in all cohesin. Would the chromosome axes even further compact and shorten? Or would there be no axes at all or aberrant synopsis between sister chromatids, since there would be no cohesion?

**Cohesin organizes the interphase nucleus**

Cohesin’s architectural function is not limited to mitotic or meiotic chromosomes. There is an increasing body of evidence that cohesin complexes play a central function in gene regulation independent of their role in holding sister chromatids together. Depletion or mutation of NIPBL or of cohesin subunits was found to have predominant effects on the expression of a number of developmental transcriptional regulators in flies and zebrafish [66-70]. In humans, the developmental disorder Cornelia de Lange syndrome (CdLS) has been linked to heterozygous loss-of-function mutations in NIPBL [71, 72] or, less frequently, to mutations in the cohesin subunits SMC1α and SMC3 [73, 74]. NIPBL+/− mice show developmental defects similar to CdLS patients [75]. Since cells of neither CdLS patients nor NIPBL−/− mice show detectable cohesion or chromosome segregation defects, it is likely that the phenotypes are the consequences of changes in gene expression.

How cohesin regulates transcription is not yet well understood. One hypothesis arises from the finding that a large fraction of cohesin bound to chromosomes co-occupies sites bound by the CCCTC-binding factor (CTCF) [76-79]. Chromosome conformation capture (3C) experiments suggest that CTCF promotes the formation of chromatin loops, for example at the imprinted IGF2-H19 locus, where it prevents the activation of the maternal allele of the IGF2 gene by a distant enhancer [80]. CTCF’s insulator function is lost and the frequency of interactions between different CTCF sites at the IGF2-H19 locus measured by 3C is reduced after cohesin depletion [76, 79, 81]. A similar decrease in long-range interactions was measured at the cytokine interferon-γ gene locus after cohesin depletion in T helper cells [82]. These findings suggest that cohesin may be required to stabilize CTCF-induced chromatin loops. It is possible that cohesin uses a similar topological mechanism for such stabilization as in sister chromatid cohesion. In this scenario, CTCF may serve to bring into close proximity two distant chromosome loci, which are then entrapped within cohesin rings that may be recruited to these loci by their direct binding to CTCF [83]. Cohesin and CTCF perform a similar insulator function at the β-globin locus [76, 79, 84, 85] and the apolipoprotein cluster [86].

In addition to assisting the insulator activity of CTCF, cohesin was also found to play a role in promoting transcription of certain genes. Chromatin Immunoprecipitation (ChIP) studies identified a significant overlap between binding sites of the estrogen receptor (ER) alpha and cohesin in breast cancer cells as well as two liver-specific transcription factors and cohesin in hepatoma-derived cells [87]. While depletion of CTCF had no effect on cohesin binding to estrogen response elements, treatment with estradiol increased cohesin association at these sites, implying that cell type-specific transcription factors such as ER may recruit cohesin independent of its localization to CTCF sites. In mouse embryonic fibroblasts, cohesin and the mediator complex bind to promoters and enhancers of actively transcribed genes, including Nanog and Oct4 [34]. ER and mediator are both thought to stimulate transcription by bringing together the core transcription machinery and specific transcription factors bound to distant enhancer sites through the formation of chromatin loops, and cohesin may help in the formation of such loops. Consistent with this notion is the finding that cohesin depletion reduces the interaction between enhancers and promoters at the Nanog locus measured by 3C [34].

Cohesin was also found to have functions in the interphase nucleus beyond regulating gene expression. Cohesin and CTCF co-bind to sites within the 2.5 Mb immunoglobulin heavy chain (IgH) locus of murine B cells [88]. Notably, the compaction of the IgH locus that can be observed by 3D fluorescence in situ hybridization (FISH) during Ig heavy chain rearrangement in pro-B cells was decreased after CTCF knock down, suggesting that CTCF–cohesin may also organize chromatin loop formation during V(D)J recombination. Moreover, cohesin or similar SMC1–SMC3-based complexes are involved in the repair of and response to DNA damage (reviewed in [89, 90]). The specific chromosome structures, if any, promoted by cohesin during these processes are not yet known. It is however very likely that during S and G2 phases, cohesin-dependent sister chromatid cohesion supports repair of DNA double-strand breaks (DSBs) through homologous recombination. Cohesin accumulates near DSBs and may stabilize cohesion at these sites, may support recruitment of repair factors, may help forming recombination intermediates such as looped or cruciform structures, and/or may perhaps even take part in the repair reaction itself by promoting homologous pairing. Cohesin mutants are often hypersensitive to g-irradiation and impaired in DNA repair. Such roles and activities were shown for RAD21 and SMC proteins even before cohesin became known [91, 92]. SMC1 and SMC3 become phosphorylated in an ATM-dependent manner upon DNA damage and contribute to the DNA damage checkpoint [93, 94]. The specific effector function of SMC1 and SMC3 in this reaction is not yet understood.

Another role for cohesin during interphase stems from the discovery that cohesin is to somewhat enriched at replication origins, to which it may be recruited by a direct association with components of the pre-replication complex
(pre-RC) [95]. To what extent the association between cohesin and NIPBL with pre-RCs is required for cohesin loading onto chromatin seems to depend on the experimental system used [96, 97]. Interestingly, single molecule and microscopy assays suggest that cohesin depletion causes a decrease in replication origin firing and an increase in the estimated length of replicons [95]. Cohesin may therefore determine the loop size of replicon units that are organized by the association of neighboring origins into one replication factory.

**Outlook**

In recent years we have seen an amazing expansion of our knowledge on how cohesin shapes chromosomes. Yet, our current insights may only be a glimpse into the real complexity of cohesin-mediated order and dynamics of chromosome architecture in various cell types, distinct organisms, different stages of the cell cycle, and in the multitude of processes cohesin is involved in. In order to understand the molecular machinery behind the multitude of cohesin functions, we need to further take into account not only the core cohesin complex but also its associated proteins, including cohesin loading factors (adherins), cohesin release factors (releasins), as well as the role of posttranslational modifications. Different modes of interaction with DNA and chromatin, resulting in stably or weakly associated cohesin complexes, may have profound consequences for chromosome structure that need to be explored. In addition, new functions for cohesin are emerging which affect chromosome structure by supporting the formation of chromosome territories, marking chromosome regions of distinct compaction levels, contributing to telomeric and sub-telomeric structure [98, 99], regulating termination of transcription [100], or mediating the interplay between microtubules and the kinetochore [101] and the centrosome cycle [102, 103].

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