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More to Cohesin than meets the eye: Complex diversity for fine-tuning of function.

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Abstract

Recent years have witnessed a dramatic expansion in our understanding of gene control. It is now widely appreciated that the spatial organization of the genome and the manner in which genes and regulatory elements are embedded therein has a critical role in facilitating the regulation of gene expression. The loop structures that underlie chromosome organization are anchored by cohesin complexes. Several components of the cohesin complex have multiple paralogs, leading to different levels of cohesin complex variants in cells. Here we review the current literature around cohesin variants and their known functions. We further discuss how variation in cohesin complex composition can result in functional differences that can impact genome organization and determine cell fate.

Cohesin introduction

Cohesin is an ancient and essential ring-shaped protein complex with fundamental roles in chromosome topology throughout the cell cycle. Cohesin is best known for its essential role in mediating sister chromatid cohesion *in trans* after DNA replication in S/G_2 phases. Sister chromatid cohesion is maintained until the onset of anaphase, at which point cohesin is fully removed from chromatin and sister chromatids can segregate into daughter nuclei [1]. Cohesin is reloaded onto chromosomes during early G_1 of the next cell cycle where it acts as a fundamental regulator of spatial genome architecture. Cohesin functions by forming or stabilizing long-range chromatin loops *in cis*, from CTCF, Mediator complex, and transcription factor binding sites [2-8]. Cohesin-anchored chromatin loops were first described at specific loci to physically tether regulatory elements to gene promoters [9-11] and have since been shown to be widely distributed throughout the genome [12-18]. Both gene-loops and larger-scale chromosomal domain loops are anchored by cohesin, together creating an extensive network of long-range contacts and thereby defining global chromosome topology.

To facilitate this diversity of cellular functions, cohesin's interactions with chromosomes are tightly regulated. The loading [19, 20], stabilization [21, 22] and removal [23-25] of cohesin from chromosomes have been intensely studied, revealing a myriad of cohesin-associated proteins and post-translational modifications (PTM) of the complex subunits as having regulatory functions [26, 27]. In this context it is known that within somatic vertebrate cells there are multiple paralogs of several components of the complex, which could lead to a wide array of cohesin complex variants. How such variants and their relative levels in different cells could contribute to cohesin's diverse functions, and specifically its roles in genome organization, is poorly understood.

Most of the regulatory mechanisms for removal and stabilization of cohesin have only been considered in the context of cohesin's sister chromatid cohesion function. How the cohesin-associated proteins, PTMs and variants contribute to cohesin's well established functions in genome organization remains to be elucidated. Here we will review the current literature around cohesin variants and their known functions. We further discuss the potential impact that varying cohesin composition could have on regulation of genome structure, gene expression and cell fate by taking inspiration from other chromatin-associated complexes that regulate these fundamental processes.

Cohesin complex variants

The core cohesin complex consists of two SMC (Structural Maintenance of Chromosomes) proteins, Smc1 and Smc3, and the alpha-kleisin protein, Scc1/Rad21. Smc proteins assume a unique structure (reviewed in [28]). They consist of two globular domains separated by 50 nm long rod-shaped antiparallel coiled coils. One of the globular domains is an ATP-binding domain built from N- and C- termini of the protein. The second, so-called 'hinge' domain, engages in heterotypic interactions with the other Smc protein in the complex, forming a V-shaped heterodimer. This heterodimer interacts with Scc1/Rad21, forming a tripartite ring that encircles and physically tethers newly replicated sister chromatids *in trans* during S-phase [29, 30] and is thought to similarly stabilize chromatin loops *in cis* that are essential for gene regulation during G_1 phase.

In somatic cells of all organisms studied so far, each of these core subunits is encoded by a single gene [1]. In contrast, there are two paralogs of the HEAT repeat-containing SA (Stag) subunit of the complex in somatic cells of human, mouse, *Drosophila,* and *Xenopus* (but not in *S. cerevisiae* and *S. pombe*) - SA1 and SA2 [31, 32]. The association of SA1 and SA2 to cohesin is mutually exclusive and therefore gives rise to two variants of the complex in somatic cells - SA1-cohesin and SA2-cohesin (Figure 1a). Existing evidence suggests that the SA proteins mediate the interaction between cohesin and CTCF via a coiled-coil domain within SA that is nearly perfectly conserved between the two paralogs (see Figure 1b) [33]. Similarly, another HEAT-repeat containing protein, Pds5 interacts with the cohesin complex and exists as two isoforms, Pds5A and Pds5B (also known as APRIN [34]). Pds5 proteins regulate the association between cohesin and chromatin by stabilizing it in the absence of the unloading protein WAPL, and promoting dissociation from chromatin in the presence of WAPL [35-39]. Like SA proteins, Pds5A and B associate with cohesin in a mutually exclusive manner [40]. Therefore, the cohesin complex is able to exist in at least four distinct variants in somatic cells: SA1-Pds5A-cohesin, SA1-Pds5B-cohesin, SA2-Pds5A-cohesin, and SA2-Pds5B-cohesin (Figure 1a). Additional variants of some of the core complex genes, such as Smc1b, Rad21L, and SA3, are expressed in human, mouse and *Xenopus* meiotic cells and play a crucial role in pairing and segregation of chromosomes in meiosis [41]. These observations emphasize the complexity of cohesins' association with chromatin and highlight the need to understand such complexity with respect to cohesin's many functions.

Cohesin complex variants – do they matter?

Diversifying composition is a common way for cells to fine-tune functions of complexes. Clear examples come from condensin, a complex structurally similar to cohesin, as well as other chromatin-associated complexes that, like cohesin, play a role in regulation of gene expression and genome structure (see below).

Condensin complexes play a major role in sister chromatid cohesion. Condensin I and II share the same SMC family proteins (Smc2 and Smc4) but differ in their kleisin and HEAT repeat containing subunits (reviewed in [42]). These structural distinctions result in differential subcellular localization, distinct dynamic properties, and ultimately different functions of the complexes [43-46]. Interestingly, the relative amounts of the two complexes present in a cell seem to have an impact on chromosome morphology [46, 47]. In *Xenopus leavis* egg extracts, where there is about five times more of condensin I compared to condensin II, chromosomes appear long and thin, whereas HeLa cells, where the ratio of the two complexes is about 1:1, have short and thick chromosomes [46]. Depletion of condensin I results in shortening and thickening of chromosomes, and depletion of condensin II in their elongation [47, 48].

Similar observations have been made for cohesin. In *Xenopus* egg extracts, SA1-cohesin is the dominant complex, whereas SA2-cohesin dominates in HeLa cells and *Xenopus* somatic cells [31] (Figure 2a). This implies that expression of SA subunits might be differentially regulated - although to date, little is known about regulation of expression of the cohesin genes themselves. The two complexes also seem to be functionally distinct. SA1-cohesin mediates telomeric cohesion, whereas SA2-cohesin controls cohesion at centromeric regions [49, 50] (Figure 2b). Chromatin immunoprecipitation (ChIP) in primary mouse embryonic fibroblasts (MEFs) showed that the two proteins also have some unique binding sites. SA1-cohesin is enriched much more strongly around transcriptional start sites compared to SA2-cohesin, and plays a role in regulation of gene expression that SA2-cohesin cannot compensate for [51] (Figure 2c). Interestingly, it is not clear at present if there is any connection between these distinct functions of individual complexes, although future studies may shine some light on this topic.

What differences on the molecular level could bring about such functional specificity? Despite their overall high degree of homology (~ 70% identical), SA1 and SA2 have distinct N- and C- termini. At the N-terminus, SA1 contains an RFX5 DNA-interacting AT-hook that has been shown to be essential for the association of SA1 with telomeric DNA [52], while this motif is absent in SA2 (Figure 1b). Perhaps there are other properties

within the amino acid code that make the variants conduct distinct functions, such as in the divergent C-terminus. Such DNA binding AT-hooks seem to also be present in Pds5B but not Pds5A, and could bring about similar functional distinctions [53]. Furthermore, despite the high degree of conservation in the region of SA proteins that has been mapped to interact with CTCF (Figure 1b), biochemical experiments suggest that there could be differences in the strength of association between individual SA proteins and CTCF [33]. These interactions could further be modulated by posttranslational modifications, or differential expression of other factors that regulate them.

As mentioned above, the Pds5 component of the complex has multiple paralogs as well. Like SA1 and SA2, Pds5A and Pds5B were shown to have distinct functions. In mice, *Pds5A* and *Pds5B* mutants have partially different phenotypes, and a double knock-out has a more severe phenotype than either individual mutant [53, 54]. This suggests that two proteins cannot completely compensate for each other, and the authors propose that this is due to a dosage effect [54]. It is however also possible that the two proteins have distinct functions. For example, Carretero and colleagues have described differential roles for Pds5 proteins, whereby Pds5B is specifically required for centromeric cohesion and accumulation of AuroraB in the centromeric regions, while both proteins are required for arm and telomere cohesion. The authors concluded that distinct cohesin complexes may have a different function at different regions along chromosomal arms [55].

Given these observations, one can readily imagine that distinct cohesin complexes might play very specific roles in different cell types, stages of differentiation, and malignancies, with their relative amounts having a profound impact on genome organization and chromatin structure.

Context-dependent regulation from variants of other chromatin-associated complexes.

Other cellular complexes with roles in chromatin structure regulation, such as Polycomb, are known to have subtypes with distinct functions. For example, Polycomb Repressive Complex 1 (PRC1) was shown to have two distinct versions in embryonic stem cells, defined by mutually exclusive presence of Cbx7 or RYBP [56]. Much like cohesin-SA1 and cohesin-SA2, the two PRC1s bind to and regulate distinct sets of genes. Even more interesting is the finding that Cbx7 is downregulated upon differentiation, concomitant with the upregulation of Cbx2, Cbx4 and Cbx8 [57, 58]. This switch results in new PRC1 subtypes, each with a distinct set of targets. Maintenance of pluripotency is dependent of Cbx7 expression and its knockdown results in differentiation, whereas

lineage commitment depends on Cbx2 and Cbx4 [57, 58]. Moreover, Cbx7 seems to suppress expression of Cbx2 and Cbx4 in ES cells, while Cbx2 and Cbx4 repress Cbx7 upon differentiation (Figure 3a).

Similarly, the SWI/SNF chromatin remodeling complex is encoded by some 25 genes which are combined into distinct complexes in different cell types [59]. Of particular interest is a switch in composition of the complex as cells differentiate from ES to neural lineage. The amount of BAF155 component is reduced, whereas BAF170, which is not expressed at all in ES cells, is upregulated [60]. BAF155 and BAF170 have a high degree of homology – over 60%, just like SA1 and SA2 and Pds5A and Pds5B, yet seem functionally quite distinct. Namely, BAF155 is required for stem cell survival, whereas the pluripotent state is incompatible with expression of BAF170 [60]. Another study ascertained the difference in ES cells levels of the two proteins, and pointed out that their relative levels are different in more differentiated cell types, like MEFs [61]. The same study showed that other components of the remodeling complex switch their expression in a similar way upon differentiation.

Context-dependent structure from cohesin complex variants

Cohesin complexes have a central role in the organization of chromosomal domain structure. Hi-C datasets have shown that chromosomal domain architecture is tightly correlated with cohesin/CTCF binding sites, and that in cells lacking functional cohesin complexes, the stability of this architecture is perturbed [12, 13, 62]. The majority of studies exploring cohesin's roles in genome architecture have analyzed structure from the perspective of one of the core components (Scc1, Smc1 or Smc3). It is known that at least the cohesin-SA1 variant can anchor chromatin loops [63], and given that SA1 and SA2 occupy distinct binding sites on chromatin [51] it is possible that separate complex variants may contribute differently to chromatin loop formation and in so doing mediate functionally distinct loops. They may interact with chromatin in different ways (handcuff vs embrace models [64]) or they may interact differently with other complexes involved in looping such as CTCF, Mediator, and transcription factors. In addition, the variants may themselves interact with chromatin in different timescales, impacting the stability of the anchored loop (Figure 3b).

Outlook

These observations prompt us to think about cohesin not as one complex, but instead several different complexes with distinct functions. In this review, we discussed

how such functional differences could be a consequence of changes in cohesin complexes' composition and/or the relative levels of distinct complexes in different cells types and developmental stages. Indeed, other multi-subunit chromatin complexes use different variants to regulate gene activity and differentiation. If and how cohesin complex variants function in a similar way and how these variants impact genome organization to support cell fate and tumorigenesis, where specific subunits (SA2 [65] and Pds5B [66, 67] are found to be commonly mutated, remains to be elucidated.

While it is clear that cohesin complex diversity exists and may in fact have important roles in gene expression and differentiation, how these different variants arise in the first place is poorly understood. An understanding of how and when the cohesin genes are themselves regulated is long overdue and will inevitably yield new insights. Similarly, cohesin has major roles in chromosome topology throughout the cell cycle, yet our understanding of how these different functions are connected is insufficient. For example, whether (and how) cohesin complexes that mediate cohesion can also contribute to its gene expression functions is a major unanswered question in the field. Finally, a central role for cohesin in cancer development is emerging. Cohesin proteins have been identified as a frequently mutated network across numerous cancers [68, 69]. In this context, it is important to consider first the properties of cohesin that make them cancer targets and second to understand the impact of complex variants to tumorigenesis.

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Figure Legends

Figure 1. Cohesin complex variant diversity. a) Four variants of the cohesin complex. b) Differences between SA1 and SA2 proteins on the molecular level. Positions of conserved domains are depicted, as determined by NCBI Conserved Domain Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Conservation between regions of the two proteins covered by amino acids 1-90, 90-1080, and 1080-end is shown, as calculated by LALIGN programme (http://embnet.vital-it.ch/software/LALIGN_form.html). Conservation is represented as percentage of amino acids that are identical between given regions. The blue bar indicates the region of SA proteins that was determined to be sufficient for interaction with CTCF in ref. 33.

Figure 2. Differences in cellular levels and chromosomal distribution between SA1- and SA2-cohesin. a) Different cell types have different levels of cohesin complex variants. b) Different variants of cohesin complex mediate cohesion at telomeric and centromeric regions. SA1-cohesin mediates telomeric cohesion, whereas SA2-cohesin mediates centromeric cohesion. c) Different variants of cohesin complex are distributed differently along the chromosome. SA1-cohesin dominates around transcriptional start sites (TSS). SA2-cohesin dominates in intergenic regions.

Figure 3. Complex variants contribute to fine-tuning of gene regulation. a) An example of a change in complex composition mediating profound changes in gene expression and cell fate. Cbx7 component of Polycomb Repressive Complex 1 (PRC1) is downregulated upon differentiation, and Cbx4 and Cbx2 are upregulated. A distinct set of genes are regulated by these different variants and associated with distinct cell fates. b) Putative examples of how cohesin complex variants may mediate genome structure and gene expression. *I.* SA1-cohesin associates with transcription factors (TFs) at TSSs. *II.* SA2-cohesin modulates genome structure in intergenic regions that are transcriptionally silent. *III.* Differential association of SA1- and SA2-cohesin with CTCF and TFs fine-tunes genome structure and gene expression.

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