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Recent discoveries of Cohesin Establishment and Maintenance in Eukaryotes

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Abstract: Cohesin is multi-subunit protein that encompasses naked DNA. In eukaryotes, cohesin is essential for accurate segregation of the sister chromatids during mitosis and meiosis. Majority of cohesin complexes are removed from chromosomes during prophase in higher eukaryotes. WAPL mediated opening of the cohesin ring at the junction between the SMC3 ATPase domain and the N-terminal domain of cohesin's α-kleisin subunit has been shown in flies and human. Cohesin complex also plays a role in DNA damage repair, regulation of gene expression and chromosome condensation. In this commentary, recent discoveries of the mode of mechanism of cohesin establishment and maintenance in higher eukaryotes has been highlighted.
Keywords: Cohesin, Mitosis, Meiosis, Cell division, Sister Chromatids.

During cell division, the entire nuclear DNA is faithfully duplicated to sister chromatids that are held together with the help of the cohesin complex. Four proteins form the core cohesin complex: Structural Maintenance of Chromosome (SMC) proteins 1 (SMC1) and 3 (SMC3), Sister Chromatid Cohesion (SSC) protein 3 (SCC3), and a α-kleisin, either SCC1 which is part of the mitotic cohesion complex, or REC8 that functions during meiosis. Among different species SMC1 and SMC3 are highly conserved. Both of the proteins share same characteristics: an N-terminal ATP binding domain, two large antiparallel coiled-coil regions separated by a hinge region, and a C-terminal DA box [1]. Cohesin structure and function has been conserved in evolution and nomenclature in different organisms summarized in table 1.

In somatic vertebrate cells, stromalin antigens 1 and 2 (SA1 and SA2) are homologs for SCC3. Both proteins are subunits of cohesin complexes that contain either SA1 or SA2 [2] (Figure 1). General mechanisms of cohesin action are conserved across species. Differences in complex member composition and the mechanistic roles of the complex have been reviewed elaborately during meiosis and mitosis [3,4].

Without chromosome cohesion, the chromosomes cannot form the bipolar spindle during mitosis and meiosis. For a long time, little was published about the how cells ensure this 'bipolar' attachment until the study in yeast came and showed that cohesins are recruited to the chromosomes and the sister chromatid cohesion is established during the S phase by Eco1/Ctf7 [5-6]. During the cohesion establishment, Eco1/Ctf7 acetylates the reserved lysine residues of Smc3, close to the ATPase domain. Acetylation of Smc3 then stabilizes its interaction with Scc1 and counteracts Rad61/Wapl dissociating cohesin from the chromosomes [7-8]. The wings apart-like (Wapl) protein was originally identified as a gene product in *Drosophila melanogaster*. Sororin is a phosphoprotein and is regulated by protein kinases, like Erk2 and Cdk1/cyclin B. The association of Sororin with chromatin requires cohesin to be preloaded to chromatin and thus modify Smc3 during DNA replication. Sororin antagonizes Wapl in cohesin releasing from S to G_2 phase and promotes cohesin release from sister chromatid arms during prophase.

Sororin, specifically in vertebrates binds to PDS5 [9]. PDS5 is essential for the establishment and maintenance of sister chromatid cohesion at centromere distall and proximal

regions. CDK1 and Aurora B kinase phosphorylates Sororin, which helps its dissociation from PDS5, and thereby WAPL unload cohesin [10]. PLK1 phosphorylates SA2, which also helps in dissociation of cohesin [11]. Two protein, Shugoshin1 (SGO1) and protein phosphatase2A (PP2A) protect centromeric cohesins. SGO1-PP2A inhibits sororin phosphorylation until anaphase [12]. At anaphase, activation of anaphase promoting complex/cyclosome (APC/C) leads to securin degradation and separase activation. The kleisin subunit cleaved by active separase and renders separation of the sister chromatids (Figure 2).

In mammalian cells, establishment of cohesion happens during S-phase when Eco1 acetyl transferase acetylates K112 and K113 on Smc3[10] and cohesion becomes sticky at the replication fork. Now a big question remained as to how these two modifications can make the cohesin protein sticky? Breakthrough discoveries about Eco1-dependent Smc3 acetylation from previous published studies [7,13], demonstrated that Eco1 also becomes dispensable for viability if PDS5 or WAPL is mutated. PDS5 is essential during mitosis and meiosis in several organisms. In contrary to its function in other species, results suggests that PDS5 is not essential for meiosis in Arabidopsis and have critical role in seed maturation and vegetative development [14, 15]. Interestingly, deletions or mutations in Rad61/Wapl, Pds5, Smc3 and Scc3 may suppress the lethal phenotype of Eco1/Ctf7 deletion, suggesting that Eco1/Ctf7 cohesion establishment activity is redundant without the anti-establishment of other genes. Alternatively, there could be other factors that modify cohesin to counteract Eco1/Ctf7 establishment activity [7,13,16,17,18].

Recent discoveries on the Wapl protein in different species has shown that it controls the mitotic sister chromatid cohesion and helps in the removal of cohesin from chromosomes. Wapl was first identified in Drosophila where they showed its involvement in the heterochromatin organization [19]. WAPL has a conserved C-terminus with divergent N-terminal domains of variable lengths in different species. Recently it has been published that in humans, the divergent N-terminus of Wapl protein contains PDS5 binding domain and the C-terminus is important for cohesion determinant [20,21]. Wapl-N–Pds5 binds to the Scc1–SA2 heterodimer, whereas the N lobe of Wapl-C likely interacts with Smc1/Smc3 heterodimer [20]. Although, the effect of Wapl inactivation on mitosis has been studied in different organisms, little to nothing is known about its role during meiosis. Recent discoveries in Arabidopsis showed that the genome contains two putative WAPL genes and plays a significant role in the removal of cohesin during the prophase pathway of meiosis[18]. Remarkably, inactivation of the two At-WAPL genes can suppress the lethal phenotype observed when the cohesion establishment factor, CTF7, is inactivated [18]. Cohesin levels from sister chromatids increase after Wapl depletion during mitosis [15,22]. The antagonistic roles of Wapl with Cohesin have been studied during meiosis [23] and mitosis [22]. Interestingly, inactivation of WAPL in Arabidopsis ctf7 mutants suppresses cohesion defects[23].

Conclusions:

All of these recent studies demonstrate that WAPL is important for the timely release of cohesion during meiosis and that inactivation of WAPL eliminates the requirement for SMC3 acetylation by CTF7 [18] . In Xenopus and HeLa cells, the acetylated Smc3 recruits Sororin, which replaces Rad61/Wapl and forms a complex with Pds5 to further stabilize the cohesion [9,24].

Future Directions:

Several questions regarding anti-establishment activity of cohesion still remain such as how do these proteins prevent cohesion establishment, how could this be overcome by Smc3 acetylation, and why would an anti-establishment activity exist in the first place? Rowland et al [13] suggest that the anti-establishment activity of Wpl1, Pds5, and Scc3 might be involved in the maintenance of cohesion after DNA replication. However, a clear understanding of these processes is needed to understand and explore the mechanism.

Mutations in Cohesin proteins result in a number of inherited diseases in humans. Mutation of CTF7/ESCO2 in human causes Robert's syndrome, which is genetical disorder characterized by limb and facial abnormalities. It has been reported that expression of human Wapl is linked to cervical carcinogenesis and tumor progression, and that it has a character of oncoproteins [25]. Therefore it is very important to decode the mechanism of how these cohesin proteins functions during sister chromatid separation.

TABLE 1:

Name	Saccharomyces cerevisiae	Drosophila	*Arabidopsis* thaliana	Vertebrates
SMC1	SMC1	DmSMC1	AtSMC1	SMC1
SMC3	SMC3	DmSMC3	AtSMC3	SMC3
ECO1	ECO1/CTF7	DECO	CTF7	ESCO1 ESCO2
WAPL	WPL1/RAD61	WAPL	WAPL1, WAPL2	WAPL
PDS5	PDS5	DmPDS5	PDS5 (A-E)	PDS5A, PDS5B

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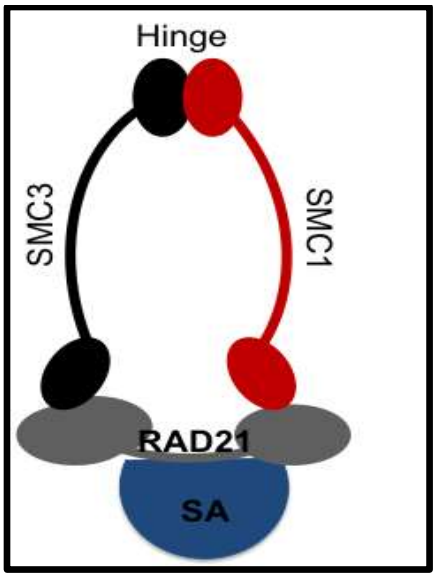

Figure 1: Structure of the cohesin complex. In somatic cells, the cohesin ring is composed of SMC1α, SMC3, RAD21, and SA1 or SA2. In germ cells, the cohesin ring can also contain substitutions with SMC1β, REC8, RAD21L, and SA3. SMC1 (red) and SMC3 (blue) together form a closed shaped structure by interaction of their hinge domains. The N-terminus and C-terminus of RAD21 (grey) interacts with SMC3 and SMC1 respectively. RAD21 interacts with SA (blue) subunit.

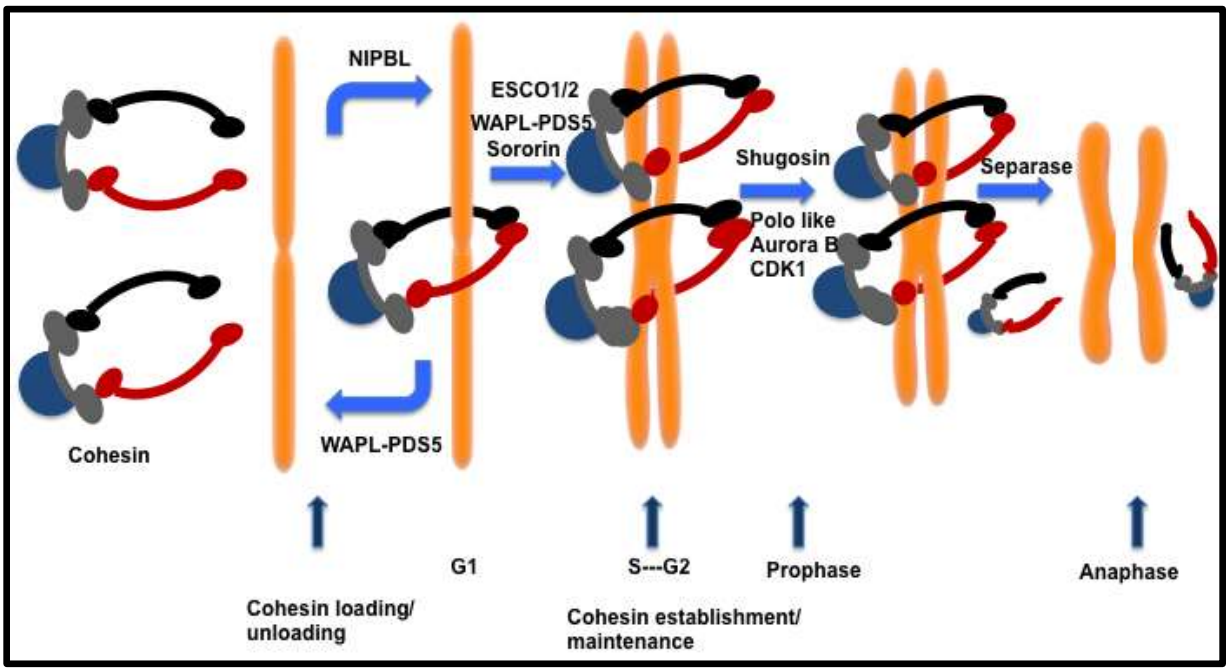

Figure 2. Cohesin loading and unloading is cell cycle dependent. In late telophase, cohesin is loaded onto the chromosome by the NIPBL/ MAU2 heterodimer. Binding of PDS5-WAPL through RAD21 and SA1/2 promotes cohesin unloading. ESCO1 and ESCO2 helps in acetylation of SMC3 to stabilize the cohesin–DNA association. Another cohesin protein, Sororin is recruited to PDS5 to antagonise WAPL function. After the onset of prophase, Aurora kinase B phosphorylates sororin and facilitate its removal. Moreover, Polo-like kinase 1 phosphorylates SA subunit to facilitate its removal cohesin from chromosome arms. Centromeric cohesin is protected during prophase by the binding of shugoshin and PP2A. At anaphase, the APC/C complex activates separase which cleaves RAD21 to release centromeric cohesin to allow sister chromatid separation.

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