Establishing and dissolving cohesion during the vertebrate cell cycle

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Abstract

Replicated chromatids are held together from the time the emerge from the replication fork until their separation in anaphase. This process, known as cohesion, promotes faithful DNA repair by homologous recombination in interphase and ensures accurate chromosome segregation in mitosis. Identification of cohesin thirty years ago solved a long-standing question about the nature of the linkage keeping together the sister chromatids. Cohesin is an evolutionarily conserved complex composed of a heterodimer of the Structural Maintenance of Chromosomes (SMC) family of ATPases, Smc1 and Smc3, the kleisin subunit Rad21 and a Huntingtin/EF3/PP2A/Tor1 (HEAT) repeat domain-containing subunit named SA/STAG. In addition to mediating cohesion, cohesin plays a major role in genome organization. Cohesin functions rely on the ability of the complex to entrap DNA topologically and in a dynamic manner. Establishment of cohesion during S phase requires coordination with the DNA replication machinery and restricts the dynamic behaviour of at least a fraction of cohesin. Dissolution of cohesion in subsequent mitosis is regulated by multiple mechanisms that ensure that daughter cells receive the correct number of intact chromosomes. We here review recent progress on our understanding of how these processes are regulated in somatic vertebrate cells.

Introduction

Cohesin was identified as the key mediator of sister chromatid cohesion in yeast [1,2] and vertebrate cells [3]. It consists of Smc1 and Smc3, Rad21 and a SA/STAG. Smc1 and Smc3 belong to the SMC family of chromosomal ATPases, conserved in all kingdoms of life. SMCs are rod-like proteins with a hinge domain on one end and an ATPase head domain on the other, separated by long anti-parallel coiled coils (**Figure 1**). Hinge-hinge interactions lead to the formation of a V-shaped SMC heterodimer. The C- and N- terminal domains of the kleisin subunit Rad21 bind near the Smc1 and Smc3 head domains, respectively, to create a tripartite ring that can entrap the DNA topologically while the SA subunit associates with the middle region of Rad21 [4–6]. The ring-shaped structure of cohesin complexes purified from cell extracts or assembled from recombinant proteins has been observed by electron microscopy [7,8]. Somatic cells contain two versions of cohesin that carry Smc1a, Smc3, Rad21 and either SA1 or SA2 [9]. Two HEAT repeat domain-containing proteins associate with cohesin during most of the cell cycle to modulate its association with chromatin, Pds5 and Wapl [10–12]. A third HEAT protein, Nipbl, loads cohesin on chromatin and may be also required for its translocation along DNA, as discussed below. In this way, cohesin resembles its related complex, condensin, formed by a heterodimer of SMC subunits (Smc2 and Smc4), a kleisin (CAP-H/H2) and two HEAT repeat proteins (CAP-D2/D3 and CAP-G/G2) [13].

Cohesion mediated by cohesin is important for faithful chromosome segregation and contributes to homologous recombination (HR)-mediated DNA repair after DNA replication. Cohesin has additional functions in chromosome organization and for this reason the complex is present in all cells, including those that do not proliferate. Together with CTCF, Mediator and other transcriptional regulators, cohesin plays a major role in genome folding and gene

regulation [14]. In this review, however, we focus on recent progress in our understanding of how cohesin mediates sister chromatid cohesion in vertebrate cells. Although many key aspects of the process are conserved in yeast and other eukaryotes, regulation of cohesion in vertebrates presents also important singularities.

Cohesin loading and unloading

Cohesin is loaded on chromatin as vertebrate cells exit from mitosis in a reaction facilitated by the heterodimer formed by Nipbl and Mau2 (Scc2 and Scc4, respectively, in budding yeast), which promotes ATP hydrolysis at the Smc heads [15–17]. In vitro, cohesin can associate topologically with DNA without the loader, although rather inefficiently [18]. Entrapment of the DNA fiber has been proposed to occur through an "entry gate" formed by the Smc1/3 hinges, at least in vivo [19] ("A" in **Figure 2**, left). The SMC coiled coils may transmit conformational changes caused by ATP hydrolysis at the head domains to promote hinge opening, as preventing post-translational modification of Lysine residues present along the coiled coils affects both association of the SMC heterodimer with the kleisin subunit and cohesin loading on DNA [20].

Pds5 and Wapl are not stoichiometric components of the cohesin complex. Wapl promotes cohesin unloading [10,11], which requires opening of an "exit gate" located in the interface between Smc3 head domain and the N terminus of Rad21 [5,8,21,22] ("C" in **Figure 2**, left). Pds5 also participates in this reaction [23], which has been reconstituted in vitro [24]. In addition to the two aforementioned "gates", a third "inner gate" is created by the interaction of Smc1 and Smc3 head domains, and is regulated by ATP binding and hydrolysis ("B" in **Figure 2**, left). In this way, DNA can be present in two different locations inside ring, between the coiled coils or between the closed inner gate and the kleisin [24,25]. It has been proposed that

DNA entrapped between the coiled coils could be sensed by two conserved Lysines located on the Smc3 head domain unless modified by acetylation and would promote ATP hydrolysis and nucleotide release, driving the two Smc heads apart to allow passage of the DNA [25–27]. Next, dissociation of the Smc3-Rad21 interaction driven by Wapl-Pds5 would occur after new ATP molecules bind the Smc heads to close the inner gate and DNA would be released from the cohesin ring. A more thorough description of this model can be found in [28].

Before DNA replication, cohesin is constantly being loaded by Nipbl-Mau2 and released by Wapl-Pds5. Chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) analyses in mammalian cells show the presence of Nipbl around promoters of actively transcribed genes [29]. The little colocalization of Nipbl and cohesin along the genome suggests that cohesin moves away from its loading sites either by itself or pushed by other translocases such as RNA polymerase II [29]. A recent hypothesis proposes that cohesin - and other SMC complexes- can extrude DNA and thereby generate DNA loops that promote long-range chromatin contacts [30,31]. Movement of cohesin along DNA by passive diffusion has been recently observed in vitro using single molecule imaging [32,33]. How well these assays reflect cohesin dynamics in vivo is uncertain since they are performed on naked DNA and do not require ATP or the cohesin loader. A third study showed cohesin complexes loaded by Nipbl-Mau2 and moving along DNA in an ATP-dependent manner [34]. Addition of Wapl-Pds5 reduced translocation but the underlying reason remains unclear. It is possible that binding of these two proteins promotes a conformation of cohesin that inhibits translocation while favouring cohesin release, e.g. with the inner gate closed.

If cohesin translocation and/or loop extrusion requires the energy of ATP hydrolysis, Nipbl could be important to enhance the inefficient cohesin ATPase. Consistent with this

possibility, Nipbl-Mau2 are required for loop extension [35] and imaging experiments suggest that Nipbl binds to cohesin as it translocates along DNA, "hopping" from one complex to another [36]. Structural data indicate that Pds5 and Nipbl bind the same region of Rad21 [37] (highlighted in **Figure 1**). Thus, interaction of Nipbl with cohesin could promote translocation also indirectly by displacing Pds5 and preventing in so doing cohesin release. This hypothesis is consistent with fluorescent recovery after photobleaching (FRAP) data showing that Pds5 and Wapl bind dynamically to cohesin [38].

Cohesion establishment

Cohesion establishment takes place during DNA replication in a process that requires acetylation of Lysines K105 and K106 in the Smc3 head domain and interaction of cohesin with a protein known as sororin [39] (**Figure 2,** middle). These two events are coupled and must take place in the context of DNA replication in order to achieve cohesion [38,40]. Sororin binds Pds5 and functions to counteract Wapl-Pds5 releasing activity [12,39]. In this way, a fraction of cohesin tethering the two sister chromatids is stabilized [41]. Since the binding of sororin to cohesin is highly dynamic - as that of Pds5- it is likely that the requirement of DNA replication for sororin recruitment depends on a property that cohesin acquires during cohesion establishment, e.g. a conformational change that allows the complex to embrace two DNA segments instead of one [38]. Whether the two sister chromatids are actually embraced by a single complex or by two interacting cohesin complexes, each one embracing a chromatid, is still controversial [4,42]. It is also unclear how tethering occurs mechanistically. Recent imaging experiments have shown that cohesin complexes bound to chromatin in G1 remain associated after passage of the replication fork [43]. Visualization of cohesin movement along DNA incubated in replication-competent

Xenopus egg extracts identified a fraction of cohesin complexes that were incorporated into replicated DNA and did not translocate while a smaller number of complexes appeared to be pushed by the replication machinery [34]. The authors of the study propose that the former "immobile fraction" could be the one responsible for cohesion. Due to size constraints, it is unlikely that the replisome can simply pass through the cohesin ring [32]. Instead, replication forks may promote transient release of the complexes that would quickly re-associate in the wake of the fork. The latter reaction may couple cohesin loading and cohesin acetylation and explain why Smc3 acetylation requires cohesin's ATPase activity [44].

Smc3 acetylation is carried out by Esco1 and Esco2. Although several studies suggest that the two cohesin acetyltransferases (CoATs) appear to contribute similarly to cohesion [38,45], some evidences favour a more prominent role of Esco2 in cohesion establishment while Esco1 would instead contribute to gene regulation [46]. Esco2 levels are low in G1 and increase during S phase while Esco1 is expressed throughout the cell cycle [40] and colocalizes largely with cohesin along the human genome [45]. However, Esco2 function must be at least partially compensated by Esco1 in human cells, since its deficiency is compatible with life although it causes a rare disease known as Roberts syndrome or RBS [47]. In contrast, Esco2-null mouse embryos die early in development [48]. Mouse embryo fibroblasts (MEFs) and human patient cells without Esco2 have centromeric cohesion defects while cohesion along the arms is maintained to variable extents. Esco2 could therefore be particularly important for establishment of cohesion around centromeres while Esco1 may provide sufficient acetylation to establish arm cohesion in the absence of Esco2 [48]. In Xenopus egg extracts, Smc3 acetylation is carried out by Esco2 at the time of DNA replication and recruitment of the CoAT depends on prereplication complex assembly [49]. Whether similar mechanisms recruit Esco2 in somatic vertebrate cells is

not known, but Esco1 recruitment depends on its interaction with Pds5 [50]. MEFs deficient in one of the two versions of Pds5 that coexist in vertebrate cells, Pds5A and Pds5B, show partial reduction in Smc3 acetylation while double knock out (KO) MEFs have undetectable levels of acetylated Smc3 [12]. Pds5 is also required for cohesin acetylation in yeast [51] although the reason for this requirement could be different than in human cells, since Eco1 was shown to be recruited through interactions with PCNA [52]. It is therefore possible that Pds5 proteins not only contribute to Esco1 recruitment in vertebrate cells, but also facilitate Smc3 acetylation by either CoAT through an evolutionarily conserved mechanism.

In yeast, there is a single CoAT and no sororin ortholog has been identified. Cohesin acetylation may be sufficient to counteract Wapl releasing activity. Smc3 acetylation inhibits the ATPase activity of the head domains, and thus interferes with the cohesin unloading mechanism described in the previous section [25–27].

Dissolution of cohesion.

Another major difference in cohesin regulation between yeast and vertebrate cells occurs in mitosis. Most vertebrate cohesin is released from chromatin when vertebrate cells enter prophase in a process regulated by phosphorylation and known as the prophase pathway. The small fraction of cohesin left on the condensed chromosomes, which is enriched around centromeres, dissociates when the kleisin subunit is cleaved by separase at the onset of anaphase [53] (**Figure 2,** right). In yeast, all cohesin is released in this way in the metaphase to anaphase transition [54]. It was hypothesized that cohesin removal in prophase would facilitate sister chromatid resolution, since the presence of cohesin hinders efficient decatenation by topoisomerase II [55].

However, volumetric analyses of differentially labeled sister chromatids to measure their overlap found no major differences between chromosomes assembled in the presence or absence of Wapl, arguing against this hypothesis [56]. Nevertheless, Wapl depleted cells do have trouble to properly separate their chromatids in anaphase [11,57]. This could be due to the presence of residual catenations, particularly around centromeres [58]. In addition to facilitate efficient chromosome separation in anaphase, the prophase pathway may have evolved to allow quick reloading of cohesin by telophase after Smc3 deacetylation by Hdac8 of the rings released from chromatin [59]. In yeast, loading of cohesin occurs in late G1, upon synthesis of the kleisin subunit.

The prophase pathway is regulated by several mitotic kinases. Phosphorylation of the SA subunit by Plk1 is necessary but no sufficient to promote cohesin release, which also requires the action of Wapl [10,11,55,60,61]. As explained in the previous section, during cohesion establishment in S phase sororin binding to Pds5 displaces Wapl to prevent its gate opening function [39]. To restore this function, sororin must be removed from cohesin in prophase. This requires sororin phosphorylation by Cdk1 and Aurora B [62]. To shield the small fraction of cohesin complexes that must hold the sister chromatids together until anaphase from the prophase pathway, the cohesin protector Shugoshin 1 (Sgo1) and its partner the protein phosphatase 2A (PP2A) antagonize the phosphorylation of cohesin and sororin, mainly at centromeres [63]. Sgo1 localization at centromeres is driven by phosphorylation of histone H2A by Bub1 kinase, whose recruitment depends, in turn, on the outer kinetochore component Knl1 once is phosphorylated by Mps1 kinase [64,65]. Mitotic transcription of centromere repeats has been proposed to promote disengagement of Sgo1 from histone H2A in the outer kinetochore so it can reach cohesin present at the inner centromere region [66]. The interaction between Sgo1

and cohesin is enhanced by Cdk1 phosphorylation of Sgo1, and competes out the binding of Wapl to SA-Rad21 [6]. In addition, two recent studies show that another mitotic kinase named Haspin also competes out Wapl binding to Pds5 and thereby contributes to prevent cohesin dissociation [67,68]. Haspin phosphorylates histone 3 on Threonine 3, a signal for the recruitment of the Chromosomal Passenger Complex (CPC) carrying Aurora B to centromeres [69]. A second CPC recruitment pathway is driven by the interaction with Sgo1 [70]. Centromeric localization of the CPC is important for mitotic checkpoint function but also for centromeric cohesion [71]. Centromeric cohesion defects are more prominent in Pds5B deficient cells than in Pds5A deficient cells and this could be due, at least in part, to defects in Haspin recruitment to centromeres [12,67,68].

A functional ortholog of Sgo1, Mesi-S32, was first identified in Drosophila in a screen for proteins essential for maintaining centromeric cohesion in meiosis. However, this protein did not play a major role in mitotic cohesion, as is the case of other shugoshin family members from yeast [72]. A study has now shown that Dalmatian, a Drosophila protein with homology to sororin in its C-terminus, actually combines the functions of both sororin and shugoshin [73]. As the authors discuss, this hybrid protein may represent an intermediate step in the co-evolution of sororin-mediated cohesion establishment and Sgo1-mediated mitotic cohesion protection.

Prometaphase chromosomes interact with spindle microtubules to achieve bi-orientation, that is, that sister kinetochores become attached to microtubules from opposite spindle poles. Cohesion at centromeres facilitates this process by promoting a back-to-back orientation of the sister kinetochores. It also stabilizes the attachment of microtubules to kinetochores by opposing the pulling forces of microtubules to generate tension. The spindle assembly checkpoint ensures that anaphase does not start until all chromosomes achieve the bioriented state. When this

happens, the Anaphase Promoting Complex/Clyclosome (APC/C) becomes activated and degrades Cyclin B and securin. Securin is an inhibitory chaperone of separase that acts as a pseudosubstrate of the protease [74–76]. Separase has a complex regulation that includes autocatalytic cleavage, phosphorylation, DNA binding and prolyl isomerization [77]. In addition to cleaving Rad21 to promote cohesin release from chromatin, separase binds and inhibits cyclin B1-Cdk1 around chromosomes to facilitate poleward movement of the disjoined sister chromatids in anaphase [78].

Conclusion and perspectives

Proper regulation of cohesion is key to genome stability and inheritance. Premature loss of cohesion leads to chromosome gains and losses that are usually detrimental for the cell, although they can provide some advantage to cancer cells. Consistent with this possibility, mutations in genes encoding cohesin subunits and regulators have been identified in several cancer types. However, they are not clearly correlated with aneuploidy, which suggests that cohesion loss is not the major contribution of cohesin dysfunction to tumourigenesis [79]. Cohesion is also essential in meiosis, not discussed here due to space constraints. In the female human oocytes, cohesion is established during premeiotic S phase that takes place in the embryo and must be maintained until ovulation, which can happen decades later in humans. Cohesion deterioration in aged oocytes increases the risk of aneuploidy that ultimately leads to infertility or genetic syndromes like Down's [80]. Our understanding of cohesion has clearly improved over the three decades that have gone by since the discovery of cohesin but it is still incomplete. Following the lead of recent efforts to recapitulate DNA replication in the test tube from purified components

[81], in vitro reconstitution of cohesion establishment and dissolution is undoubtedly one major goal of current research in this field.

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Figure Legends

Figure 1. Ring-shaped structure of cohesin complex in vertebrates. Smc proteins fold back on themselves to form a rod-shape molecule with the hinge domain on one side and the ATPase domain on the other connected by the coiled-coil domain. Smc1 and Smc3 interact through their hinges while the kleisin subunit Rad21 bridges the two head domains. The SA subunit associates with the middle region of the kleisin. Main interaction sites for regulatory factors Nipbl, Pds5 and Wapl are indicated. Nipbl and Pds5 bind the same region in Rad21 (darker tone).

Figure 2. Cohesin dynamics throughout the cell cycle in vertebrate cells.

Left: In G1 phase, cohesin loading on chromatin is facilitated by Nipbl and Mau2 in a process that requires ATP hydrolysis at the Smc heads (left). The proposed "entry gate" is located at the Smc1/3 hinge interface (A). Engagement of the two Smc head domains through ATP binding (green balls) creates an "inner gate", which is opened by ATP hydrolysis and regulates both

cohesin loading and unloading (B). The unloading reaction is mediated by Wapl and Pds5. DNA passes through and "exit gate" located at the interface between Smc3 coiled coil region next to the head domain and the N terminal domain of Rad21 (C). Middle: In S phase, cohesion establishment is coordinated with DNA replication and requires acetylation of Lysines K105 and K106 in the Smc3 head domain (represented by a flag) by CoATs Esco1 and Esco2 and subsequent binding of sororin to counteract Wapl. A fraction of cohesive cohesin carrying sororin maintains cohesion throughout G2. Right: Dissolution of cohesion in mitosis occurs in a stepwise manner. In prophase, most cohesin is removed from chromosome arms in a Wapl-dependent manner. This process requires phosphorylation of cohesin and sororin by mitotic kinases. At centromeres, cohesin is protected by the action of Sgo1-PP2A complex, which promotes dephosphorylation of cohesin and sororin, and the action of Sgo1 and Haspin that competes out Wapl binding to cohesin and Pds5 respectively. Finally, at the onset of anaphase, cohesin dissociates from centromeres when the kleisin subunit is cleaved by separase, allowing the separation of the sister chromatids

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Of special interest

***Beckouët 2016**

By means of chemical crosslinking, immunoprecipitation and fluorescence microscopy, this study provides evidence to support that Pds5-Wapl unloading activity disrupts the Smc3-kleisin interface.

***Elbatsh 2016**

This study shows that the two ATPase active sites at the Smc1-Smc3 head domains are asymmetrically required for cohesin release while they are both used during cohesin loading. Importantly, the requirement is observed also in human cells.

****Murayama and Uhlmann 2015**

After developing an in vitro assay to load cohesin topologically on a circular plasmid DNA (Murayama and Uhlmann 2014), the same authors explore here the role of Pds5 and Wapl. In addition to showing the importance of ATP hydrolysis for loading and unloading cohesin, they propose that for both events DNA may traverse the Smc3-kleisin interface, an exciting but still controversial idea that will need further validation.

***Ouyang 2017**

An insightful essay on how Pds5 and Wapl release cohesin from chromosomes.

***Stigler 2016**

***Davidson 2016**

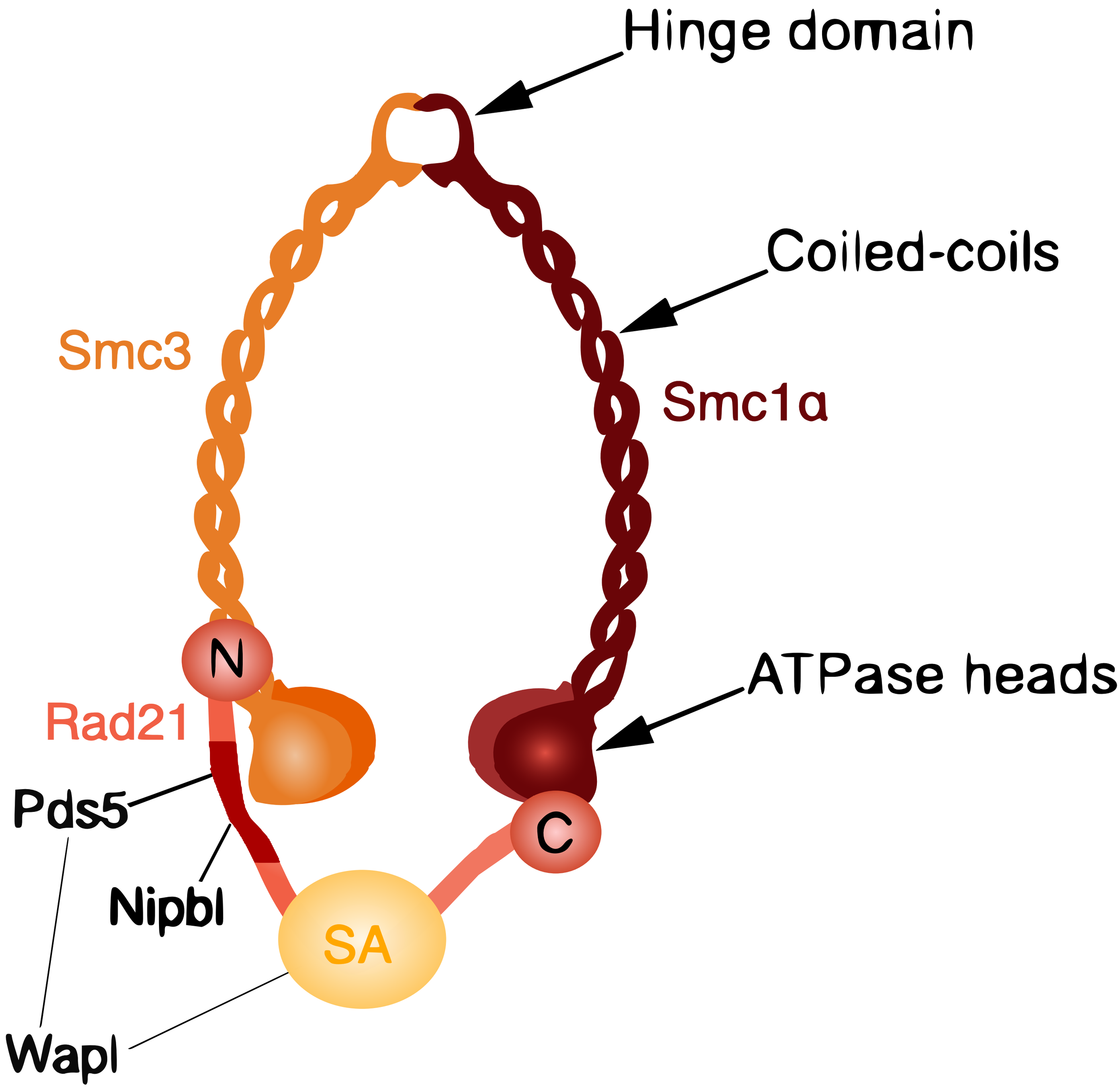
Using single molecule analysis by TIRF microscopy, these two studies show purified cohesin moving along DNA.

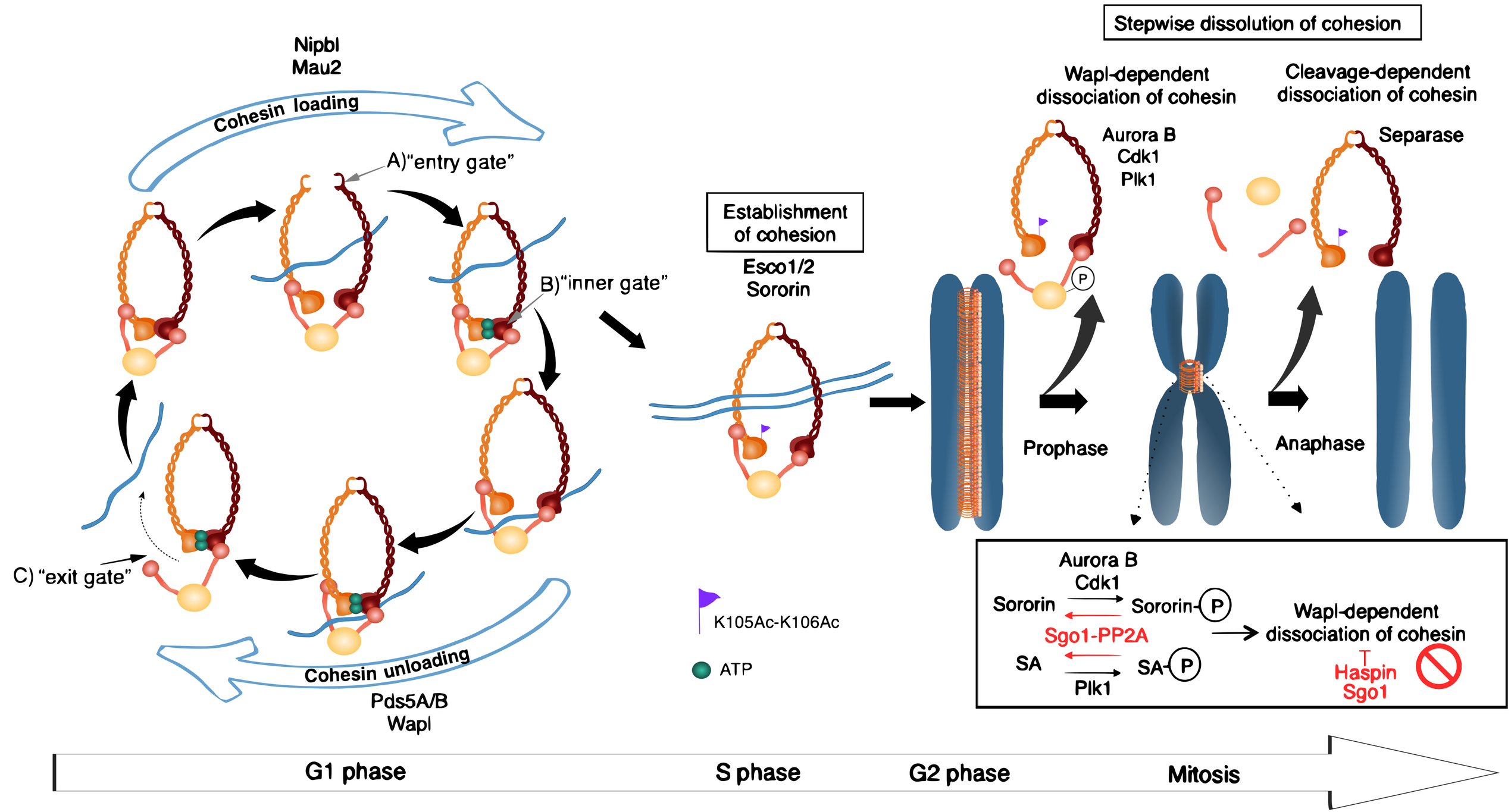
****Kanke 2016**

This study goes beyond the two previous ones by showing that cohesin translocation depends ATP and is affected by the presence of Pds5-Wapl, Smc3 acetylation and sororin. Moreover, it also shows cohesin moving on chromatin undergoing DNA replication in Xenopus egg extracts.

***Kikuchi 2016**

A structural study of the cohesin loader (Nipbl/Scc2) that highlights its resemblance to Pds5 and SA as well as the competition of Pds5 and Nipbl for binding Rad21.


Hinge domain
Coiled-coils
Smc3
Smc1α
N
ATPase heads
Rad21
Pds5
C
Nipbl
SA
Wapl


Nipbl
Mau2
Cohesin loading
A)"entry gate"
B)"inner gate"
C) "exit gate"
Cohesin unloading
Pds5A/B
Wapl
Establishment of cohesion
Esco1/2
Sororin
K105Ac-K106Ac
ATP
Stepwise dissolution of cohesion
Wapl-dependent dissociation of cohesin
Aurora B
Cdk1
Plk1
P
Prophase
Cleavage-dependent dissociation of cohesin
Separase
Anaphase
Aurora B
Cdk1
Sororin
Sororin-P
Sgo1-PP2A
SA
SA-P
Plk1
Wapl-dependent dissociation of cohesin
Haspin
Sgo1
G1 phase
S phase
G2 phase
Mitosis