Centromeric Cohesin: Molecular Glue and Much More

Mihailo Mirkovic and Raquel A. Oliveira

Abstract Sister chromatid cohesion, mediated by the cohesin complex, is a prerequisite for faithful chromosome segregation during mitosis. Premature release of sister chromatid cohesion leads to random segregation of the genetic material and consequent aneuploidy. Multiple regulatory mechanisms ensure proper timing for cohesion establishment, concomitant with DNA replication, and cohesion release during the subsequent mitosis. Here we summarize the most important phases of the cohesin cycle and the coordination of cohesion release with the progression through mitosis. We further discuss recent evidence that has revealed additional functions for centromeric localization of cohesin in the fidelity of mitosis in metazoans. Beyond its well-established role as "molecular glue", centromeric cohesin complexes are now emerging as a scaffold for multiple fundamental processes during mitosis, including the formation of correct chromosome and kinetochore architecture, force balance with the mitotic spindle, and the association with key molecules that regulate mitotic fidelity, particularly at the chromosomal inner centromere. Centromeric chromatin may be thus seen as a dynamic place where cohesin ensures mitotic fidelity by multiple means.

1 The Importance of Gluing DNA Molecules

Mitosis is one of the most dynamic periods in the life of the cell. In a short period of time, the cell condenses its DNA into discrete chromosomes, aligns them on the metaphase plane, and finally, destroys the forces that hold equal-DNA molecules together, creating two identical daughter nuclei in the process. The fidelity of this process relies on cells' ability to keep the two identical sister chromatids together from the moment of DNA replication until the later stages of mitosis, once (and only when) the conditions for their separation are met.

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Sister chromatid cohesion provides cells with the ability to determine chromosome identity, as cohesed sister chromatids are identical and therefore need to be pulled to opposite poles. Moreover, sister chromatid cohesion provides the counterforce that resist the pulling force of the spindle, thus preventing premature sister chromatid separation (Oliveira et al. 2010; Tanaka et al. 2000), and random chromosome segregation. Cohesin is also essential for the correct geometry of the kinetochore region which promotes effective, stable capture of the kinetochores by the mitotic spindle, leading to the biorientation of chromosomes during metaphase (Ng et al. 2009; Sakuno et al. 2009; Stephens et al. 2013).

Therefore, to align chromosomes at the metaphase plane and segregate them symmetrically, chromosomal cohesive state must be maintained until anaphase at all cost. Premature separation of chromosomes renders the cell unable to align chromosomes correctly, causing random segregation of the genetic material and

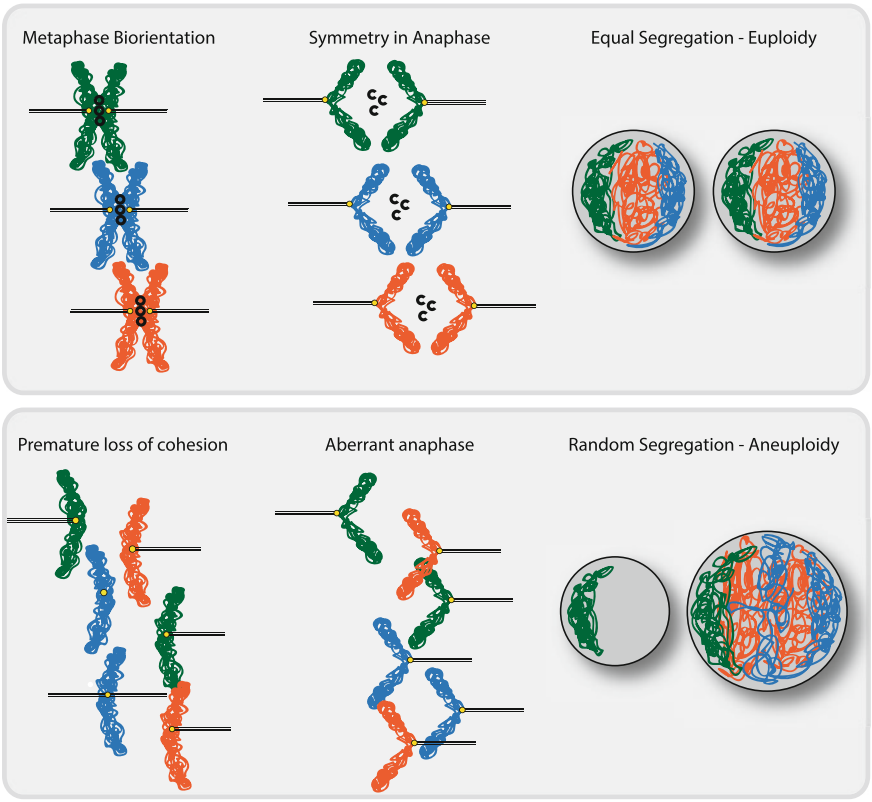

Fig. 1 Sister chromatid cohesion during mitosis. Cohesin is essential for biorientation of chromosomes on the metaphase plane and the symmetry of subsequent anaphase. Defects in sister chromatid cohesion result in premature separation of sister chromatids, resulting in random chromosome segregation and aneuploidy

consequent aneuploidy (Fig. 1), which is usually lethal and a common cause of human pathological conditions (Box 1).

Box 1—Sister chromatid cohesion defects and human disease

Proteins involved in keeping the two sister DNAs together have been linked to several human-health and reproduction conditions. Defects in cohesion and mechanisms regulating cohesin are common amongst **cancer** cells (De Koninck and Losada 2016; Losada 2014). Cancer cells display Chromosomal Instability (CIN) characterized by frequent gain or loss of chromosomes (Holland and Cleveland 2009). CIN enhances the speed at which the cancer cells can evolve, by gaining or losing whole chromosomes, making them highly adaptable to any possible treatment. Interestingly, recent studies have been able to reverse the CIN of multiple cancer-derived cells lines by reinstating the network associated with protection of cohesion (Tanno et al. 2015).

Age-related **female infertility** has also been proposed to relate with cohesion decay in aged oocytes, giving rise to genetic abnormalities such as Down's syndrome (Reviewed in Webster and Schuh 2016). "Cohesion fatigue", evidenced by decreased levels of cohesion is followed by segregation defects and decreased fertility in oocytes (Patel et al. 2015; Zielinska et al. 2015). It is currently thought that the meiotic cohesin variant is loaded into an oocyte only during the germ-line development (pre-meiotic S phase) without significant turnover (Burkhardt et al. 2016; Tachibana-Konwalski et al. 2013). This would mean that oocytes solely rely on cohesion established during their creation, and maintain it throughout the entire reproductive life cycle of the female, which lasts for decades in humans. Studies in human oocytes have revealed an increased distance between bivalents in meiosis of older females, leading to aberrant kinetochore attachments and segregation errors (Patel et al. 2015; Zielinska et al. 2015).

Other rare developmental disorders have also been linked to the cohesion process and are now known as "***Cohesinopathies***" (reviewed in references Dorsett 2007; Liu and Krantz 2008; Remeseiro et al. 2013). Most of these diseases are linked to the non-mitotic roles of the cohesion apparatus (e.g. regulation of transcription and genome architecture). However, a certain number of Cohesinopathies, such as the Roberts or Warsaw breakage syndromes exhibit cohesion defects between replicated chromatids during mitosis, resulting in aneuploidy and mitotic defects (Tomkins et al. 1979; van der Lelij et al. 2010).

In order to understand how defects in chromosome cohesion take place, it is fundamental to understand the molecular structure of the cohesin complex, as well as the principle mechanisms underlying its loading, establishment, and release during the cell cycle. Here we summarize our current knowledge on the regulation

of sister chromatid cohesion. We further highlight the importance of such dynamic regulation for the efficiency of mitosis, in mechanisms that go far beyond cohesin's primary role in sister chromatid cohesion.

2 Cohesin: The Molecular Glue that Holds Chromosomes Together

The protein complex responsible for the pairing of replicated chromosomes is called cohesin (Guacci et al. 1997; Michaelis et al. 1997) (Fig. 2). Cohesin is a tripartite ring complex, which topologically entraps replicated DNA molecules keeping them together until the onset of anaphase (Haering et al. 2008; Ivanov and Nasmyth 2005). The core of this ring complex is composed out of three molecules: SMC 1 and SMC 3 (belonging to the Structural Maintenance of Chromosomes protein family) and the kleisin subunit Rad21/Scc1, which connects them (Nasmyth and Haering 2009; Peters et al. 2008) (Fig. 2). Additional proteins directly associate with the cohesin complex (Scc3/SA, Pds5, WAPL, Sororin) and are thought to have critical roles in cohesin dynamics, and consequently mitotic fidelity (summarized in Box 2).

Box 2—Cohesin and its regulators

See Fig. 3 and Table 1.

The most popular, and soundly tested cohesin model postulates that cohesin keeps sister chromatids together by entrapping sister DNA fibers within the same cohesin ring (Haering et al. 2008). EM-studies support that cohesin rings are about 40 nm in diameter (Haering et al. 2002) thus providing sufficient space for enclosing two 11 nm chromatin fibers. Other models have been proposed, such as the "handcuff" model, in which cohesion is mediated by two interlinked cohesin complexes, each entrapping its own DNA fiber (Diaz-Martinez et al. 2008; Guacci 2007). In either case, solid evidence supports that cohesin's interaction with DNA is of a topological nature (Haering et al. 2008; Ivanov and Nasmyth 2005),

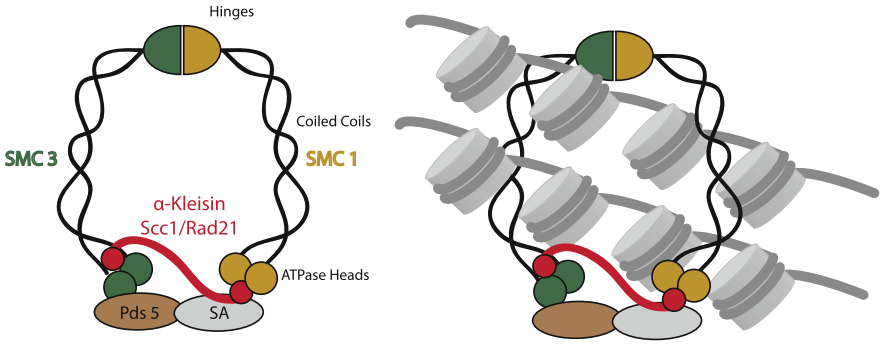

Fig. 2 The cohesin complex. Cohesin complex forms a ring-shaped molecule that topologically embraces sister DNA molecules inside its ring

emphasizing that regulation of cohesin binding and function relies on the opening and closing the interphases between the core components (discussed below).

Besides its role in sister chromatid cohesion, cohesin also regulates transcription, contributes to the DNA repair mechanisms, and participates in the organization of the genome in mitotic and post-mitotic tissues (Reviewed in Nasmyth and Haering 2009; Peters et al. 2008).

The distribution and presence of cohesin on chromatin during the cell cycle coincides with its multiple roles (Fig. 4). Cohesin is loaded onto chromatin during G1 phase in budding yeast (Guacci et al. 1997), and already in telophase in vertebrates (Losada et al. 1998). Fluorescence Recovery After Photobleaching (FRAP) studies have shown that during G1 phase cohesin is dynamically interacting with the DNA (Gerlich et al. 2006). Similar dynamics was observed in cells that are not undergoing mitotic divisions, for example endocycling *Drosophila melanogaster* salivary glands (Eichinger et al. 2013). This highly dynamic nature of cohesin–DNA interaction in non-dividing or non-replicated cells is believed to relate to cohesin's role in transcription regulation and interphase genome architecture.

Following the onset of S phase, a fraction of cohesin molecules establishes cohesion between newly replicated sister chromatids. Specific changes on the

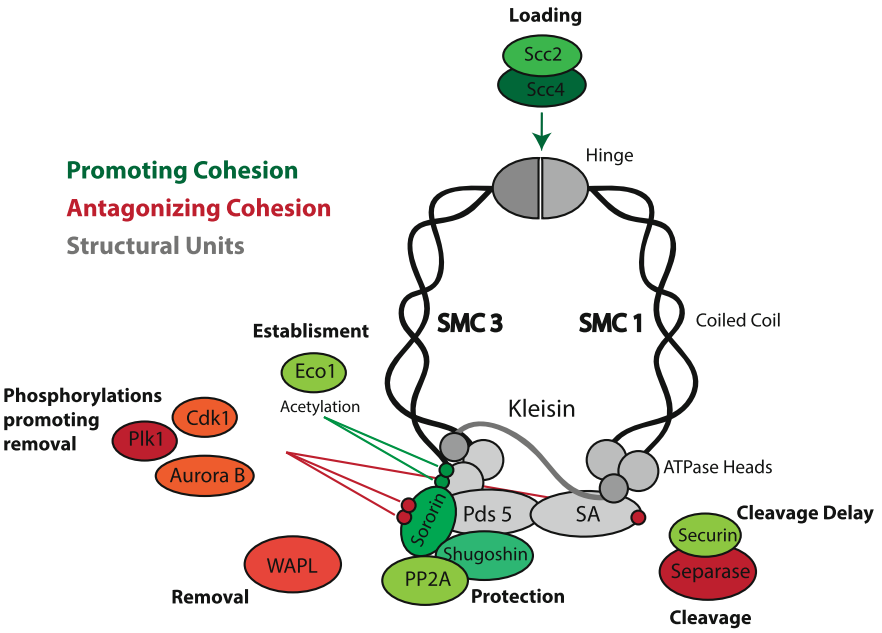

Fig. 3 Cohesin and associated molecules. The cohesin complex and the different associated molecules that modulate cohesin's function. Molecules are color-coded according to their influence on the stability of cohesin's association with chromatin (molecules that promote cohesion are in *green*; cohesion antagonists in red and proteins with dual effect in *orange*)

Table 1 Cohesin and its regulators

Name	Function	Mode of operation
SMC1	Structural/ATP binding and hydrolysis	Part of the ring; interacts with SMC3 through the hinge and the α-kleisin through the ATPase heads; binds and hydrolysis ATP
SMC3	Structural/ATP binding and hydrolysis	Part of the ring; interacts with SMC1 through the hinge and the α-kleisin through its coiled-coil; binds and hydrolysis ATP
α-kleisin/Rad21/Scc1	Structural/scaffold	Closes the ring by bridging the heads of SMC1/3; serves as a scaffold to other regulatory proteins
Pds5	Structural/scaffold	Connects the ring with various other molecules: (e.g. Sororin and WAPL)
SA/Scc3	Structural/scaffold	Essential for the ring structure; phosphorylated by Plk1 during prophase pathway
Scc2/NIPB/NIPBL	Loading	Required for cohesin loading; possibly opens SMC1/3 hinges
Scc4/Mau-2	Loading	Required for cohesin loading; possibly opens SMC1/3 hinges
Eco1/ESCO1	Establishment	Acetylates SMC3 heads, promoting sororin recruitment
WAPL	Removal	Interacts with Pds5 and disrupts the SMC3/kleisin interface, opening the ring
Sororin	Protection	Blocks WAPL interaction with Pds5
Cdk1/CycB	Removal/protection/cleavage inhibitor	Removes sororin; promotes shugoshin localization; inhibits separase
Aurora B	Removal/protection	Removes sororin; promotes shugoshin localization
Plk1	Removal/cleavage	Promotes cohesin release through SA phosphorylation; enhances Rad21/Scc1 cleavage by separase
Shugoshin	Protection	Recruits PP2A to the cohesin complex
PP2A	Protection	Dephosphorylates sororin
Securin	Cleavage inhibitor	Inhibits separase activation
Separase	Cleavage	Cleaves the Rad21/Scc1 subunit and opens the ring

cohesin complex (discussed in Sect. 3.2) ensure the post-replicative stabilization of cohesin–DNA interaction concomitantly or right after replication fork passage. This cohesive state is then maintained until the subsequent mitosis.

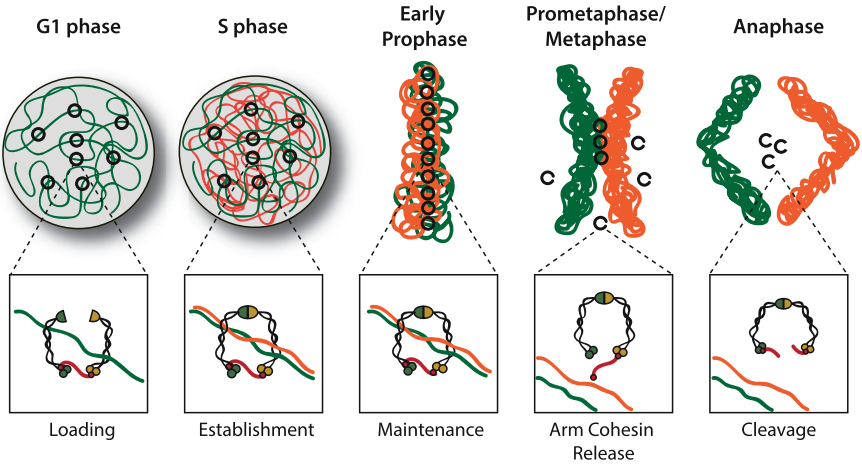

Fig. 4 Overview of the cohesin cycle. Cohesin is loaded in telophase or G1, and is dynamically associated with chromatin. Upon replication, cohesion is established, connecting two replicated strands. Non-centromeric cohesin is removed from chromosome arms during prophase in metazoans, resulting in X-shaped chromosomes in metaphase. Finally, cohesin is cleaved during anaphase, allowing for the separation of sister chromatids

In early mitosis, the majority of the cohesin complexes are released from chromosome arms. By the time cells reach metaphase, cohesion is solely maintained by a small pool of cohesin molecules retained at the centromeric and pericentromeric regions (Losada et al. 1998; Waizenegger et al. 2000; Warren et al. 2000), providing chromosome their characteristic "X-shape".

At the onset of anaphase, remaining centromeric cohesin is destroyed in a rapid and acute manner by a cysteine protease named separase, allowing the separation of sister chromatids by the spindle (Uhlmann et al. 1999). Separase cleaves the kleisin subunit Rad21/Scc1, releasing sister chromatids from topological entrapment. The destruction of cohesin during anaphase marks the point of no return for the mitotic cell: once cohesin is cleaved, separation of the chromatids is rapid and irreversible. Consequently, release of cohesin from mitotic chromosomes is a highly regulated affair.

The key surveillance mechanism governing cohesin release is the Spindle Assembly Checkpoint (SAC) (Reviewed in Musacchio and Salmon 2007). The SAC regulates cohesin cleavage by delaying the onset of anaphase until all the chromosomes are bioriented on the metaphase plane, with sister chromatids correctly oriented towards the opposite poles by the spindle (biorientation). SAC mediates this delay by directly inhibiting the Anaphase Promoting Complex/Cyclosome (APC/C), whose activity is needed for anaphase events. APC/C mediates cohesin cleavage through indirect activation of separase, the protease responsible for proteolytic opening of the cohesin ring.

Loss of cohesin or its regulators in virtually all organisms results in premature separation of sister chromatids (Guacci et al. 1997; Losada et al. 1998; Michaelis et al. 1997; Mirkovic et al. 2015; Sumara et al. 2000; Vagnarelli et al. 2004),

arguing that cohesin is the most significant force that counteracts spindle forces. Nevertheless, it is conceivable that other forces may additionally play a role in chromosome cohesion. In particular, DNA–DNA intertwines (catenation) have long been argued to contribute to cohesion during mitosis (Reviewed in Diaz-Martinez et al. 2008; Guacci 2007; Liu et al. 2009b). Due to the helical nature of the DNA molecule, the replication fork passage creates tangles between replicated DNA molecules. These catenations need to be resolved before the onset of anaphase; otherwise, the entanglements will cause chromosome bridges and breakages in the DNA molecule. Topoisomerase II is the molecule responsible for decatenation of these linkages and inhibition of this enzyme leads to accumulation of catenations, which are sufficient to confer cohesion even in the absence of cohesin proteins (Toyoda and Yanagida 2006; Vagnarelli et al. 2004).

How much residual catenation contributes to cohesion during normal mitosis is a matter of debate. Although residual catenation has been observed even in anaphase segregating chromatids (Baumann et al. 2007), inhibition of topoisomerase II specifically during metaphase has only a small effect on the efficiency of chromosome segregation (Oliveira et al. 2010). This suggests that residual catenation may contribute to chromosome cohesion; yet, it is insufficient to resist the drastic spindle forces affecting chromosomes during mitosis. More importantly, unlike cohesin's destruction, which requires SAC silencing and APC/C activation, there is little to no evidence that removal of residual catenation is delayed by cell cycle progression checkpoints which control mitosis. SUMOylation of topoisomerase II has been proposed to restrict centromeric decatenation during mitosis (Bachant et al., 2002; Dawlaty et al. 2008; Ryu et al. 2010), but there is no evidence that this reaction is under surveillance of the SAC.

Thus, regulation of the cohesive state of chromosomes is mechanistically linked to the control of cohesin's association with chromatin throughout the cell cycle, which will be discussed below.

3 The Cohesin Cycle

3.1 The Cohesin Cycle I: Chromatin Loading

Cohesin loading onto chromatin is dependent on a two-protein complex known as Scc2/4, also known as NIPBL/MAU-2 (Ocampo-Hafalla and Uhlmann 2011) (Fig. 5). The Scc2/Scc4 loading complex is essential for sister chromatid cohesion during G1/S phase, but not during G2 (Ciosk et al. 2000; Uhlmann and Nasmyth 1998). This would entail that the Scc2/Scc4 has a primary function of loading cohesin onto the chromatin, but not in its stabilization or maintenance. Given the ring-like architecture of cohesin, its loading onto chromatin requires opening of the ring. Elegant experiments with fusion of interfaces between different cohesin components support that the entry gate for cohesin loading resides at the interface of the SMC1 and SMC3 hinge domains, in an ATP-dependent process

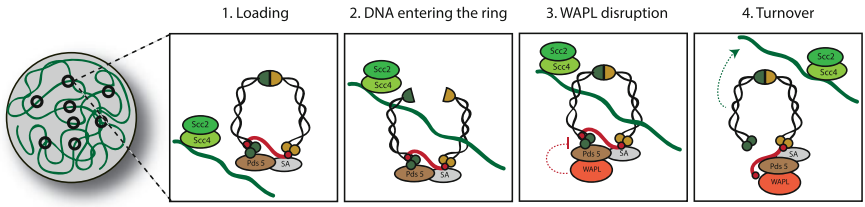

Fig. 5 Cohesin loading and turnover. Cohesin loading onto DNA depends on the Scc2/4 complex. DNA loading involves opening of the SMC1/3 interface, the hinge. Before replication, this interaction is dynamic, as loaded cohesin can be destabilized by WAPL, which opens the SMC3/Kleisin interface and releases cohesin from the chromatin

(Arumugam et al. 2003; Gruber et al. 2006; Weitzer et al. 2003). Nevertheless, the molecular mechanism by which Scc2/4 promote cohesin's loading remains unknown.

Sites of cohesin loading do not necessarily coincide with cohesin's accumulation sites. This is mostly due to the fact that once loaded, cohesin complexes can slide on the DNA molecule (Hu et al. 2011; Lengronne et al. 2004; Ocampo-Hafalla et al. 2016; Stigler et al. 2016). Additionally, before DNA replication, the cohesin molecules display a highly dynamic association with DNA (Gerlich et al. 2006). Dissociation of cohesin from un-replicated DNA molecules is mediated by Wings-apart like protein (WAPL) (Gandhi et al. 2006; Kueng et al. 2006; Verni et al. 2000). Upon binding to the cohesin complex, WAPL removes cohesin from chromatin by disrupting the interface between SMC3 and Rad21/Scc1 subunits (Buheitel and Stemmann 2013; Eichinger et al. 2013).

Cohesin loading is not a uniform event across the chromatin landscape and is found to be enriched at the centromeric/pericentromeric regions in most species studied so far (Blat and Kleckner 1999; Glynn et al. 2004; Hahn et al. 2013; Oliveira et al. 2014). Studies in budding yeast support that cohesin enrichment at the centromere is dependent on centromeric DNA sequences as well as proteins involved in kinetochore assembly (Megee and Koshland 1999; Tanaka et al. 1999; Weber et al. 2004). However, species with longer centromeric sequences, such as fission yeast, rely on heterochromatin rather than centromeric sequences for cohesin enrichment (Bernard et al. 2001; Nonaka et al. 2002). In accordance, recent studies in *D. melanogaster* showed that cohesin enrichment at ectopic regions of peri-centromeric heterochromatin occurs in the absence of a proximal centromere, most likely due to preferential binding of the cohesin loading factor Scc2/Nipped B (Oliveira et al. 2014). The preferential activity of Nipped B at the centromeric region is thought to be due to the specific state of pericentromeric heterochromatin, mainly H4K20 and H3K9 methylations and the presence of HP1 protein, tough clear links have been controversial (Hahn et al. 2013; Koch et al. 2008; Oliveira et al. 2014).

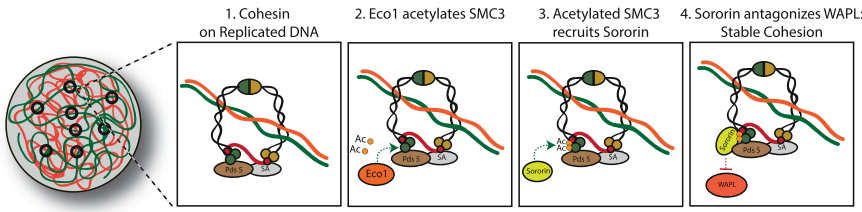

Fig. 6 Cohesion establishment during S phase. Upon DNA replication, a fraction of cohesin becomes stable on the chromatin. This happens due to SMC3 acetylation by Eco1 and recruitment of Sororin, protecting the cohesin complex from WAPL removal. This stable fraction of cohesin is considered "cohesive" cohesin, stably binding sister chromatids until the end of mitosis

3.2 The Cohesin Cycle II: Cohesion Establishment

Cohesin establishment occurs during replication, at the time the newly replicated DNA molecule is being formed. Disruption of cohesin loading during G1 results in sister chromatid defects, while disruption during G2 does not. This means that the "effective" cohesion is established during S phase, during DNA replication (Uhlmann and Nasmyth 1998). At the onset of replication, the dynamic properties of cohesin turnover change and a new pool of stable, "cohesive" cohesin can be identified by FRAP (Gerlich et al. 2006).

Stabilization of cohesin complexes upon replication depends on the Eco1 acetyl transferase (Skibbens et al. 1999; Toth et al. 1999) (Fig. 6). This enzyme acetylates cohesin associated with replicated DNA at specific lysine residues on SMC3 and failure to acetylate leads to cohesion defects and cell death. The mechanism by which SMC3 lysine acetylation prevents cohesin de-association once it is bound to chromatin is contentious (reviewed in Rudra and Skibbens 2013). Some studies propose models in which the acetylation locks the SMC3/kleisin interface, effectively closing the ring; however, these findings are inconsistent with the fact that SMC3 can be acetylated before replication (Rudra and Skibbens 2013). SMC3 acetylation during the S phase has also been shown to confer cohesin protection by aiding the recruitment of Sororin, which favors cohesion establishment by protecting acetylated cohesin complexes from WAPL-mediated removal (Nishiyama et al. 2010).

These stably associated cohesin molecules [$\sim$30% of total nuclear cohesin (Gerlich et al. 2006)] are responsible for sustaining cohesion from the time of DNA replication until the subsequent mitosis.

3.3 The Cohesin Cycle III: Cohesin's Prophase Release and Retention at the Centromere

Once the cell enters mitosis, profound changes in the distribution of cohesin begin to take place. Cohesin at the chromosome arms is removed while centromeric cohesion is retained (Losada et al. 1998; Waizenegger et al. 2000; Warren et al. 2000). The loss of

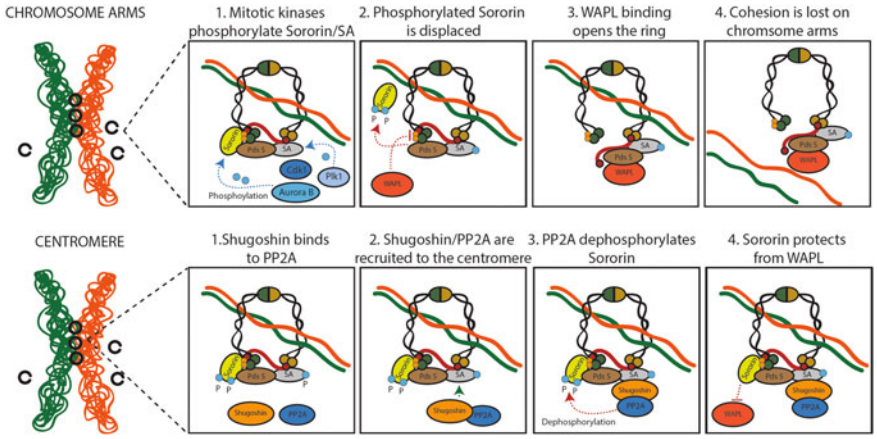

Fig. 7 Cohesin release during early mitosis and centromeric protection. In metazoans, cohesin is removed from the arms by the prophase pathway. Mitotic kinases phosphorylate sororin and SA. Phosphorylation induces sororin displacement, which allows WAPL to destabilize cohesin. Centromeric cohesin complex are protected from this removal process as Shugoshin/PP2A complex protects centromeric cohesion from WAPL-mediated removal

arm cohesion, coupled with centromeric retention gives the characteristic "X" shape to the metaphase chromosomes. The removal of cohesin from the arms in early mitosis is a consequence of the "prophase pathway" which mainly relies on action of WAPL protein (Gandhi et al. 2006; Kueng et al. 2006).

WAPL imposes opening of the cohesin ring by disrupting the interface between SMC3 and Rad21/Scc1 subunits (Buheitel and Stemmann 2013; Eichinger et al. 2013) (Fig. 7). Consequently, WAPL mutations or knockdown leads to the loss of the characteristic X shape of chromosomes, with cohesin remaining all over chromosome arms (Gandhi et al. 2006; Haarhuis et al. 2013; Kueng et al. 2006).

Several mitotic kinases contribute to the process of cohesin removal, by phosphorylating key proteins involved in the cohesin cycle. Aurora B and Cyclin-Dependent Kinase 1 (Cdk1) were shown to antagonize Sororin by phosphorylation, resulting in its dissociation from chromosome arms during prophase (Dreier et al. 2011; Nishiyama et al. 2013). WAPL and Sororin directly compete for the binding to the cohesin-associated protein Pds5 (Nishiyama et al. 2010). The removal of Sororin from chromosome arms during prophase favors WAPL binding, and consequently the removal of cohesin complexes from chromosome arms. In addition to antagonizing Sororin, Aurora B seems to participate in WAPL activation, thus directly promoting cohesin removal (Nishiyama et al. 2013). Polo-Like kinase (Plk) is another key mitotic kinase participating in the cohesin cycle. The phosphorylation activity of Plk1 is crucial for the release of cohesin during the prophase pathway by phosphorylation of SA (Hauf et al. 2005; Lenart et al. 2007; Sumara et al. 2002). The net result of these changes in the cohesin complex results in the removal of most of cohesin from chromosome arms but not from the centromeric region.

How are centromeric complexes protected from prophase pathway removal? A key molecule in the protection of centromeric cohesion is called Shugoshin, meaning "Guardian Spirit" in Japanese. Shugoshin confers protection of cohesin specifically at the centromere of both mitotic and meiotic cells (Kerrebrock et al. 1992; Kitajima et al. 2004; McGuinness et al. 2005).

Shugoshin is moved to the centromeric chromatin in complex with the PP2A phosphatase at the onset of mitosis (Kitajima et al. 2006; Liu et al. 2013b). Sugoshin/PP2A complex protects centromeric cohesin from WAPL-mediated removal by several means: It antagonizes the Aurora B-/Cdk1-mediated phosphorylation of Sororin and thereby favors Sororin interaction with Pds5, shifting the WAPL/Sororin competition for cohesin binding towards Sororin, preventing WAPL-mediated removal (Dreier et al. 2011; Liu et al. 2013b; Nishiyama et al. 2013). Aurora B and Cdk1 also phosphorylate and aid in the centromeric localization and activation of Shugoshin (Kitajima et al. 2006; Liu et al. 2013b; Tanno et al. 2010). This means that Cdk1 and Aurora B have conflicting roles in cohesin maintenance. They destabilize Sororin and thereby promote cohesin dissociation along chromosome arms, while at the same time localize and activate Shugoshin at the centromere, allowing for cohesin protection. Shugoshin also counteracts the effect of Plk-1 mediated phosphorylation of SA (Hauf et al. 2005; Kitajima et al. 2006; McGuinness et al. 2005). This was initially thought to rely on de-phosphorylation of this subunit by PP2A. However, recent evidence reveals that Shugoshin-bound centromeric cohesin complexes contain a phosphorylated form of SA (Liu et al. 2013b), suggesting alternative mechanisms may exist. In accordance, structural analyses propose that Shugoshin may additionally work by a direct competition with WAPL for the binding to cohesin (Hara et al. 2014).

This protection mechanism is of outmost importance as centromeric cohesin complexes are the only ones that suffice cohesion maintenance during prometaphase and metaphase, while chromosomes are under drastic pulling and pushing forces exerted by the mitotic spindle to accomplish chromosome alignment.

3.4 The Cohesin Cycle IV: The Final Cut

Mitosis is a process of trial and error, with a few decisive breakpoints. Mitotic events of chromosome attachment, substrate phosphorylation and biorientation are mostly redundantly regulated, and reversible. This allows for ample error correction in an otherwise error prone process. However, once the metaphase is formed, and chromosomes are bioriented, the cell reaches the point of no return: cohesin cleavage.

The cleavage of cohesin at the metaphase-to-anaphase transition is conducted by a large cysteine protease called separase, which cleaves the kleisin subunit, distancing the heads of SMC1 and SMC3 subunits (Lin et al. 2016; Uhlmann et al. 2000). This opens the cohesin ring, releasing sister DNA molecules from the proteinaceous cage (Fig. 8).

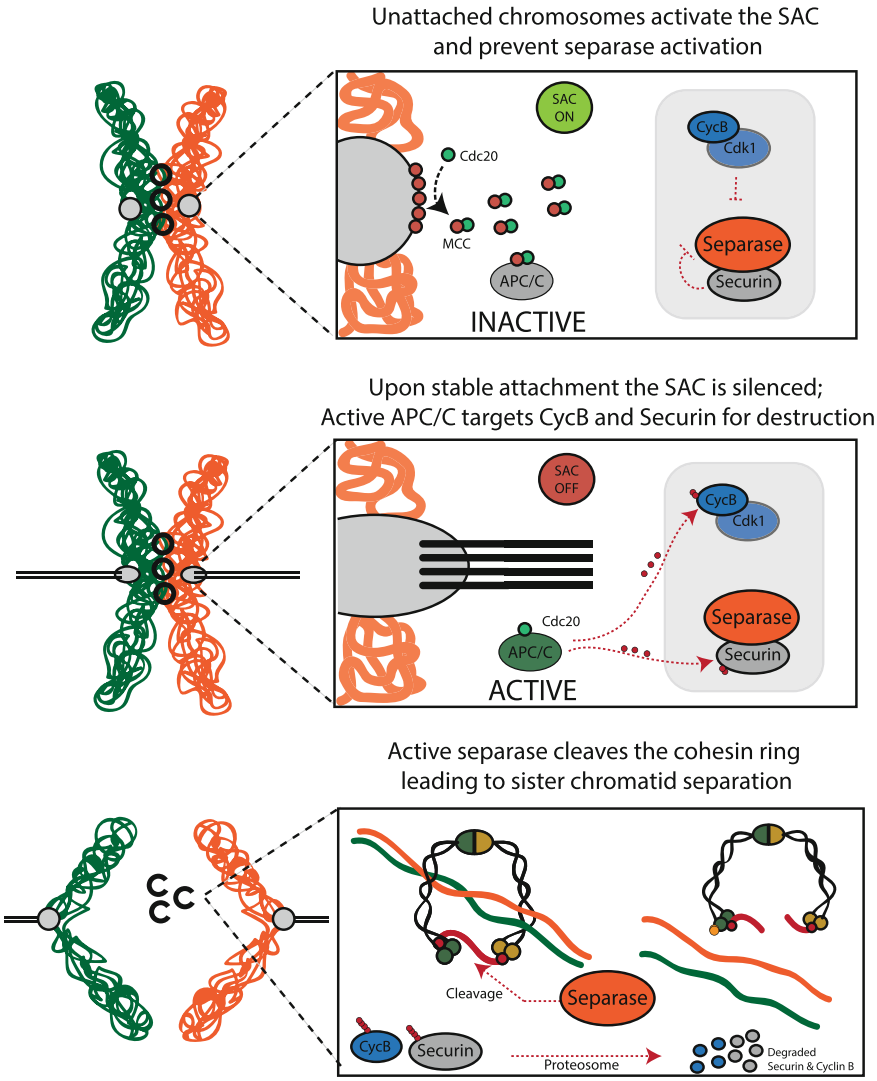

Fig. 8 Cohesin cleavage at the metaphase-to-anaphase transition. **a** In the presence of unattached kinetochores, the spindle assembly checkpoint is activated and generates the formation of the mitotic checkpoint complex (*MCC*) that prevents anaphase promoting complex/cyclosome activation. Separase is kept inactive by securin and Cdk1/CyclinB binding. **b** Upon bipolar attachment, the SAC signal is extinguished and the APC/C is activated. Active APC/C ubiquitinates securin and Cyclin B and targets them for degradation. **c** Active separase cleaves the Rad21/Scc1 subunit and causes ring opening. This opening allows the spindle to drag sister chromatids to opposite poles

Once the forces that hold chromosomes together are released, there is no going back: therefore, centromeric cohesin cleavage must occur only after multiple safeguard mechanisms have been satisfied. Separase activity is thus tightly regulated and inhibited through multiple mechanisms until the onset of anaphase.

First, separase is inhibited by the binding of Securin, whose degradation is a prerequisite for sister chromatid separation (Ciosk et al. 1998; Hirano et al. 1986; Zou et al. 1999). Securin inhibits separase by binding to its active site and abolishing its interaction with other substrates (Hornig et al. 2002; Lin et al. 2016). However, mutants for Securin in several organisms do not suffer from premature loss of cohesion, evidencing that other mechanisms of separase inhibition must be in place (Alexandru et al. 2001; Hellmuth et al. 2015) (see below). Furthermore, Securin has been proposed to work as a separase chaperone by binding to the nascent separase and aiding in its proper folding and activity (Jallepalli et al. 2001). Consequently, Securin was shown to be required for sister chromatid separation in fission yeast and *D. melanogaster* (Funabiki et al. 1996; Stratmann and Lehner 1996).

The second layer of separase inhibition is mediated by the Cdk1-Cyclin B complex. Cyclin B-Cdk1 phosphorylates separase and this phosphorylation promotes Cdk1-CycB-separase binding, preventing separase activation until the onset of anaphase (Gorr et al. 2005; Stemmann et al. 2001). The dual inhibition of separase by CycB-Cdk1/securin is lifted by the APC/C, an E3 ubiquitin ligase, which is the main effector of anaphase (reviewed in (Primorac and Musacchio 2013; Sullivan and Morgan 2007)). The APC/C ubiquitinates both securin and Cyclin B, which targets them for degradation by the proteasome and releases separase from its double leash. This, in turn, leads to cohesin cleavage and the onset of anaphase (Oliveira and Nasmyth 2010).

Given the importance of this transition, the APC/C itself is tightly regulated during mitosis by a surveillance mechanism known as the Spindle Assembly Checkpoint (SAC) (Musacchio and Salmon 2007; Sullivan and Morgan 2007) (Fig. 8a). The key effector of this mechanism is the Mitotic Checkpoint Complex (MCC). Unattached kinetochores catalyze the formation of this inhibitory complex, which sequesters Cdc20, a key activator required for APC/C activity (Musacchio and Salmon 2007; Sullivan and Morgan 2007). The MCC complex is composed of Mad2, BubR1, Bub3 and Cdc20 that form a complex that actively binds and inactivates the APC/C (Primorac and Musacchio 2013). As long as the SAC is active and the MCC is being produced at unattached kinetochores, the APC/C will not be activated by Cdc20, Cyclin B and Securin will remain intact, Separase inactive, and cohesin will not be cleaved.

This equilibrium changes once metaphase is achieved and chromosomes are bioriented (Fig. 8b). Stable chromosome attachments result in SAC satisfaction and the release of Cdc20 from the inhibitory MCC complex (Primorac and Musacchio 2013; Sullivan and Morgan 2007). Once this happens, APC/C binds Cdc20 becoming active to ubiquitinate Cyclin B and Securin. Ubiquitination promotes the proteasome-mediated degradation of these targets and consequently the release of Separase from its inhibition. Anaphase is imminent.

Since chromosome biorientation and microtubule attachment are highly dynamic processes, once all the chromosomes are bioriented, the decision to commit to anaphase must be rapid and the execution swift. Indeed, live imaging analysis revealed that separase-mediated cohesin cleavage happens within a few minutes during the metaphase-to-anaphase transition (Gerlich et al. 2006; Oliveira et al. 2014; Yaakov et al. 2012).

In order to achieve this sharp metaphase-to-anaphase transition and rapid cohesin cleavage, multiple positive feedback mechanisms are needed to create a molecular switch. First, Separase has autocatalytic activity, and once released from its Cyclin B-Cdk/Securin inhibition, it is able to cleave itself, and convert to an even more enzymatically potent form (Waizenegger et al. 2002). Furthermore, APC/C is constantly ubiquitinating the MCC and trying to pry away the Cdc20 subunit away from it, weakening the SAC signal in the process (He et al. 2011; Uzunova et al. 2012). In this way APC, accelerates its own release from SAC inhibition during anaphase.

In addition (or in parallel) to separase-mediated cleavage, the cohesin protection machinery is also released from centromeres at the metaphase-to-anaphase transition, which may accelerate cohesin release. Release of Shugoshin/PP2A from the centromeres may additionally promote the Plk1-mediated phosphorylation of Rad21/Scc1 (Plk1-mediated), which enhances its cleavage by the Separase (Alexandru et al. 2001; Hornig and Uhlmann 2004). Moreover, both Shugoshin and Sororin, two key molecules involved in cohesin protection, are directly targeted for degradation by the APC/C (Karamysheva et al. 2009; Rankin et al. 2005). Whether or not removal of the mechanisms involved in cohesin protection actively contribute to the sharp cohesion release at the metaphase-to-anaphase transition remains to be determined.

As discussed above, cohesin cleavage is only initiated once chromosome biorientation is achieved. Thus, given that chromosomes at this stage are being pulled by mitotic spindle, release of cohesin is on its own sufficient to trigger pole-ward chromosome movement (Oliveira et al. 2010; Uhlmann et al. 2000). This, however, is insufficient for efficient anaphase chromosome movement. Sister chromatid separation, when triggered alone, results in ~ 1/3 slower movements, and concomitant re-activation of the SAC and error-correction mechanisms (Mirchenko and Uhlmann 2010; Oliveira et al. 2010). Uncoupling cohesin cleavage from Cyclin B destruction leads to similar failures in chromosome segregation (Parry et al. 2003; Vazquez-Novelle and Petronczki 2010; Vazquez-Novelle et al. 2014). Successful anaphase onset thus relies not only on a sharp anaphase transition but also on a synchrony between sister chromatid cohesion release and cell cycle progression. The fact that cohesin cleavage is regulated by the APC/C, which cleaves both securin (cohesin release) and Cyclin B (cohesin release + cell cycle transition) should in principle provide this synchrony. Additional feedbacks, however, further ensure that sister chromatid separation occurs in synchrony with inactivation of Cdk1 (reviewed in Kamenz and Hauf 2016).

4 Functional Implications for a Multiple-Step Cohesin Removal

Cohesin binding and release is a dynamic and multi-step process whose mechanisms are mostly conserved across species. Exception goes for the dual-step removal for cohesin during mitosis. In budding yeast, unlike in metazoans, arm cohesion is not removed at the onset of mitosis and the entire cohesin pool is removed at the metaphase-to-anaphase transition by Separase. The question does arise as to why do metazoans have a two-step removal of cohesin? Does accumulation and retention of cohesin specifically at the centromeric region play any specific function in metazoans? When considering the biological significance for cohesion removal during mitosis, one must have interphase functions of cohesin in mind. During prophase removal of cohesin, the Rad21/Scc1 subunit is not cleaved, but disengaged from SMC3 (see Sect. 3.3), leaving intact cohesin complexes in the cytoplasm. This cohesin is not reloaded during mitosis, possibly due to the dissociation of the Scc2/4 loading complex from chromosomes (Watrin et al. 2006; Woodman et al. 2014). However, these intact cohesin complexes can load freely during the impending telophase/G1 and preform roles in transcription regulation and interphase genome architecture early in the subsequent cell cycle. Thus, the prophase pathway may be seen as a recycling mechanism, protecting the majority of cohesin from cleavage during anaphase. It is nevertheless becoming more and more evident, however, that the concentration of cohesin specifically around the centromere fulfills important functions for the efficiency of mitosis, as outlined below.

4.1 Sister Chromatid Resolution

During replication, sister DNA molecules become heavily intertwined as a consequence of the unwinding of parental DNA strands and colliding replication forks. In order to segregate these tangled sister molecules into two daughter cells, their catenations must be resolved. Failure to resolve such DNA intertwines by topoisomerase II leads to breaks in the DNA molecules during anaphase, when chromosomes are pulled to the poles by the spindle.

Cohesin was shown to block the action of topoisomerase II (Farcas et al. 2011; Sen et al. 2016), possibly by keeping the two sisters in such close proximity that disfavors their efficient decatenation. Thus, cohesin removal from chromosome arms during prophase is believed to aid sister chromatid resolution along chromosome arms, providing topoisomerase II with enough space to resolve catenations (Fig. 9).

The degree to which sister chromatid resolution can occur in the presence of chromosome-bound cohesin has been hard to estimate. A recent study has elegantly shown that in the absence of WAPL, when cohesin is retained all over chromosome

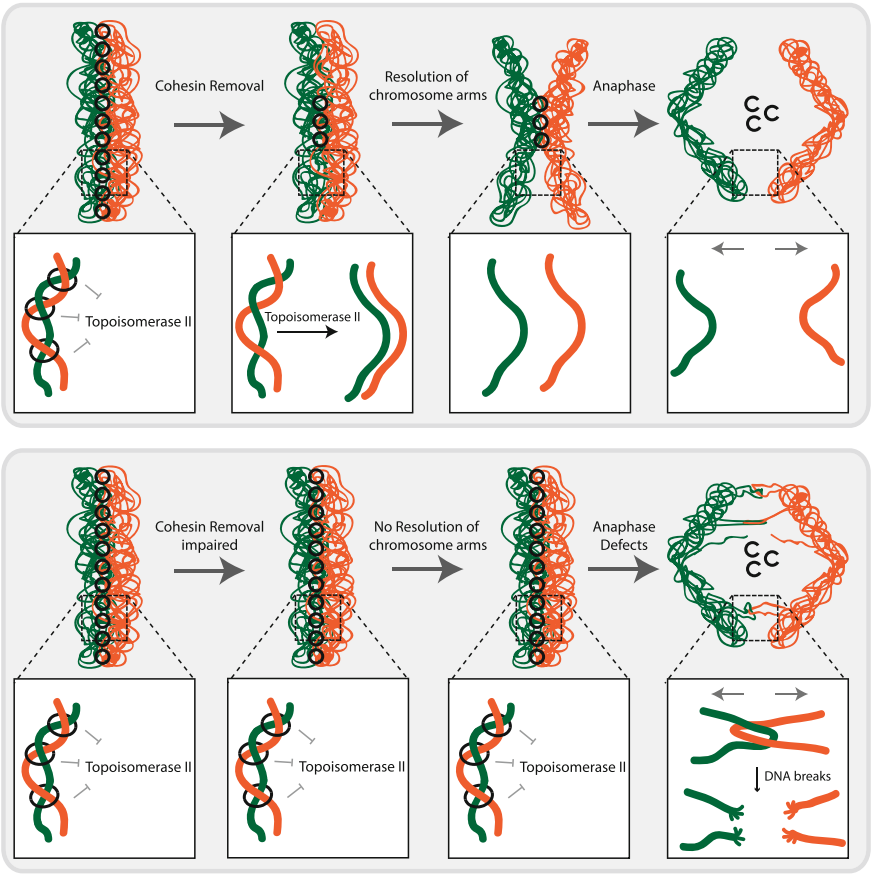

Fig. 9 Cohesin and sister chromatid resolution. Cohesin entrapment prevents efficient decatenation by topoisomerase II. Cohesin removal from chromosome arms ensures proper sister chromatid resolution. Abnormal retention of cohesin on the arms results in residual entanglements and consequently mitotic defects

arms, sister chromatids can be mostly resolved, at least at the limit of the cytological method applied to image differentially labeled sister chromatids (Nagasaka et al. 2016). Thus, although cohesin may impair efficient decatenation, the degree of chromosome intertwines even in the presence of cohesin must be residual.

These residual levels of chromosome intertwines are nevertheless sufficient to impair efficient chromosome segregation. When cohesin is not removed from chromosome arms in a timely manner, which happens if WAPL is downregulated and the prophase pathway inhibited, chromosomes lose their characteristic "X-shape" and cells undergo an erroneous anaphase, marked by detectable chromosome bridges during anaphase (Haarhuis et al. 2013; Tedeschi et al. 2013). Similar results were observed in cells expressing a modified version of Sororin that

lacks its Cdk1-phosphorylation site. This version is not removed from chromosomes arms at the onset of mitosis leading to over-cohesion of metaphase chromosome arms and lagging chromosomes during anaphase (Nishiyama et al. 2013). Moreover, chromosome rearrangements that misplace pericentromeric heterochromatin away from the centromere were shown to abnormally accumulate non-centromeric cohesin (Oliveira et al. 2014). These chromosomes also exhibit chromatin stretching during anaphase, specifically at ectopic cohesin-retention sites. Thus, the spatial and temporal positioning of cohesin on the mitotic chromosome is crucial for timely chromosome resolution. Any disturbance, such as prolonged retention or enrichment of cohesin along chromosome arms leads to incomplete sister chromatid separation, followed by mitotic errors.

4.2 *Inner-Centromere Defining Platform*

Centromeric cohesin has recently emerged as a core component of the inner-centromere network and thereby influences the localization of important machinery that regulates mitotic fidelity (Fig. 10).

Kinetochore-microtubule attachments are regulated by the actions of Aurora B, a key mitotic kinase that destabilizes erroneous kinetochore-microtubule attachments. It is well established that Aurora B destabilizes attachments that are not under tension through the phosphorylation of key kinetochore substrates (Biggins and Murray 2001). This phosphorylation results in microtubule detachment and the

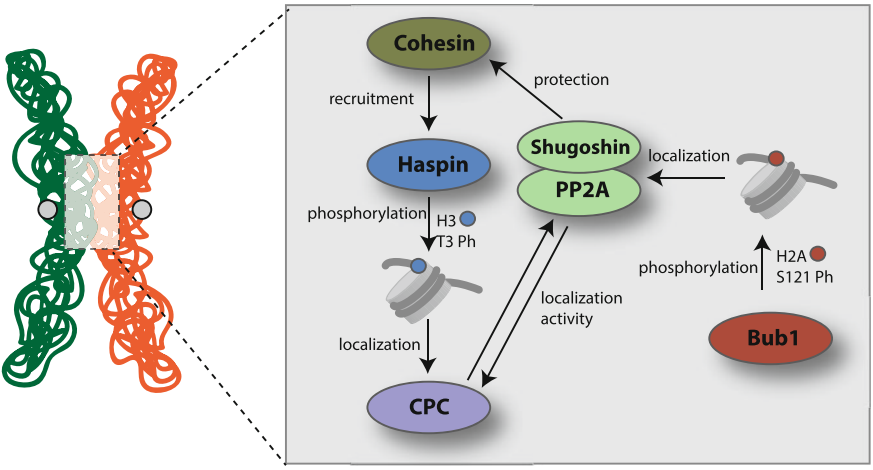

Fig. 10 The inner centromere network. Cohesin sets the blueprint for the inner centromere network, regulating chromosome architecture and microtubule attachment. Cohesin is needed for the recruitment of Haspin kinase, which triggers the cascade resulting in recruitment of CPC and Shugoshin to the pericentromeric region

creation of unattached kinetochores that can trigger SAC signaling. Aurora B, together with its regulatory partners INCENP, Borealin and Survivin, forms the Chromosome Passenger Complex (CPC). This complex decorates the entire chromosome length during early mitotic stages but dynamically shifts its localization towards prometaphase/metaphase, becoming highly enriched at the inner-centromeric region (Reviewed in (Carmena et al. 2012).

Cohesin's importance for CPC localization has been documented in several studies (Carretero et al. 2013; Haarhuis et al. 2013; Kenney and Heald 2006; Mirkovic et al. 2015; Sonoda et al. 2001; Vass et al. 2003) but only recently the mechanistic details for this interaction are being elucidated. CPC localization to the inner centromere was shown to depend on two histone marks: Histone H3 phosphorylation on Threonine 3 (H3pT3) and histone 2A-serine 121 (equivalent to H2A Threonine 120 in humans) phosphorylation (Yamagishi et al. 2010). The cohesin subunit PDS5A interacts with the Haspin Kinase, which is the kinase responsible for H3T3 phosphorylation (Yamagishi et al. 2010). Depletion of Pds5 or cohesin subunits result in delocalized Aurora B and possibly impaired error correction (Carretero et al. 2013; Mirkovic et al. 2015; Yamagishi et al. 2010). Interestingly enough, "too much" cohesin produces a similar phenotype, as WAPL depleted cells also exhibit delocalized Aurora B signals and defective error-correction capacity (Haarhuis et al. 2013).

In addition to CPC localization, cohesin also plays a role in the localization of another key inner centromere component: Shugoshin. Shugoshin interacts directly with cohesin and requires this interaction for its activity (Liu et al. 2013a, b). In this way, cohesin enhances its own centromeric protection but also contributes to other events that are governed by Sgo1 at the centromeres, namely biorientation of sister chromatids, localization of the CPC and SAC silencing (reviewed in Marston 2015).

Thus, while enhancing its own protection, cohesin plays a pivotal role in the establishment of the inner centromere network.

4.3 Force Balance

The binding and stability of microtubule attachments to the kinetochore is enhanced by the tension between the spindle and the kinetochore, both in vivo and in vitro (reviewed in Biggins 2015). Tension-dependent stabilization of kinetochore-microtubule interactions depends on an intrinsic stabilization ability of the mechanical force exerted by the microtubule pulling forces (Akiyoshi et al. 2010), as well as on biochemical changes that promote the stabilization of kinetochore-microtubule interactions. The latter are regulated by Aurora B kinase, responsible for the correction of erroneous microtubule-kinetochore interactions through the phosphorylation of key kinetochore substrates. Upon bipolar attachment, i.e. maximal tension, the increase in the distance between the inner-centromeric Aurora B and the kinetochore is believed to displace Aurora B away from its targets

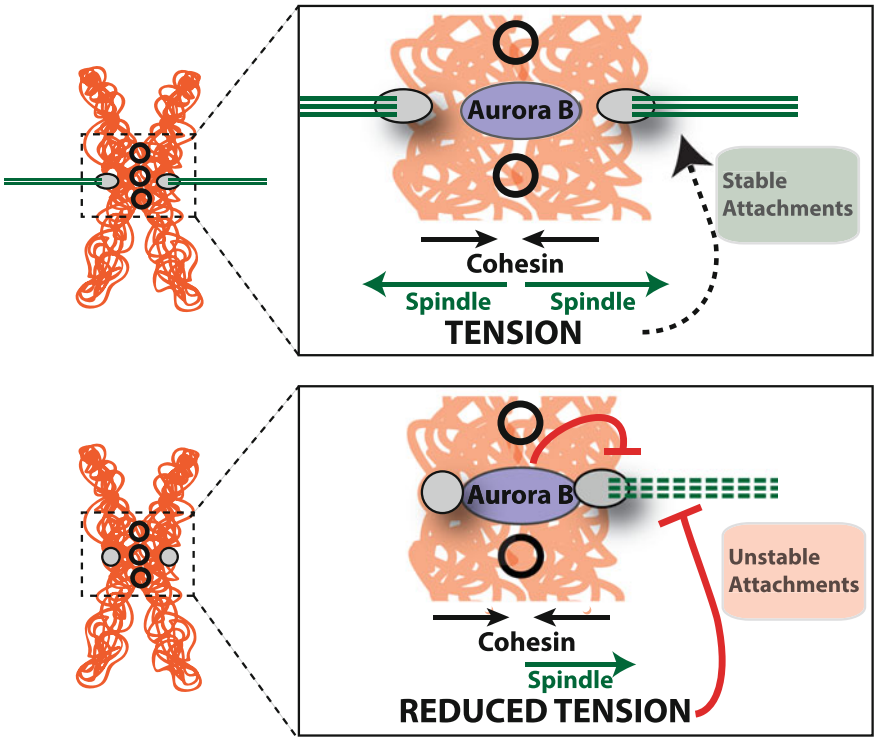

Fig. 11 Force balance. Cohesin is the major force resisting the mitotic spindle during metaphase. The antagonism between cohesin and the spindle results in sufficient tension that is required to stabilize the attachments of microtubules to the kinetochore. Erroneous attachments (e.g. mono-oriented chromosomes or chromosome with the two kinetochores bound to the same pole) are not under sufficient tension. This reduced tension leads to destabilization of these interactions by Aurora B kinase

thus reverting Aurora B-mediated destabilization of microtubule attachments (Liu et al. 2009a) (Fig. 11).

How chromosome tension is established, sensed and ultimately regulates kinetochore-microtubule interactions has been widely investigated. Bipolar attachment increases tension across the entire pericentromeric domain (inter-kinetochore tension), but also within each individual kinetochore, marked by the increase in the distance between the proteins of inner and outer kinetochore (reviewed in (Maresca and Salmon 2010). Both intra- and inter-kinetochore stretch require a counterforce to the spindle to generate stable microtubule attachment and tension. The cohesin ring presents the only force at the centromere that is able to resist the pulling forces of the spindle. Thus, centromeric cohesion contributes to the generation of tension needed for stable chromosome biorientation on the metaphase plane (Fig. 11). It provides the counterforce necessary to maintain a force-equilibrium between with the mitotic spindle, which can generate forces of up

to hundreds of piconewtons (Nicklas et al. 1995; Ye et al. 2016). In agreement with cohesin's major role in the establishment of kinetochore tension, loss of cohesin prior to or during metaphase leads to extensive chromosome shuffling along the spindle, as attachments to isolated single sisters are highly unstable (Drpic et al. 2015; Mirkovic et al. 2015; Oliveira et al. 2010) (Fig. 11).

Whether or not cohesin could also contribute to tension sensing has also been speculated. Upon bipolar attachment, tension across sister chromatids will influence the entire pericentromeric domain and evidence suggests that this alone can lead to removal of centromeric cohesin complexes (Eckert et al. 2007; Ocampo-Hafalla et al. 2007). More distal pericentromeric domains would then provide the necessary antagonistic force to the spindle. This dynamic change on the cohesive forces could alone provide a cue to sense bipolar attachment. In agreement, cohesin-associated molecules, particularly Shugoshin, have been proposed to contribute to tension sensing and SAC silencing at the metaphase-to-anaphase transition (reviewed in Marston 2015).

However, inter-kinetochore stretch does not seem to be necessary for tension sensing as chromosomes in which two neighboring kinetochores were artificially tethered, preventing the inter-kinetochore stretch, still resulted in normal metaphase attachment. These experiments imply that mechanical tension exerted on the single kinetochore might be more important than the stretching between kinetochore pairs itself to stabilize chromosome attachments (Nannas and Murray 2014).

Regardless of the exact location that senses chromosome tension, the structure of the pericentromeric domain will likely play a major influence on the force provided by the chromosomes (Stephens et al. 2013). Does this force balance require a specific amount of cohesin at chromosomes and does centromeric accumulation play a role? It is conceivable that reaching the right spindle counterforce requires a fine-tuning of cohesin levels at chromosomes. This has been difficult to tackle experimentally as manipulating cohesin levels is not a trivial task. Metazoan chromosomes with artificial high levels of cohesin (e.g. WAPL knockdown) do display defects in chromosome attachment. Although these have been largely attributed to defects in the localization of the machinery that regulates microtubule-kinetochore attachments (see Sect. 4.2), it remains to be determined the consequences of too much cohesion on tension establishment and sensing, independently of Aurora B localization.

4.4 Anaphase Sharpness

Cohesin destruction marks the onset of anaphase, a point of no return for every dividing cell. As discussed above (see Sect. 3.4), several feedback loops operate at this stage to ensure efficient cohesin cleavage at this crucial transition. Restricting cohesin to centromeric region may be an additional mechanism to ensure fast anaphase onset and promote synchrony of anaphase movements, particularly in organisms containing variable chromosome sizes. Separase is functionally active

along the entire chromosome, as evidenced by complete cohesin cleavage in WAPL mutants, in which cohesin is now all over chromosome arms, or in cells expressing Separase sensors targeted to the entire chromosomes (Haarhuis et al. 2013; Oliveira et al. 2014; Shindo et al. 2012; Yaakov et al. 2012).

Whether or not the efficiency of cohesin cleavage is the same all over the chromatin mass has been quite controversial. Direct measurements of Separase activity using engineered sensors at different chromosome loci in budding yeast, failed to detect any delay of cleaving telomeric versus centromeric sites (Yaakov et al. 2012). In contrast, other studies support that removal of cohesin at regions distal to the centromere is less efficient than at centromere-proximal ones (Oliveira et al. 2014; Renshaw et al. 2010). These studies thus suggest that although separase is capable of cleaving cohesin all over chromosome arms, coupling residual cohesion to the centromere may be an efficient way to accelerate cohesin degradation. This could be due to the pulling force of the spindle that could aid in cohesin release, or enhanced Separase activity at the centromeric region.

5 Concluding Remarks

In the cell biology field, centromeric cohesin is mostly viewed as an architectural molecule, a molecular glue linking sister chromatids and preventing random chromosome segregation. However, it is crucial to shift such a viewpoint in order to encompass all the diverse functions of cohesin during nuclear division. Restricting cohesion to the centromeric region during mitosis is of paramount importance for efficient chromosome resolution and segregation. Cohesin itself provides the main elastic force necessary to resist the metaphase spindle and establish biorientation of the chromosomes during metaphase. Cohesin is also crucial for the establishment of an inner-centromere network thus contributing to the localization and function of proteins involved in the regulation of chromosome attachments and spindle assembly checkpoint. As such, mitotic cohesin is way more than a pure "architectural" molecule and should be viewed as a dynamic scaffold for multiple mitotic processes, rather than a hinge keeping chromosomes together.

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