Chapter 5

Linking Chromosome Duplication and Segregation via Sister Chromatid Cohesion

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Abstract

DNA replication during S phase generates two identical copies of each chromosome. Each chromosome is destined for a daughter cell, but each daughter must receive one and only one copy of each chromosome. To ensure accurate chromosome segregation, eukaryotic cells are equipped with a mechanism to pair the chromosomes during chromosome duplication and hold the pairs until a bi-oriented mitotic spindle is formed and the pairs are pulled apart. This mechanism is known as sister chromatid cohesion, and its actions span the entire cell cycle. During G1, before DNA is copied during S phase, proteins termed cohesins are loaded onto DNA. Paired chromosomes are held together through G2 phase, and finally the cohesins are dismantled during mitosis. The processes governing sister chromatid cohesion ensure that newly replicated sisters are held together from the moment they are generated to the metaphase–anaphase transition, when sisters separate.

Key words Sister chromatid cohesion, SMC proteins, Cohesin, Adherin/kollerin, DNA replication, Cohesinopathy, Replication fork, Genomic integrity, S phase, Chromosome segregation

1 Introduction

During the cell cycle, new organelles, membranes, cytosol, and genetic materials are all generated to give rise to two new cells. Even during processes that promote asymmetrical cell divisions, arguably, the most important cell-cycle processes revolve around duplication and segregation of the entire genome, so that both daughter cells inherit the exact same genetic material.

During S phase, genomic DNA is replicated and packaged into chromatin. The identical copies of each chromosome are known as sister chromatids, and they are tightly associated together through G2 phase and early mitosis. During metaphase of mitosis, sister chromatids are associated with the mitotic spindle, aligned along the central axis of the cell, and one sister from each pair is associated with a separate spindle pole under tension. At the metaphase–anaphase transition, sister chromatid cohesion is relieved, and the

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microtubule spindle-pulling forces separate each sister chromatid pair and move one copy of the entire genome to one pole. The spindle-pulling forces continue until the cell is divided, and two separate cells are generated at cytokinesis.

Sister pairing calls for the physical tethering of the sister chromatids to each other. The primary proteins responsible for this tethering comprise the cohesin complex. Cohesins are well conserved throughout eukaryotes, and the processes governing cohesion are generally conserved as well. The cohesin complex must be loaded onto DNA during G1 phase prior to DNA replication. For sister chromatid cohesion to be established, the newly replicated DNA copies are encircled by the cohesin complex in S phase. Finally, owing to the elaborate regulation during mitosis, cohesin-mediated sister chromatid-pairing ends, allowing for equal segregation of the genome to each daughter cell. In this review, we discuss the structure of the cohesin complex, how it is loaded onto DNA, the link between DNA replication and cohesion establishment, and finally how the cohesin is released from sister chromatids during mitosis.

2 The Cohesion Complex

Cohesin is a four-subunit complex comprising "structural maintenance of chromosomes (SMC)"-type proteins and non-SMC-type proteins. SMC-type proteins exist in all three domains of life (eukaryota, prokaryota, and archaea), and eukaryotic cells have several SMC proteins that help govern a variety of cellular processes [1]. Smc1 and Smc3 form the cohesin complex with non-SMC subunits, Scc1 and Scc3, and function in sister chromatid cohesion [2]. We focus on this cohesin complex in this review; however, various other complexes containing SMC proteins are involved in the preservation of genome integrity. The Smc2–Smc4 complex is known as condensin and works during mitosis to compact chromosomes [3, 4]. The Smc5–Smc6 complex contributes to various genome maintenance processes including homologous recombination [5–7]. Furthermore, Rad50, a component of the MRN complex, is also an SMC family member and initiates DNA double-strand break processing [8]. While these SMC family proteins are involved in diverse roles in genomic integrity, their structures are remarkably similar (reviewed in ref. 9).

These proteins are characterized by a conserved modular structure, possessing a long coiled-coil region interrupted by a dimerization domain (also known as a hinge domain) and the amino (N)- and carboxyl (C)-termini domains that contain Walker A and Walker B ATP-binding motifs, respectively. Because the coiled-coil region folds at the hinge domain, and the N- and C-termini are brought together to create a nucleotide-binding domain (NBD), each monomer of SMC proteins forms a structure

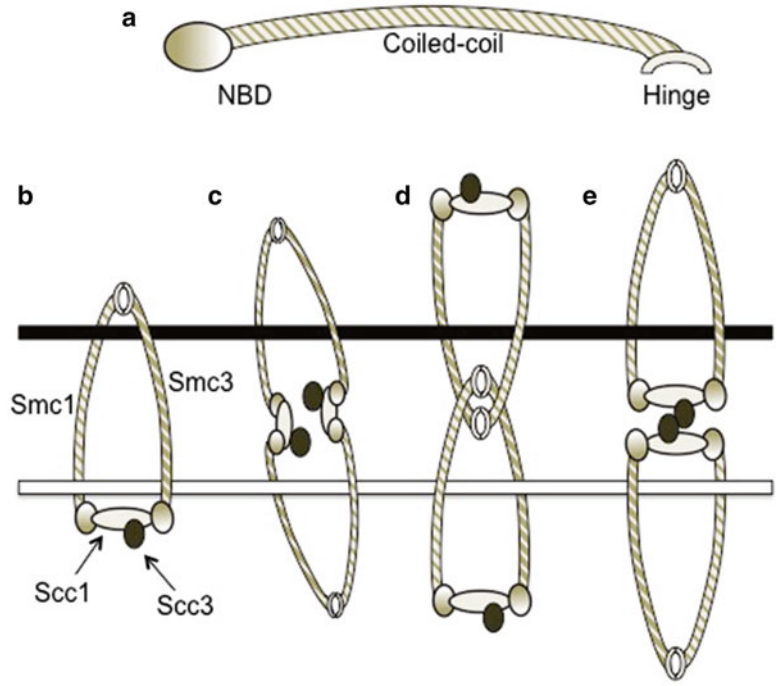

Fig. 1 Models of cohesin structure. (**a**) The general structure of the Smc1/3 proteins. A nucleotide-binding domain with ATPase activity (NBD) connected by a coiled-coil domain to a hinge domain. (**b**) The cohesin complex as a ring. (**c**) The cohesin ring as a 2:2:2 complex of Smc1/Smc3/Scc1. (**d**) The cohesin complex as a bracelet or links in a chain. (**e**) The cohesin complex "handcuff" model. The *black* and *white bars* denote the sister chromatids

reminiscent of two spheroid objects connected by a flexible chain (Fig. 1a) [10, 11].

The overall structure of the cohesion SMC proteins helps to define the function of the cohesin complex and its role in tethering sister chromatids. While the precise shape of the cohesion complex has yet to be fully sorted out (discussed below), the structures of the major components of the cohesion complex have been determined. The cohesin SMC proteins exist as an Smc1–Smc3 heterodimer in the cell, with two 50-nm extensions protruding from the interacting hinge domain (Fig. 1a) [2]. The heterodimer is brought to a closed form by Scc1, which is a member of kleisin protein family [2, 12, 13]. Scc1 interacts with NBD domains of both Smc1 and Smc3, generating a ring with a diameter of approximately 45–50 nm (Fig. 1b) [2, 13, 14]. Within this tripartite complex, paired sister chromatids are trapped and physically kept in close proximity. The cohesins then keep the sister chromatids juxtaposed within multiple complexes loaded on every chromosome. Localization analyses place the cohesin complex at intervals of ~20 kb throughout the genome [15, 16].

Apart from the core ring-forming subunits, the functional cohesin complex requires the Scc3 subunit [17, 18]. In vertebrates, there are two Scc3 homologs, known as stromal antigen (SA) or STAG proteins, SA1/STAG1 and SA2/STAG2 [19]. Although Scc3 is not a structural subunit of cohesin, it is essential for cell growth in yeast and is required for proper cohesion processes [18, 20]. Scc3 binds directly to Scc1, and together these proteins mediate cohesin interaction with other proteins required for regulation of sister chromatid cohesion throughout the cell cycle [2, 21].

The structure of the cohesin complex in vivo is still a topic for discussion in the field. Several different models of cohesin complex entrapment of DNA have been proposed, and each has different implications for the overall cohesin complex stoichiometry. The most prominent model of cohesin complex structure is that of a tripartite ring made of Smc1–Smc3–Scc1 [2, 22]. This ring encircles sister chromatid pairs within its diameter with a 1:1:1 stoichiometry (Fig. 1b). As an alternative possibility, it has been proposed that these rings could be concatenated to increase the ring diameter. In this model, the interacting faces each still associates with the same subunit, but on a different molecule (with a stoichiometry of 2:2:2, 3:3:3, etc.) (Fig. 1c) [23]. Instead of one ring holding two chromosomes, another model posits that the rings can form links such as on a chain. In this model, each ring holds one chromosome and also another cohesin ring (Fig. 1d) [24]. Finally, a handcuff model has been proposed in which closed cohesin rings are bridged by an Scc3 molecule, a shape reminiscent of handcuff around two arms [25, 26] (Fig. 1e). The strongest evidence so far has been for 1:1:1 tripartite rings forming cohesin complexes in vivo on circular minichromosomes in yeast, but further work will define whether this conformation is universal [27].

3 Loading Cohesin Prior to DNA Replication

To properly pair chromosomes and to reduce pairing errors, sister chromosomes need to be held together as soon as they are duplicated. Rather than loading cohesin complexes after DNA replication, the rings are loaded onto the parental DNA prior to DNA replication. Vertebrates perform this process almost immediately after the parental DNA is separated from its sister copy, during telophase at the end of mitosis. In fungi, the cohesin loading occurs during G1 phase. In both cases, a conserved protein complex performs the loading. In yeast, two proteins, Scc2 and Scc4, form a complex and are responsible for cohesin loading (Fig. 2) [28]. In mammals, orthologs of these proteins are known as NIPBL and MAU2, respectively, and form a cohesin-loading complex. In the literature, this complex is often referred to as adherin, but it

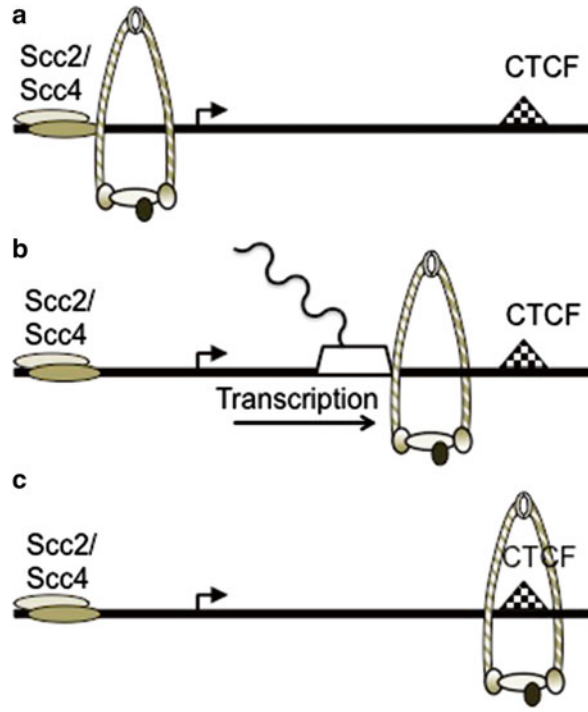

Fig. 2 Redistribution of cohesins by transcription. (**a**) The Scc2/Scc4 complex loads cohesin complexes onto chromatin. (**b**) The transcription machinery pushes some cohesin complexes to the end of open reading frames. (**c**) Cohesins interact with CTCF proteins bound at the edge of open transcription

was recently proposed to rename the complex kollerin to avoid confusion with cadherin proteins [23]. Neither subunit in the loading complex has enzymatic function. It is thought that the function of the adherin/kollerin complex is to facilitate or stimulate the ATPase activity of the Smc1 and Smc3 proteins to load them onto DNA. The ATPase activity of the Smc1/3 proteins is involved in loading of cohesin rings onto DNA [29, 30]. However, an ATPase mutation, which permits ATP binding but ATP hydrolysis, in Smc1 and Smc3 still allows for the loading of the cohesin ring complex onto DNA, although the association is not stable. This indicates that the cohesin complex can be recruited to chromatin without ATPase activity, but likely not in the conformation required for proper chromosome cohesion [31]. Strikingly, the localization of these mutant cohesin complexes is similar to that of Scc2/4, suggesting that ATP binding by SMCs is involved in initial recruitment and association of cohesins with chromatin, but transition to a stable cohesin loading on DNA and subsequent relocalization of cohesins require ATP hydrolysis.

What might the hydrolysis of ATP do to load cohesin rings onto DNA? Interestingly, the answer may come at the opposite

ends of Smc1/3 molecules, the hinge domain. There are several lines of evidence that the complex opens at the Smc1–Smc3 hinge domains to trap or encircle chromatin. Gruber et al. fused Smc1 and Smc3 hinge domains. This fusion construct was lethal to budding yeast, whereas fusion constructs that permanently connect SMC subunits and Scc1 (Smc1–Scc1 and Smc3–Scc1) were not [32]. These experiments suggest that the ring opens at the hinge domain. How then could ATP hydrolysis at the NBD, which is at the opposite end of each SMC subunit, affect the hinge domain interface? Investigation of Rad50, an SMC family protein involved in DNA double-strand break processing, revealed that ATP hydrolysis at the NBD induces a conformational change of the entire protein [33]. The dimerization- and nucleotide-binding domains of Rad50 are separated by a long coiled coil of about 50 nm [34]. In spite of this long distance, upon ATP hydrolysis at its NBD, Rad50 undergoes a conformational change that rotates and releases Rad50 from dimerization at the hinge-domain equivalent [35]. Therefore, a similar conformational change may occur in the Smc1–Smc3 hinge interaction upon ATP hydrolysis at the NBD, leading to ring opening. Once the ring is opened, the next step is to trap sister chromatids and close the ring. The dimerization of two hinges from Smc1 and Smc3 is dependent on two independent interaction surfaces of each hinge. This configuration creates a small donut-like structure at the hinge [2]. Mutational analysis of the hinge domains of Smc1/3 has shown that the interaction between two separate faces on each hinge domain is required for stable association of cohesin with chromatin. Mutations within either interface resulted in lethal defects of sister chromatid cohesion, presumably due to the inability to close the ring and stably load cohesins onto chromatin [36]. Therefore, cohesin loading involves two processes: ATP hydrolysis to open the complex at the hinge interface, followed by securing interactions of the hinge domains of Smc1/3 to close the ring for stable chromatin association.

To successfully tether sister chromatids together, cohesin complexes must be loaded at many sites on each chromosome. Therefore, cohesin loading occurs throughout the genome. However, the loading sites are species specific, although there is no major difference in the quality of sister chromatid cohesion. In yeast, cohesin loading is especially concentrated at centromeres and telomeres [37]. Interestingly, in *Xenopus*, the pre-replication complexes (pre-RCs) forming at replication origins recruit the Scc2–Scc4 complex [38, 39]. This recruitment links origins of replication to cohesin loading. Other factors at both origin and cohesin-loading sites facilitate the loading of cohesins. In yeast, kinetochore proteins as well as replication fork proteins are required for proper loading of cohesins at the centromere and for subsequent stability of pericentromeric cohesion [40]. Strikingly, kinase activity known to regulate origin firing is also required for cohesin loading in *Xenopus*. The recruitment of the adherin/kollerin

complex to the pre-RC is dependent upon the DDK (Dbf4–Cdc7) kinase, which acts on pre-RCs [41]. It appears that mechanisms linking cohesin loading to other processes have somewhat diverged in evolution; however, each leads to successful loading of cohesin complexes.

Although cohesins are loaded at Scc2–Scc4 sites, some cohesins do not stay localized at the same sites for the duration of the cell cycle. In yeast, large numbers of cohesin rings can be "pushed" or "slid" away from their original loading sites and moved into intergenic regions (Fig. 2) [37, 42]. Since these sites are often at the end of open reading frames or at regions of convergent transcription, the model is that the transcription elongation complex facilitates the translocation of cohesins away from transcriptionally active sites to heterochromatin regions, usually proximal to euchromatin. In mammalian cells, the outcome is the same, but the mechanism may be different. The majority of NIPBL/Scc2-binding sites colocalize with cohesin rings; however, the cohesin ring sites far outnumber NIPBL sites, and most cohesin ring sites do not have coincident NIPBL binding [43, 44]. These results are consistent with the model that mammalian cohesin can be loaded at NIPBL/Scc2–Mau2/Scc4 sites and then relocated elsewhere (Fig. 2). However, in higher eukaryotes, cohesin rings might not be displaced by transcription machinery as seen in yeast. For example, *Drosophila* genes contain cohesins even when actively transcribed [45]. Therefore, the relocation of cohesin rings after loading may occur by a different process. In metazoans, the transcriptional repressor CTCF uses its zinc-finger domains to recognize DNA sequences containing CCCTC repeats. CTCF is found in numerous sites on the genome and has a variety of roles in chromatin architecture and transcription regulation (reviewed in ref. 46). Interestingly, CTCF has a role in determining cohesin ring sites on DNA (Fig. 2). Cohesin loading is not dependent on CTCF, but the localization of a large subset of cohesin complexes is dictated by CTCF [16]. The tethering of cohesin rings to CTCF appears to act through SA2 (Scc3), which binds the CTCF C-terminus, and this interaction appears to contribute to CTCF functions in transcription insulation [47]. Although this study explains how cohesin complexes are associated with CTCF sites, no clear mechanism has been found for translocating cohesin rings from NIPBL sites to CTCF sites.

4 Establishment of Sister Chromatid Cohesion During DNA Replication

Sister chromatid cohesion is established during DNA replication and maintained until the two sisters separate in mitosis. Cohesin complexes are loaded onto DNA and associated with chromatin prior to DNA replication. However, these cohesins are not yet engaged in sister chromatid cohesion. Initially, it was unclear

whether cohesin paired chromatids during DNA replication or after replication was completed. To test whether sister chromosome cohesion could be established during S phase or during G2 (after the genome has been duplicated), Uhlmann and Nasmyth placed the *SCC1* gene under an inducible promoter and restricted Scc1 production to G1 or G2 phase in budding yeast [48]. When Scc1 was expressed in G1 phase (before DNA replication), the cells paired their chromosomes properly. However, when Scc1 expression was turned on only in G2 (after DNA replication), cells failed to pair their chromosomes, leading to chromosome missegregation and cell death [48]. The temporal requirement for Scc1 is consistent with the requirement of the adherin/kollerin cohesin loader complex, which is dispensable after G1 [49]. Thus, the cohesin ring subunits must be present when Scc2–Scc4 mediates their loading. Further, mutation in a critical arginine finger within the ATPase-active site suggests that Smc1/3-mediated ATP hydrolysis only occurs during cohesin loading during G1 in yeast [49]. Thus, the complete cohesin complex must be loaded onto chromatin prior to DNA replication to establish sister chromatid cohesion [48]. In addition, cohesion establishment requires involvement of replication factors moving with the replication fork in order to pair the sister chromosomes during S phase without displacing the cohesin ring from the chromatin [49]. Therefore, cohesin complexes are loaded prior to DNA replication, remain associated with chromatin during DNA replication, and then fully establish sister chromatid cohesion during DNA replication. Because sister chromatids are in close proximity immediately after DNA replication at the replication fork, cells are able to eliminate the need to search for sister chromatids, thus increasing the fidelity of sister chromatid cohesion.

Upon DNA replication, the cohesin complex undergoes a transition, leading to a more secure association with chromatin. Fluorescent recovery after photobleaching (FRAP) experiments show that, after cohesion is established during S phase in mammalian cells, cohesin complexes are far more stably associated with DNA [50]. One of the major S-phase factors involved in establishment of sister chromatin cohesion is the acetyltransferase Eco1, which is also known as Ctf7 [18, 51]. In animals, two genes encode for the acetyltransferase. In humans, the Eco1 homologs are known as Esco1/2 (or EFO1/2), and in *Drosophila*, they are called san and deco; both acetyltransferases are required for cohesion in animals [52–54]. In yeast, Eco1 appears to progress with the replication fork during DNA replication [37]. Although the Eco1 acetyltransferase activity leads to the establishment of cohesion, Eco1 does not promote a direct interaction of cohesin and DNA. Instead, it appears that Eco1-dependent acetylation leads to a stabilization of cohesin complexes on chromatin. The target of Eco1/Ctf7 acetylation activity is known to be two lysine residues near the NBD on the Smc3 [55–57].

The exact mechanism by which Eco1/Ctf7-dependent Smc3 acetylation stabilizes cohesion is unknown, but several models have been put forward. In the first model, Smc3 acetylation negatively regulates ATP binding by Smc3 and breaks the ATP-loading and -hydrolysis cycle of cohesin loading. This leads to stabilization of the cohesin complex on chromatin while preventing oligomerization of SMC proteins that could negatively affect sister chromatid cohesion. This is supported by the fact that acetyl-mimetic mutations of Smc3 restore viability of cells with lethal ATP-hydrolysis mutations of the same molecule [58]. In the second model, Smc3 acetylation appears to have an effect on suppressing anti-cohesion factors, such as Wapl and Pds5, that bind to the cohesin complex [59, 60]. Mutations in budding yeast Wpl1 (Wapl homolog) rescue a mutant allele of Eco1 (*eco1-1*), demonstrating that these proteins have counteracting activities in cohesion [55]. Furthermore, when Wpl1 and Pds5 are deleted in yeast, the requirement for Eco1-dependent acetylation of Smc3 is abolished, and cells remain viable. This is presumably due to the suppression of the anti-cohesion establishment activity of the Wpl1–Pds5 complex during S phase [61, 62]. Interestingly, Eco1/Ctf7 activity is continuously required to maintain Smc3 acetylation, but it is dispensable after DNA replication, further supporting the model that the acetylated form of Smc3 is only required for cohesion establishment during S phase, but not for cohesion maintenance after DNA replication has been completed [18, 51, 61]. Thus, Smc3 acetylation by Eco1/Ctf7 may counteract an antiestablishment activity of Wapl and Pds5 in order to establish cohesion. In vertebrate animals, this appears to occur through recruitment of an essential cohesion protein, Sororin, which stabilizes cohesin on chromatin [63, 64]. Sororin is required only in the presence of Wapl, suggesting that Sororin counteracts Wapl after it is recruited to acetylated cohesins [65]. However, this mechanism may not be universal, because no Sororin homolog has been identified in yeast.

5 Establishing Cohesion at the Replication Fork

The establishment of cohesion at the replication fork is, as mentioned previously, a clever mechanism to pair sister chromatids as soon as they are generated. While most work has focused on how replication fork proteins impact chromosome cohesion phenotypes, proper establishment of cohesion also plays an important role in DNA replication. It has been shown that by restricting Smc3 acetylation, DNA replication speed is reduced [66]. Eco1 is a replisome-associated acetyltransferase and travels with the replisome during DNA replication (Fig. 3) [49, 67, 68]. Overexpression of the polymerase clamp PCNA rescues temperature-sensitive mutants of Eco1 in budding yeast [51]. PCNA is a heterotrimeric

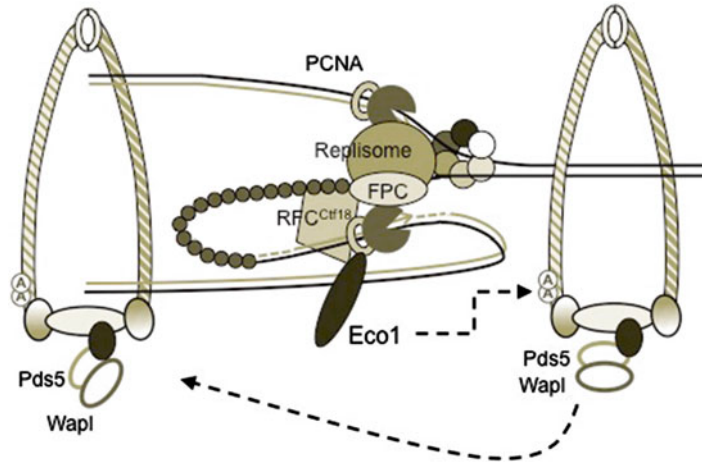

Fig. 3 Interactions between the cohesins and the replisome establish sister chromatid cohesion. Localizing the Eco1 acetyltransferase to the replisome through PCNA interaction allows for cohesion establishment at the replication fork. Factors such as the FPC and RFC^{Ctf18} stabilize the replisome and ensure that the replisome structure is amenable to cohesion establishment

clamp that coordinates a myriad of interactions between replication and other processes [69]. Eco1 binds PCNA at its PCNA-interacting protein (PIP) box domain. The PIP box domain is conserved throughout Eco1 homologs, including the human variant Esco2 [68]. Since Eco1 interacts with PCNA, one can imagine a model in which Eco1 travels with the replication fork and acetylates Smc3 subunits of cohesin complexes as they are encountered by the replication fork, establishing cohesion as the replisome progresses (Fig. 3).

During DNA replication, PCNA is loaded onto DNA continuously by a five-subunit clamp loader known as replication factor C (RFC) complex [70]. An alternative RFC complex containing Ctf18 (RFC^{Ctf18}) is capable of loading PCNA onto DNA and is required for proper sister chromatid cohesion [49, 66, 71–75]. Indeed, PCNA localization to chromatin is dramatically reduced in *ctf18* mutants, rendering these cells more sensitive to genotoxic agents [49]. However, replication and cohesion establishment still occur in *ctf18* cells, indicating that this function is not essential for cohesion establishment. An open question is how RFC^{Ctf18}-mediated PCNA loading enhances cohesin establishment in a manner different from the canonical RFC complex. It is possible that RFC^{Ctf18} loads PCNA specifically at sites of cohesin localization or loads a modified PCNA that interacts more efficiently with Eco1. RFC^{Ctf18}-dependent promotion of cohesion establishment may be indirect, in which RFC^{Ctf18} might serve to increase replisome integrity or maintain the replisome in a confirmation in such a way that the replisome can smoothly progress through cohesin-associated chromosome sites.

Proteins involved in replisome structure and stability also play a role in proper cohesion establishment during DNA replication (Fig. 3). Representative of these include Mrc1/Claspin, Ctf4/And-1, and the replication fork protection complex (FPC). Mrc1/Claspin, which interacts with DNA polymerase ε and the MCM helicase, moves with the replication fork and mediates the signal of stalled replication forks to activate the replication checkpoint [76–78]. The Ctf4/And-1 protein acts as a linker between the MCM helicase and the DNA polymerase α-primase complex while promoting proper cohesion establishment [79–82]. The FPC, which consists of the Timeless and Tipin proteins in metazoans, plays a critical role in replisome stabilization and replication checkpoint signaling and is also involved in promoting sister chromatid cohesion (reviewed in ref. 83). Although it is still largely unknown how this complex serves as a cohesion-promoting factor, depletion or mutation of FPC components in a variety of eukaryotic organisms leads to a cohesion defect [84–91]. It has been proposed that the FPC coordinates leading- and lagging-strand DNA synthesis processes at the replication fork [92, 93]. Inefficient lagging-strand synthesis may cause a long stretch of single-stranded DNA, generating a large loop structure at the replication fork. Such a large replication fork structure with the replisome components would render the replisome unable to pass through the cohesin ring complex [49]. Consistent with this notion, lagging-strand processing has been linked with Smc3 acetylation. Eco1 interacts with Fen1, a flap endonuclease required for Okazaki fragment maturation, possibly positioning the acetyltransferase to act on Smc3 as it localizes to the lagging-strand processing machinery [94, 95].

As mentioned above, efficient lagging-strand synthesis appears to be a key determinant of sister chromatid cohesion establishment. In both mammalian cells and budding yeast, Fen1 associates with the ChlR1 (Chl1 in yeast) protein, a member of the FANCJ DNA helicase family [95, 96]. The loss of ChlR1 leads to sister chromatid cohesion defects in yeast and mammalian cells [97–101]. Biochemical studies revealed that ChlR1 stimulates Fen1 flap endonuclease activity in vitro, and loss of Fen1 itself also leads to cohesion defects with striking similarity to the cohesion defects associated with ChlR1 depletion [96], indicating the intimate link between lagging-strand processing at the replication fork and sister chromatid cohesion. It appears that ChlR1 and FPC operate in the same pathway to promote sister chromatid cohesion. Studies in human cells show that ChlR1 co-purifies with the FPC and that both the FPC and ChlR1 are found to interact with cohesin complexes by immunoprecipitation [89, 101]. ChlR1 overexpression rescues cohesion defects caused by FPC depletion, while Chl1 overexpression suppresses the sensitivity of FPC mutants to genotoxic agents in fission yeast [86, 89]. Furthermore, downregulation of FPC or ChlR1 causes profound defects in replication

recover after replication stress [89, 102]. Considering that ChlR1 interacts with Fen1 [96], it is highly possible that the FPC and ChlR1 act together to facilitate lagging-strand synthesis to accommodate proper establishment of sister chromatid cohesion at the replication fork.

In addition to the direct involvement of lagging-strand synthesis in sister chromatid cohesion, the replisome itself may also need to be stabilized when it passes through the cohesin-bound chromosome regions. It is proposed that the fork stalls transiently at the sites of cohesin complexes, necessitating fork stabilization [103, 104]. Indeed, the FPC and ChlR1/Chl1 are involved in maintaining replisome stability when the replication fork stalls, probably at the lagging strand [83, 102]. It is also important to note that RFC^{Ctf18} is involved in both fork stabilization and sister chromatid cohesion [86]. Interestingly, loss of RFC^{Ctf18} results in reduced levels of Smc3 acetylation [66]. In addition, RFC^{Ctf18} stimulates the helicase activity of ChlR1, suggesting the role of RFC^{Ctf18} in lagging-strand processing. Therefore, it is possible that fork stabilization and efficient lagging-strand synthesis are required for efficient acetylation of Smc3 by Eco1 acetyltransferase at the replication fork. Since Eco1 physically associates with PCNA [68], the localization of Eco1 at the fork may be dependent on PCNA loaded by RFC^{Ctf18} and also on Fen1 engaged at the lagging strand. Such a molecular configuration may provide a condition for efficient Smc3 acetylation that promotes fork progression through cohesin-bound chromosome regions. An alternative explanation is that uncoupling the lagging strand from the leading strand creates a structure that is incompatible with passage through the cohesin complex. Additional studies are needed to disentangle these possibilities and determine the relationship between replisome progression and cohesion establishment.

6 G2/M Phase: Maintaining and Disassembling Chromosome Cohesion

Once sister chromatid cohesion is established, it must be maintained until cells segregate sister chromosomes at anaphase. Upon the completion of DNA replication in budding yeast, cohesion establishment is ended by the Clb2–Cdk1 complex-dependent phosphorylation of Eco1 [105]. This phosphorylation greatly enhances the targeting of Eco1 to the SCF^{Cdc4} ubiquitin ligase complex, leading to the degradation of Eco1 [106]. There is one notable exception: in response to DNA damage, Eco1 is stabilized, and cohesin complexes need to be loaded at the sites of DNA damage for a proper DNA damage response [107–109]. Therefore, in the absence of DNA damage, chromosome cohesion must be maintained on chromosomes after DNA replication, since Eco1 is not available to reestablish cohesion.

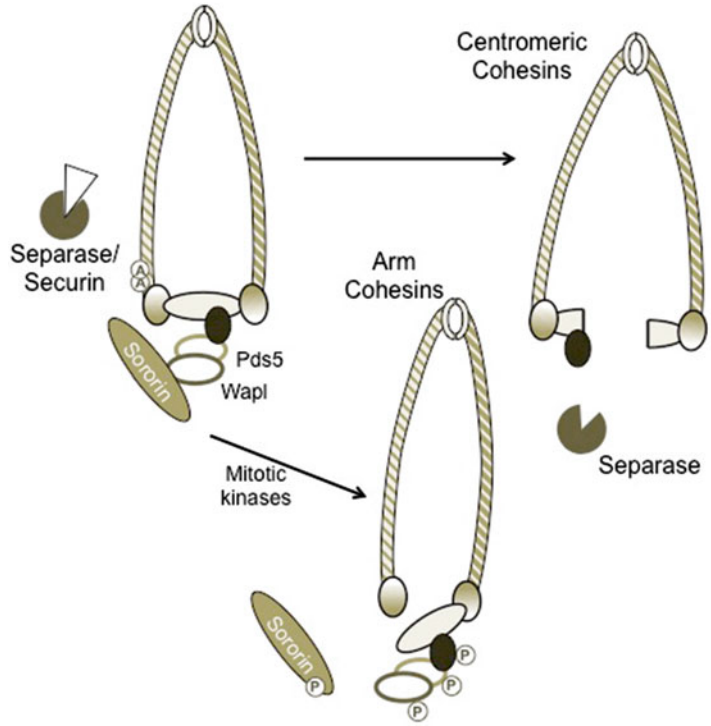

Fig. 4 The cohesin complex is opened by two mechanisms at mitosis. G2 cohesins are stabilized and protected from dissociation and complex opening. On chromosome arms, mitotic kinases phosphorylate multiple targets, reducing Sororin inhibition of Wapl and opening the cohesion complex at the Smc3–Scc1 interface. At centromeres and pericentromeres, the APC destroys securin, activating separase and leading to Scc1 degradation and complex opening

After DNA replication, cohesin complexes are rendered far more stable on chromatin than prior to S phase. FRAP studies in both yeast and humans show that the turnover of cohesin complexes on chromatin is greatly reduced in G2 [36, 50, 110]. In human cells perhaps one-third of cohesin complexes are stably associated with chromatin for the duration of G2 phase, a dramatic increase from the ~25-min residence time of G1 cohesin complexes [50]. In this state, cohesins stably pair sister chromatids until mitosis where the process of removing cohesin complexes is highly regulated (Fig. 4).

How is then cohesion maintained? It appears that Smc3 acetylation has a key role in this mechanism. Major factors involved in cohesion maintenance include Scc3 and Pds5. In the absence of functional Scc3 or Pds5, the levels of cohesin on DNA are reduced [18, 111]. As described below, Pds5 appears to protect Smc3 from deacetylation by Hos1/HDAC8 deacetylase during G2 phase through early mitosis. Since cohesion maintenance and subsequent cohesin removal are tightly coordinated,

the mechanisms that stabilize cohesin complexes on chromatin must be efficiently deactivated to facilitate removal of cohesin complexes during mitosis.

Cohesin complexes distributed over each chromatid pair in the eukaryotic genome must be disassembled at the proper time every cell cycle. The dismantling of cohesion completes the task of the cohesin complexes that pair the sister chromatids until they are separated equally to two daughter cells. Two distinct processes are initiated during mitosis to remove cohesin complexes (Fig. 4). First, during prophase and metaphase much of the cohesin complexes localized to chromosome arms are released. This action is followed by destroying cohesin complexes at pericentromeric regions at the metaphase–anaphase transition, allowing the segregation of sister chromatids to opposing poles. Together, these processes remove all functional cohesin complexes from the DNA.

First, removal of cohesin complexes from the chromosome arms requires the antiestablishment factor Wapl [59, 60]. Interestingly, Wapl-mediated alleviation of cohesion does not require degradation of cohesins, whereas later stage cohesin removal does. Instead, Wapl-associated cohesin removal involves opening of the cohesin complex at the Smc3–Scc1 interface [112]. This is counteracted by Smc3 acetylation, which represses Wapl-mediated cohesin opening [112, 113]. Therefore, maintaining Smc3 acetylation is vital to preserving cohesion until prometaphase. By preventing the Wapl-dependent cohesin opening, Pds5, in concert with Scc3, protects Smc3 acetylation from a deacetylase known as HDAC8 (Hos1 in budding yeast) [114].

It appears that the concerted effort of several kinases on cohesin complexes effectively deactivate the protective activity of Pds5 and Sororin, the latter of which stabilizes acetylated cohesins by counteracting Wapl activity [63, 65, 115, 116]. Consistent with this idea, phosphorylation of SA2 (Scc3 ortholog) is required for the dissociation of cohesins during prophase and prometaphase [117]. Mitotic cyclin–CDK complexes phosphorylate *Xenopus* XSA1/2 (Scc3 orthologs) in vitro [19]. Plk1 activity is required for alleviation of cohesion during mitosis, where *Xenopus* Scc3 orthologs are phosphorylated in a Plk1-dependent manner [118]. Furthermore, proteomics approaches indicate that both Pds5 and Wapl are phosphorylated by mitotic kinases [116]. Sororin-dependent antagonization of Wapl is also regulated by mitotic kinases. During mitosis, aurora B and cyclin–CDK complexes phosphorylate Sororin, thus freeing Wapl from its inhibition [119]. From these studies, one could imagine a mechanism by which mitotic kinases further stimulate Wapl activity (by removal of Sororin) while deactivating Pds5 to allow deacetylation of Smc3 and opening of cohesin complexes. It is also possible that phosphorylation of cohesin complexes promotes Smc3 deacetylation (Fig. 4). These actions lead to robust cohesin complex release from DNA on chromosome arms.

Interestingly, the process of cohesin removal at centromeric/pericentromeric regions is Wapl independent. The centromeric regions of sister chromosomes are protected during metaphase by the Shugoshin (Sgo1) protein [120]. When SA2 mitotic phosphorylation-site mutants were expressed, cohesion defects and the mitotic arrest phonotype of Sgo1-deficient cells were alleviated, suggesting that Shugoshin prevents phosphorylation of Scc3 to preserve cohesion until Shugoshin is destroyed [121]. Indeed, Shugoshin is activated by mitotic cyclin–CDK activity and associates with centromeres during mitosis [122]. At the centromere, Shugoshin recruits PP2A, a phosphatase that prevents the phospho-regulation of cohesin subunits [123]. The localization of PP2A to centromeres prevents the Wapl-mediated mechanism of cohesin removal by preventing phosphorylation of key cohesin components. Therefore, another mechanism must control cohesin removal at the centromeric and pericentromeric regions of chromosomes.

Sister chromatid separation should ideally occur during anaphase as this is when sister chromosomes migrate to opposite poles of the mitotic spindle. Cohesion of chromosome arms is removed before the metaphase–anaphase transition, leaving only centromeric cohesion to tether sister chromatids to each other. This leaves a relatively small area of each chromosome held by cohesins left to remove. At the onset of anaphase, the Scc1 subunit of the tripartite cohesin complex undergoes a proteolytic cleavage by a protein known as separin or separase [124, 125]. The separin protein is bound and rendered inactive by securin, preventing premature activity [126, 127]. Securin is a target of the anaphase-promoting complex (APC), a ubiquitin ligase complex that becomes active at the metaphase–anaphase transition [128, 129]. Securin has been characterized as one of the major targets (along with the mitotic cyclin) for the APC^{Cdc20} complex in yeast cell-cycle regulation [130]. Once securin is degraded, separin/separase is free to cleave Scc1 and relieve chromosome cohesion, allowing sister chromatids to be pulled to their respective poles by the mitotic microtubule spindle (Fig. 4). At this point, chromosomes are unpaired and free of cohesin complexes. Prior to the next DNA replication round, cohesins are loaded again and the cycle is iterated.

7 Cohesinopathies: Broken Rings That Compromise Genomic Integrity

The regulation of genetic inheritance is critical for the reproductive and cellular health of humans. Although not discussed in this review, meiotic chromosome cohesion uses a similar mechanism to that of mitotic sister chromatid cohesion, and failure to properly pair chromosomes during meiosis can lead to trisomy disorders such as Downs, Edwards, or Patau syndromes [131]. However, most types of aneuploidy are incompatible with development.

In analyses of human spontaneous abortions, it has been observed that over 35 % are trisomic or monosomic [132]. It has been proposed that this high rate of chromosomal abnormalities is due to chromosome cohesion defects during meiosis, probably due to the fact that cohesion must be maintained for many years in human oocytes (reviewed in ref. 133).

Autosomal or spontaneous mutations in the cohesion establishment and maintenance pathways can also lead to syndromes in humans that are collectively known as cohesinopathies. The severity of these disorders underlines the importance of maintaining proper sister chromosome cohesion during development and cell proliferation in tissue maintenance. Cornelia de Lange syndrome (CdLS) is a human disease characterized by short stature, craniofacial/limb abnormalities, seizures, and mental retardation. In addition, many CdLS patients die of gastrointestinal problems or pneumonia, suggesting immune-system problems in these patients [134]. CdLS is caused by mutations in cohesion proteins NIPBL (the human Scc2 homolog), Smc1, or Smc3 [135–138]. Mutations in NIPBL, the cohesin loader, have a stronger effect and lead to a more serious form of CdLS. Recently, mutations in HDAC8, the Smc3 deacetylase, have also been identified in some CdLS patients with previously uncharacterized mutations [139].

Interestingly, cells derived from CdLS patients display strong sensitivity to DNA-damaging agents [140]. This suggests that some phenotypes of the disease could result from improper DNA repair responses, yet most CdLS patients do not have increased tumor incidence. Roberts syndrome has a similar clinical presentation to CdLS, although it is caused by mutations in Esco2 acetyltransferase [141].

A recently characterized disease, Warsaw breakage syndrome (WABS), has been attributed to the loss of functional DDX11/ChlR1 DNA helicase, which plays a critical role during S phase to establish proper sister chromatid cohesion [101, 102, 142, 143]. Mutations to both alleles of the *DDX11/CHLR1* gene lead to WABS, which is characterized by severe developmental defects, including microcephaly, growth and mental retardation, and facial dysmorphy [143]. The first WABS patient was reported to carry biallelic mutations in the *DDX11/CHLR1* gene, including a splice-site mutation and a carboxyl-terminal deletion [143]. More recently, a new homozygous mutation in *DDX11/CHLR1* was identified in siblings with many of the symptoms associated with WABS, confirming the role of *DDX11/CHLR1* mutations in WABS [144]. Interestingly, the phenotypic presentation of WABS is a combination of those seen in patients with mutations in cohesion establishment proteins (such as Roberts syndrome or CdLS) and in Fanconi anemia pathway, which plays a critical role in the repair of DNA interstrand cross-links during DNA replication [145], further confirming the role of DDX11/ChlR1 in sister chromatid cohesion during S phase.

The processes of cohesin establishment, maintenance, and dissolution are tightly regulated through the cell cycle. The ability to coordinate chromosome cohesion with DNA replication is critical for proper sister chromatid pairing during S phase, thereby allowing for their equal segregation at mitosis. Unlocking these mechanisms is an important research focus of genome maintenance mechanisms. However, much work remains to understand how the processes occurring at the replication fork are linked to cohesin complexes. Importantly, CdLS cells have increased genotoxic sensitivity [140], and WABS cells show combined phenotypes of Fanconi anemia and the cohesinopathies, including abnormal chromosome segregation and sensitivities to interstrand crosslinking agents [143]. These findings indicate the inseparable connection between sister chromatid cohesion and DNA replication/repair pathways. By studying the mechanisms of these diseases and developing possible therapeutic strategies, we will have a unique opportunity to further characterize the complicated interplay between DNA replication and cohesion processes.

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