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The roles of cohesins in mitosis, meiosis, and human health and disease

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Summary

Mitosis and meiosis are essential processes that occur during development. Throughout these processes, cohesion is required to keep the sister chromatids together until their separation at anaphase. Cohesion is created by multi-protein subunit complexes called cohesins. Although the subunits differ slightly in mitosis and meiosis, the canonical cohesin complex is composed of four subunits that are quite diverse. The cohesin complexes are also important for DNA repair, gene expression, development, and genome integrity. Here we provide an overview of the roles of cohesins during these different events, as well as their roles in human health and disease, including the cohesinopathies. Although the exact roles and mechanisms of these proteins are still being elucidated, this review will serve as a guide for the current knowledge of cohesins.

Keywords

cohesin; mitosis; meiosis; sister chromatid cohesion; cell cycle; chromosome segregation; aneuploidy; human health; cohesinopathies; maternal age effect

1. Introduction

During the S phase of the cell cycle, DNA replication generates a pair of sister chromatids with identical genetic content. The sister chromatids must be physically connected through the G2 phase and will only begin to separate during the transition from metaphase to anaphase during mitosis. The separation is completed in anaphase owing to the loss of cohesion between the sister chromatids. The end result is two daughter cells that are identical to each other and to the parent cell. Separation of sister chromatids in mitosis is the most important event during the cell cycle and this process must be monitored effectively.

Meiosis occurs strictly in germ cells and differs between males and females. The key difference between meiosis and mitosis is that meiotic cells undergo two cell divisions, meiosis I and meiosis II, without an intervening S phase. During meiosis I, the chromatin condenses as in mitosis and the sister chromatids are held together through a process called cohesion. In prophase I, however, DNA crossovers form between paired homologous chromosomes, called bivalents. This involves chromosomal synapsis and formation of a

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tripartite protein complex, the synaptonemal complex (SC), as well as formation of chiasmata. Prophase I is divided into five distinct sub-stages: leptonema, zygonema, pachynema, diplonema, and diakinesis. The bivalents, which are attached to microtubules through their kinetochores and centromeres, align on the metaphase plate during metaphase I. Unlike in mitosis, the sister chromatids remain attached at their centromeres by cohesion, and only the homologous chromosomes segregate during anaphase I. The second meiotic division is exactly like the division in mitosis, with separation of the sister chromatids. However, the end result is four haploid spermatids or one haploid oocyte (and two or three polar bodies) that are not identical to each other or to the parent cell.

Both mitosis and meiosis require cohesion to keep the sister chromatids together until separation is imminent at anaphase. Cohesion is established during DNA replication before both mitosis and meiosis by multiprotein subunit complexes called cohesins. Although the subunits differ slightly in mitosis and meiosis, the canonical cohesin complex is composed of four subunits. In mammals these are the following: two structural maintenance of chromosomes (SMC) subunits (SMC1α or SMC1β and SMC3); one stromalin, HEAT-repeat domain subunit (STAG1or STAG2 or STAG3 also called SA1 or SA2 or SA3, respectively); and one kleisin subunit protein (RAD21 or REC8 or RAD21L) (Figure 1). Because these subunits are quite diverse, a wide variety of cohesin complexes with different subunit compositions exists in mitotic and in meiotic cells. These cohesin complexes are important for chromosome segregation, DNA repair, gene expression, development, and genome integrity.

Although cohesins have been studied extensively, the exact roles and mechanisms of these proteins are still being elucidated. Recent interest focuses on the roles of cohesins in genome integrity during mitosis and meiosis. The role sister chromatid cohesion plays in replication fork maintenance is still unclear, but several mechanisms have been proposed. Cohesins are also important in double strand break (DSB) repair and are implemented in cellular responses to DNA damage. Exactly how these processes occur is still unknown, but recent work is illuminating them. This review highlights the importance of cohesins during mitosis and meiosis by distinguishing different aspects of cohesin complexes and their functions. We include the structure of cohesins, the tempo-spatial association of cohesin subunits with chromosomes, recent mammalian studies involving targeted deletion of cohesin subunits, and the importance of cohesins in genome integrity. We also discuss the roles and mechanisms of cohesins in human health and disease, highlighting the cohesinopathies and the maternal age effect.

2. Mitosis

During somatic cell division, several key events occur before a cell can complete the cell cycle and divide into two identical cells. The specific phases of the cell cycle and its checkpoints allow healthy cells to divide, and prevent abnormal cells from replicating. In some instances, however, problems occur and the regulation of the cell cycle is dysfunctional, leading to aberrant cell division. The G1 checkpoint is designed to identify these errors, halt the cell cycle, and to allow only functional cells to progress into S phase. The G2 checkpoint ensures that the cell has replicated its DNA correctly so it can progress

into mitosis and begin cell division. During S phase of the cell cycle, the chromosomes undergo DNA replication in order to produce identical sister chromatids. The sister chromatids must be held together throughout G2 phase and into mitosis by cohesin complexes, most of which are conserved among eukaryotes. During prophase, the loosely coiled chromatin begins to condense into distinct chromosomes while the spindle apparatus migrates to opposite poles of the cell. In early metaphase the condensed chromosomes align on the equatorial plate, and then begin to separate in late metaphase as the cell transitions into early anaphase. Cohesion between the sister chromatids is maintained until this point, known as the metaphase-to-anaphase transition. During early anaphase, the sister chromatids begin to separate to opposite poles via kinetochore attachment to the spindle microtubules. Normally, sister kinetochores attach to microtubules with opposite orientations, known as amphitelic attachment. Attachment of kinetochores to microtubules with the same orientation is called syntelic. Failure to correct erroneous syntelic attachment during mitosis will lead to improper segregation of sister chromatids, and the gain or loss of chromosomes. Once sister chromatids have separated in late anaphase, the final steps of telophase and cytokinesis yield two daughter cells, which are identical to the parent cell.

2.1 What is cohesion?

It is critical that cohesion between sister chromatids be maintained until chromosome segregation occurs during both mitosis and meiosis. Disruption of cohesion can lead to genome instability, such as aneuploidy, defects in DNA repair, and chromosomal translocations. Cohesion exists along the sister chromatid arms and at centromeres. In late metaphase, the microtubules at the spindle begin to contract to opposite poles of the cell, biorienting the sister chromatids. Sister chromatid cohesion is an essential part of this process and it also provides a force that counteracts that exerted by the microtubules [1]. Separation of sister chromatids occurs only after chromosomes have bioriented on the metaphase plate, triggering the dissolution of cohesion and subsequent migration to the spindle poles [2] (Figure 2). Cohesion between sister chromatids results in a tight association that is not released until the metaphase-to-anaphase transition (Figure 2). The linkage between the sister chromatids is especially crucial at centromeres because it ensures correct microtubule attachment to the kinetochores.

2.2 Cohesins create cohesion between sister chromatids

Sister chromatids are held together by multisubunit complexes called cohesins, which were first identified in the budding yeast, *Saccharomyces cerevisiae*, and in *Xenopus* (Table 1). The cohesin complex is evolutionarily conserved among eukaryotes and consists of four main proteins. The core subunits of the cohesin complex in budding yeast contain two subunits of the SMC family, Smc1 and Smc3; a kleisin subunit protein Scc1/Mcd1; and a stromalin, HEAT-repeat domain protein Scc3/Irr1 [3–6]. Homologues of the cohesin subunits have been identified in a variety of eukaryotic organisms from yeast to humans (Table 1). Higher eukaryotes have three homologues of Scc3 termed SA1, SA2, and SA3, also known as STAG1, STAG2, and STAG3 [7]. SA1/STAG1 and SA2/STAG2 are present in mitosis while SA3/STAG3 is specific to meiosis. Both SA1 and SA2 associate with the other cohesin subunits to create a diverse group of cohesin complexes in vertebrates [7–9]. Two mammalian homologues of Smc1 are termed SMC1α, found in both mitosis and

meiosis, and SMC1β, which is specific to meiosis. Fission yeast Psc3 and Rec11 are also homologues of Scc3, but Rec11 is required for cohesion during meiosis.

A model of the cohesin complex has been frequently proposed in which each proteinaceous ring entraps two sister chromatids [6,10,11]. The Smc1 and Smc3 molecules consist of long, rodshaped proteins that fold back on themselves at N and C terminal domains to form long stretches of intramolecular and antiparallel coiled-coils [10,12] (Figure 1). A characteristic ABC (ATP binding cassette)-like ATPase is found at one end of the monomer and a half-hinge domain at the other of each Smc1 and Smc3 molecule [12]. The ABC-like ATPase is a member of the protein superfamily that utilizes the energy of ATP hydrolysis to carry out certain functions. One Smc1 and one Smc3 molecule join together through their hinge domains to form a heterodimer [10] when ATP binds. This complex is then joined together by the Scc1/Mcd1/Rad21 subunit, effectively closing the ring [5,6]. The Scc1 N-terminus binds Smc3 while the C-terminus of Scc1 binds Smc1. Scc3/SA1/SA2 binds to the C-terminus of Scc1 and does not make direct contact with Smc1 or Smc3. Together these cohesin proteins form a very distinct ring structure that are distinguished from other associated proteins.

Biorientation of sister chromatids is tightly regulated and requires several proteins that work in concert to allow the metaphase-to-anaphase transition to occur. Separase is a mammalian cysteine protease; it is the homologue of Esp1 in budding yeast and Cut1 in fission yeast. When the centromeres are under tension in metaphase, the mitotic checkpoint prevents separase activation through Mad2 and Aurora B (Ipl1 in budding yeast) [1]. When activated, Mad2 and Aurora B inhibit APC^{Cdc20}, a ubiquitin ligase for securin, which in turn inhibits separase [13,14]. This tension is relaxed once all the pairs have aligned correctly on the metaphase plate. Aurora B/Ipl1 plays a crucial role in promoting biorientation of sister chromatids [1,15,16]. In the absence of Ipl1, attachment of sister kinetochores is syntelic, leading both sister chromatids to segregate to the same daughter cell [16]. Aurora B plays a similar role in humans by destabilizing defective kinetochore attachments, but only when there is no tension on the kinetochores.

Several studies utilizing cohesin mutants have helped to elucidate the role of cohesins in sister chromatid cohesion; the mutants were all incapable of keeping sister chromatids together during metaphase [3–5,17,18]. In eukaryotic cells lacking cohesin, sister chromatids separate precociously, leading to inefficient biorientation and errors in segregation [19–21]. Mutations in cohesins have also been shown to result in an increased distance between sister centromeres [3,4]. Cohesin function has been studied in higher eukaryotes by employing different techniques including gene deletion in *Xenopus* and chickens, and RNA interference (RNAi) in *Drosophila* and humans. Scc1-deficient cells in chickens show chromosome misalignment at metaphase, resulting in mitotic arrest or delay with aberrant disjunction at anaphase [21]. Sonoda *et al.* also observed a significant increase in distance between sister chromatids in Scc1-deficient cells, but not full separation. Cells with separated sisters and aberrant anaphases were also observed in *Drosophila* cells depleted of DRAD21 by RNAi [22]. This phenotype, however, was not observed in cells depleted of DSA1, the *Drosophila* homologue of Scc3. These cells had cohered sisters and were able to progress through anaphase normally, despite a slight increase in distance

between the sisters. In order to release the cohesin complexes from the DNA, RAD21 is cleaved by separase in mammals. When a deficiency in a cleavable form of RAD21 was expressed in human cells, no loss of centromeric cohesion was observed in prophase or prometaphase [23]. Anaphase, however, occurred aberrantly because the separation of chromosome arms was perturbed. This finding indicates that separation of the chromosome arms is promoted by RAD21 cleavage and that cohesion-independent forces maintain cohesion at centromeres until anaphase. Although the structure of the cohesin complex forms a tripartite ring [6,10], how the complex associates with the DNA is not well understood. Different ring models have been described, but two types are most common (Figure 3). One ring model predicts that both sister chromatids are entrapped within a single cohesin ring [6,24]. This model proposes that the connection between the sisters is topological rather than biochemical. The model would explain why cohesin does not bind strongly to DNA on its own [25] and why cohesin is readily released once the Scc1 subunit is cleaved [2]. Another type of ring model, the “handcuff” model, suggests that each of two cohesin rings entraps one sister chromatid, either by binding a single Scc3 subunit or topological interconnection between rings [26] (Figure 3). The exact method by which the cohesin complex associates with DNA has yet to be elucidated, but a few models have been proposed.

2.3 The association and dissociation of cohesins

Sister chromatids are tightly associated through cohesion, which prevents the separation of sisters before the metaphase-to-anaphase transition (Figure 2). As early as S phase of the cell cycle, cohesion components are present in eukaryotes. For example, Scc1 in budding yeast associates with chromosomes during S phase and remains tightly associated until the metaphaseto- anaphase transition [4] (Figure 2). When Scc1 expression is induced experimentally during G2, it is ineffective at promoting cohesion because it is needed at the time of DNA replication to establish sister chromatid cohesion [27]. Cohesion is also needed throughout G2 to facilitate the repair of DSBs by homologous recombination between sister chromatids [28]. Cohesins are recruited to DSBs in G2 and are implicated in holding the sister chromatid with a DSB near its undamaged sister template. Preventing cohesins from localizing to the DSBs actually abolishes DNA repair [29]. The loading of cohesins is extremely important from S phase through mitosis, but the dissociation signals the beginning of segregation between the sister chromatids. In budding yeast, the cohesin dissociation and destruction process begins with proteolytic cleavage of the Scc1 subunit at specific residues by Esp1, a separin and protease [2,30,31] (Figure 2). This triggers the dissociation of cohesins from chromosomes that is essential for the segregation of the sister chromatids to opposite poles of the cell in anaphase [2]. This important step is disrupted in Scc1 mutants as demonstrated by the premature separation of sister chromatids [4]. Sister chromatids in yeast that express a non-cleavable form of Scc1 resistant to Esp1, are unable to separate [2]. Conversely, artificially targeting a different protease to Scc1 can still result in premature separation of sister chromatids [30]. In fission yeast only a small amount of the Scc1 homologue, Rad21, is cleaved at the metaphase-to-anaphase transition to promote sister chromatid separation [32]. A bulk of Rad21 associated with the chromosomes remains during anaphase and may be necessary for the establishment of cohesion at the next S phase.

In lower eukaryotes the dissociation process occurs in one step, but higher eukaryotes require additional steps.

In vertebrate cells, cohesin dissociation is regulated by two distinct pathways. A bulk of cohesins is removed from sister chromatid arms during prophase by a separase- and cleavage-independent pathway [9,33,34] through phosphorylation by Polo-like kinases (PLK) and Aurora B [35–38]. This occurs when chromosomes begin to condense and also when they biorient on the mitotic spindle during prometaphase. Phosphorylation of SA2/STAG2 by Plk1 and Aurora B is essential for cohesion dissociation during these stages, but it is not required in the next stage of removal [39]. Hauf *et al*. have also shown that although RAD21 phosphorylation is not essential for cohesin dissociation in early mitosis, it enhances the ability of separase to be cleaved during the metaphase-to-anaphase transition. The cohesins remain at the centromeres and are responsible for holding the sisters together while they biorient during prometaphase. They are removed, however, at the metaphase-to-anaphase transition when all the chromosomes have correctly bioriented and the spindle assembly checkpoint has been fulfilled. This occurs through an APC/C (anaphase-promoting complex or cyclosome)- and separin-dependent pathway by cleavage of RAD21 [34]. In human cells, RAD21 is cleaved by separase, a step required to progress into anaphase [40]. Separase is also required for cleavage of the remaining cohesin complexes at sister chromatid arms during metaphase in human cells [41].

Until the metaphase-to-anaphase transition, separase is kept inactive by an inhibitory chaperone called securin [42], also known as Pds1 in budding yeast [31,43] and Cut2 in fission yeast [44]. Securin is controlled by the ubiquitin protein ligase APC/C. It is destroyed via ubiquitination by the APC/C only after all the chromatid pairs have aligned correctly on the mitotic spindle, allowing separase to become active. Once separase is activated in vertebrate cells by the APC/C, it undergoes autocleavage, similar to that of caspases. Separase cleaves RAD21 and the cohesin ring opens, allowing the release of cohesion and separation of sister chromatids. Sister chromatids do not separate in the presence of non-cleavable Scc1, which suggests that separase may be the only mode of cohesin removal from the sister chromatid arms.

2.4 Accessory cohesion factor components

Proteins that are essential for sister chromatid cohesion, but not structural components of the cohesin complex, are known as accessory or cofactor proteins (Table 1). Scc2 and Scc4 function together in a complex to load cohesins onto chromosomes; they are conserved among budding yeast and humans, and are required for initial cohesin binding to chromosomes [45,46]. Cohesin is initially loaded onto the Scc2-Scc4 complex at centromeres and at cohesion-associated regions along sister chromatid arms (Figure 2). Scc2 is conserved in most eukaryotes; the fission yeast homologue is Mis4 and the *Drosophila* homologue is Nipped-B, while the Scc4 homologue in fission yeast is Ssl3. Metazoan Scc2 contains a heterochromatin protein 1 (HP1) binding domain that has been shown to interact with HP1α, raising the possibility that Scc2 is directly involved in the establishment and maintenance of heterochromatic domains [47]. Depletion of Scc4 results in severe premature sister chromatid separation, suggesting that Scc4 is critical for chromosome cohesion in

actively dividing metazoan cells [46,48]. Both Scc2 and Scc4 are essential for cohesin loading onto chromosomes during S phase.

Pds5 [49,50], WAPL [51], sororin [52], and haspin [53] are involved in the regulation of cohesin complex association to and dissociation from chromatin. These proteins physically associate either directly or indirectly with the cohesin complex and they are involved in cohesion maintenance. In humans, PDS5 interacts with SA1/STAG1 and SA2/STAG2-containing complexes [9], and in *Caenorhabditis elegans* PDS5 also has an important role in sister chromatid cohesion during mitosis and meiosis [54]. Two vertebrate PDS5 proteins have been characterized, PDS5A and PDS5B, and depletion of these proteins from *Xenopus* extracts results in partial defects in sister chromatid cohesion, but not in mammals [55,56]. Human WAPL regulates the resolution of sister chromatid cohesion and promotes cohesin complex dissociation during and after anaphase by direct interaction with the RAD21 and SA/STAG subunits [51,57]. WAPL has also been found on axial and lateral elements (AE/LE) in some prophase I stages in mouse spermatocytes and oocytes, colocalizing with SYCP2 [58,59]. Sororin was first identified in vertebrates during a screen for substrates of the APC/C, but no homologues have been characterized in other organisms [52]. Sororin is ubiquitinated and degraded after cohesion is dissolved between sister chromatids. Recently, however, sororin has been shown to be necessary for maintaining sister chromatid cohesion in mitotic cells, as well as for the stable binding of cohesin to chromatin and efficient repair of DSBs in G2 [52,60]. Haspin is a histone H3 threonine-3 kinase that colocalizes with the cohesin complex at inner centromeres during vertebrate mitosis. Depletion of haspin in human cells results in premature separation of sister chromatids, suggesting a role in the maintenance of centromeric cohesion prior to anaphase [53]. Thus, PDS5, WAPL, sororin, and haspin are all important mediators of cohesin complex function during mitosis.

2.5 Role of cohesins in genome integrity

Mutations and deletions in replication machinery components result in defects in sister chromatid cohesion, suggesting a functional relationship between processes that involve DNA replication and cohesion establishment. This requires not only the cohesin complex but also a number of accessory protein factors. Initial studies in budding yeast demonstrated that the Eco1/Ctf7 acetyltransferase is required during S phase for cohesion establishment [5,61,62] (Figure 2). Eco1/Ctf7 mutations are synthetically lethal with proliferating cell nuclear antigen (PCNA) mutations. The synthetically lethal phenotype can be rescued, however, by overexpressing PCNA. Recent work has shown that Eco1/Ctf7 is also necessary to establish sister chromatid cohesion in G2/M in response to DSBs [63]. The acetyltransferase domain of Eco1/Ctf7 and its activity are required to generate cohesion during G2/M, as well as during S phase. Thus, cohesion can be generated outside of S phase. Homologues in fission yeast, *Drosophila*, and humans have been termed Eso1, deco, and Esco2, respectively (Table 1).

Another group of proteins involved in establishing cohesion in budding yeast are components of the replication machinery. Investigators have suggested that stabilization of stalled replication forks may be essential for proper establishment of cohesion. Ctf18 is a protein subunit of the alternative replication factor C-like complex (Ctf18-RLC), a seven

subunit complex (Ctf18-Ctf8-Dcc1-Rfc2-Rfc3-Rfc4-Rfc5). Ctf18-RLC establishes sister chromatid cohesion and has been shown to load and unload PCNA onto and off of DNA [64–67]. Eco1/Ctf7, Ctf4, and Ctf18-RLC all act in close proximity to the replication fork and are essential for cohesion [68] (Figure 2). Ctf4 associates with replication origins and with DNA polymerase α and moves with the replication machinery along chromosomes [66,67]. Recent work has suggested that Eco1/Ctf7 and Ctf18-RLC colocalize with replication forks, but it is not known whether they move with the replication machinery. In their absence, however, sister chromatid cohesion is compromised. Stabilization or "protection" of stalled replication forks and proper sister chromatid cohesion involves proteins Swi1-Swi3, Ctf18-RLC, and Chl1 in fission yeast [69]. The Swi1-Swi3 complex plays an important role in efficient activation of Cds1, a replication checkpoint kinase. The complex moves with replication forks and is required to prevent accumulation of singlestranded DNA structures near the replication fork [70]. Homologues of Swi-Swi3 exist as the Timeless-Tipin complex in humans and the Tof1-Csm3 complex in budding yeast. The DNA helicase activity of Chl1 is evolutionarily conserved and appears to be involved in sister chromatid cohesion. In fission yeast, Chl1 has been shown to stabilize replication forks and to promote proper establishment of sister cohesion [69] and in budding yeast Chl1 associates with Eco1/Ctf7 for critical involvement in chromatid cohesion [71]. ChlR1, the homologue of Chl1 in mammals, binds cohesin and is required for normal sister chromatid cohesion [72]. Depletion of ChlR1 results in abnormal sister cohesion and a delay at prometaphase. These proteins are critical for cohesion between sister chromatids but their functions have not been fully elucidated. In this same context, Ctf18-RLC has been suggested to control the speed, spacing, and restart activity of replication forks in human cells and is also required for robust acetylation of SMC3 and sister chromatid cohesion [73]. Terret *et al*. also found that cohesin acetylation itself is a "central determinant of fork processivity", because slow-moving replication forks were found in human cells expressing a form of non-acetylatable SMC3, and in cells lacking the Eco1-related acetyltransferases, ESCO1 or ESCO2. The defect was a consequence of the strong interaction between cohesin and the regulatory cofactors WAPL and PDS5A because removal of either cofactor allowed forks to progress rapidly without ESCO1, ESCO2, or Ctf18-RLC. Although only demonstrated in human cells, these findings suggest a possible new mechanism for clamploader- dependent fork progression, resulting from the posttranslational modification and structural remodeling of the cohesin ring [73].

Several mechanisms have been proposed for the role of replication fork maintenance in sister chromatid cohesion. One model proposes that cohesin bound to chromosomes before arrival of the replication fork is sufficient to establish sister chromatid cohesion [68]. Therefore, it is thought that the replication machinery slides through the cohesin rings. However, Lengronne *et al*. have also proposed that the cohesin complex may transiently dissociate upon fork passage through the rings. Fork components, such as Ctf18-RLC and/or Swi1-Swi3, may tether cohesinrelated proteins to DNA when forks pass through the cohesin ring [69]. CHTF18, the gene product of the human Ctf18 homologue, has been shown to interact with several cohesin proteins, supporting this idea [64]; recent work also supports a possible interaction of CHTF18 with cohesins during mammalian meiosis [74]. Another model suggests that the cohesin ring may be an obstacle for replication fork progression and

causes stalling of the fork [69]. This would require stabilizing proteins, such as Swi1-Swi3 and Ctf18-RLC, at cohesin sites. A third model proposes that Ctf18-dependent unloading of PCNA might loosen the replication fork structure in order for the forks to pass through the cohesin ring without its dissociation [65]. A very recent model proposes that sister chromatid cohesion is established simultaneously with cohesin loading behind the replication fork in close proximity to processing of the lagging strand [75]. Although several models have been proposed, the exact mechanism for replication fork maintenance in sister chromatid cohesion remains unknown.

Cohesins are also involved in cellular responses to DNA damage [76]. Mammalian cohesins are recruited to DSBs; they take part in the ataxia-telangiectasia mutant (ATM) DNA damage signal transduction pathway and are important for survival after irradiation [76]. Two different populations of cohesins contribute to the repair process: cohesins engaged in holding sisters together at the time of the break and cohesins subsequently recruited to chromatin surrounding the break itself [29,77]. After induction of DSBs, cohesins are recruited to these sites via the DNA damage response pathway. Because recombination between sister chromatids is generally more efficient than between homologous chromosomes, cohesin might inhibit recombination between the homologues. Suppressing recombination between homologues is important in preventing chromosome instability and rearrangements such as non-allelic recombination and/or loss-of-heterozygosity. In budding yeast the cohesin complex encoded by *MCD1* genes plays a dual role in protecting chromosome and genome integrity [78]. Even a small reduction in the levels of cohesin subunits decreases DSB repair and significantly increases damage-induced recombination between homologous chromosomes. Thus, cohesin levels appear to be a limiting factor in controlling genome integrity [78].

Phosphorylation of cohesin SMC subunits has also been found to be implemented in the cellular response to DNA damage. In response to ionizing radiation, the phosphorylation of S957 and S966 of human SMC1 by ATM kinase is required for the activation of the S-phase checkpoint [79]. Mutant cells defective in SMC1 phosphorylation still exhibited formation of DNA damage foci after exposure to ionizing radiation [80]. However, these cells showed decreased survival, chromosomal anomalies, and a defective S-phase checkpoint after DNA damage. Investigators have also reported that SMC3 is phosphorylated at two specific serine residues as well as by two different kinases [81]. Human SMC3 S1083 phosphorylation is inducible and ATM-dependent by ionizing radiation, while S1067 is constitutively phosphorylated by CK2 kinase and not increased by ionizing radiation. Phosphorylation of both of these sites, however, is required for the S-phase checkpoint. The roles of cohesins in genome integrity are still being elucidated, but it is well known that cohesins play a larger role during mitosis than originally thought.

3. Meiosis

Although the process of meiosis is similar to mitosis, haploid gametes are generated instead of diploid cells. Several distinct differences between the two processes have been established and cohesins play a vital role in many aspects of meiosis. Meiosis begins in diploid germ cells following one round of DNA replication in which maternal and paternal

homologous chromosomes have been duplicated, each chromosome consisting of two sister chromatids (4C DNA content). Ultimately, these duplicated pairs of sister chromatids are separated into four different nuclei by two rounds of cell division without any intervening DNA replication. In mammals, male meiosis gives rise to four different haploid gametes (spermatids) whereas female meiosis gives rise to ultimately one haploid gamete (oocyte) and two or three polar bodies. During the first meiotic division (meiosis I), pairs of maternal and paternal homologous chromosomes ultimately segregate in opposite directions. This reduces the chromosome number and also ensures that each gamete will inherit a complete copy of the genome. Pairs of sister chromatids then separate in the second meiotic division (meiosis II) as in mitosis.

Meiosis I is unique in the manner of chromosome segregation and in the distinct processes that occur during prophase I. Homologous recombination is an essential phenomenon during meiosis because it physically joins the maternal and paternal homologues before segregation, and ultimately generates new combinations of alleles and genetic variation. Homologous recombination during meiosis I (also called meiotic recombination) results in the exchange of DNA between maternal and paternal chromatids, and the sites of DNA exchange are called crossovers. Crossovers are seen cytologically as structures called chiasmata. Chiasmata and cohesion along sister chromatid arms hold homologous chromosomes together prior to their segregation in anaphase I. Attachment of sister kinetochores to microtubules with the same polarity, called syntelic attachment, is another feature that is unique to meiosis I. This type of attachment of sister kinetochores is also known as mono-orientation, and it differs from the biorientation of sister kinetochores during mitosis. Because the chiasmata physically link homologous chromosomes, tension is generated and a new form of equilibrium is established during metaphase I. Chiasmata ensure that the tension will be generated if both maternal centromeres attach to microtubules with one orientation and both paternal centromeres attach to microtubules with the opposite orientation. The spindle machinery senses this bipolar attachment-like tension between homologous chromosomes and not sister chromatids in metaphase I. Although tension on homologues of maternal and paternal centromeres pulls them in opposite directions, they are prevented from disjoining during prophase I by the presence of chiasmata and cohesion between sister chromatids. Cells systematically suppress amphitelic attachment and promote syntelic attachment of the sister chromatids during the first meiotic division to prevent aneuploidy. During the second meiotic division sister kinetochores attach to microtubules in an amphitelic manner and the sisters are segregated to opposite poles during the metaphase-to-anaphase transition, as in mitosis. Only sister chromatid arm cohesion is destroyed during anaphase I, leaving centromeric cohesion to persist. This process, along with resolution of chiasmata, results in the separation of homologues only and not sister chromatids during anaphase I. Centromeric cohesion in meiosis II is essential to ensure the bipolar attachment of sister kinetochores as in mitosis.

Cohesion between sister chromatids is established during premeiotic DNA replication and differs from its mitotic counterparts (Figure 4). Meiotic cohesins must participate in the recombination process as well as persist at centromeres through the first division. However, cohesion along sister chromatid arms must dissolve during meiosis I to allow the homologues, joined by chiasmata, to separate (Figure 4). The cohesion along sister

chromatid arms ensures correct chromosome alignment during the first division, and cohesion at the centromeres ensures proper segregation at the second division [82,83]. Once cohesion between sister chromatid arms is released, the microtubules pull maternal and paternal centromere pairs to opposite poles of the cell. These different types of cohesion are extremely important during meiosis because the chromosomes must undergo two distinct rounds of segregation. Cohesion at the centromeres ensures biorientation of chromatids on the spindle and accurate segregation during meiosis II, as in mitosis. The destruction of centromeric sister chromatid cohesion triggers their disjunction and segregation to opposite poles of the cell, yielding haploid cells. The two steps involved in cohesin removal during meiosis are similar to the steps in prophase and anaphase of mitosis. Meiotic recombination has been most well characterized in yeast. The process begins with generation of DNA DSBs by Spo11 endonuclease [84]. This occurs in early prophase I at multiple locations along each of the four chromatids. The 5′ ends resulting from Spo11 cleavage are resected in yeast by Rad50, Mre11, and Com1/Sae2 to form single-stranded 3′ overhangs on each side of the break [85–87]. First end capture occurs by one 3′ overhang invading the homologous non-sister chromatid [88]. The invading 3′ end becomes paired with the complementary strand from the other chromatid, creating a template for repair. The displaced strand will then pair with the second 3′ overhang on the original chromatid. The ends are ligated to the newly synthesized DNA, creating a joint molecule. At this point, the non-sister chromatids (one maternal and one paternal) will have recombined homologues and crossing over will be complete, creating a double Holliday junction (DHJ). The final step in the recombination process is the resolution of DHJs by cleaving of a pair of chromosome strands at each end and their reciprocal ligation. The cleavage can be either horizontal or vertical, but crossover occurs only when one junction is resolved horizontally and the other vertically. Most organisms create several of these exchanges per chromosome, but only one chiasma is needed to hold a pair of homologous chromosomes together.

3.1 Unique meiotic cohesin characteristics

The cohesin complex in germ cells differs from somatic cells, and distinct meiosis-specific subunits have been characterized in various organisms. In both fission and budding yeast, Rad21 is involved in mitosis and Rec8 is the meiotic paralogue of Scc1 [82,89,90]. Fission yeast has two Scc3 homologues, Rec11 and Psc3 (Table 1). Rec11 is meiosis-specific and forms a complex with Rec8, mainly along the chromosome arm regions, and the complex is critical for recombination [91]. Psc3, however, is expressed in mitosis and meiosis and associates with Rec8 mainly at the centromeres. Although inactivation of Rec11 impairs sister chromatid cohesion specifically along the arm and reduces the rate of recombination, Psc3 is dispensable for these functions but it is required for centromeric cohesion persisting throughout meiosis I. In mammals, the meiotic paralogues of SMC1, SCC1/RAD21, and SA/STAG1/2 are SMC1β, REC8, and SA3/STAG3, respectively [92–96] (Table 1). Although these three subunits are strictly expressed in germ cells, SMC1α, RAD21, and SA2/STAG2 are also implemented in meiotic chromosome dynamics [97]. Recently, a third kleisin subunit in mammals, named RAD21L, has been identified in meiotic cells and localizes along the AE/LEs of the SC throughout meiosis I [98–101]. This subunit may be involved in synapsis initiation and crossover formation between homologous chromosomes. RAD21L has also been shown to be a functionally relevant meiotic kleisin subunit that is

essential for male fertility and maintenance of fertility during natural aging in females [99]. Evidence for participation of different cohesin complexes during mammalian meiosis suggests a variety of putative cohesin complexes formed by combinations of cohesin subunits (Figure 5). Several distinct complexes are thought to exist, showing differences in spatiotemporal distribution throughout the meiotic divisions.

3.2 Cohesins in genome integrity during meiosis

In yeast Chl1, Ctf4, and Ctf18-RLC are necessary for sister chromatid cohesion in both mitosis and meiosis, and they are are essential for chromosome segregation during meiosis. In fact, they contribute significantly to the establishment of cohesion in the region of centromeres. Deletion of *CTF18,* or *CHL1,* or *CTF4* in budding yeast leads to severe defects in chromosome segregation, aneuploidy in the spores, and meiosis II nondisjunction at a high frequency [102]. In yeast, frequent errors in meiosis II, rather than homologue nondisjunction in meiosis I, predominantly contribute to the mis-segregation phenotype in meiotic mutant cells.

Cohesin is particularly important in meiotic cells to hold bivalents together during homologous recombination and DSB repair. Whether cohesin is actively recruited to sites of DSBs during meiosis, as it is in mitotic cells, is not well known. A conserved DNA damage checkpoint, known as the pachytene checkpoint, also monitors the efficient repair of meiotic DSBs and induces apoptosis when DSBs are not repaired in a timely fashion. The involvement of cohesin in repair of meiotic DSBs and activation of the pachytene checkpoint have been demonstrated in the *C. elegans* germline [103]. Loading of cohesin onto chromatin during S phase, and also in response to DSBs in post-replicative cells, depends on a conserved complex composed of Scc2 and Scc4 proteins. Meiotic cohesin is loaded by Scc2 and in the absence of meiotic cohesin, recombination intermediates accumulate extensively but fail to trigger the apoptotic response of the pachytene checkpoint [103]. Meiotic cohesion is required for early DSB processing and for efficient recruitment of DNA damage sensors [103]. This suggests that cohesin is involved in early events of the meiotic DNA damage response.

3.3 Specific events in meiosis I and II

Prophase I is prolonged in mammalian meiosis and it is divided into substages according to chromatin changes based on cytological studies. The most important event during prophase I is formation of the synaptonemal complex (SC), which forms between homologous chromosomes. This structure supports meiotic recombination, and it represents an essential difference between mitosis and meiosis. Meiosis-specific cohesin complexes are believed to form a scaffold to which components of the SC can attach.

During leptonema of prophase I, the axial elements (AE) form along each chromosome. SYCP2 and SYCP3 create a bipartite polymer along the bivalent axes and are the main structural protein components of the AE/LE [104–107]. Then in zygonema, homologues begin to pair and central elements (CE) are deposited between the AE (now called lateral elements or LE). Zip1 in yeast and SYCP1 in mammals, known as transverse filaments, form the center of the SC, or the central elements. In pachynema, homologues synapse along

their length, the SC fully forms, and DNA recombination takes place. This close association between maternal and paternal axes along the entire length of the bivalent is called synapsis, and it is achieved by the SC. The onset of diplonema is characterized by the disassembly of the SC and homologue desynapsis. The final stage of prophase I is diakinesis, which quickly progresses into metaphase I. Homologues remain connected at chiasmata, which can now be seen cytologically at this stage, and cohesion between sister chromatids prevents premature segregation. Immunocytological studies have helped characterize the spatiotemporal localization of cohesins during meiosis.

3.3.1 Leptonema—During prophase I in spermatocytes, cohesin subunits are observed at different stages and in different quantities. SMC1β can be observed along the asynapsed AE and STAG3 is found along the AE during leptonema [95,96]. REC8 is localized along asynapsed, synapsed, and desynapsed AE/LE throughout prophase I [92]. RAD21, like REC8, also appears at the AE/LE during all stages of prophase I [108,109]. RAD21L is expressed from premeiotic S phase and localizes along the AE in leptonema, with some conflicting reports as to whether it persists to midpachynema or diplonema and into metaphase I [98–101].

3.3.2 Zygonema—In the zygotene stage of prophase I, SMC1β is found along the asynapsed AE and also the synapsed LE [96]. SMC1α and SMC3 are observed in a distinct punctate pattern along the synapsed LE in late zygonema and are found to interact with SYCP2 and SYCP3, structural proteins of the synaptonemal complex [110]. STAG3 is observed along the AE/LE as in leptonema [95]. RAD21L localizes along the AE/LEs in zygonema in a punctate or continuous linear pattern depending on the report [98–101].

3.3.3 Pachynema—During pachynema, SMC1α and SMC3 are still seen immunocytologically in a distinct punctate pattern along the synapsed LE and interact with SYCP2 and SYCP3 [96,110]. SMC1β and STAG3 are also found along the synapsed LE. Although RAD21L is distributed along the SC through at least mid-pachynema, reports of its localization vary. Some groups have reported that RAD21L is evenly distributed along the AE/LE, while other groups have reported that it is discontinuous [98–101]. In addition, two groups have reported that RAD21L localizes in a mutually exclusive pattern with REC8, perhaps suggesting inherent loading sites for these cohesins [100,101].

3.3.4 Diplonema/Diakinesis—SMC1α is lost from the desynapsed LE during diplonema and it is not detected on bivalents in diakinesis or metaphase I. SMC3, however, persists at the desynapsed LE but is progressively lost and accumulates at centromeres during diakinesis. SMC1β is found along the desynapsed LE, most of it dissociating in late diplonema, and accumulating at the centromeres during diakinesis. STAG3 is still visible along the LE but is observed as patches along the contact surface between sister chromatids, called the “interchromatid domain,” during diakinesis [95,111]. This subunit is maintained at the chromosome arms and centromeres until metaphase I [95]. During late diplonema, RAD21 appears along desynapsed LE but also accumulates in areas where it is colocalized with SYCP3. By late diplonema to diakinesis, RAD21 is partially released from the LE [108]. REC8 has been found at the interchromatid domain along chromosome arms and

centromeres during diakinesis and metaphase I bivalents [92,110]. RAD21L disappears by mid-pachynema or diplonema as it accumulates at centromeres [98–101].

Based on the studies mentioned here, several different cohesin complexes are present during mammalian prophase I (Figure 5). The complex, SMC1α/SMC3/RAD21/STAG1 or STAG2, is present during premeiotic S phase. SMC1α/SMC3/RAD21L/STAG3 and SMC1β/SMC3/RAD21L/STAG3 are present along the AE/LE from premeiotic S phase through diplonema. The canonical meiotic complex, SMC1β/SMC3/REC8/STAG3 and the SMC1β/SMC3/RAD21/STAG3 complex are likely present throughout prophase I. These complexes ensure that at the end of prophase I homologous chromosomes remain connected at chiasmata despite dissolution of the SC.

3.3.5 Metaphase I—In metaphase I mammalian spermatocytes, STAG3 is seen as discontinuous bright patches lining the interchromatid domain along sister chromatid arms, but not at chiasmata [95]. STAG3 is also present at the centromere domain just below the closely associated sister kinetochores. The same pattern of labeling has also been reported for REC8 [92,110]. SMC3 was initially reported to be concentrated at centromeres and absent from chromosome arms [96,112]. However, recent work has suggested that SMC3, like STAG3 and REC8, is distributed along the interchromatid domain and centromere domains of metaphase I bivalents [114]. The distribution of RAD21is distinctive; it accumulates at the inner centromere domain in a “double cornet-like” configuration with SYCP2 and SYCP3, and is also seen as small patches at the interchromatid domain [108]. SMC1β also localizes with SYCP2 and SYCP3 to mainly the centromeres of metaphase I spermatocytes, but the exact configuration at the inner centromere domain has not been studied [113]. Studies suggest that RAD21L remains in residual amounts, partly colocalized with SYCP3 at or near centromeres, although reports are conflicting [98–101].

3.3.6 Anaphase I to Metaphase II—The exact localization pattern of cohesin subunits from anaphase I to metaphase II is not known. REC8, STAG3, RAD21, SMC3, and SMC1β, persist at centromeres during anaphase I, although their patterns differ [92,95,108,109,112]. The dynamics of these subunits are unknown during telophase I and interkinesis, but some information is known about a few of the subunits. RAD21 changes its distribution to a bar-like pattern in between sister kinetochores at telophase I centromeres [108]. These bars are also seen during interkinesis at “heterochromatic chromocenters,” which represent closely associated centromeres [108,114]. This pattern disappears at prophase II. STAG3 and REC8 have also been reported to disappear from centromeres during telophase I and are no longer seen in interkinesis nuclei [95,114,115].

3.3.7 Metaphase II—Reports regarding the appearance and distribution of cohesin subunits at centromeres in metaphase II are conflicting. Original studies in rodent surface spread spermatocytes indicated that RAD21, SMC1β, and SYCP3 appeared as rod-shaped aggregates between sister centromeres [96,109]. However, RAD21 and SYCP3 were not visualized at centromeres in squashed spermatocytes [108,115]. The conflicting results obtained are attributed to differences in the techniques used as well as possible differences in the ability to detect small amounts of the cohesins [108,114,115].

3.3.8 Female Meiosis—Although features of meiosis are similar in male and female mammals, important gender-specific differences exist in the onset, timing, duration, and outcome of meiotic processes. Female germ cells enter meiosis as oocytes during fetal development and arrest at the end of the diplotene stage of prophase I, known as dictyate. Dictyate arrest lasts from the late stages of fetal development until resumption of meiosis just prior to ovulation. Information regarding chromosome cohesion during this extended time frame and whether cohesin complexes established during fetal life are present decades later is not known. Localization patterns of several meiotic cohesins have been compared to SYCP3 during the formation and dissolution of the SC in fetal oocytes during human and murine prophase I [116]. Results from this study suggested that STAG3, REC8, SMC1β and SMC3 associate with chromatin to form a "cohesin axis" prior to AE formation during female meiosis in mammals [116]. In human fetal oocytes STAG3 and REC8 are scattered throughout preleptotene nuclei but become more organized in leptonema and partially colocalize with SYCP3. By zygonema, however, REC8 and STAG3 colocalize with SYCP3 and persist into early diplonema. In mouse oocytes expression of STAG3, SMC3, and SMC1β first appears as fibers in leptonema prior to AE formation, similar to the timing of cohesin axis formation in human oocytes. The cohesin fibers become more prominent in zygonema with AE formation, then colocalize with SYCP3 in pachynema. During dictyate arrest in mouse oocytes there is a gradual loss of both SYCP3 and the cohesin axis [116].

A recent study analyzed the distribution of SMC3, REC8, SMC1β, STAG3, and SYCP3 in human oocytes throughout meiosis [117]. As meiosis progresses into leptonema in oocytes, the cohesins appear as thin threads and their staining completely overlaps with SYCP3 and remains colocalized through diplonema. Unlike mouse oocytes, cohesins do not appear to be lost during dictyate arrest in human oocytes. REC3, STAG3, and SMC3 appear as short filaments with a diffuse pattern of distribution in the nucleoplasm and cytoplasm [117]. SMC1β, however, appears intensely all over the oocyte, including the nucleus and cytoplasm. In fully grown germinal vesicle oocytes STAG3 appears as cohesin threads all over the chromatin, including intense staining at the nucleolus. In metaphase I oocytes, cohesins are seen as bright patches along the interchromatid domain and the centromeric area of all bivalents. From early anaphase I, cohesins are no longer seen at the arms of sister chromatids and are confined to the centromeric area. At metaphase II, REC8, STAG3, SMC1β, and SMC3 are observed in the space between sister kinetochores, and SYCP3 appears as small dots partially colocalizing with each sister kinetochore.

3.3.9 Synaptonemal Complex – Central Elements and Cohesin Function—One component of the CE unique to mammals is FK506-binding protein 6 (FKBP6), which belongs to the FKBP family of proteins and is expressed in mouse male and female germ cells during prophase I [118]. FKBP6 localizes to SYCP1 of synapsed chromosome cores and also coimmunoprecipitates with SYCP1, suggesting a role in the assembly and maintenance of the SC [118,119]. FKBP6 appears to interact with NEK1, a NIMA (never-in-mitosis A)-related kinase-1 dual-specificity serine-threonine and tyrosine kinase [119]. NEK1 is highly expressed in spermatogonial cells and spermatocytes during prophase I in mice. SMC3 staining decreases and becomes more diffuse in spermatocytes of wild-type mice during diplonema. However, SMC3 persists in diplotene *Nek1*-deficient spermatocytes,

consistent with a role for NEK1 in removal of the meiotic cohesin SMC3 from chromosome cores at the end of prophase I [119]. Similar findings are observed in *Fkbp6*-null spermatocytes, suggesting that the FKBP6-NEK1 pathway may be involved in cohesin removal at the end of prophase I. However, normal accumulation of SC and DSB repair proteins are seen in *Nek1*-deficient spermatocytes [119].

3.4 Loss of cohesion through destruction of cohesins

Destruction of cohesion distal to chiasmata is mediated by the same mechanism that triggers disjunction of chromatids in mitosis. Rec8 is present along sister chromatid arms during metaphase I but disappears from the arms at the onset of anaphase I in budding yeast and mice [82,83,92]. In budding yeast resolution of chiasmata and removal of Rec8 from sister chromatid arms depend on cleavage by separase, just like Scc1 in mitosis [120]. However, Rec8 remains in the area of centromeres until the onset of anaphase II in budding yeast [82], fission yeast [83], *C. elegans* [121], and mouse spermatocytes [92]. These findings suggest that eukaryotic organisms maintain sufficient cohesion around centromeres during meiosis II by protecting Rec8 from separase cleavage during meiosis I. Mutations in *rec8* result in precocious separation of sister chromatids during anaphase I. In fission yeast, Rad21 ectopically expressed at centromeres cannot rescue this defect, suggesting that Rec8 is responsible for the persisting centromeric cohesion until meiosis II and it cannot be replaced by Rad21[89]. Protection of centromeric Rec8 is lost after anaphase I, as indicated by the dissociation of Rec8 from chromosomes with reactivation of separase at the onset of anaphase II. If the protection were to dissolve prior to inactivation of separase, premature disjunction of sister centromeres would occur. It is interesting, however, that exchange of Scc1 for Rec8 during mitosis does not prevent cohesin cleavage at the centromere, suggesting that other meiosis-specific factors are involved [120]. In *C. elegans*, where separase is also required for meiosis I, the phosphorylation of Rec8 by the Aurora B protein Air2 might ensure that only Rec8 distal to chiasmata is cleaved at the first division [122–124]. In budding and fission yeast, the expression of a nondegradable form of Rec8 that carries mutations at the separase target sites dominantly blocks the onset of anaphase I. This phenotype is suppressed by the elimination of chiasmata, suggesting that the separase-mediated cleavage of Rec8 triggers homologue separation by resolving chiasmata on the arm regions [120,125]. An accumulation of securin, the inhibitory chaperone of separase, has been observed not only in meiosis I but also in meiosis II, indicating separase activation at both meiotic divisions [120,125]. The same observation has been made in *C. elegans* and in mice, where the activation of securin is crucial for the progression of meiosis I [122,126,127].

Identification of a protein that protects centromeric cohesion during prophase I has revealed why centromeric Rec8 is only cleaved during meiosis II and not during meiosis I. In fission yeast this protector of Rec8 centromeric cohesion is a gene product that when coexpresssed with Rec8 causes toxicity during mitotic growth [128]. The gene encodes a meiosis-specific protein named shugoshin (Sgo1), a homologue of the *Drosophila* protector Mei-S332 [129–131]. Shugoshin associates with protein phosphatase 2A (PP2A) and forms a complex at centromeres, which blocks the cohesin phosphorylation necessary for removal of cohesion and also prevents premature loss of centromere cohesion [132,133]. Fission yeast Sgo1

localizes exclusively at the site where Rec8 is predicted to have a role in centromeric protection during meiosis I [91]. Budding yeast shugoshin is also thought to have the same effect on Rec8 during meiosis I [128,130,131]. Fission yeast and mammals also possess paralogues of Sgo1 called Sgo2 and SGOL2, respectively. Their proteins are ubiquitously expressed throughout the mitotic and meiotic cell cycle in yeast [128,129], but only SGOL2 is essential for meiosis in mammals [134]. However, both SGOL1 and SGOL2 are expressed in mouse germ cells, and SGOL1-depleted oocytes also show meiotic defects [135,136]. During metaphase II, SGOL2 relocates in a tension-dependent way to the centromeres in mouse spermatocytes and oocytes [115,135]. In the absence of Sgo1, fission yeast sister chromatids co-segregate to the same pole, implying that monopolar attachment is intact, but they start to separate precociously during anaphase I. Thus because Rec8 is no longer protected without Sgo1 during meiosis I, the sister chromatids separate prematurely in anaphase I.

The finding that shugoshins protect centromeric cohesion by recruiting PP2A suggests that the phosphorylation of a protein is needed for Rec8 cleavage. In mitotic yeast cells, cohesin cleavage is promoted through phosphorylation of Scc1 by PLK (Cdc5 in yeast), which also participates in the phosphorylation of Rec8 [36]. Replacement of alanine for Rec8 residues that are thought to be phosphorylated by Cdc5 has no significant effect on the kinetics of cohesin cleavage at meiosis I [137]. Recent work has shown that casein kinase 1δ/ε (CK1δ/ε), Hrr25 in yeast, and Dbf4-dependent Cdc7 kinase (DDK) are essential for Rec8 cleavage, not Cdc5 [138]. Investigators have proposed that Hrr25- and DDK-dependent phosphorylation of Rec8 promotes cohesin cleavage in meiosis I, whereas dephosphorylation of Rec8 by PP2A bound to Sgo1 protects it from separase at centromeres.

3.5 Characterization of cohesin subunit mutants

The characterization of mice deficient in meiosis-specific subunits has helped us to understand the function of these proteins in mammalian meiosis. Both male and female *SMC1*β-deficient mice are sterile and show defects in SC formation and premature loss of sister chromatid cohesion [139]. *SMC1*β-deficient spermatocytes undergo pachytene arrest whereas mutant oocytes reveal premature loss of cohesion at metaphase II. *REC8*-deficient male and female mice are also sterile and display severe defects in synapsis and sister chromatid cohesion, but the phenotypes are different than those of *SMC1*β mutant mice [140]. SC formation occurs aberrantly in *REC8* mutant spermatocytes between sister chromatids instead of between homologous chromosomes. AE-like structures are formed, even though synapsis does not occur correctly. Rec8 deletion mutants in budding yeast and *C. elegans* also cause sister chromatids to lose cohesion and to separate early, yielding aneuploid gametes [82,121]. However, in fission yeast Rec8 mutants lose cohesion only at centromeres because Rad21 provides cohesion along sister chromatid arms [83]. RAD21L-deficient male mice show a defect in chromosome synapsis at prophase I, which leads to meiotic arrest at a zygotene-like stage [99]. Deficient females, however, are initially fertile but develop an age-dependent sterility.

Absence of SYCP2 or SYCP3 in mice results in a sexually dimorphic phenotype: males are sterile and females are subfertile [141,142]. Males show display a disruption in

chromosomal synapsis and meiotic arrest in prophase I, but females have reduced litter size and embryo death due to chromosome mis-segregation from aneuploid oocytes. *Sycp3*-deficient male mice show defects in AE formation, chromosomal synapsis, and SC assembly [141]. A null mutation of *Sycp1* causes sterility in homozygous male and female mice. Most of *Sycp1*-deficient spermatocytes display defects in meiotic recombination and arrest at the pachytene stage, and mutant ovaries reveal a paucity of oocytes and growing follicles [143]. Male *Fkbp6*$^{-/-}$ mice are sterile, whereas mutant females are fertile. The mutant spermatocytes show severe defects in pairing and synapsis and arrest at pachytene of prophase I [118]. Similar to *Fkbp6*-null mice, *Nek1*-null male mice show severely impaired fertility consistent with an absence of epididymal sperm and a reduction in testis weight and size [119]. Holloway *et al*. also demonstrated that *Nek1*-null mice show defects in cohesin SMC3 removal during diplonema, suggesting that NEK1 plays a role in cohesin unloading at the end of prophase I.

4. Human Health and Disease

4.1 Cohesinopathies

Human diseases caused by mutations in primary genes associated with the cohesin network are termed cohesinopathies. All the cohesinopathies that have been identified manifest as multisystem developmental disorders, but they have distinct phenotypes. Although mutations in the cohesin network might be expected to generate defects in chromosome segregation and/or the ability to repair DNA, mutations of this nature are probably lethal and have not been reported. Instead, cohesinopathies are characterized by a variety of developmental defects, including growth and mental retardation, limb deformities, and craniofacial anomalies. These phenotypes are consistent with a role for cohesins in gene expression during embryogenesis. Although downregulating cohesin sufficiently to cause significant sister chromatid cohesion defects is lethal in eukaryotes, the mechanism of action by which cohesin effects developmental processes appears to be through a noncanonical role as a regulator of gene expression and other genomic processes. The molecular mechanisms underlying the changes in gene expression that result in cohesinopathies are not well known. Mechanisms have been proposed, such as actions of cohesin in transcriptional activation, transcriptional repression, transcript termination, and long-distance enhancer–promoter interactions, none of which are mutually exclusive.

4.2 Cornelia de Lange syndrome

Cornelia de Lange syndrome (CdLS) is a dominantly inherited, multisystem developmental disorder characterized by classic facial anomalies, upper extremity malformations, hirsutism, cardiac defects, growth and cognitive retardation, and gastrointestinal abnormalities. Behavioral and cognitive defects display a wide range of severity, as do limb malformations, which can range from small digits to both upper and lower limb defects. CdLS is caused by point mutations or small deletions/insertions in one of the two alleles of *SMC1, SMC3*, or most commonly, *NIPBL* (Nipped-B-like and the human orthologue of *SCC2*) [144–147]. Mutations in NIPBL, the vertebrate homologue of the yeast Scc2 protein and a regulator of cohesin loading and unloading, are responsible for approximately 50% of cases of CdLS [144,145,148]. Two other mutations in SMC1 and SMC3 were shown to

result in an X-linked form of CdLS that is milder than the syndrome caused by NIPBL mutations [146]. The mutations in the SMC proteins have been identified within the coiled coil of the ATPase head domain, and near the interface of the coiledcoil with the hinge domain [147]. Mutations in this region disrupt DNA binding and ATP hydrolysis involved in loading cohesins. Mutations in *NIPBL* have been identified throughout the coding and noncoding regions of the gene. Alternative splicing of *NIPBL* is consistent with multiple transcripts detected by Northern blot analysis, and some types of mutations tend to result in more severe forms of CdLS [144,148]. Mutations have been identified only in the context of the genomic copy and may affect particular splice variants, potentially affecting the severity of the disease phenotype.

The mutations in the SMC proteins could weaken interactions between cohesin subunits or between chromatin and cohesin. However, the mutations most likely do not abolish complex formation or chromatin association completely because patients do not exhibit severe defects in chromosome cohesion, DNA damage response, or chromosome segregation [149,150]. Sister chromatid cohesion has been reported to be mildly affected in cell lines derived from individuals with mutations in *NIPBL* [149], but no defects in precocious sister chromatid separation have been observed in cells with a mutation in *SMC1* or *SMC3* [151]. CdLS mutations could affect the dynamics of cohesin subunit–chromatin interaction, resulting in mild destabilization of the complex on chromatin without affecting the overall function of the complex for cohesion. Interestingly, *NIPBL* expression in human embryonic tissue sections is consistent with affected tissues and organs seen in patients [145]. Molecular studies of cohesins in this disease will help elucidate the defects underlying the mechanism of the mutated cohesins.

A mouse model of CdLS has been developed in which the mice are heterozygous for a *Nipbl* mutation [152]. These mice show similar defects that are characteristic of the syndrome, including small size, craniofacial anomalies, delayed bone maturation, microbrachycephaly, behavioral disturbances, and high mortality during the early weeks of postnatal life. The *Nipbl* deficiency in heterozygous mice leads to small but significant transcriptional dysregulation of many genes. Expression changes at the protocadherin β locus, which encodes synaptic cell adhesion molecules for neural tube and CNS development, as well as other loci, support the notion that *NIPBL* influences long-range chromosomal regulatory interactions. Although this model has proven to be beneficial in studying CdLS, closer scrutiny of cohesins in the disease is still needed.

4.3 Roberts syndrome/SC phocomelia

Roberts syndrome and SC phocomelia are rare, recessively inherited, multisystem disorders involving craniofacial, cardiac, limb, other systemic abnormalities, and neurocognitive dysfunction. Roberts syndrome and SC phocomelia are similar disorders, but SC phocomelia represents a milder phenotype of Roberts syndrome. Chromosomal features in metaphase spreads of patients with Roberts syndrome reveal a lack of cohesion in heterochromatic areas around centromeres and at the distal region on the long arm of the Y chromosome, known as heterochromatin repulsion or puffing or premature centromere separation [153,154]. Mitotic chromosomes have a railroad track–like appearance; although this

resembles a cohesion defect, it does not appear to cause chromosome segregation defects. Roberts syndrome/SC phocomelia is caused by a mutation in both alleles of *ESCO2*, the human orthologue of yeast *ECO1*. In most cases the mutations are truncating, but at least two mutations that disrupt the acetyltransferase activity of ESCO2 have been identified [155]. The majority of mutations identified result in low or undetectable levels of mRNA compared with wild-type *ESCO2* expression. Although there are two genes that encode *ECO1* paralogues, *ESCO1* and *ESCO2*, only *ESCO2* has been implicated in Roberts syndrome and SC phocomelia. This is interesting because the *ESCO1* and *ESCO2* genes share a C-terminal acetyltransferase domain and a zinc-finger motif but differ in their N-termini [156].

Although ESCO2 is required for the establishment of sister chromatid cohesion, processivity of DNA replication forks in cells from patients with Roberts syndrome is reduced, suggesting a role for *ESCO2* in replication-coupled cohesion [73]. Decreased *ESCO2* activity may lead to some loss of cohesion that manifests as heterochromatic repulsion, but there may be sufficient protection of centromeric cohesion through the activity of shugoshin and PP2A so that chromosome segregation is not disturbed. As in CdLS, *ESCO2* is expressed in human embryonic tissues in a pattern that is consistent with the systems and organs affected in patients with this syndrome [155].

4.4 PDS5 deficiencies

Two copies of the *Pds5* gene, *Pds5A* and *Pds5B*, are found in mammals and differ in expression [55]. Both *Pds5A*- and *Pds5B*-deficient mice are born with multiple congenital abnormalities, including growth retardation, cleft palate, and congenital heart defects, similar to the abnormalities found in humans with CdLS, and they die at birth [56,157]. Surprisingly, *Pds5B*-deficient mouse embryonic fibroblasts lack defects in sister chromatid cohesion, but expression is detected in postmitotic neurons in the brain [157], suggesting an alternate role for cohesins. This expression pattern is similar to that of *Smc1*, *Rad21*, *Pds5B*, and *Smc3* in zebrafish [158], and in conjunction with the neurological phenotypes of the mutants the pattern suggests a crucial role for cohesin in the development and migration of neurons. Because this regulatory cohesin protein has not been well characterized in the human disease, examining these deficiencies more closely would be beneficial to better understanding the mechanisms underlying PDS5A and PDS5B function.

4.5 α-Thalassemia/mental retardation syndrome, X-linked

α-Thalassemia/mental retardation syndrome, X-linked (ATRX) is a multisystem disorder of postnatal growth deficiency, mental retardation, microcephaly, dysmorphic craniofacial features, genital abnormalities in males, seizures, and a mild form of hemoglobin H disease. ATRX is caused by mutations in the *ATRX* gene on the X chromosome and was recently found to lead to a cohesion defect in *ATRX*-depleted mammalian cells. The *ATRX* gene encodes a chromatin remodeling enzyme that is highly enriched at pericentromeric heterochromatin in mouse and human cells and associates with heterochromatin protein 1α (HP1α), just like NIPBL [159]. In mammalian cells, defects in sister chromatid cohesion, chromosome congression at the metaphase plate, and mitotic defects were described. Defects in the *ATRX* gene are thought to result from perturbed cohesin targeting or loading/

unloading. ATRX is believed to play a dual role in the regulation of cohesion during mitosis and in the control of gene expression in interphase, which is reminiscent of cohesin complex function. Investigators have recently found that ATRX is required for normal recruitment of cohesin in mouse brain cells and alters expression of imprinted genes in the postnatal brain [160]. Therefore, ATRX along with cohesin, may regulate expression of this imprinted gene network by controlling higher-order chromatin structure. Defects in the *ATRX* gene disrupt the cohesin targeting and/or loading/unloading, resulting in ATRX syndrome phenotypes.

4.6 Warsaw Breakage Syndrome

Only one patient with Warsaw breakage syndrome has been reported who displayed severe microcephaly, pre- and postnatal growth retardation, and abnormal skin pigmentation. The patient displayed two mutations in the *ChlR1* helicase, also called DDX11: a splice-site mutation in intron 22 of the maternal allele and a three-base pair deletion in exon 26 of the paternal allele [161]. The maternal allele mutation leads to a deletion of the last 10 base pairs of exon 22 from the cDNA, and the paternal allele mutations results in deletion of a highly conserved lysine residue in the ChlR1 protein. Cells from this patient reveal chromosomal instability characterized by sister cohesion defects, chromosomal breakage, and sensitivity to DNA cross-linking agents and topoisomerase inhibitors. Investigators have suggested that Warsaw breakage syndrome represents a unique disease with cellular features of both Fanconi anemia and Roberts syndrome, but with a distinct clinical phenotype. Other patients have yet to be identified with these same characteristics, and the defects underlying the *ChlR1* mutations have yet to be revealed.

4.7 Maternal aging and chromosome segregation

Chromosome abnormalities represent not only the leading cause of birth defects in humans but also the major cause of pregnancy loss. Approximately 0.2% to 0.3% of newborn infants are trisomic, and a majority of these errors result from fertilization of a chromosomally abnormal egg by a normal sperm (reviewed in [162]). For this reason, attention has focused on why human female meiosis is so error-prone. It is widely understood that the number of pregnancies involving trisomies increases drastically among women in their 40s to 35%, compared with women in their 20s, in whom the rate is 2% to 3% (reviewed in [163]). Little is known about the basis of this increased frequency of aneuploidy with age, but cohesins are becoming increasingly implicated because these complexes are essential for proper chromosome segregation in mitosis and meiosis. Because S phase takes place during fetal development in the oocyte and cell division does not occur until resumption of meiosis beginning at puberty, cohesins may in part responsible for these errors. Sites of DNA crossover are also established decades before they function as physical mediators of chromosome segregation (reviewed in [163]). The correlation between age and aneuploidy in humans has been postulated to result from age-related weakening of cohesion.

SMC1β-deficient female mice provided the first direct evidence of an age-related decline in chromosome cohesion in mammalian oocytes [139]. Revenkova *et al*. demonstrated that *SMC1*β-deficient mice in both sexes were sterile, but male meiosis was blocked in pachynema, whereas in females meiosis progressed until metaphase II. AEs are markedly shortened, chromatin extends further from the AEs, chromosome synapsis is incomplete,

sister chromatid cohesion at chromosome arms and centromeres are lost prematurely, and crossovers are absent or reduced owing to this deficiency. A recent study observed that when the *SMC1*β gene is deleted in mice after the neonatal period and the protein is produced only during fetal development, fertility is not affected [164]. This finding suggests that meiotic cohesin is sufficiently robust that once cohesion is established in fetal oocytes, little or no turnover of the cohesin protein occurs until fertilization at reproductive maturity. The pronounced age effect observed in *SMC1*β-deficient mice suggests that the cause may not be related to recombination itself, but instead to defective cohesion [165]. Weakened cohesion in these mice may accelerate the normal aging process, but severe abnormalities occur if cohesin complexes are absent [139]. Loss of cohesion may explain human age-related nondisjunction, but it raises a question about the fate of cohesins during prophase I arrest in women.

The possible association between age-related degradation of cohesion and increasing rate of aneuploidy was also examined in older, naturally aged female mice [166,167]. Centromere cohesion was assessed by examining the distances between sister kinetochores in old compared to young oocytes [166]. Studies of metaphase I and II oocytes revealed an increase in distance between sister kinetochores from old compared to young mice, suggesting an age-related loss of centromere cohesion. Immunofluorescence staining of chromosome-associated REC8 was also analyzed and levels were significantly reduced in old compared to young oocytes [166]. Thus, loss of cohesion with age could predispose oocytes to meiotic errors involving the premature separation of homologues and sister chromatids. In a similar study, 14-month-old female mice showed increased interkinetochore distances, reduction in REC8 staining, and increases in anaphase defects compared to 2-month-old mice [167]. An age-related depletion of SGO2, a protein necessary for preventing degradation of centromere cohesin at anaphase I, was also observed, suggesting another cause of aneuploidy. These studies provide a plausible explanation for nondisjunction events, including not only abnormalities involving homologous chromosomes at the first meiotic division but also abnormalities involving missegregation of sister chromatids. Human oogenesis is an extremely error-prone process, which leads to a high percentage of aneuploid oocytes compared to spermatocytes. The percentage of aneuploid oocytes increases with age, known as the “maternal age effect,” and loss of sister chromatid cohesion has been postulated as a culprit for this phenomenon [168]. A recent study has shed light on cohesins in human oocytes and provides surprising counterpoints to the mouse data above [117]. In oocytes from women aged 18 to 34 years, no age-related changes were identifiable in immunolocalization patterns of REC8, SMC3, STAG3, or in levels of *SMC1*β gene expression. Direct evidence linking age-related cohesin degradation to human oogenesis is therefore lacking and the physiological basis of maternal age-related aneuploidy is unknown, although loss of cohesion could still be an important contributing factor.

5. Conclusions

Cumulative studies from many model organisms have established that cohesins play a key role in sister chromatid cohesion and the maintenance of genome integrity during cell division. During meiosis, distinct cohesin complexes, composed of different subunits

including those that are meiosis-specific, regulate chromosome dynamics and are essential for normal germ cell development and precise chromosome segregation. The recent discovery that cohesins are involved with the replication machinery and other factors necessary for proper DNA replication during mitosis and meiosis barely touches the surface in shedding light on these complex proteins. The question of how cohesin complexes associate with DNA has yet to be answered. Debate over the different models continues and conclusive data are needed to settle the issue. Only in the past several years have cohesinopathies been recognized and mutations in the cohesin subunits characterized. The maternal age effect is unresolved but it is thought to be due to loss of cohesion between sister chromatids with age, leading to premature chromosome separation and ultimately to aneuploidy. Although the roles of cohesins and their mechanisms of action have yet to be fully elucidated, research continues to move forward and progress so far has been remarkable.

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References

1. Tanaka T, Fuchs J, Loidl J, Nasmyth K. Cohesin ensures bipolar attachment of microtubules to sister centromeres and resists their precocious separation. Nat Cell Biol. 2000; 2:492–499. [PubMed: 10934469]
2. Uhlmann F, Lottspeich F, Nasmyth K. Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. Nature. 1999; 400:37–42. [PubMed: 10403247]
3. Guacci V, Koshland D, Strunnikov AV. A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of *MCD1* in S. cerevisiae. Cell. 1997; 91(1):47–57. [PubMed: 9335334]
4. Michaelis C, Ciosk R, Nasmyth K. Cohesins: chromosomal proteins that prevents premature separation of sister chromatids. Cell. 1997; 91:35–45. [PubMed: 9335333]
5. Toth A, Ciosk R, Uhlmann F, Galova M, Schleiffer A, Nasmyth K. Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. Genes Dev. 1999; 13:320–333. [PubMed: 9990856]
6. Gruber S, Haering CH, Nasmyth K. Chromosomal cohesin forms a ring. Cell. 2003; 112(6):765–777. [PubMed: 12654244]
7. Carramolino L, Lee BC, Zaballos A, Peled A, Barthelemy I, Shav-Tal Y, Prieto I, Carmi P, Gothelf Y, Gonzalez de Buitrago G, Aracil M, Marquez G, Barbero JL, Zipori D. SA-1, a nuclear protein encoded by one member of a novel gene family: molecular cloning and detection in hemopoietic organs. Gene. 1997; 195(2):151–159. [PubMed: 9305759]
8. Losada A, Yokochi T, Kobayashi R, Hirano T. Identification and characterization of SA/Scc3p subunits in the *Xenopus* and human cohesin complexes. J Cell Biol. 2000; 150(3):405–416. [PubMed: 10931856]
9. Sumara I, Vorlaufer E, Gieffers C, Peters BH, Peters J-M. Characterization of vertebrate cohesin complexes and their regulation in prophase. J Cell Biol. 2000; 151(4):749–761. [PubMed: 11076961]
10. Haering CH, Lowe J, Hochwagen A, Nasmyth K. Molecular architecture of SMC proteins and the yeast cohesin complex. Mol Cell. 2002; 9(4):773–788. [PubMed: 11983169]

11. Anderson DE, Losada A, Erickson HP, Hirano T. Condensin and cohesin display different arm conformations with characteristic hinge angles. J Cell Biol. 2002; 156(3):419–424. [PubMed: 11815634]
12. Melby TE, Ciampaglio CN, Briscoe G, Erickson HP. The symmetrical structure of structural maintenance of chromosomes (SMC) and MukB proteins: long, antiparallel coiled coils, folded at a flexible hinge. J Cell Biol. 1998; 142(6):1595–1604. [PubMed: 9744887]
13. Biggins S, Murray AW. The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. Genes Dev. 2001; 15(23):3118–3129. [PubMed: 11731476]
14. Tanaka TU, Rachidi N, Janke C, Pereira G, Galova M, Schiebel E, Stark MJR, Nasmyth K. Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. Cell. 2002; 108:317–329. [PubMed: 11853667]
15. He X, Rines DR, Espelin CW, Sorger PK. Molecular analysis of kinetochore-microtubule attachment in budding yeast. Cell. 2001; 106(2):195–206. [PubMed: 11511347]
16. Biggins S, Severin FF, Bhalla N, Sassoon I, Hyman AA, Murray AW. The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. Genes Dev. 1999; 13(5):532–544. [PubMed: 10072382]
17. Strunnikov AV, Larionov VL, Koshland D. *SMC1*: an essential yeast gene encoding a putative head-rod-tail protein is required for nuclear division and defines a new ubiquitous protein family. J Cell Biol. 1993; 123(6):1635–1648. [PubMed: 8276886]
18. Strunnikov AV, Hogan E, Koshland D. *SMC2* a *Saccharomyces cerevisiae* gene essential for chromosome segregation and condensation, defines a subgroup within the SMC family. Genes Dev. 1995; 9:587–599. [PubMed: 7698648]
19. Hoque MT, Ishikawa F. Cohesin defects lead to premature sister chromatid separation, kinetochore dysfunction, and spindle-assembly checkpoint activation. J Biol Chem. 2002; 277(44):42306–42314. [PubMed: 12200439]
20. Britton RA, Lin DC, Grossman AD. Characterization of a prokaryotic SMC protein involved in chromosome partitioning. Genes Dev. 1998; 12(9):1254–1259. [PubMed: 9573042]
21. Sonoda E, Matsusaka T, Morrison C, Vagnarelli P, Hoshi O, Ushiki T, Nojima K, Fukagawa T, Waizenegger IC, Peters J-M, Earnshaw WC, Takeda S. Scc1/Rad21/Mcd1 is required for sister chromatid cohesion and kinetochore function in vertebrate cells. Dev Cell. 2001; 1:759–770. [PubMed: 11740938]
22. Vass S, Cotterill S, Valdeolmillos AM, Barbero JL, Lin E, Warren WD, Heck MMS. Depletion of Drad21/Scc1 in *Drosophila* cells leads to instability of the cohesin complex and disruption of mitotic progression. Curr Biol. 2003; 13:208–218. [PubMed: 12573216]
23. Diaz-Martinez LA, Gimenez-Abian JF, Clarke DJ. Cohesin is dispensable for centromere cohesion in human cells. PLoS One. 2007; 2(3):e318. [PubMed: 17389909]
24. Chang C-R, Wu C-S, Hom Y, Gartenberg MR. Targeting of cohesin by transcriptionally silent chromatin. Genes Dev. 2005; 19(24):3031–3042. [PubMed: 16319193]
25. Losada A, Hirano T. Intermolecular DNA interactions stimulated by the cohesin complex in vitro: Implications for sister chromatid cohesion. Curr Biol. 2001; 11(4):268–272. [PubMed: 11250156]
26. Zhang N, Kuznetsov SG, Sharan SK, Li K, Rao PH, Pati D. A handcuff model for the cohesin complex. J Cell Biol. 2008; 183(6):1019–1031. [PubMed: 19075111]
27. Uhlmann F, Nasmyth K. Cohesion between sister chromatids must be established during DNA replication. Curr Biol. 1998; 8(20):1095–1101. [PubMed: 9778527]
28. Sjogren C, Nasmyth K. Sister chromatid cohesion is required for postreplicative double-strand break repair in *Saccharomyces cerevisiae* . Curr Biol. 2001; 11:991–995. [PubMed: 11448778]
29. Strom L, Lindroos HB, Shirahige K, Sjogren C. Postreplicative recruitment of cohesin to double-strand breaks is required for DNA repair. Mol Cell. 2004; 16:1003–1015. [PubMed: 15610742]
30. Uhlmann F, Wernic D, Poupart M-A, Koonin EV, Nasmyth K. Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. Cell. 2000; 103:375–386. [PubMed: 11081625]

31. Ciosk R, Zachariae W, Michaelis C, Shevchenko A, Mann M, Nasmyth K. An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. Cell. 1998; 93(6):1067–1076. [PubMed: 9635435]
32. Tomonaga T, Nagao K, Kawasaki Y, Furuya K, Murakami A, Morishita J, Yuasa T, Sutani T, Kearsey SE, Uhlmann F, Nasmyth K, Yanagida M. Characterization of fission yeast cohesin: essential anaphase proteolysis of Rad21 phosphorylated in the S phase. Genes Dev. 2000; 14:2757–2770. [PubMed: 11069892]
33. Losada A, Hirano M, Hirano T. Identification of *Xenopus* SMC protein complexes required for sister chromatid cohesion. Genes Dev. 1998; 12(13):1986–1997. [PubMed: 9649503]
34. Waizenegger IC, Hauf S, Meinke A, Peters J-M. Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. Cell. 2000; 103:399–410. [PubMed: 11081627]
35. Sumara I, Vorlaufer E, Stukenberg PT, Kelm O, Redemann N, Nigg EA, Peters J-M. The dissociation of cohesin from chromosomes in prophase is regulated by Polo-like kinase. Mol Cell. 2002; 9:515–525. [PubMed: 11931760]
36. Alexandru G, Uhlmann F, Mechtler K, Poupart M-A, Nasmyth K. Phosphorylation of the cohesin subunit Scc1 by Polo/Cdc5 kinase regulates sister chromatid separation in yeast. Cell. 2001; 105(4):459–472. [PubMed: 11371343]
37. Hoque MT, Ishikawa F. Human chromatid cohesin component hRad21 is phosphorylated in M phase and associated with metaphase centromeres. J Biol Chem. 2001; 276(7):5059–5067. [PubMed: 11073952]
38. Gimenez-Abian JF, Sumara I, Hirota T, Hauf S, Gerlich D, de la Torre C, Ellenberg J, Peters J-M. Regulation of sister chromatid cohesion between chromosome arms. Curr Biol. 2004; 14(13): 1187–1193. [PubMed: 15242616]
39. Hauf S, Roitinger E, Koch B, Dittrich CM, Mechtler K, Peters J-M. Dissociation of cohesin from chromosome arms and loss of arm cohesion during early mitosis depends on phosphorylation of SA2. PLoS Biol. 2005; 3(3):0419–0432.
40. Hauf S, Waizenegger IC, Peters J-M. Cohesin cleavage by separase required for anaphase and cytokinesis in human cells. Science. 2001; 293(5533):1320–1323. [PubMed: 11509732]
41. Nakajima M, Kumada K, Hatakeyama K, Noda T, Peters J-M, Hirota T. The complete removal of cohesin from chromosome arms depends on separase. J Cell Sci. 2007; 120:4188–4196. [PubMed: 18003702]
42. Zou H, McGarry TJ, Bernal T, Kirschner MW. Identification of a vertebrate sisterchromatid separation inhibitor involved in transformation and tumorigenesis. Science. 1999; 285:418–422. [PubMed: 10411507]
43. Cohen-Fix O, Peters J-M, Kirschner MW, Koshland D. Anaphase inititation in *Saccharomyces cerevisiae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. Genes Dev. 1996; 10:3081–3093. [PubMed: 8985178]
44. Funabiki H, Yamano H, Kumada K, Nagao K, Hunt T, Yanagida M. Cut2 proteolysis required for sister-chromatid separation in fission yeast. Nature. 1996; 381(6581):438–441. [PubMed: 8632802]
45. Ciosk R, Shirayama M, Shevchenko A, Tanaka T, Toth A, Shevchenko A, Nasmyth K. Cohesin's binding to chromosomes depends on a separate complex consisting of Scc2 and Scc4 proteins. Mol Cell. 2000; 5(2):243–254. [PubMed: 10882066]
46. Watrin E, Schleiffer A, Tanaka K, Eisenhaber F, Nasmyth K, Peters J-M. Human Scc4 is required for cohesin binding to chromatin, sister-chromatid cohesion, and mitotic progression. Curr Biol. 2006; 16:863–874. [PubMed: 16682347]
47. Lechner MS, Schultz DC, Negorev D, Maul GG, Rauscher FJ III. The mammalian heterochromatin protein 1 binds diverse nuclear proteins through a common motif that targets the chromoshadow domain. Biochem Biophys Res Commun. 2005; 331(4):929–937. [PubMed: 15882967]
48. Seitan VC, Banks P, Laval S, Majid NA, Dorsett D, Rana A, Smith J, Bateman A, Krpic S, Hostert A, Rollins RA, Erdjument-Bromage H, Tempst P, Benard CY, Hekimi S, Newbury SF, Strachan T. Metazoan Scc4 homologs link sister chromatid cohesion to cell and axon migration guidance. PLoS Biol. 2006; 4(8):e242. [PubMed: 16802858]

49. Hartman T, Stead K, Koshland D, Guacci V. Pds5p is an essential chromosomal protein required for both sister chromatid cohesion and condensation in *Saccharomyces cerevisiae*. J Cell Biol. 2000; 151(3):613–626. [PubMed: 11062262]
50. Panizza S, Tanaka T, Hochwagen A, Eisenhaber F, Nasmyth K. Pds5 cooperates with cohesin in maintaining sister chromatid cohesion. Curr Biol. 2000; 10:1557–1564. [PubMed: 11137006]
51. Gandhi R, Gillespie PJ, Hirano T. Human Wapl is a cohesin-binding protein that promotes sister-chromatid resolution in mitotic prophase. Curr Biol. 2006; 16(24):2406–2417. [PubMed: 17112726]
52. Rankin S, Ayad NG, Kirschner MW. Sororin, a substrate of the anaphase-promoting complex, is required for sister chromatid cohesion in vertebrates. Mol Cell. 2005; 18:185–200. [PubMed: 15837422]
53. Dai J, Sullivan BA, Higgins JMG. Regulation of mitotic chromosome cohesion by Haspin and Aurora B. Dev Cell. 2006; 11(5):741–750. [PubMed: 17084365]
54. Wang F, Yoder J, Antoshechkin I, Han M. *Caenorhabditis elegans* EVL-14/PDS5 and SCC-3 are essential for sister chromatid cohesion in meiosis and mitosis. Mol Cell Biol. 2003; 23(21):7698–7707. [PubMed: 14560015]
55. Losada A, Yokochi T, Hirano T. Functional contribution of Pds5 to cohesin-mediated cohesion in human cells and *Xenopus* egg extracts. J Cell Sci. 2005; 118(Pt 10):2133–2141. [PubMed: 15855230]
56. Zhang B, Chang J, Fu M, Huang J, Kashyap R, Salavaggione E, Jain S, Shashikant K, Deardorff MA, Giovannucci Uzielli ML, Dorsett D, Beebe DC, Jay PY, Heuckeroth RO, Krantz ID, Millbrandt J. Dosage effects of cohesin regulatory factor PDS5 on mammalian development: implications for cohesinopathies. PLoS One. 2009; 4(5):1–17.
57. Kueng S, Hegemann B, Peters BH, Lipp JJ, Schleiffer A, Mechtler K, Peters J-M. Wapl controls the dynamic association of cohesin with chromatin. Cell. 2006; 127(5):955–967. [PubMed: 17113138]
58. Kuroda M, Oikawa K, Ohbayashi T, Yoshida K, Yamada K, Mimura J, Matsuda Y, Fujii-Kuriyama Y, Mukai K. A dioxin sensitive gene, mammalian *WAPL* is implicated in spermatogenesis. FEBS Journal. 2005; 579(1):167–172.
59. Zhang J, Hakansson H, Kuroda M, Yuan L. Wapl localization on the synaptonemal complex, a meiosis-specific proteinaceous structure that binds homologous chromosomes, in the female mouse. Reproduction in Domestic Animals. 2008; 43(1):124–126. [PubMed: 18199270]
60. Schmitz J, Watrin E, Lenart P, Mechtler K, Peters J-M. Sororin is required for stable binding of cohesin to chromatin and for sister chromatid cohesion in interphase. Curr Biol. 2007; 17:630–636. [PubMed: 17349791]
61. Skibbens RV, Corson LB, Koshland D, Hieter P. Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. Genes Dev. 1999; 13:307–319. [PubMed: 9990855]
62. Ivanov D, Schleiffer A, Eisenhaber F, Mechtler K, Haering CH, Nasmyth K. Eco1 is a novel acetyltransferase that can acetylate proteins involved in cohesion. Curr Biol. 2002; 12(4):323–328. [PubMed: 11864574]
63. Unal E, Heidinger-Pauli JM, Koshland D. DNA double-strand breaks trigger genomewide sister-chromatid cohesion through Eco1 (Ctf7). Science. 2007; 317:245–248. [PubMed: 17626885]
64. Bermudez VP, Maniwa Y, Tappin I, Ozato K, Yokomori K, Hurwitz J. The alternative Ctf18-Dcc1-Ctf8-replication factor C complex required for sister chromatid cohesion loads proliferating cell nuclear antigen onto DNA. PNAS. 2003; 100(18):10237–10242. [PubMed: 12930902]
65. Bylund GO, Burgers PM. Replication protein A-directed unloading of PCNA by the Ctf18 cohesion establishment complex. Mol Cell Biol. 2005; 25(13):5445–5455. [PubMed: 15964801]
66. Hanna JS, Kroll ES, Lundblad V, Spencer FA. *Saccharomyces cerevisiae* CTF18 and CTF4 are required for sister chromatid cohesion. Mol Cell Biol. 2001; 21(9):3144–3158. [PubMed: 11287619]
67. Mayer ML, Gygi SP, Aebersold R, Hieter P. Identification of RFC(Ctf18p, Ctf8p, Dcc1p): an alternative RFC complex required for sister chromatid cohesion in *S. cerevisiae* . Mol Cell. 2001; 7:959–970. [PubMed: 11389843]

68. Lengronne A, McIntyre J, Katou Y, Kanoh Y, Hopfner K-P, Shirahige K, Uhlmann F. Establishment of sister chromatid cohesion at the *S. cerevisiae* replication fork. Mol Cell. 2006; 23(6):787–799. [PubMed: 16962805]
69. Ansbach AB, Noguchi C, Klansek IW, Heidlebaugh M, Nakamura TM, Noguchi E. RFCCtf18 and the Swi1-Swi3 complex function in separate and redundant pathways required for the stabilization of replication forks to facilitate sister chromatid cohesion in *Schizosaccharomyces pombe* . Mol Biol Cell. 2008; 19(2):595–607. [PubMed: 18045993]
70. Noguchi E, Noguchi C, McDonald WH, Yates JRI, Russell P. Swi1 and Swi3 are components of a replication fork protection complex in fission yeast. Mol Cell Biol. 2004; 24(19):8342–8355. [PubMed: 15367656]
71. Skibbens RV. Chl1p, a DNA helicase-like protein in budding yeast, functions in sisterchromatid cohesion. Genetics. 2004; 166:33–42. [PubMed: 15020404]
72. Parish JL, Rosa J, Wang X, Lahti JM, Doxsey SJ, Androphy EJ. The DNA helicase ChlR1 is required for sister chromatid cohesion in mammalian cells. J Cell Sci. 2006; 119(4857–4865)
73. Terret ME, Sherwood R, Rahman S, Qin J, Jallepalli PV. Cohesin acetylation speeds the replication fork. Nature. 2009; 462(7270):231–234. [PubMed: 19907496]
74. Berkowitz KM, Sowash AR, Koenig LR, Urcuyo D, Khan F, Yang F, Wang PJ, Jongens TA, Kaestner KH. Disruption of CHTF18 causes defective meiotic recombination in male mice. PLoS Genet. 2012; 8(11):e1002996. [PubMed: 23133398]
75. Rudra S, Skibbens RV. Sister chromatid cohesion establishment occurs in concert with lagging strand synthesis. Cell Cycle. 2012; 11(11):2114–2121. [PubMed: 22592531]
76. Kim S-T, Xu B, Kastan MB. Involvement of the cohesin protein, Smc1, in Atm-dependent and independent responses to DNA damage. Genes Dev. 2002; 16(5):560–570. [PubMed: 11877376]
77. Unal E, Arbel-Eden A, Sattler U, Shroff R, Lichten M, Haber JE, Koshland D. DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. Mol Cell. 2004; 16:991–1002. [PubMed: 15610741]
78. Covo S, Westmoreland JW, Gordenin DA, Resnick MA. Cohesin is limiting for the suppression of DNA damage-induced recombination between homologous chromosomes. PLoS Genet. 2010; 6(7):1–16.
79. Yazdi PT, Wang Y, Zhao S, Patel N, Lee EY-HP, Qin J. SMC1 is a downstream effector in the ATM/NBS1 branch of the human S-phase checkpoint. Genes Dev. 2002; 16:571–582. [PubMed: 11877377]
80. Kitagawa R, Bakkenist CJ, McKinnon PJ, Kastan MB. Phosphorylation of SMC1 is a critical downstream event in the ATM-NBS1-BRCA1 pathway. Genes Dev. 2004; 18(12):1423–1438. [PubMed: 15175241]
81. Luo H, Li Y, Mu J-J, Zhang J, Tonaka T, Hamamori Y, Jung SY, Wang Y, Qin J. Regulation of intra-S phase checkpoint by ionzing radiation (IR)-dependent and IR-independent phosphorylation of SMC3. J Biol Chem. 2008; 283(28):19176–19183. [PubMed: 18442975]
82. Klein F, Mahr P, Galova M, Buonomo SBC, Michaelis C, Nairz K, Nasmyth K. A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell. 1999; 98(1):91–103. [PubMed: 10412984]
83. Watanabe Y, Nurse P. Cohesin Rec8 is required for reductional chromosome segregation at meiosis. Nature. 1999; 400(6743):461–464. [PubMed: 10440376]
84. Keeney S, Giroux CN, Kleckner N. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell. 1997; 88(3):375–384. [PubMed: 9039264]
85. Alani E, Padmore R, Kleckner N. Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synpasis and recombination. Cell. 1990; 61:419–436. [PubMed: 2185891]
86. Nairz K, Klein F. *mre11S*- a yeast mutation that blocks double-strand break processing and permits nonhomologous synapsis in meiosis. Genes Dev. 1997; 11:2272–2290. [PubMed: 9303542]
87. Prinz S, Amon A, Klein F. Isolation of *COM1*, a new gene required to complete meiotic double-strand break induced recombination in *Saccharomyces cerevisiae*. Genetics. 1997; 146:781–795. [PubMed: 9215887]

88. Hunter N, Kleckner N. The single-end invasion: an asymmetric intermediate at the double-strand break to double-holliday junction transition of meiotic recombination. Cell. 2001; 106(1):59–70. [PubMed: 11461702]

89. Yokobayashi S, Yamamoto M, Watanabe Y. Cohesins determine the attachment manner of kinetochores to spindle microtubules at meiosis I in fission yeast. Mol Cell Biol. 2003; 23(11): 3965–3973. [PubMed: 12748297]

90. Kateneva AV, Konovchenko AA, Guacci V, Dresser ME. Recombination protein Tid1p controls resolution of cohesin-dependent linkages in meiosis in *Saccharomyces cerevisiae*. J Cell Biol. 2005; 171(2):241–253. [PubMed: 16230461]

91. Kitajima TS, Yokobayashi S, Yamamoto M, Watanabe Y. Distinct cohesin complexes organize meiotic chromosome domains. Science. 2003; 300(5622):1152–1155. [PubMed: 12750522]

92. Lee J, Iwai T, Yokota T, Yamashita M. Temporally and spatially selective loss of Rec8 protein from meiotic chromosomes during mammalian meiosis. J Cell Sci. 2003; 116(Pt 13):2781–2790. [PubMed: 12759374]

93. Parisi S, McKay MJ, Molnar M, Thompson MA, van der Spek PJ, van Drunen-Schoenmaker E, Kanaar R, Lehmann E, Hoeijmakers JHJ, Kohli J. Rec8p, a meiotic recombination and sister chromatid cohesion phosphoprotein of the Rad21p family conserved from fission yeast to humans. Mol Cell Biol. 1999; 19(5):3515–3528. [PubMed: 10207075]

94. Pezzi N, Prieto I, Kremer L, Jurado LAP, Valero C, del Mazo J, Martinez-A C, Barbero JL. *STAG3* a novel gene encoding a protein involved in meiotic chromosome pairing and location of *STAG3*-related genes flanking the Williams-Beuren syndrome deletion. FASEB J. 2000; 14(3):581–595. [PubMed: 10698974]

95. Prieto I, Suja JA, Pezzi N, Kremer L, Martinez-A C, Rufas JS, Barbero JL. Mammalian STAG3 is a cohesin specific to sister chromatid arms in meiosis. Nat Cell Biol. 2001; 3:761–766. [PubMed: 11483963]

96. Revenkova E, Eijpe M, Heyting C, Gross B, Jessberger R. Novel meiosis-specific isoform of mammalian SMC1. Mol Cell Biol. 2001; 21(20):6984–6998. [PubMed: 11564881]

97. Prieto I, Pezzi N, Buesa JM, Kremer L, Barthelemy I, Carreiro C, Roncal F, Martinez A, Gomez L, Fernandez R, Martinez-A C, Barbero JL. STAG2 and Rad21 mammalian mitotic cohesins are implicated in meiosis. EMBO. 2002; 3(6):543–550.

98. Gutierrez-Caballero C, Herran Y, Sanchez-Martin M, Suja JA, Barbero JL, Llano E, Pendas AM. Identification and molecular characterization of the mammalian alpha-kleisin RAD21L. Cell Cycle. 2011; 10(9):1477–1487. [PubMed: 21527826]

99. Herran Y, Gutierrez-Caballero C, Sanchez-Martin M, Hernandez T, Viera A, Barbero JL, De Alava E, de Rooij DG, Suja JA, Llano E, Pendas AM. The cohesin subunit RAD21L functions in meiotic synapsis and exhibits sexual dimorphism in fertility. EMBO. 2011; 30(15):3091–3105.

100. Ishiguro K, Kim J, Fujiyama-Nakamura S, Kato S, Watanabe Y. A new meiosis-specific cohesin complex implicated in the cohesin code for homologous pairing. EMBO Rep. 2011; 12(3):267–275. [PubMed: 21274006]

101. Lee J, Hirano T. RAD21L, a novel cohesin subunit implicated in linking homologous chromosomes in mammalian meiosis. J Cell Biol. 2011; 192(2):263–276. [PubMed: 21242291]

102. Petronczki M, Chwalla B, Siomos MF, Yokobayashi S, Helmhart W, Deutschbauer AM, Davis RW, Watanabe Y, Nasmyth K. Sister-chromatid cohesion mediated by the alternative RF-C$^{Ctf18/Dcc1/Ctf8}$, the helicase Chl1 and the polymerase-a-associated protein Ctf4 is essential for chromatid disjunction during meiosis II. J Cell Sci. 2004; 117(16):3547–3559. [PubMed: 15226378]

103. Lightfoot J, Testori S, Barroso C, Martinez-Perez E. Loading of meiotic cohesin by SCC-2 is required for early processing of DSBs and for the DNA damage checkpoint. Curr Biol. 2011; 21(17):1421–1430. [PubMed: 21856158]

104. Sym M, Engebrecht J, Roeder GS. ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. Cell. 1993; 72:365–378. [PubMed: 7916652]

105. Meuwissen RLJ, Offenberg HH, Dietrch AJJ, Riesewijk A, van Iersel M, Heyting C. A coiled-coil related protein specific for synapsed regions of meiotic prophase chromosomes. EMBO. 1992; 11(13):5091–5100.

106. Schmekel K, Meuwissen RLJ, Dietrch AJJ, Vink ACG, van Marle J, van Veen H, Heyting C. Organization of SCP1 protein molecules within synaptonemal complexes of the rat. Exp Cell Res. 1996; 226:20–30. [PubMed: 8660935]

107. Schalk JAC, Dietrch AJJ, Vink ACG, Offenberg HH, van Aalderen M, Heyting C. Localization of SCP2 and SCP3 protein molecules within synaptonemal complexes of the rat. Chromosoma. 1998; 107:540–548. [PubMed: 9933407]

108. Parra MT, Viera A, Gomez R, Page J, Benavente R, Santos JL, Rufas JS, Suja JA. Involvement of the cohesin Rad21 and SCP3 in monopolar attachment of sister kinetochores during mouse meiosis I. J Cell Sci. 2004; 117:1221–1234. [PubMed: 14970259]

109. Xu H, Beasley M, Verschoor S, Inselman A, Handel MA, McKay MJ. A new role for the mitotic RAD21/SCC1 cohesin in meiotic chromosome cohesion and segregation in the mouse. EMBO. 2004; 5:378–384.

110. Eijpe M, Heyting C, Gross B, Jessberger R. Association of mammalian SMC1 and SMC3 proteins with meiotic chromosomes and synaptonemal complexes. J Cell Sci. 2000; 113:673–682. [PubMed: 10652260]

111. Suja JA, Antonio C, Debec A, Rufas JS. Phosphorylated proteins are involved in sister-chromatid arm cohesion during meiosis I. J Cell Sci. 1999; 112:2957–2969. [PubMed: 10444390]

112. Eijpe M, Offenberg HH, Jessberger R, Revenkova E, Heyting C. Meiotic cohesin REC8 marks the axial elements of rat synaptonemal complexes before cohesins SMC1b and SMC3. J Cell Biol. 2003; 160(5):657–670. [PubMed: 12615909]

113. Kouznetsova A, Novak I, Jessberger R, Hoog C. SYCP2 and SYCP3 are required for cohesin core integrity at diplotene but not for centromere cohesion at the first meiotic division. J Cell Sci. 2005; 118:2271–2278. [PubMed: 15870106]

114. Suja JA, Barbero JL. Cohesin complexes and sister chromatid cohesion in mammalian meiosis. Genome dynamics. 2009; 5:94–116. [PubMed: 18948710]

115. Gomez R, Valdeolmillos AM, Parra MT, Viera A, Carreiro C, Roncal F, Rufas JS, Barbero JL, Suja JA. Mammalian SGO2 appears at the inner centromere domain and redistributes depending on tension across centromeres during meiosis II and mitosis. EMBO Reports. 2007; 8(2):173–180. [PubMed: 17205076]

116. Prieto I, Tease C, Pezzi N, Buesa JM, Ortega S, Kremer L, Martinez A, Martinez-A C, Hulten MA, Barbero JL. Cohesin component dynamics during meiotic prophase I in mammalian oocytes. Chromosome Res. 2004; 12:197–213. [PubMed: 15125634]

117. Garcia-Cruz R, Brieno MA, Roig I, Grossmann M, Velilla E, Pujol A, Cabero L, Pessarrodona A, Barbero JL, Garcia Caldes M. Dynamics of cohesin proteins REC8, STAG3, SMC1b and SMC3 are consistent with a role in sister chromatid cohesion during meiosis in human oocytes. Hum Reprod. 2010; 25(9):2316–2327. [PubMed: 20634189]

118. Crackower MA, Kolas NK, Noguchi J, Sarao R, Kikuchi K, Kaneko H, Kobayashi E, Kawai Y, Kozieradzki I, Landers R, Mo R, Hui C-C, Nieves E, Cohen PE, Osborne LR, Wada T, Kunieda T, Moens PB, Penninger JM. Essential role of Fkbp6 in male fertility and homologous chromosome pairing in meiosis. Science. 2003; 300(5623):1291–1295. [PubMed: 12764197]

119. Holloway K, Roberson EC, Corbett KL, Kolas NK, Nieves E, Cohen PE. NEK1 Facilitates Cohesin Removal during Mammalian Spermatogenesis. Genes (Basel). 2011; 2(1):260–279. [PubMed: 21931878]

120. Buonomo SB, Clyne RK, Fuchs J, Loidl J, Uhlmann F, Nasmyth K. Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. Cell. 2000; 103(3):387–398. [PubMed: 11081626]

121. Pasierbek P, Jantsch M, Melcher M, Schleiffer A, Schweizer D, Loidl J. A *Caenorhabditis elegans* cohesion protein with functions in meiotic chromosome pairing and disjunction. Genes Dev. 2001; 15:1349–1360. [PubMed: 11390355]

122. Siomos MF, Badrinath A, Pasierbek P, Livingstone D, White J, Glotzer M, Nasmyth K. Separase is required for chromosome segregation during meiosis I in *Caenorhabditis elegans*. Curr Biol. 2001; 11:1825–1835. [PubMed: 11728305]

123. Rogers E, Bishop JD, Waddle JA, Schumacher JM, Lin R. The aurora kinase AIR-2 functions in the release of chromosome cohesion in *Caenorhabditis elegans* meiosis. J Cell Biol. 2002; 157(2):219–229. [PubMed: 11940606]

124. Kaitna S, Pasierbek P, Jantsch M, Loidl J, Glotzer M. The aurora B kinase AIR-2 regulated kinetochores during mitosis and is required for separation of homologous chromosomes during meiosis. Curr Biol. 2002; 12:798–812. [PubMed: 12015116]

125. Kitajima T, Miyazaki Y, Yamamoto M, Watanabe Y. Rec8 cleavage by separase is required for meiotic nuclear division in fission yeast. EMBO. 2003; 22(20):5643–5653.

126. Herbert M, Levasseur M, Homer H, Yallop K, Murdoch A, McDougall A. Homologue disjunction in mouse oocytes requires proteolysis of securin and cyclin B1. Nat Cell Biol. 2003; 5(11):1023–1025. [PubMed: 14593421]

127. Terret ME, Wassmann K, Waizenegger IC, Maro B, Peters J-M, Verlhac M-H. The meiosis I-to-meiosis II transition in mouse oocytes requires separase activity. Curr Biol. 2003; 13:1797–1802. [PubMed: 14561405]

128. Kitajima TS, Kawashima SA, Watanabe Y. The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. Nature. 2004; 427(6974):510–517. [PubMed: 14730319]

129. Rabitsch KP, Gregan J, Schleiffer A, Javerzat J-P, Eisenhaber F, Nasmyth K. Two fission yeast homologs of *Drosophila* Mei-S332 are required for chromosome segregation during meiosis I and II. Curr Biol. 2004; 14:287–301. [PubMed: 14972679]

130. Marston AL, Tham W-H, Shah H, Amon A. A genome-wide screen identifies genes required for centromeric cohesion. Science. 2004; 303:1367–1370. [PubMed: 14752166]

131. Katis VL, Galova M, Rabitsch KP, Gregan J, Nasmyth K. Maintenance of cohesin at centromeres after meiosis I in budding yeast requires a kinetochore-associated protein related to MEI-S332. Curr Biol. 2004; 14(7):560–572. [PubMed: 15062096]

132. Kitajima TS, Sakuno T, Ishiguro K, Iemura S, Natsume T, Kawashima SA, Watanabe Y. Shugoshin collaborates with protein phosphatase 2A to protect cohesin. Nature. 2006; 441(7089): 46–52. [PubMed: 16541025]

133. Ridel CG, Katis VL, Katou Y, Mori S, Itoh T, Helmhart W, Galova M, Petronczki M, Gregan J, Cetin B, Mudrak I, Ogris E, Mechtler K, Pelletier L, Buchholz F, Shirahige K, Nasmyth K. Protein phosphatase 2A protects centromeric sister chromatid cohesion during meiosis I. Nature. 2006; 441(7089):53–61. [PubMed: 16541024]

134. Llano E, Gomez R, Gutierrez-Caballero C, Herran Y, Sanchez-Martin M, Vazquez-Quinones L, Hernandez T, de Alava E, Cuadrado A, Barbero JL, Suja JA, Pendas AM. Shugoshin-2 is essential for the completion of meiosis but not for mitotic cell division in mice. Genes Dev. 2008; 22:2400–2413. [PubMed: 18765791]

135. Lee J, Kitajima TS, Tanno Y, Yoshida K, Morita T, Miyano T, Miyake M, Watanabe Y. Unified mode of centromeric protection by shugoshin in mammalian oocytes and somatic cells. Nat Cell Biol. 2008; 10(1):42–52. [PubMed: 18084284]

136. Yin S, Ai J-S, Shi L-H, Wei L, Yuan J, Ouyang Y-C, Hou Y, Chen D-Y, Schatten H, Sun Q-Y. Shugoshin 1 may play important roles in separation of homolgous chromosomes and sister chromatids during mouse oocyte meiosis. PLoS One. 2008; 3(10):1–7.

137. Brar GA, Kiburz BM, Zhang Y, Kim JE, White F, Amon A. Rec8 phosphorylation and recombination promote the step-wise loss of cohesins in meiosis. Nature. 2006; 441(7092):532–536. [PubMed: 16672979]

138. Katis VL, Lipp JJ, Imre R, Bogdanova A, Okaz E, Habermann B, Mechtler K, Nasmyth K, Zachariae W. Rec8 phosphorylation by casein kinase 1 and Cdc7-Dbf4 kinase regulates cohesin cleavage by separase during meiosis. Dev Cell. 2010; 18(3):397–409. [PubMed: 20230747]

139. Revenkova E, Eijpe M, Heyting C, Hodges CA, Hunt PA, Liebe B, Scherthan H, Jessberger R. Cohesin SMC1b is required for meiotic chromosome dynamics, sister chromatid cohesion and DNA recombination. Nat Cell Biol. 2004; 6(6):555–562. [PubMed: 15146193]

140. Xu H, Beasley MD, Warren WD, van der Horst GTJ, McKay MJ. Absence of mouse REC8 cohesin promotes synapsis of sister chromatids in meiosis. Dev Cell. 2005; 8:949–961. [PubMed: 15935783]

141. Yuan L, Liu J-G, Zhao J, Brundell E, Daneholt B, Hoog C. The murine *SCP3* gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. Mol Cell. 2000; 5:73–83. [PubMed: 10678170]

142. Yang F, De La Fuente R, Leu NA, Baumann C, McLaughlin KJ, Wang PJ. Mouse SYCP2 is required for synaptonemal complex assembly and chromosomal synapsis during male meiosis. J Cell Biol. 2006; 173(4):497–507. [PubMed: 16717126]

143. de Vries FAT, de Boer E, van den Bosch M, Baarends WM, Ooms M, Yuan L, Liu J-G, van Zeeland AA, Heyting C, Pastink A. Mouse *Sycp1* functions in synaptonemal complex assembly, meiotic recombination, and XY body formation. Genes Dev. 2005; 19(11):1376–1389. [PubMed: 15937223]

144. Krantz ID, McCallum J, DeScipio C, Kaur M, Gillis LA, Yaeger D, Jukofsky L, Wasserman N, Bottani A, Morris CA, Nowaczyk MJM, Toriello H, Bamshad MJ, Carey JC, Rappaport E, Kawauchi S, Lander AD, Calof AL, Li H, Devoto M, Jackson LG. Cornelia de Lange syndrome is caused by mutations in *NIPBL* the human homolog of *Drosophila melanogaster Nipped-B*. Nat Genet. 2004; 36(6):631–635. [PubMed: 15146186]

145. Tonkin ET, Wang T-J, Lisgo S, Bamshad MJ, Strachan T. *NIPBL* encoding a homolog of fungal Scc2-type sister chromatid cohesion proteins and fly Nipped-B, is mutated in Cornelia de Lange syndrome. Nat Genet. 2004; 36(6):636–641. [PubMed: 15146185]

146. Musio A, Selicorni A, Focarelli ML, Gervasini C, Milani D, Russo S, Vezzoni P, Larizza L. X-linked Cornelia de Lange syndrome owing to *SMC1L1* mutations. Nat Genet. 2006; 38(5):528–530. [PubMed: 16604071]

147. Deardorff MA, Kaur M, Yaeger D, Rampuria A, Korolev S, Pie J, Gil-Rodriguez C, Arnedo M, Loeys B, Kline AD, Wilson M, Lillquist K, Siu V, Ramos FJ, Musio A, Jackson LG, Dorsett D, Krantz ID. Mutations in cohesin complex members SMC3 and SMC1A cause a mild variant of Cornelia de Lange syndrome with predominant mental retardation. Am J Hum Genet. 2007; 80(3):485–494. [PubMed: 17273969]

148. Gillis LA, McCallum J, Kaur M, DeScipio C, Yaeger D, Mariani A, Kline AD, Li H, Devoto M, Jackson LG, Krantz ID. *NIPBL* mutational analysis in 120 individuals with Cornelia de Lange syndrome and evaluation of genotype-phenotype correlations. Am J Hum Genet. 2004; 75(4): 610–623. [PubMed: 15318302]

149. Kaur M, DeScipio C, McCallum J, Yaeger D, Devoto M, Jackson LG, Spinner NB, Krantz ID. Precocious sister chromatid separation (PSCS) in Cornelia de Lange syndrome. Am J Med Genet. 2005; 138A(1):27–31. [PubMed: 16100726]

150. Vrouwe MG, Elghalbzouri-Maghrani E, Meijers M, Schouten P, Godthelp BC, Bhuiyan ZA, Redeker EJ, Mannens MM, Mullenders LHF, Pastink A, Darroudi F. Increased DNA damage sensitivity of Cornelia de Lange syndrome cells: evidence for impaired recombinational repair. Hum Mol Genet. 2007; 16(12):1478–1487. [PubMed: 17468178]

151. Revenkova E, Focarelli ML, Susani L, Paulis M, Bassi MT, Mannini L, Frattini A, Delia D, Krantz ID, Vezzoni P, Jessberger R, Musio A. Cornelia de Lange syndrome mutations in SMC1A or SMC3 affect binding to DNA. Hum Mol Genet. 2009; 18(3):418–427. [PubMed: 18996922]

152. Kawauchi S, Calof AL, Santos R, Lopez-Burks ME, Young CM, Hoang MP, Chua A, Lao T, Lechner MS, Daniel JA, Nussenzweig A, Kitzes L, Yokomori K, Hallgrimsson B, Lander AD. Multiple organ system defects and transcriptional dysregulation in the *Nipbl*$^{+/-}$ mouse, a model of Cornelia de Lange syndrome. PLoS Genet. 2009; 5(9):1–17.

153. Vega H, Waisfisz Q, Gordillo M, Sakai N, Yanagihara I, Yamada M, van Gosliga D, Kayserili H, Xu C, Ozono K, Jabs EW, Inui K, Joenje H. Roberts syndrome is caused by mutations in *ESCO2* a human homolog of yeast *ECO1* that is essential for the establishment of sister chromatid cohesion. Nat Genet. 2005; 37(5):468–470. [PubMed: 15821733]

154. Schule B, Oviedo A, Johnston K, Pai S, Francke U. Inactivating mutations in *ESCO2* cause SC phocomelia and Roberts syndrome: no phenotype-genotype correlation. Am J Hum Genet. 2005; 77(6):1117–1128. [PubMed: 16380922]

155. Vega H, Trainer AH, Gordillo M, Crosier M, Kayserili H, Skovby F, Giovannucci Uzielli ML, Schnur RE, Manouvrier S, Blair E, Hurst JA, Forzano F, Meins M, Simola KOJ, Raas-Rothschild A, Hennekam RCM, Jabs EW. Phenotypic variability in 49 cases of *ESCO2* mutations, including

novel missense and codon deletion in the acetyltransferase domain, correlates with *ESCO2* expression and establishes the clinical criteria for Roberts syndrome. J Med Genet. 2010; 47(1): 30–37. [PubMed: 19574259]

156. Hou F, Zou H. Two human orthologues of Eco1/Ctf7 acetyltransferases are both required for proper sister-chromatid cohesion. Mol Biol Cell. 2005; 16(8):3908–3918. [PubMed: 15958495]
157. Zhang B, Jain S, Song H, Fu M, Heuckeroth RO, Erlich JM, Jay PY, Millbrandt J. Mice lacking sister chromatid cohesion protein PDS5B exhibit developmental abnormalities reminiscent of Cornelia de Lange syndrome. Development. 2007; 134:3191–3201. [PubMed: 17652350]
158. Monnich M, Banks S, Eccles M, Dickinson E, Horsfield J. Expression of cohesin and condensin genes during zebrafish development supports a non-proliferative role for cohesin. Gene Expression Patterns. 2009; 9:586–594. [PubMed: 19723591]
159. Ritchie K, Seah C, Moulin J, Isaac C, Dick F, Berube NG. Loss of ATRX leads to chromosome cohesion and congression defects. J Cell Biol. 2008; 180(2):315–324. [PubMed: 18227278]
160. Kernohan KD, Jiang Y, Tremblay DC, Bonvissuto AC, Eubanks JH, Mann MRW, Berube NG. ATRX partners with cohesin and MeCP2 and contributes to developmental silencing of imprinted genes in the brain. Dev Cell. 2010; 18(2):191–202. [PubMed: 20159591]
161. van der Lelij P, Chrzanowska KH, Godthelp BC, Rooimans MA, Oostra AB, Stumm M, Zdzienicka MZ, Joenje H, de Winter JP. Warsaw breakage syndrome, a cohesinopathy associated with mutations in the XPD helicase family member DDX11/ChlR1. Am J Hum Genet. 2010; 86:262–266. [PubMed: 20137776]
162. Hassold TJ, Jacobs PA. Trisomy in man. Annu Rev Genet. 1984; 18:69–97. [PubMed: 6241455]
163. Hunt PA, Hassold TJ. Female meiosis: coming unglued with age. Curr Biol. 2010; 20(17):R699–R702. [PubMed: 20833308]
164. Revenkova E, Hermann K, Adelfalk C, Jessberger R. Oocyte cohesin expression restricted to predictyate stages provides full fertility and prevents aneuploidy. Curr Biol. 2010; 20:1529–1533. [PubMed: 20817531]
165. Hodges CA, Revenkova E, Jessberger R, Hassold TJ, Hunt PA. SMC1b-deficient female mice provide evidence that cohesins are a missing link in age-related nondisjunction. Nat Genet. 2005; 37(12):1351–1355. [PubMed: 16258540]
166. Chiang T, Duncan FE, Schindler K, Schultz RM, Lampson MA. Evidence that weakened centromere cohesion is a leading cause of age-related aneuploidy in oocytes. Curr Biol. 2010; 20(17):1522–1528. [PubMed: 20817534]
167. Lister LM, Kouznetsova A, Hyslop LA, Kalleas D, Pace SL, Barel JC, Nathan A, Floros V, Adelfalk C, Watanabe Y, Jessberger R, Kirkwood TB, Hoog C, Herbert M. Age-related meiotic segregation errors in mammalian oocytes are preceded by depletion of cohesin and Sgo2. Curr Biol. 2010; 20(17):1511–1521. [PubMed: 20817533]
168. Chiang T, Schultz RM, Lampson MA. Meiotic origins of maternal age-related aneuploidy. Biol Reprod. 2012; 86(1):1–7. [PubMed: 21957193]

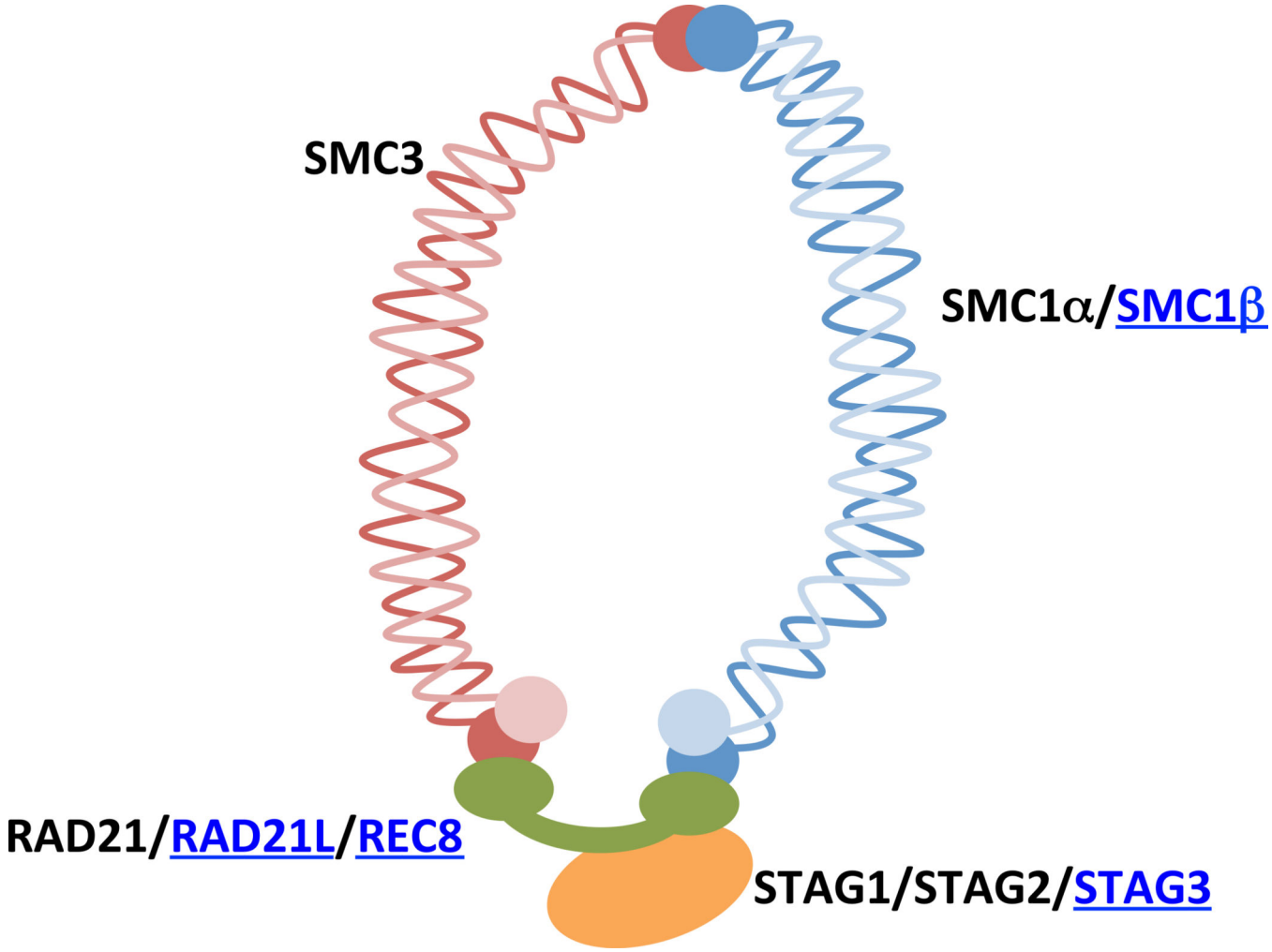

Figure 1.

Cohesin subunits form a ring-like structure. SMC1 and SMC3 form a heterodimer, interacting through their hinge regions. The SMC1 and SMC3 head domains, which contain ATPase motifs, interact with the C- and N-termini of the REC8 or RAD21 or RAD21L kleisin subunit, effectively closing the ring. The STAG1 or STAG2 or STAG3 (also called SA1/SA2/SA3) subunit interacts with RAD21 or RAD21L or REC8, contributing to maintenance of the ring structure. Mammalian subunits are shown. Meiosis-specific subunits are depicted as underlined.

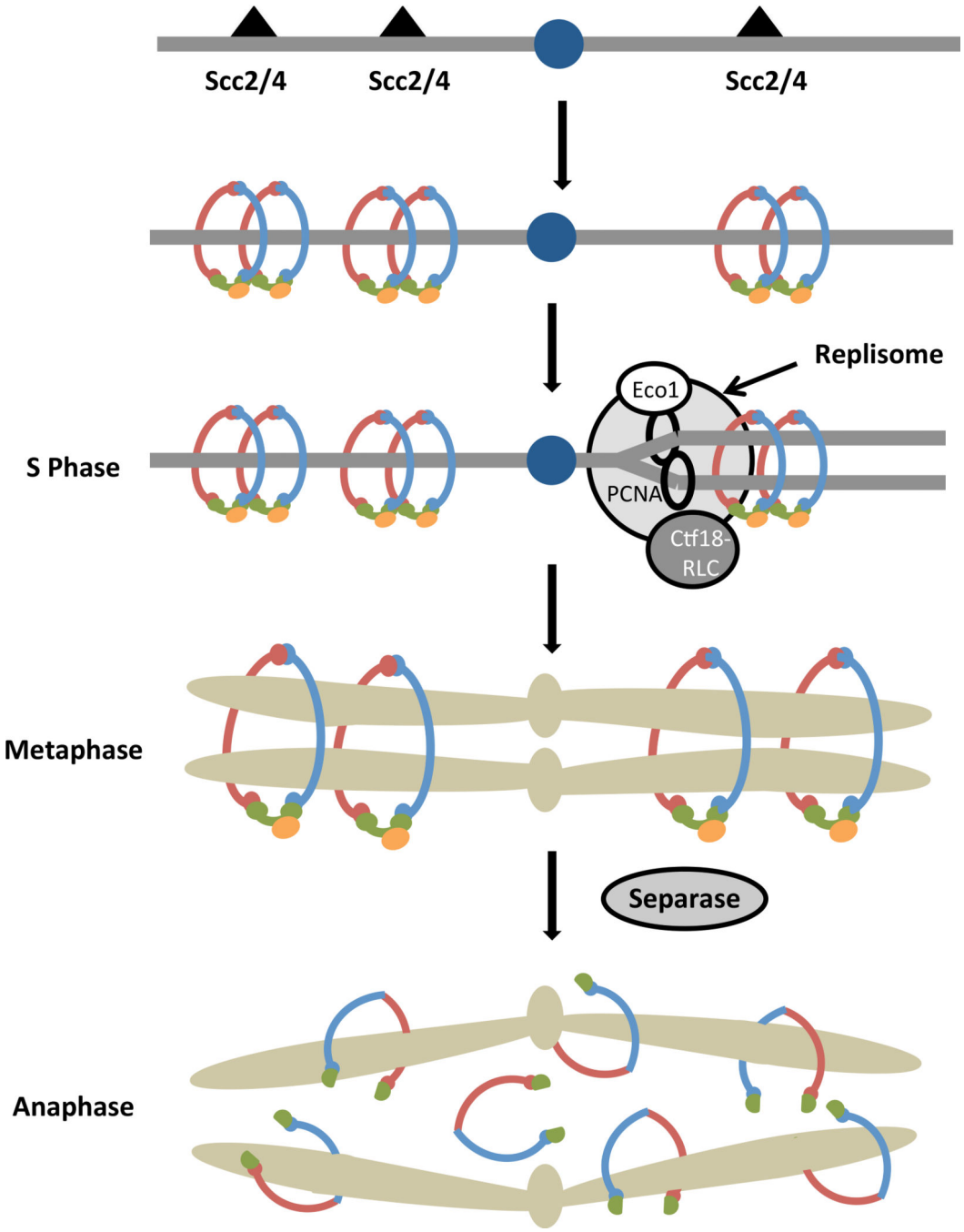

Figure 2.

Cohesion in yeast mitosis. Cohesin complexes require the Scc2/Scc4 protein complex in order to be loaded on chromosomes. Several proteins act together to establish cohesion during DNA replication. These proteins include Eco1 acetyltransferase, the CTF18-RLC complex, and the polymerase-associated protein Ctf4. Tension at centromeres is generated by the bipolar attachment of kinetochores to the mitotic spindle. Following biorientation of sister chromatids, separase is activated to cleave the Scc1 subunit resulting in removal of cohesin complexes, loss of cohesion, and separation of sister chromatids.

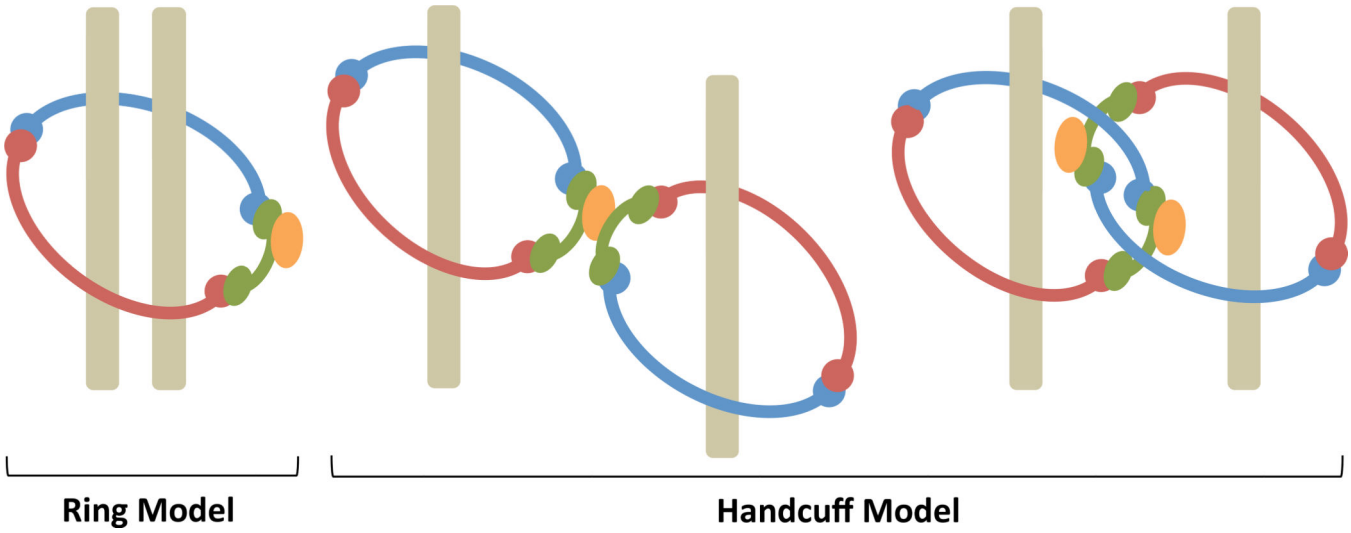

Figure 3.

Models of cohesin rings. **(A)** One ring model predicts that both sister chromatids are entrapped within a single cohesin ring. **(B)** Another type of ring model, the “handcuff” model, proposes that each of two cohesin rings entraps one sister chromatid, either by binding a single Scc3 subunit or topological interconnection between rings.

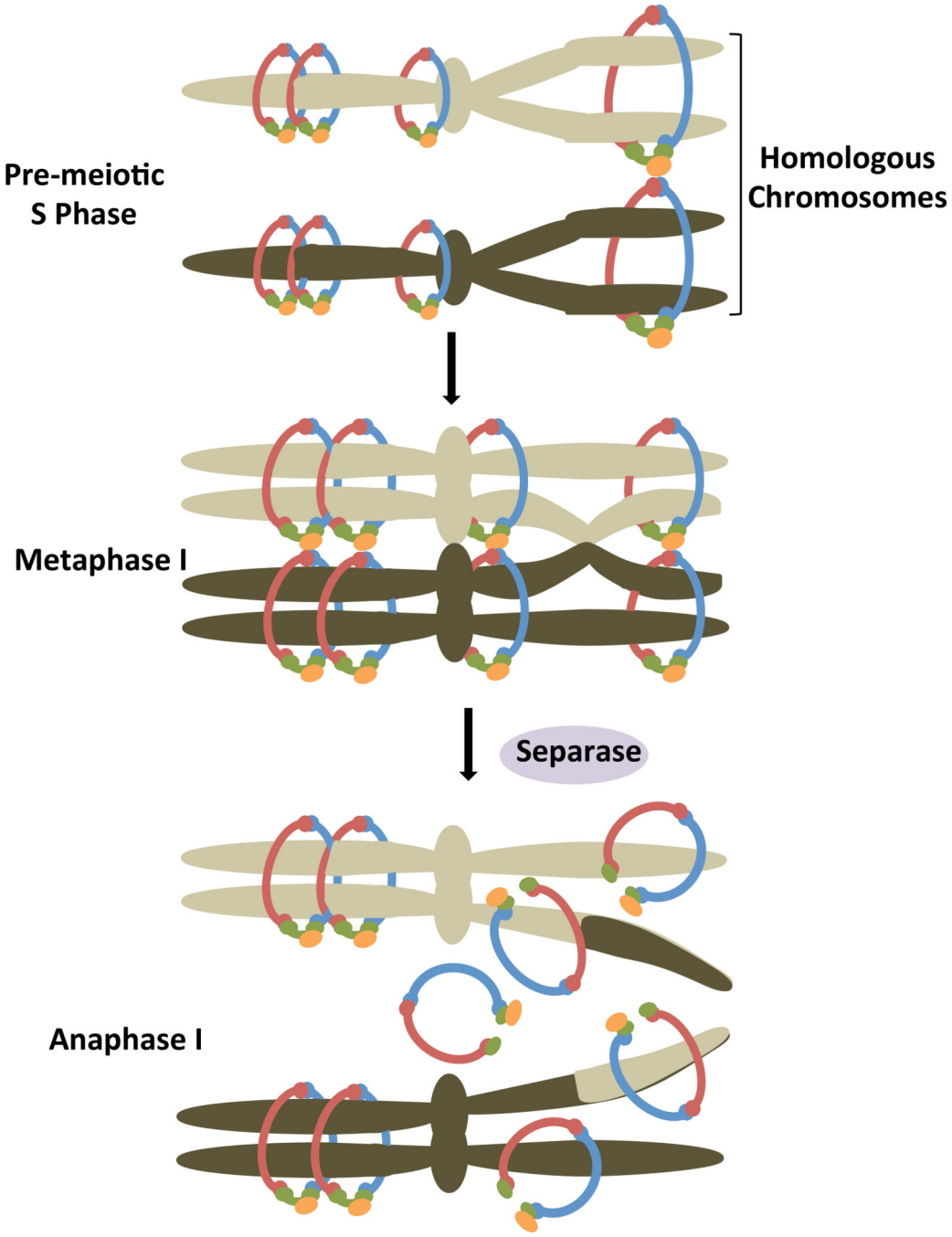

Figure 4.

Cohesion in yeast meiosis I. Rec8 replaces Scc1 of the cohesin complex in S phase. During prophase I homologous chromosomes pair and meiotic recombination leads to DNA crossovers between non-sister chromatids. In order for homologous chromosomes to segregate, kinetochores of sister chromatid pairs must each be mono-oriented to opposite poles during metaphase I. Separase cleavage of Rec8 during anaphase I, much like that during mitosis, resolves the cohesion distal to crossovers to allow segregation of

homologues. In order to allow for the proper biorientation and segregation of sister chromatids during meiosis II, cohesion proximal to centromeres is preserved.

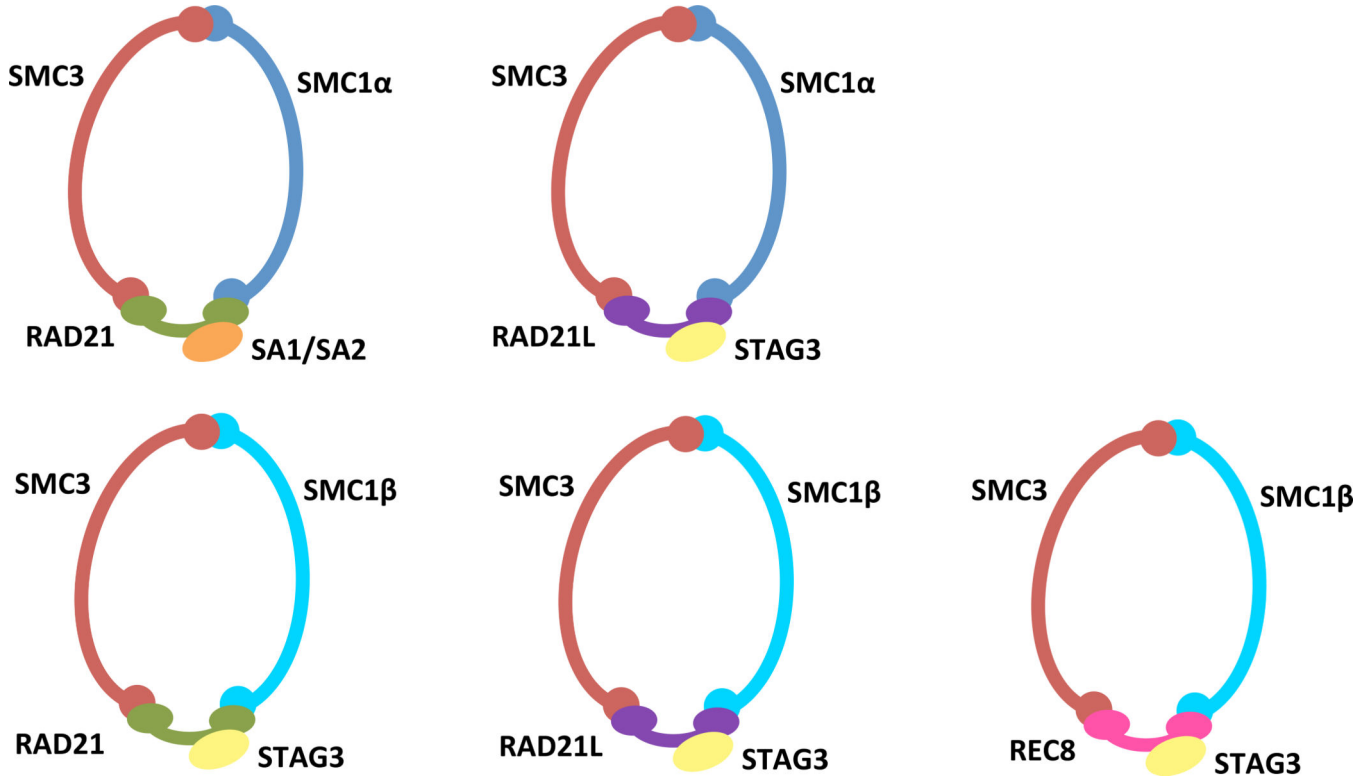

Figure 5.
Putative subunit compositions of some of the cohesin complexes in mammals. Differences in spatiotemporal distribution occur throughout the meiotic divisions.

Table 1

Mitotic, meiotic, and regulatory protein homologues

	Budding yeast	**Fission yeast**	**Mammals**	***Xenopus***	***C. elegans***	***Drosophila***
Cohesin Subunits	Smc1	Psm1	SMC1α, **SMC1β**	XSMC1	Him1	DCAP
	Smc3	Psm3	SMC3	XSMC3	Smc3	Smc3
	Scc1/Mcd1	Rad21 **Rec8**	RAD21, **RAD21L** **REC8**	XRAD21 **XREC8**	Coh2/Scc1 **Rec8**	DRAD21 **DREC8**
	Scc3/Irr1	Psc3 **Rec11**	SA1/STAG1 SA2/STAG2 **SA3/STAG3**	XSA1, XSA2	Scc3	DSA1 **DSA2/MNM**
Loading	Scc2	Mis4	NIPBL	SCC2	Pqn-85	Nipped-B
	Scc4	Ssl3	Mau2/Scc4	XSCC4	Mau2	Mau2
Establishment	Ctf7/Eco1	Eso1	ESCO1, ESCO2	XECO1, XECO2		Deco/San
Maintenance	Pds5	Pds5	PDS5A, PDS5B	PDS5A, PDS5B	Pds5/Evl14	Pds5
	Rad61	Wpl1	WAPL			
Dissolution	Pds1	Cut2	Securin	Securin		PIM
	Esp1	Cut1	Separase	Separin		SSE/THR
	Cdc5	Plo1	PLK1	PLX1		POLO
	Sgo1	Sgo2 **Sgo1**	Shugoshin/SGOL1 SGOL2	Shugoshin-like 1 (xSGO1)		MEI-S332

*
Bold denotes meiosis-specific.