Journal of Andrology, Vol. 26, No. 1, January/February 2005

From the XXVII North American Testis Workshop: The Function of SMC and Other Cohesin Proteins in Meiosis

Minireview

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To develop into haploid gametes, germ cells must undergo meiosis, a 2-step cell division process that is characterized by a sophisticated design in chromosome architecture and highly specific dynamic behavior of chromosomes. After premeiotic duplication, homologous chromosomes, each with 2 connected sister chromatids, pair and synapse, exchange genetic material, and then align on the metaphase plate to be segregated in anaphase of the first meiotic division. In the second step, the remaining pairs of sister chromatids are separated, and 4 daughter cells, haploid gametes each with 1 chromatid per chromosome, are generated. An elaborate set of proteins promotes and regulates these structures and processes. Complexes built on the structural maintenance of chromosome (SMC) protein family take center stage in these events. In recent years, important new data were gathered on these complexes and their partners, and hypotheses were developed that carry us significantly further on our road toward an understanding of the meiotic process. This review summarizes the most significant developments in this field.

Meiosis

The purpose of meiosis is to create haploid germ cells from diploid parental cells (Figure 1). Chromosomes are duplicated during premeiotic S-phase, which generates cells with 4 chromatids of each type of chromosome—2 maternal and 2 paternal—similar to the mitotic S phase. In contrast to mitosis, meiotic DNA replication is followed by 2 successive rounds of nuclear divisions called meiosis I and II, which result in haploid gametes containing 1 copy of each chromosome. At meiosis I, the reductional division, the homologous chromosomes move toward opposite poles, while the sister chromatids of each homolog remain connected, generating cells with 2 chromatids. This is followed by a second mitosislike equational division in which sister chromatids separate from each other and the haploid cells are generated. Errors in meiosis result in the production of aneuploid zygotes with devastating consequences. Aneuploidy is a key factor in ~35% of spontaneous pregnancy losses and the most commonly recognized cause of mental retardation (Hassold and Hunt, 2001; Page and Hawley, 2003).

During prophase of meiosis I, various chromatin rearrangements take place, which are, like the reductional division itself, unique to meiosis (Roeder, 1997; van Heemst and Heyting, 2000; Page and Hawley, 2003; Petronczki et al, 2003). Homologous chromosomes, each consisting of 2 sister chromatids, find each other and pair to form the ''bivalent,'' a structure containing all 4 sister chromatids. Maternal and paternal chromosomes pair and recombine to generate new combinations of alleles. Recombination occurs between 1 sister chromatid of each homolog and also serves to keep the homologs physically connected until metaphase I. The pairing between homologous chromosomes in prophase I is stabilized by tight axial associations called synapsis and is possible within the synaptonemal complex (SC), a proteinaceous ladderlike structure that consists of 2 axial elements (AEs) connected all along their length by transverse filaments (TF). In mammals, 3 proteins were identified as components of the SC: SYCP1 (named SCP1 in the rat) is a component of the TF, and SYCP2 (SCP2) and SYCP3 (SCP3) are constituents of the AE (reviewed in Moens et al, 1998). Thus, the 4 sister chromatids are specifically structured and behave uniquely during meiosis. SMC protein complexes are central in determining these features. Unless otherwise noted, this review focuses on mammalian (mouse) cohesins and their functions.

Mitotic and Meiotic Sister Chromatid Cohesion

Generally, cohesion is essential to hold sister chromatids together until they are separated at nuclear division. During the last few years, protein components of the mitotic

Work in the authors' laboratory was supported by a grant from the National Institutes of Health to R.J. (GM62517).

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Received for publication June 6, 2004; accepted for publication August 30, 2004.

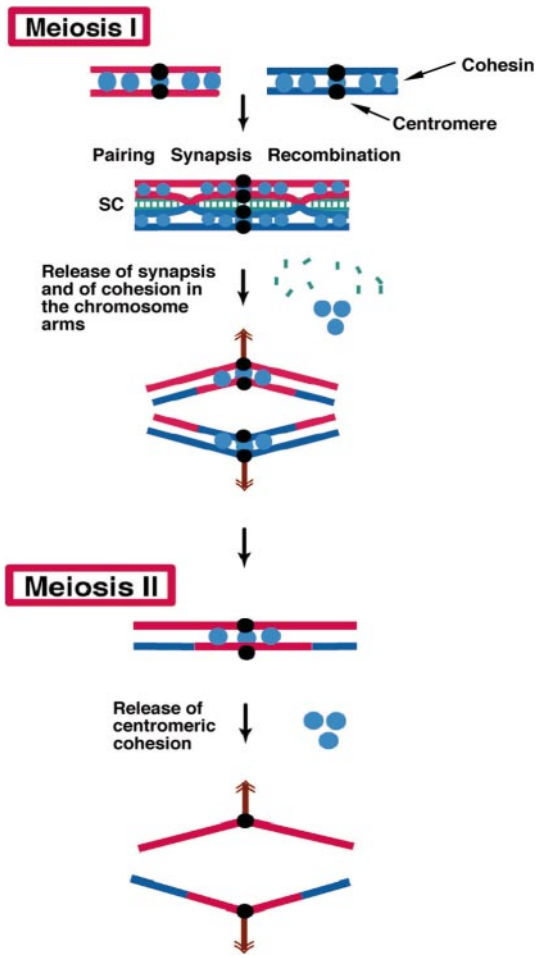

Figure 1. Chromosome dynamics in meiosis. Simplified scheme of both meiotic divisions with an emphasis on synaptonemal complex formation, recombination, and sister chromatid cohesion.

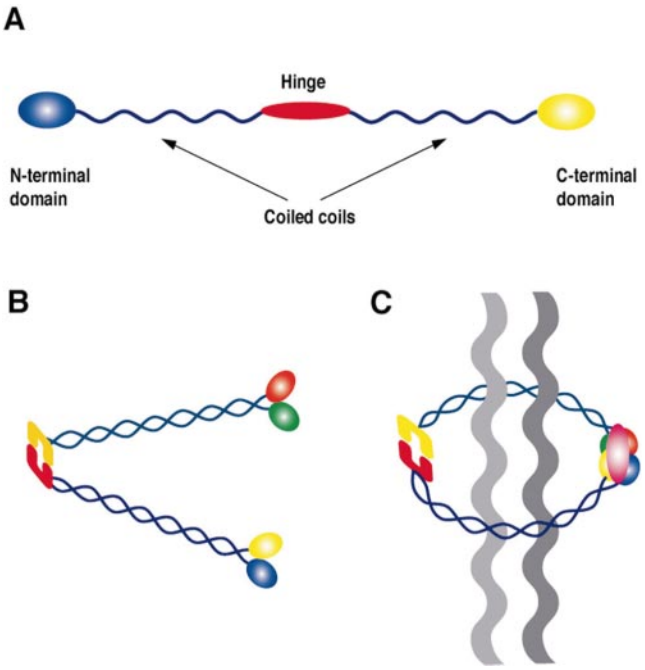

Figure 2. Structural maintenance of chromosome (SMC) proteins. **(A)** Monomeric SMC; molecular mass between 130 and 170 kd. **(B)** Heterodimer of 2 SMC proteins (eg, SMC1 and SMC3) in their most frequent V-shaped configuration. Note that dimerization occurs through interaction of hinge domains. N- and C-terminal domains of the same SMC molecules interact. **(C)** Model for a ringlike cohesin embracing the 2 sister chromatids (according to Gruber et al, 2003).

cohesin complex were identified (for recent reviews, see Jessberger, 2002, 2003; Hagström and Meyer, 2003). In mammalian somatic cells, the cohesin complex consists of at least 4 different subunits, 2 of them belong to the SMC family of proteins (Figure 2). SMC proteins are highly dynamic proteins that modulate chromosome structures. Six members of the family, usually called SMC1 to SMC6 fall into 2 subgroups. One member of a subgroup heterodimerizes with 1 SMC of the other subgroup (Figure 2B). These dimers associate with other proteins to form large complexes and act in various chromosome-related processes (Table). For example, the SMC2-SMC4 heterodimer is part of the condensin complex, which contributes to chromosome condensation. The SMC1-SMC3 heterodimer constitutes the core of the cohesin complex. Cohesin is an essential multiprotein complex required for sister chromatid cohesion (ie, the ordered arrangement of the newly replicated daughter DNA duplices in S, G_2, and early M phase of the cell cycle; Figure 2C). In the mitotic cohesin complex, the heterodimer associates with 2 non-SMC subunits: the Rad21 (Scc1) protein, a member of the kleisin protein family (Schleiffer et al, 2003), and an Scc3-type protein, of which there are several variants. In mitotic cells, these are either the SA1 or the SA2 proteins. Homologs of all these proteins were identified in many organisms, and they appear to be essential. If defective, chromosomes are not segregated properly during mitosis, aneuploidy is generated, and the cells die. There is also impaired DNA recombinational repair, which under normal circumstances after S phase usually uses the aligned sister chromatids. The meiotic cohesin significantly differs from its mitotic counterparts and is specifically adapted to accommodate the unique structural and dynamic needs of meiotic chromosomes.

Although the protein composition of the mitotic cohesin complex is well understood, it is not yet entirely clear how cohesin interacts with the 2 sister chromatids. Recent data derived from the crystal structure of bacterial SMC hinge domains (Haering et al, 2002) and supported by biochemical protein interaction studies on yeast cohesin subunits (Gruber et al, 2003) suggest that cohesin forms a large proteinaceous ring within which sister chromatids could be entrapped after DNA replication (Figure 2C). Embracing of DNA strands by the cohesin ring creates a link between sister chromatids, which might be more to-

*Mitotic and meiotic structural maintenance of chromosome (SMS) and associated proteins in vertebrates**

Mitosis			Meiosis		
Protein	Complex	Function	Protein	Complex	Function
SMC1	Cohesin	SCC, Rec Rep	SMC1α	Cohesin	SCC, ?
			SMC1β	Cohesin	SCC, Rec, Chr Struct
SMC3	Cohesin	SCC, Rec Rep	SMC3	Cohesin	SCC, Rec, Chr Struct
RAD21	Cohesin	SCC, Rec Rep	RAD21	Cohesin	SCC, ?
			REC8	Cohesin	SCC, ?
SA1 or SA2	Cohesin	SCC, ?	STAG3	Cohesin	SCC, ?
SMC2	Condensin	M Chr Struct	SMC2	Condensin	M Chr Struct
SMC4	Condensin	M Chr Struct	SMC4	Condensin	M Chr Struct
CAP-D2	Condensin	M Chr Struct	?	Condensin	M Chr Struct
CAP-G	Condensin	M Chr Struct	?	Condensin	M Chr Struct
CAP-H	Condensin	M Chr Struct	?	Condensin	M Chr Struct
CAP-D3	Condensin II	M Chr Struct	?	Condensin	M Chr Struct
CAP-H2	Condensin II	M Chr Struct	?	Condensin	M Chr Struct
CAP-G2	Condensin II	M Chr Struct	?	Condensin	M Chr Struct
SMC5		DNA repair	SMC5	?	?
SMC6		DNA repair	SMC6	?	?
Nse1		DNA repair	?		
Nse2		DNA repair	?		
Nse3		DNA repair			Meiotic segregation, Rec
RAD60		DNA repair	?		

* The same SMC protein might be involved in several different complexes with related or different functions; only the most prominent is named; several different cohesion complexes exist in meiotic cells. The same complex or protein might have different functions; only functions for which there is significant evidence are indicated. SCC indicates sister chromatid cohesion; Rec Rep, recombinational repair; Rec, recombination; M Chr Struct, metaphase chromosome structure; Chr Struct, chromosome structure; ?, unknown but suspected additional proteins, complexes, or functions.

pological than chemical (ie, through protein-DNA binding). In vivo evidence for this elegant model still needs to be substantiated.

The meiotic cohesin, however, differs significantly from its mitotic counterparts. In fact, not just 1 cohesin but several cohesin complexes are found in meiotic cells (Table). All are built around the SMC1-SMC3 heterodimer. In mammalian meiotic cells, the SMC1 protein exists in 2 variants: the canonical SMC1, called SMC1α, and a meiosis-specific variant, SMC1β (Revenkova et al, 2001). SMC1β has only been found in vertebrate cells. There is no published evidence for SMC3 variants, but the RAD21 protein coexists with a meiosis-specific paralog named REC8. REC8 is highly conserved through evolution, and orthologs from yeast, plants, and vertebrates are described (DeVeaux et al, 1992; Klein et al, 1999; Watanabe and Nurse, 1999; Cai et al, 2003; Lee et al, 2003). The SA1 and SA2 proteins coexist with their meiosis-specific variant STAG3 (Pezzi et al, 2000; Prieto et al, 2001).

Establishing Meiotic Sister Chromatid Cohesion

In somatic cells, cohesion between sister chromatids is established during S phase, and only the fully assembled cohesin complex can provide cohesion. Data obtained by studying the yeast *Schizosaccharomyces pombe* suggested that at least Rec8 associates with chromosomes during the last premeiotic round of replication, just before entry into meiosis (Watanabe and Nurse, 1999). In mice, the equivalent might be spermatogonial cells that are about to enter preleptotene. Although REC8 was not observed in spermatogonia, immunofluorescence studies showed its presence in preleptotene cells (Eijpe et al, 2003). Interestingly, SMC1β was not found at this early stage of meiosis but appeared later in leptotene cells (Figure 3). Therefore, REC8, possibly within a complex with SMC1α, might provide an initial basis for sites of meiotic cohesion, onto which the complex assembles. In preleptotene REC8 forms AE-like filamentous chromatin structures, which are, however, still quite diffuse. SMC1α exists in preleptotene cells; thus, other cohesin components presumably are present in those cells as well, including SMC3 (Figure 3). In leptotene, zygotene, and pachytene, all known cohesin components localize to the AEs, although with different characteristics. Unlike SMC1β or SMC3, the SMC1α protein, which is detectable only until the end of prophase I, does not localize as uniformly throughout the chromosomal axes; rather, it appears concentrated in certain areas, displaying a dotlike pattern (Eijpe et al, 2000). This, together with its absence in later meiosis, led to the suggestion that SMC1β rather than SMC1α is primarily responsible for sister chromatid cohesion during meiosis (Revenkova et al, 2001).

Some data suggest physical interactions between components of the SC and cohesin proteins (Eijpe et al, 2000; Lee et al 2003), but the assembly of filamentous cohesin structures on meiotic chromatin does not depend on SC

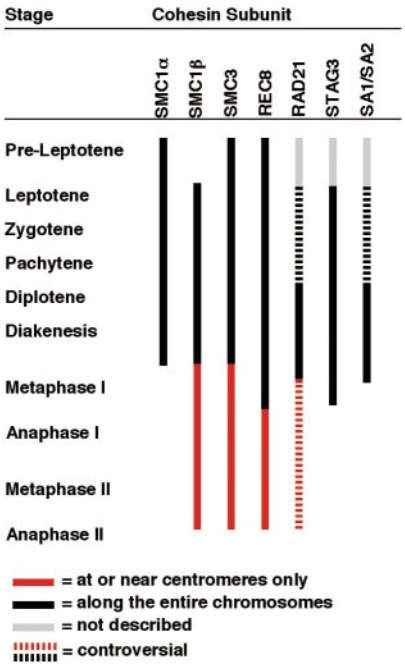

Figure 3. Presence of cohesin components at different stages throughout meiosis. Localization on chromosome arms or at centromeres (if known) is indicated, as are controversial data.

formation. Mice lacking the SYCP3 protein, a component of the AE, still assemble filamentous cohesin structures, although the SC is absent (Pelttari et al, 2001). Thus a ''cohesin core'' exists that is believed to layer itself on top of and parallel to the AE. This cohesin core might, together with SC proteins, regulate the structure of meiotic chromosomes (eg, their compaction).

In early meiosis, there are at least 2 types of cohesin complexes, probably more, but the situation is not yet clear. There is certainly an SMC1α- and at least 1 SMC1β-based complex. Both SMC1 variants dimerize with SMC3 but might associate with different non-SMC proteins. Recent coimmunoprecipitation experiments indicate the existence of 2 SMC1α-based complexes in testis, bringing the total number of cohesinlike complexes to at least 3 (Revenkova et al, 2004). One SMC1α complex seems to reflect the mitotic RAD21-type complex (Prieto et al, 2002; Eijpe et al, 2003; Revenkova et al, 2004; Xu et al, 2004), whereas the other contains the meiotic STAG3 and REC8 proteins (Revenkova et al, 2004). However, in another report no coimmunoprecipitation of SMC1α with REC8 was observed (Lee et al, 2003). Reports also conflict on the chromosomal localization and stage-specific existence of RAD21. One paper suggests that a mammalian complex made of RAD21, SMC3, SMC1β, and an undetermined fourth subunit persists at the centromeres until the metaphase to anaphase II transition (Xu et al, 2004). Another publication claims that RAD21 is displaced from the centromeres in telophase I and is not present at meiosis II centromeres (Parra et al, 2004). In fission yeast, Rec8 was found to associate either with a STAG3 ortholog (Rec11) or with an ortholog of the mitotic SA1 or SA2 cohesin subunits (Psc3; Kitajima et al, 2003). Thus it appears as if many of the potential combinations of the various cohesin subunits—meiosis-specific or ubiquitous—are realized in germ cells. The exact composition of each complex and their chromosomal localization; stage-specific occurrence; and relative contribution to sister chromatid cohesion, AE formation, and meiotic recombination are a matter of current debate and will be further discussed below.

It is important to distinguish between sister chromatid cohesion in chromosome arms and centromeric cohesion. Although arm cohesion along with chiasmata is dissolved in metaphase I, centromeric cohesion is maintained until metaphase II to keep the sister chromatids connected for their shared journey toward a pole in anaphase I. Only in anaphase II are sister chromatids separated to allow their migration to opposite poles and formation of the haploid cells.

REC8 might not only provide an early basis for cohesin assembly but seems also to be involved in ensuring cohesion throughout meiosis. In mammalian cells, SMC1α, SMC1β, and SMC3 are initially found all along the prophase chromosomes, possibly contributing to ubiquitous cohesion along the chromosomal axis. Evidence gathered from analysis of a mouse deficient in SMC1β points to an essential role of SMC1β in sister chromatid cohesion starting in pachytene (Revenkova et al, 2004). Without SMC1β, the mutant pachytene spermatocytes enter into apoptosis but can be driven prematurely into metaphase by okadaic acid treatment. Here they visibly lack all sister chromatid cohesion, whereas mutant zygotene cells treated with okadaic acid maintained cohesion. SMC1β and SMC3 dissociate from the chromosome arms in late prophase I, whereas REC8 persists along the chromosome arms until metaphase I (Eijpe et al, 2003; Lee et al, 2003). At least in *S pombe*, the 2 different Scc3-like subunits with which Rec8 associates mark differently located Rec8 complexes: the Rec11-Rec8 complex localizes to chromosome arms, and the Psc3-Rec8 complex to the centromeres (Kitajima et al, 2003).

Perhaps the most prominent role for the SMC1β complex is in centromeric cohesion because the protein, along with REC8 and SMC3, remains associated with the centromeres until anaphase II (Revenkova et al, 2001). In SMC1β-deficient mice, spermatocytes die in pachytene of apoptosis. Oocytes develop further but terminate in metaphase II, in which the total absence of sister chromatid cohesion leads to complete separation of individual chromatids. Expectedly, neither male nor female mutant mice are fertile (Revenkova et al, 2004).

If the SMC1β complex becomes critical for cohesion

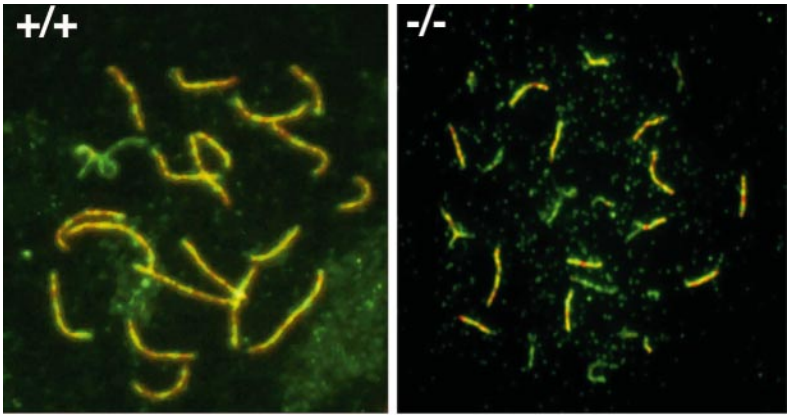

Figure 4. Absence of the meiosis-specific variant of a structural maintenance of chromosome protein, SMC1β, reduces the length of prophase I chromosomes to about 50%. Spermatocyte pachytene chromosome spreads of wild-type (+/+) or SMC1β-deficient (−/−) mice were stained by immunofluorescence for the synaptonemal complex component SCP1 (red) and the cohesin SMC3 (green).

in spermatocytes around pachytene, then other cohesin complexes should support cohesion early in meiosis. The SMC1α-based complexes likely provide this function. SMC1α is detected early, and along with STAG3, disappears gradually in late prophase to metaphase I (Prieto et al, 2001) when arm cohesion is dissolved. RAD21 also dissociates from chromosome arms but, according to one report, stays at the centromeres until the metaphase II to anaphase II transition, similar to REC8, SMC3, and SMC1β (Xu et al, 2004). This poses the question of whether 2 SMC1β-SMC3 complexes are at the centromeres—1 with REC8 and 1 with RAD21 as a kleisin subunit—or whether some other cohesinlike entity is still present. Because removal of Rec8 causes premature sister chromatid dissociation in yeast (Klein et al, 1999), Rad21p alone seems not to be sufficient to maintain centromeric cohesion. However, recent data on different localization of REC8 and RAD21 in the centromeric region suggest that these proteins perform different functions (Xu et al, 2004). Because REC8 localizes to sites that flank the kinetochores, it could allow monopolar attachment of kinetochores to the spindle microtubules for segregation of homologs in meiosis I, similar to the proposal for fission yeast Rec8 (Yokobayashi et al, 2003). According to Xu et al (2004) RAD21 localizes to the centromeres in meiosis II and thus could be involved in bipolar attachment of the kinetochores to the tubules for segregation of sister chromatids. In contrast, Parra et al (2004) suggest the presence of RAD21 only at meiosis I centromeres and its contribution to monopolar rather than bipolar spindle attachment. In an earlier paper, Prieto et al (2002) proposed that RAD21, within an SMC1α-SMC3-SA2 complex only localizes to chromosomes late in prophase I and disappears in metaphase I when chromosomes condense. Accordingly, in early prophase, RAD21 and SA2 were seen only loosely attached to chromosomes or dispersed in the nuclei (Eijpe et al, 2000). This contrasts with the paper by Xu et al (2004), in which the presence of RAD21 was reported along the chromosome arms in parallel with SCP3 from leptotene.

Another dissenting view on the involvement of cohesins in centromeric cohesion until meiosis II is presented by Parra et al (2004). Because these authors do not observe SMC1, SMC3, REC8, STAG3, and RAD21 at the centromeres of meiosis II, they suggest that cohesins might not be required at all for centromeric cohesion in meiosis II. This is in contrast to studies in *Saccharomyces cerevisiae* and *S pombe* (Klein et al, 1999; Watanabe and Nurse, 1999) and to reports on the presence of SMC1β, SMC3, REC8, and RAD21 at meiosis II centromeres in mammalian meiocytes (Eijpe et al, 2000, 2003; Revenkova et al, 2001; Lee et al, 2003; Xu et al, 2004). It is also difficult to reconcile with the loss of all cohesion, including centromeric cohesion in SMC1β-deficient meiocytes (Revenkova et al, 2004). These differences likely are based on different techniques used to analyze chromosomes: squashing (Parra et al, 2004) vs spreading (the other groups).

As confusing as these different reports might be, they agree on a meiotic role for RAD21 and REC8 and on the formation of a number of different cohesinlike complexes in mammalian meiocytes. The need to clarify this rather complex and dynamic, and still quite diffuse, picture of the function of individual cohesins is obvious.

Recently, the SMC1β cohesin complex was also shown to be essential for a number of other events in meiosis (Revenkova et al, 2004). Spermatocytes or oocytes deficient in this protein fail to form AEs and SCs of proper length: in a unique phenotype, their chromosomes are about 50% shorter than those of wild-type meiocytes (Figure 4). Thus, it appears as if this cohesin codetermines the length compaction of meiotic chromosomes. It might do so by supporting the packaging of chromatin into the AEs (eg, at the base of chromatin loops that em-

anate from the AEs). If so, chromatin loops in SMC1β-deficient mice should be enlarged. Measurements by fluorescent in situ hybridization (FISH) of chromatin surrounding the AEs support this hypothesis: the chromatin clouds in the mutant are about 2-fold more extended from the AEs than those of wild-type spermatocytes. Assuming that the intrinsic compaction of DNA in the loops is the same in both wild-type and mutant spermatocytes, a role for SMC1β in determining the ratio of chromatin in loops vs chromatin in the filamentous core in early meiosis is indicated. The shortened chromosomes fail in several other respects. Their telomeres do not properly attach to the nuclear periphery as they do in wild-type spermatocytes. Bouquet formation (ie, bundling of the telomeres at one spot on the nuclear periphery characteristic for early prophase I) is impaired. Furthermore, synapsis of homologs is incomplete in the mutant. Both phenotypes could be a consequence of aberrant chromosome structure. For example, the shortened chromosomes might fail to reach the nuclear periphery because of steric problems, and those who do succeed might have similar steric problems in finding the homolog partner for synapsis. Finally, sister chromatid cohesion gets lost in late prophase at both the chromosome arms and the centromeres. Together, it became clear that the meiosis-specific SMC1β protein plays essential roles in meiotic chromosome dynamics and structure (Revenkova et al, 2004).

Why has the vertebrate meiocyte acquired an additional SMC1 protein when other organisms perform meiosis fine with just SMC1α? In human, mouse, rat, and presumably other vertebrates, the SMC1α gene is located on the X chromosome and might thus be silenced during early meiosis. SMC1β, whose gene resides on chromosome 15 in mouse (on chromosome 22 in human), could represent a transposed variant generated during evolution to ensure availability of an SMC1 protein all throughout meiosis. SMC1β might later then have acquired additional functions even early in meiosis, as illustrated by the roles described here.

Maintenance and Dissolution of Meiotic Sister Chromatid Cohesion

Maintenance of sister chromatid cohesion at the centromeres and destruction of cohesion along the arms is achieved through differential removal of cohesins from the arms and centromeres. The question arises as to the mechanism that triggers this stepwise dissolution of cohesion. During mitosis in yeast, sister chromatids are separated after cleavage of Rad21p by separase, a cysteine protease that belongs to the same protease family as caspases (reviewed in Uhlmann, 2001; Haering and Nasmyth, 2003). Separase becomes active after dissociation from its inhibitor called securin, which is degraded at the metaphase to anaphase transition. In vertebrates, however, the majority of cohesin is removed by a separase-independent mechanism as chromosome condensation starts in prophase. Only a small fraction remains associated with the centromeric region and dissociates at the metaphase to anaphase transition. Dissociation of cohesin during the first stage in prophase requires a Polo-like/Cd5 kinase, which phosphorylates RAD21 (Waizenegger et al, 2000; Sumara et al, 2002).

In meiotic yeast cells, Rec8p disappears from chromosome arms at the onset of anaphase I, but centromeric Rec8p persists until the metaphase II to anaphase II transition (Watanabe and Nurse, 1999), as observed in mouse spermatocytes (Eijpe et al, 2003). Dissociation of yeast Rec8p from chromosome arms is a result of cleavage by separase (Buonomo et al, 2000). Cleavage of Rec8 along chromosome arms and at the centromeres by separase is also required in fission yeast for segregation of homologs and of sister chromatids in meiosis I and II, respectively (Kitajima et al, 2003). Similarly, in *Caenorhabditis elegans* mutants, the chromosomes fail to separate during meiosis I because of inactivation of separase (Siomos et al, 2001) or of APC/C needed for separase activation (Golden et al, 2000). In contrast, it seems that segregation of homologs and of sister chromatids are differentially regulated in higher eukaryotes. Both the separase and the APC/C are dispensable for progression from meiosis I to II in *Xenopus laevis* oocytes (Peter et al, 2001; Taieb et al, 2001). However, in yeast and nematodes, APC/C activity is required for the metaphase I to anaphase I transition. The authors suggest that either differences based on the enormous size of the oocyte or divergent evolution might have caused the different experimental outcomes. Mechanisms that rule cohesion dissociation in mammalian meiosis resemble those shown for yeast and *C elegans.* In mouse oocytes, the meiosis I to meiosis II transition also depends on APC/C activity. Inactivation of separase results in perturbed chromosome segregation in meiosis I (Terret et al, 2003).

If separase activity removes REC8 from chromosomal arms at the metaphase I to anaphase I transition, how are REC8 and cohesin protected at the centromere, where it must stay until anaphase II? On the basis of studies in *C elegans,* it has been proposed that aurora-B kinase AIR-2 regulates the selective release of chromosome cohesion during both meiotic divisions, probably by phosphorylation of REC-8 (Rogers et al, 2002). In mouse spermatocytes, aurora-B relocalizes along the chromosome during meiosis, being present at the right place and time for regulating sister chromatid cohesion during both meiotic divisions (Parra et al, 2003). Therefore, it is possible that differential phosphorylation of cohesin complexes on arms and centromeres could result in 2-step cohesion dissolution in the first and second meiotic divisions. Proteins localizing to centromeres could also act as factors pro-

tecting centromeric cohesion. The meiosis-specific Spo13p in budding yeast (Shonn et al, 2002) and the Mei-S322 protein of *Drosophila melanogaster* (Moore et al, 1998) have been suggested as potential candidates. The recent identification of the meiosis-specific protein Sgo1 and its characterization as a protector of the centromeric Rec8 cohesin in fission and budding yeasts brought more insight into the molecular mechanism responsible for protecting centromeric cohesion during meiosis I (Kitajima et al, 2004; Katis et al, 2004). The amount of centromeric Rec8 is reduced and meiotic cohesion fails to persist at centromeres after meiosis I in mutant Sgo1 cells. It was suggested that Sgo1 acts as a shield for Rec8, protecting it physically from separase action.

Cohesins in Meiotic Recombination and Segregation of Homologs

Reciprocal exchange of DNA between the maternal and paternal chromosomes is essential for creating diversity and is a major driving force of evolution. The formation of crossovers gives rise to a protein-DNA structure called chiasmata, which also functions to physically connect the homologs in prophase I so that they can be properly aligned in metaphase for subsequent segregation. Without chiasmata, no orderly chromosome segregation is possible. Evidence is accumulating for an important role of cohesins in meiotic DNA recombination. An early indication for such a role originated from studies in *S cerevisiae* (Klein et al, 1999) since *Smc3* or *Rec8* mutants are recombination-deficient. In *C elegans*, silencing of REC-8 expression by RNA interference (RNAi) not only caused defects in chromosome synapsis at pachytene, but also accumulation of unrepaired DNA double-strand breaks (Pasierbeck et al, 2001), pointing to a DNA repair function of REC-8. In vertebrates, the localization of SMC1β and SMC3 to bridges between chromosomes, which are visible in pachytene and diplotene and are thought to represent sites of chiasmata, suggested that these cohesins have some role in meiotic recombination in higher organisms as well (Revenkova et al, 2001; Eijpe et al, 2003). Notably, the cohesin REC8 appears to be absent from these chromosomal bridges, posing the question of whether there exists a specialized SMC complex that localizes to and possibly acts on chiasmata. Very recently, data obtained through analysis of the SMC1β-deficient mouse support the idea of a role of cohesins in meiotic recombination (Revenkova et al, 2004). Sites of chiasmata can be visualized by labeling the mismatch repair proteins MLH1 and MLH3 in pachytene meiocytes. The absence of SMC1β eliminates MLH1 or MLH3 signals in spermatocytes and significantly reduces their number in oocytes. Interestingly, proteins that appear at early steps during meiotic recombination (ie, at initiation when double-strand breaks are formed) appear unaffected by the SMC1β deficiency. Reduced numbers of chiasmata can be observed also in metaphase I oocytes, in which some homologs fall apart instead of being kept together via their chiasmata. Thus, the SMC1β complex functions in DNA recombination after its initiation to allow stable formation of chiasmata. How could this cohesin complex possibly support chiasmata? It might, for example, be directly involved in the assembly of proteins required for processing of the early intermediates in the DNA recombination process, such as holiday junction processing enzymes. Or cohesin might compose (part of) the ''roadblocks'' that prevent chiasmata from migrating and falling off the ends of chromosomes.

Because there is increasing evidence for function(s) of cohesins in DNA repair, in particular the repair of double-strand breaks through homologous recombination between sister chromatids (Jessberger, 2002), meiotic SMC proteins could also be involved in DNA repair in meiocytes.

This may not only be true for the SMC1-SMC3 heterodimer in the context of cohesin or a related complex, but also for the third SMC heterodimer, the SMC5-SMC6. That dimer, which is known to act in DNA repair in mitotic cells and associates with other repair proteins, probably has additional chromosomal housekeeping functions and is highly expressed in the testis (Taylor et al, 2001; McDonald et al, 2003). However, a recent paper (Pebernard et al, 2004) suggests that in fission yeast an SMC5-SMC6 complex with associated proteins Nse1, Nse2, and Nse3 is important for meiotic chromosome segregation and recombination.

Is Chromosome Condensation in Meiosis Mediated by SMC Proteins?

Chromosome condensation during cell division leads from diffuse interphase chromatin to a rod-shaped metaphase chromosome (for review, see Swedlow and Hirano, 2003) and is required for separation of sister chromatids in mitosis, or of homologs and later of sister chromatids in meiotic anaphase I and II, respectively. The axial and lateral compaction of chromosomes is coordinated with decatenation of chromosomes and sister chromatids. Not much is known about proteins and processes that contribute specifically to meiotic chromosome condensation, but it is instructive to very briefly summarize the most important features of mitotic chromosome condensation.

In 1997, a 5-subunit protein complex called condensin was isolated and subsequently characterized as a major protein component of mitotic chromosomes (Hirano et al, 1997). Besides 3 non-SMC proteins, the evolutionarily highly conserved condensin harbors 2 SMC proteins—that is, a heterodimer of SMC2 (CAP-C) and SMC4 (CAP-E; Table).

Deficiencies in individual condensin subunits, all of

which are essential in *S cerevisiae*, lead to severe defects in chromosome segregation. Examination of yeast or *D melanogaster* mutants revealed failures of chromosome condensation, including a more diffuse appearance from prophase to anaphase, and the persistence of chromosome bridges in anaphase and telophase, resulting from impaired sister chromatid resolution (Strunnikov et al, 1995; Freeman et al, 2000; Steffensen et al, 2001). Interestingly, chromosome compaction is delayed but still maintained in the fly mutant. Similarly, RNAi depletion of SMC4 or SMC2 (called MIX-1 in *C elegans*) suggests that condensin is not crucial for chromosome compaction but rather indispensable for separation of the sister chromatids (Hagström et al, 2002). Recently, a conditional ''knockout'' of the SMC2 (ScII) subunit of condensin was created in a chicken preB cell line (Hudson et al, 2003). Again, chromosome condensation was delayed but not eliminated, sister chromatids failed to segregate, and the distribution of topoisomerase II was altered. Similar observations were reported also for *D melanogaster* cells deficient in SMC4 (Coelho et al, 2003). Because topoisomerase II is important for resolving entanglements between sister chromatids, its mislocalization or failure to be recruited could be a prime reason for the defect in chromosome separation. Vertebrates contain a second condensin complex, built on the same SMC2 and SMC4 proteins, that associates however with an entirely different set of other proteins (Ono et al, 2003). Both condensin complexes contribute to the formation of the proper shape of chromosomes (Ono et al, 2003). However, because they share the SMC2 subunit, both complexes are affected by the SMC2 deletion in the chicken cell line. It emerges from these recent studies that condensin, although important for promoting a chromosome structure supportive of chromosome segregation, is not essential for chromosome condensation in vertebrate cells.

How exactly condensin establishes such structures, how it promotes formation of mitotic chromosomes is not yet understood. In vitro, condensin changes the topology of certain DNA substrates (Kimura and Hirano, 1997; Kimura et al, 1999). This activity might contribute to the function of condensin in shaping the mitotic—and likely meiotic—chromosome structure.

The involvement of condensin in meiosis is not well understood. Meiotic functions of condensin in *S cerevisiae* were recently elucidated by the work of Yu and Koshland (2003), who characterized meiotic aberrations in conditional condensin mutants. In the absence of functional condensin subunits in meiosis I, the homologs paired and chromosomes condensed, but both pairing and condensation were significantly less efficient than in the wild type. Assembly of the SC was highly inefficient, demonstrating a role of condensin in recruitment of meiosis-specific chromosome proteins. In anaphase I and II, the mutants exhibited a high incidence of bridges between chromosomes. Importantly, this phenotype was dependent on recombination because in double mutants, defective also in initiation of recombination, this chromosome bridging was reduced to approximately the wild-type level. Thus the physical linkages between chromosomes, which stay unresolved in condensin mutants, appear to be generated by incomplete or erroneous processing of recombination intermediates.

The above-mentioned RNAi depletion of SMC2 (MIX-1) and SMC4 in the germline of *C elegans* embryos (Hagström et al, 2002) allowed observation of the consequences of condensin deficiency in meiotic cells. Meiosis I seems to proceed normally, but at anaphase of meiosis II, the chromosomes failed to segregate, leaving chromatin bridges between the second polar body and the maternal pronucleus. Probably, as in the other systems already mentioned, condensin is required for sister chromatid resolution, but it remains unclear why condensin deficiency has no striking effect on the first meiotic division. One explanation invokes the existence of a second condensinlike complex in meiosis I.

Little more is known about SMC2 or SMC4 in meiosis or related processes. In the plant *A thaliana*, 2 highly homologous SMC2 genes exist. Mutations in both genes cause impaired male and female gametogenesis and embryonic lethality (Siddiqui et al, 2003). Expression of the non-SMC condensin subunit XCAP-D2 in *X laevis* oocytes was analyzed, and the protein was shown to massively accumulate during oocyte maturation (Watrin et al, 2003). Inhibition of expression by injection of antisense oligonucleotides did not affect overall compactness of the chromosomes in the subsequent metaphase, but caused aberrant, ovoid-shaped chromosomes and prevented spontaneous separation of sister chromatid arms, which occurs in untreated oocytes.

Conclusions

Over the last few years, evidence has been generated that clearly demonstrates important functions of cohesin and related SMC protein complexes in meiosis. Specific SMC complexes are essential for 1) chromosome segregation in both meiotic divisions, 2) establishing the proper chromosome structure with full-length AEs and SCs in prophase I, 3) sister chromatid cohesion in meiosis I and II, 4) chromosome movements such as the leptotene/zygotene telomere attachment to the nuclear periphery and bouquet formation, and 5) progression of DNA recombination (ie, reciprocal exchange).

Most of these processes are essential for proper chromosome segregation. If defective, nondisjunction of chromosomes could result, and it is thus not far-fetched to propose that malfunctioning of an SMC complex in meiotic cells constitutes one of the causes for the frequent

human meiotic missegregation syndromes, such as the well-known trisomies (Hassold and Hunt, 2001).

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