SYCP2 and SYCP3 are required for cohesin core integrity at diplotene but not for centromere cohesion at the first meiotic division

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Summary

Much of the organization of the meiotic prophase-I chromosome axis is attributed to two groups of proteins: the axial element proteins, SYCP2 and SYCP3; and the cohesin-complex proteins. Although the cohesin-complex proteins ensure that sister chromatids remain paired during meiosis, the role of SYCP2 and SYCP3 is not clear. Interestingly, it has been shown that SYCP3 and SYCP2 associate with the centromere regions of male, but not female, metaphase-I chromosomes, suggesting a sex-specific function for the two proteins. We have analysed the spatial distribution of cohesin-complex proteins associated with meiotic chromosomes in germ cells derived from Sycp3-deficient female and male mice. We show that, in the absence of SYCP3, the cohesin cores associated with the female meiotic chromosomes disassemble prematurely at the diplotene stage of meiosis. We also show that SYCP3 and SYCP2 are not required for centromere cohesion at the metaphase-I stage in male germ cells. We conclude that SYCP3 has a temporally restricted role in maintaining, but not establishing, cohesin-core organization during prophase I. This finding supports a model in which the removal of bulk cohesin from paired sister chromatids at late prophase in both meiotic and mitotic cells ensures proper chromosome compaction and segregation.

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Introduction

The generation of haploid germ cells requires a distinct chromosomal segregation process termed meiosis (for a review, see Petronczki et al., 2003). Meiosis involves one round of DNA replication followed by one round of chromosome segregation and one round of chromatid separation. During the first division of the meiotic cell cycle, the homologous chromosomes (each consisting of two sister chromatids) synapse, recombine and then separate, whereas the paired sister centromeres do not segregate from each other until the second meiotic division. The paired sister chromatids are organized into parallel linear arrays of chromatin loops, the bases of which define a protein-rich chromosomal axis (Kleckner et al., 2004; Moens and Spyropoulos, 1995). The most prominent structural feature of the synapsed homologous chromosomes is their trilaminar organization, which is generated through the formation of the synaptonemal complex (SC), a meiosis-specific proteinaceous structure (for reviews, see Page and Hawley, 2004; Zickler and Kleckner, 1999). The SC is composed of two axial elements (AEs) and many transverse filaments. The AEs are found at the interchromatid domains of the paired sister chromatids, whereas the transverse filaments are found between the two homologous chromosomes and connect the AEs along their entire length. The AEs were initially defined at an ultrastructural level as proteinaceous structures that stained with silver or uranyl acetate. Several proteins in different organisms have subsequently been localized to the AE using immunocytological methods (Page and Hawley, 2004), including Red1p in Saccharomyces cerevisiae (Smith and Roeder, 1997) and HIM-3 in Caenorhabditis elegans (Couteau et al., 2004), as well as SYCP2 and SYCP3 in mice (Dobson et al., 1994; Lammers et al., 1994; Schalk et al., 1998). Red1p recruits other proteins to the AE and is essential for AE formation, as well as for synapsis, whereas HIM-3 has been suggested to take part in homologue alignment, synapsis and recombination. One additional group of proteins, the cohesin-complex proteins, have also been localized to the AE (Eijpe et al., 2003; Klein et al., 1999). Cohesin-complex proteins are of critical importance for sister-chromatid pairing and separation during mitosis and meiosis (for a review, see Petronczki et al., 2003) and are likely to be key organizers of the chromatin loop arrays along the meiotic chromosome axes (Revenkova et al., 2004; Zickler and Kleckner, 1999). In mammalian meiotic cells, three meiosis-specific cohesin subunits – REC8 (Eijpe et al., 2003), STAG3 (Prieto et al., 2001) and SMC1β (Revenkova et al., 2001) – have been identified in addition to the cohesin subunits that are also produced in mitotic cells, which include SMC1α, SMC3 and RAD21 (Eijpe et al., 2000; Xu et al., 2004).

What is the role of the mammalian AE proteins SYCP2 and
SYCP3? Several experimental results suggest that these proteins are structural elements of the AE (for review, see Page and Hawley, 2004). Ectopic production of SYCP3 in mammalian somatic cell cultures gives rise to extended AE-like fibres (Yuan et al., 1998). Furthermore, ultrastructural light-microscopy studies of Sycp3-deficient male and female germ cells have revealed a complete loss of the nuclear silver-stained filaments normally associated with the SC (Yuan et al., 2002; Yuan et al., 2000). SYCP3 has been shown in both male and female germ cells to be required for the recruitment of SYCP2 to the AE (Pelttari et al., 2001; Yuan et al., 2002). However, SYCP2 remains associated with the telomeres of male meiotic chromosomes whereas SYCP3 is depleted (Liebe et al., 2004). Similarly, SYCP2 foci are found at the distal ends of pachytene chromosomes in Sycp3-deficient female germ cells, and approximately half the number of the foci are associated with the centromeres (Yuan, 2002). This suggests that SYCP2 also remains localized to the telomeres in Sycp3-deficient female germ cells. Despite the accumulated evidence for a role at the MI stage, SYCP3 and SYCP2 from the axis of meiotic chromosomes, electron-microscopy studies have identified a residual axial core that organizes the transverse filament and the central element structures of the SC (Liebe et al., 2004). The cohesin complex proteins SMC1, SMC3 and STAG3 have been localized to this residual axial core in male meiotic germ cells using immunocytochemical methods (Kolas et al., 2004; Pelttari et al., 2001). Not only is SYCP3 required for chromosome compaction at zygotene and pachytene (Kolas et al., 2004; Yuan et al., 2002) but not for sister-chromatid cohesion, homologue alignment or synapsis (Liebe et al., 2004; Yuan et al., 2002).

The absence of SYCP3 leads to an arrest of spermatocyte development at a zygotene/pachytene-like stage of meiotic prophase I, resulting in male infertility (Yuan et al., 2000). Remarkably, loss of SYCP3 only subtly affects female germ-cell development and fertility, demonstrating a sexually dimorphic response to the absence of this protein (Yuan et al., 2000). Interestingly, SYCP3 and SYCP2 are produced differently during meiosis in males and females. The production of the two AE proteins is not detectable beyond the dictyate stage of female germ-cell development (Hodges et al., 2001), whereas both proteins label the centromere regions of metaphase I (MI) chromosomes in male germ cells (Moens and Spyropoulos, 1995; Parra et al., 2004). Based on the localization of SYCP3 and SYCP2 to male MI chromosomes, it has been suggested that the two proteins provide centromere and kinetochore cohesion (Moens and Spyropoulos, 1995; Parra et al., 2004).

Here, we have used Sycp3-deficient female mice to investigate additional structural roles for SYCP3 in supporting the meiotic chromosome axis, as defined by cohesin protein localization. We have focused on the late stages of meiosis to examine further the proposed functional differences for SYCP3 and SYCP2 in male and female germ cells. We show that the axial cohesin cores prematurely disassemble in the absence of SYCP3 at the diplotene stage of female meiosis. This result supports a model in which SYCP3 has a structural role in maintaining, but not establishing, cohesin core organization. Studies of Sycp3-deficient spermatocytes show that SYCP3 is required for recruitment of SYCP2 to the centromere regions, but we find no evidence that SYCP3 or SYCP2 are required for cohesin protein distribution or centromere cohesion at the MI stage. Thus, we find no evidence for a sexually dimorphic role for SYCP3 or SYCP2 at the MI stage.

Materials and Methods

Generation of antibodies

Antibodies against SYCP1, SYCP2, STAG3, REC8 and SMC1β were raised in guinea pigs using short peptides coupled to keyhole limpet haemocyanin (KLH). Anti-SYCP1 antibodies were raised against amino acids 1-15, 878-893 and the last 15 amino acids of mouse SYCP1 (Q62209 SwissProt). Anti-SYCP2 antibodies were raised against amino acids 455-475, 620-665 and 1337-1360 of mouse SYCP2 (Q9CUU3 SwissProt). Anti-STAG3 antibodies were raised against amino acids 1-14, 911-927 and the last 13 amino acids of mouse STAG3 (O70576 SwissProt). An anti-REC8 antibody was raised against amino acids 474-500 of mouse REC8 (NP_064386 GenBank) and an anti-SMC1β antibody was raised against amino acids 121-146 of mouse SMC1β (NP_536718 GenBank). The individual antisera were affinity purified on columns coupled to the corresponding peptide. For immunoblot analysis of the antibodies, 15 µl of the cell or tissue extract were loaded on a 15 well 7.5% Ready Tris-HCl Gel (Bio-Rad), separated and blotted onto nitrocellulose membranes. Primary antibodies were used at 1:100-1:1000 dilutions. Binding of the primary antibodies to the blot was detected using a donkey anti-guinea-pig horseradish peroxidase (HRP) conjugated secondary antibody (diluted 1:10,000; Jackson Immunoresearch Laboratories). Blots were probed with a mouse anti-α-tubulin antibody (diluted 1:10,000, Sigma) detected by a donkey anti-mouse HRP-conjugated antibody (diluted 1:10,000, Jackson Immunoresearch Laboratories) (see supplementary material, Fig. S1).

Preparation of oocytes and spermatocytes

Animals heterozygous for Sycp3 (Yuan et al., 2000) were mated to obtain oocytes at different stages of prophase I of meiosis. After genotyping (Yuan et al., 2000) oocytes from embryonic-day 18.5 (E18.5) embryos, as well as from newborn and 4- and 7-day-old female mice were fixed in 0.8% paraformaldehyde (PFA) using a ‘drying-down’ technique (Peters et al., 1997). To obtain oocytes at the MI stage of meiosis ovariates from 3.5- to 4-week-old mice were dissected, and the oocytes were fixed in 1% PFA (Hodges and Hunt, 2002). Testicular cell suspensions were resuspended in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 5% foetal calf serum to a density of 1×10⁶ ml⁻¹ to 5×10⁶ ml⁻¹, cultured with 3 µM okadaic acid (OA) at 32°C for 4 hours and fixed in 1% PFA.

Immunofluorescence microscopy

For immunolocalization of oocytes and spermatocytes, we used the following antibodies and dilutions: rabbit anti-SMC3 (Eije et al., 2000), 1:25; rabbit anti-SMC4 (Bethyl Laboratories), 1:2000; rabbit anti-SYCP3 (Liu et al., 1996), 1:50; rabbit anti-SYCP1 (Liu et al., 1996), 1:50; human anti-CREST antiserum, 1:4000; guinea-pig anti-SYCP1, 1:400; guinea-pig anti-SYCP2, 1:100; guinea-pig anti-REC8, 1:100; guinea-pig anti-STAG3, 1:100; guinea-pig anti-SMC1β, 1:100 (for the guinea-pig antibodies, see supplementary material, Figs S1, S2). Secondary antibodies used were: swine anti-rabbit conjugated to fluorescein isothiocyanate (FITC) (DAKO), 1:100; donkey anti-guinea-pig conjugated to Cy3 (Jackson Labs), 1:1000; donkey anti-guinea pig conjugated to Cy2 (Jackson Labs), 1:500; donkey-anti-mouse Cy3 (Jackson Labs), 1:1200; goat anti-human conjugated to Cy5 (Amersham), 1:2500. All slides were stained with 0.5 µg ml⁻¹ DAPI to control the quality of the fixation procedures and only cells with undisrupted morphology were analysed. After staining, cells were mounted in Prolong mounting medium (Molecular Probes). Images were captured using a Leica DMRXA microscope at 1000×.
magnification and a Hamamatsu C4880-40 CCD camera. Images were processed using Openlab 3.1.4 software (Improvision) and Adobe Photoshop 8.0.

Staging of the oocytes
Meiotic progression in females differs from that in males in timing and stage appearance. Staging of mouse oocytes were based on nuclear morphology (DAPI staining), centromere numbers (CREST staining) and the extent of core synopsis (SYCP1 staining). SYCP1 fibres first become visible at early zygotene and increase in size as cells progress through zygotene and homologous chromosomes pair; centromeres (CREST foci) are usually the last to pair. At pachytene, SYCP1 fibres are fully colocalized with SYCP2, SYCP3 and cohesin cores, and cells display 20 distinct CREST foci, corresponding to the paired centromeres. SYCP1 cores disappear as chromosomes desynapse at diplotene; the number of CREST foci is between 20 and 40. Unlike the previous stages, male and female diplotene differ. Spermatocytes go straight into compaction at diakinesis, whereas oocytes enter dictyate with decondensed chromatin. Meiosis in females is initiated in a synchronous manner during foetal development: most of the oocytes in both wild-type and SYCP3-null embryos at E16.5 had entered zygotene, whereas they reached pachytene and early diplotene by E18.5. All oocytes reached dictyate during the first week after birth.

Results
Integrity of the STAG3 cohesin core at the diplotene stage of meiosis is dependent on SYCP3 and SYCP2
We initially analysed the organization of the diplotene-dictyate meiotic chromosome axis in female meiotic germ cells derived from wild-type animals. Nuclear spreads of oocytes derived from embryos at different stages of meiotic prophase were stained with antisera against STAG3, SYCP1 and CREST (Fig. 1). SYCP1 is a major transverse filament protein of the SC and a marker for synopsis (Dobson et al., 1994; Meuwissen et al., 1992), whereas the CREST antiserum visualizes centromeres. STAG3 immunostaining of pachytene and late diplotene oocytes derived from wild-type E18.5 embryos defined cohesin cores that colocalize with SYCP1 (Fig. 1A,B,E,F) and SYCP3 (not shown). Such core-like cohesin structures have also been noted in spermatocytes at the diplotene stage (Prieto et al., 2001). However, as the oocytes entered dictyate during the first week of postnatal development, the STAG3 cohesin cores disappeared and were replaced by an irregular pattern of nuclear foci (Fig. 1I,J). SYCP3 and SYCP2 have been shown to disappear completely during the first week of postnatal oocyte development (Hodges et al., 2001), suggesting to us that the loss of these two proteins and the cohesin cores might be

Fig. 1. The absence of SYCP3 results in disassembly of the STAG3 cohesin core. Wild-type (A,B,E,F,I,J) or Syce3−/− (C,D,G,H,K,L) oocytes were spread and triple-stained with antisera against STAG3 (red), SYCP1 (green) and CREST (white); the stages of meiosis are indicated to the left. An integral axial core labelled by STAG3 antiserum is observed in wild-type pachytene (A,B) and late diplotene (E,F) oocytes. By contrast, the STAG axial core is prematurely fragmented at late diplotene in Syce3-deficient oocytes (G,H). Yellow labelling indicates co-staining of STAG3 and SYCP1. Arrows in G,H mark STAG3 filaments that are retained in regions that have not yet desynapsed. Bars, 10 µm.
linked mechanistically. In agreement with this, we found that, in nuclei of early dictyate oocytes (derived from newborn animals), short filamentous STAG3 structures were retained almost exclusively at sites where SYCP3 was still present on chromatin (Fig. 2). A direct comparison to male germ cells is complicated by the fact that the dictyate stage is unique to females. However, analysis of cells at the stage that follows diplotene during spermatogenesis (prometaphase I) also identifies a fragmented axial cohesin core (Prieto et al., 2001).

To test the hypothesis that SYCP3 modulates cohesin core integrity, the chromosomal distribution of STAG3 was analysed in Syca3−/− oocytes. We observed that STAG3 forms cohesin cores in zygotene (not shown) and pachytene (Fig. 1C,D) oocytes deficient for SYCP3, as described previously for male germ cells (Kolas et al., 2004; Pelttari et al., 2001). Interestingly, the STAG3 cohesin cores became severely fragmented in the absence of SYCP3 as meiosis progressed into the diplotene stage (compare Fig. 1G,H with 1E,F). Exceptions were found at chromosomal regions that had yet to undergo desynapsis (SYCP1 positive), where residual short

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**Fig. 2.** STAG3 cores and SYCP3 filaments colocalize and are fragmented simultaneously at the early dictyate stage in wild-type oocytes. Oocytes in the early dictyate stage (from newborn animals) were labelled with antisera against SYCP3 (red) and STAG3 (green). A merged picture (centre) shows overlap between the two proteins (yellow). White arrows indicate colocalization of STAG3 and SYCP3, whereas red arrowheads indicate large SYCP3 aggregates, presumably representing self-assembled complexes of dissociated but not yet degraded SYCP3 protein. Bars, 10 µm.

**Fig. 3.** Fragmentation of the cohesin core occurs at diplotene in the absence of the SYCP3. Late-diplotene oocytes were stained with antisera against SYCP1 (B,D, green; F,H,J,L, red) and CREST (white) in combination with anti-SMC1β (red), anti-REC8 (green) and anti-SMC3 (green) antibodies. (A,B,E,F,I,J) Wild-type oocytes. (C,D,G,H,K,L) Syca3−/− oocytes; arrows indicate some of the centromeres. Notice the persistence of cohesin signal at all centromeres (see supplementary material, Fig. S2 for more detail). Bars, 10 µm.
STAG3-positive filaments were seen (Fig. 1G,H, arrows). We conclude that SYCP3 and SYCP2 are required for maintaining the integrity of the desynapsed STAG3 cohesin cores at the diplotene stage and that, in the absence of these two AE proteins, the STAG3 cohesin core disassembles prematurely.

SYCP3 is required to retain the cohesin cores until the dictyate stage

We then investigated whether other cohesin subunits also require SYCP3 to be maintained as part of a cohesin core during the diplotene stage of meiosis in oocytes. Wild-type and Sycp3−/− diplotene oocytes derived from E18.5 embryos were stained with antibodies against the cohesin subunits SMC1β, REC8 or SMC3, as well as against SYCP1 and CREST. We found that loss of SYCP3 has drastic consequences for the integrity of the desynapsed STAG3 cohesin cores at the diplotene stage and that, in the absence of these two AE proteins, the STAG3 cohesin core disassembles prematurely.

Premature disassembly of cohesin cores does not affect MI chromosome organization

The premature disassembly of the cohesin cores at diplotene in Sycp3−/− oocytes could affect cohesin-complex distribution and functions at later meiotic stages. We therefore characterized the distribution of STAG3, REC8 and SMC1β on MI chromosomes derived from oocytes. The localization of these meiosis-specific cohesin proteins on MI chromosomes has previously only been reported for male germ cells (Eijpe et al., 2000; Lee et al., 2003; Prieto et al., 2001; Revenkova et al., 2001). We found that STAG3, REC8 and SMC1β localization on female Sycp3−/− and wild-type MI chromosomes (Fig. 4A-P) closely followed what has been shown for male germ cells (see supplementary material, Fig. S1). We also monitored the distribution of SMC4, a condensin subunit produced in mitotic and meiotic cells that regulates chromosome condensation (Hirano, 2002) and axial compaction (Yu and Koshland, 2003), to investigate whether SYCP3 deficiency affects the organization of this protein on mammalian meiotic chromosomes. It was found that SMC4...
accumulates at the end of prophase I and then localizes to the MI chromosomes in a pattern that is indistinguishable between wild-type and Sycp3–/– oocytes (Fig. 4Q-T). In addition, we score the same number of centromeres in Sycp3–/– and wild-type MI oocytes (Fig. 4B,H). We conclude that the premature disassembly of the cohesin cores at diplotene in Sycp3–/– oocytes does not affect the distribution of the condensin subunit SMC4 or cohesin complex proteins along MI chromosomes, or centromere cohesion.

SYCP3 and SYCP2 are not required for sister-centromere cohesion at the MI stage in male germ cells

Based on the observation that SYCP3 localizes to the centromeres of MI chromosomes in spermatocytes, it has been proposed that this protein is essential for sister-centromere and/or kinetochore cohesion (Moenes and Spyropoulos, 1995; Parra et al., 2004). The functional role for SYCP3 at MI in Sycp3–/– spermatocytes cannot be studied directly because they arrest at a zygotene/pachytene-like stage (Yuan et al., 2000). To circumvent this arrest point, we exposed such spermatocytes to the phosphatase inhibitor OA in vitro. It has been shown that OA prematurely initiates cell-cycle progression and chromosome condensation in pachytene spermatocytes in vitro, forcing them into an MI-like stage (Tarsounas et al., 1999; Wiltshire et al., 1995). We found that a large proportion of the OA-treated wild-type testicular cells entered an MI-like stage and displayed 20 bivalents held together by chiasmata, each bivalent having two pairs of closely associated centromeres (Fig. 5A). Immunostaining of ‘MI’ chromosomes derived from OA-treated wild-type testicular cells using antisera against STAG3 (Fig. 5B), REC8 and SMC1β (see supplementary material, Fig. S1) results in a localization pattern very similar to what has been reported for normal cells (Eijpe et al., 2000; Lee et al., 2003; Prieto et al., 2001; Revenkova et al., 2001). By contrast, analysis of OA-treated Sycp3–/– mutant spermatocytes revealed 40 univalents in the resulting MI-like cells (Fig. 5C,D). This suggests that crossover formation is not completed between homologous chromosomes before the early pachytene arrest point in cells lacking SYCP3. We found that, whereas SYCP2 failed to accumulate at the centromeres at MI in OA-treated Sycp3–/– spermatocytes (Fig. 5J,K), the distributions of STAG3, REC8 and SMC1β along the univalents (Fig. 5D,F,I) were not affected by SYCP3 or SYCP2 deficiency. Furthermore, we found that the shape and number of centromeres (40 foci) as
shown by CREST staining are identical in wild-type and SYCP3-deficient cells (Fig. 5B,D, compare also with 5L,M showing a mitotic cell in which the sister chromatids are separated from each other, visualizing 80 distinct CREST foci). The close physical association of centromeres and kinetochores ensures that CREST also provides a marker for kinetochore cohesion. We conclude from these experiments that SYCP3 and SYCP2 are not required for sister-chromatid centromere or kinetochore cohesion at the MI stage in male meiotic cells. Thus, there is no evidence for sexually dimorphic functions for SYCP3 or SYCP2 at MI.

Discussion
SYCP3 and SYCP2 support cohesin-core integrity
SYCP3 has been considered to be an essential component of the meiotic chromosomal axis. Previous studies have, however, defined only one distinct structural chromosomal abnormality in meiotic cells deficient for this protein – a twofold extension of the chromosome axis (Yuan et al., 2002). Cohesin-complex proteins still form an organized cohesin core associated with both asynapsed and synapsed meiotic chromosomes despite the absence of SYCP3 and SYCP2 in male (Pelttari et al., 2001) and female zygote (data not shown) and pachytene (Fig. 1) germ cells. We have here described a second structural role for SYCP3 in reinforcing the cohesin cores, because we find that the absence of SYCP3 and SYCP2 drastically affects the integrity of the desynapsed cohesin cores at the diplotene stage of female meiosis. A similar dependency on SYCP3 and SYCP2 for cohesin core integrity is seen at the dictyate stage in wild-type oocytes, during which the removal of SYCP3 and SYCP2 from the meiotic chromosomes parallels the loss of the cohesin cores. The dependency of cohesin core integrity on SYCP3 and SYCP2 suggests that the two protein complexes interact at least transiently, a proposal supported by in vitro protein-interaction studies (Eijpe et al., 2000; Lee et al., 2003). We found that sister-chromatid centromer cohesion (based on the number of centromeres detected by the CREST antisera) and the distribution of cohesin and condensin complex subunits on MI chromosomes was similar in wild-type and Sycp3−/− oocytes, suggesting that the premature fragmentation of the cohesin core that occurs in Sycp3+/- diplotene oocytes has limited functional consequences. We speculate that the role of the cohesin cores is to promote transverse filament formation between the homologous chromosomes, thereby promoting synopsis. We find in support of this that short residual cohesin filaments colocalized with SYCP1 at the diplotene stage in Sycp3+/- oocytes. This model is further supported by recent results that show that the mouse REC8 protein is required for proper synopsis of homologous chromosomes (Bannister et al., 2004). Once desynthesis is complete at late diplotene, the cohesin cores are no longer required and are therefore disassembled, leaving behind only a subset of cohesin complexes required for maintaining arm and centromere cohesion at the first meiotic division. The loss of the cohesin cores at late prophase I in meiotic cells could also be a prerequisite for the resolution of sister chromatids, as described for mitotic cells (Losada et al., 2002; Sumara et al., 2002). It has been suggested that Polo-like kinases and Aurora B regulate the process that results in cleavage-independent dissociation of bulk cohesin at prophase in somatic cells (Losada et al., 2002; Sumara et al., 2002). Our results suggest that the dissociation of the cohesin cores in meiotic prophase-I cells is regulated in part by SYCP3. The function of SYCP3 could be to protect cohesin core components from exposure to an external activity that acts to release most cohesin from the meiotic chromosome axis. Alternatively, the cohesin core could have become destabilized before diplotene but has its integrity maintained by the AE. Additional studies are required to find out the mechanism for removal of SYCP3 from the meiotic chromosome axis.

Absence of SYCP3 in spermatocytes does not affect cohesin protein localization or cohesion between sister centromeres or kinetochores at MI
We have shown that the absence of SYCP2 and SYCP3 from the meiotic chromosome axis during female meiotic prophase does not have a detectable effect on cohesin protein distribution or centromere cohesion at MI. Similarly, ‘MI’ chromosomes derived from wild-type or SYCP3-deficient spermatocytes by OA treatment did not exhibit differences in sister-chromatid centromere/kinetochore cohesion, or in cohesin protein localization along the chromosomes. We found, however, that SYCP2 required SYCP3 in order to associate with the centromere region of MI chromosomes in male germ cells. The fact that SYCP3 and SYCP2 are not produced beyond the dictyate stage of female meiosis, together with the absence of a detectable cohesion defects in male MI cells in the absence of SYCP3 and SYCP2, strongly questions the proposals that these two proteins are essential for centromere or kinetochore cohesion at the MI stage in male germ cells (Moens and Spyropoulos, 1995; Parra et al., 2004). Our results do not support a sexually dimorphic role for SYCP3 and SYCP2 at meiotic MI.

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