BRCA1-mediated chromatin silencing is limited to oocytes with a small number of asynapsed chromosomes

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Summary
Transcriptional silencing of the sex chromosomes during male meiosis is regarded as a manifestation of a general mechanism active in both male and female germ cells, called meiotic silencing of unsynapsed chromatins (MSUC). MSUC is initiated by the recruitment of the tumor suppressor protein BRCA1 to the axes of unsynapsed chromosomes. We now show that Sye3, a structural component of the chromosome axis, is required for localization of BRCA1 to unsynapsed pachytene chromosomes. Importantly, we find that oocytes carrying an excess of two to three pairs of asynapsed homologous chromosomes fail to recruit enough BRCA1 to the asynapsed axes to activate MSUC. Furthermore, loss of MSUC function only transiently rescues oocytes from elimination during early postnatal development. The fact that the BRCA1-dependent synapsis surveillance system cannot respond to higher degrees of asynapsis and is dispensable for removal of aberrant oocytes argues that MSUC has a limited input as a quality control mechanism in female germ cells.

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Key words: Meiosis, Oocytes, BRCA1, Meiotic silencing of unsynapsed chromatin

Introduction
Meiosis is a specialized cell division process in germ cells that reduces the chromosome complement by half and generates haploid gametes (Page and Hawley, 2004; Zickler and Kleckner, 1999). The integrity of the meiotic process is crucial as errors may result in chromosomal missegregation and thus contribute to aneuploidy in mature germ cells and future offspring (Hassold and Hunt, 2001). Quality assurance mechanisms monitor several of the developmental transitions that take place during meiosis, including DNA recombination and repair, synopsis of the homologous chromosomes and the meiosis I and II divisions (Homer et al., 2005; Morelli and Cohen, 2005). Synapsis between homologous chromosomes at the pachytene stage of meiosis is essential for the formation of crossovers – recombination events that promote bi-orientation of the chromosomes at the first meiotic division (Nasmyth and Haering, 2005; Page and Hawley, 2004; Zickler and Kleckner, 1999). In mammals, the unsynapsed regions of the XY bivalent in normal pachytene spermatocytes activate a mechanism known as ‘meiotic sex chromosome inactivation’ (MSCI), which triggers chromatin changes and results in transcriptional silencing of sex chromosome-linked genes (Turner, 2007). The MSCI response is conserved in most organisms that possess heteromorphic sex chromosomes and a failure to activate MSCI results in midpachytene apoptosis and spermatocyte death (Burgoyne et al., 2009; Turner et al., 2005). The MSCI response depends on the tumor suppressor protein BRCA1 that accumulates on the unsynapsed axes of the sex chromosomes (Mahadevaiah et al., 2008; Turner et al., 2005), but the mechanism for targeting BRCA1 to the axes of unsynapsed chromosomes is presently unknown. BRCA1 then recruits the protein kinase ATR (ataxia telangiectasia and Rad3 related), which phosphorylates histone H2A.X, triggering the MSCI response (Bellani et al., 2005; Celeste et al., 2002; Mahadevaiah et al., 2008; Turner et al., 2004). The male-specific role of MSCI suggests that sex-specific surveillance mechanisms have evolved to protect the integrity of the germ cells (Morelli and Cohen, 2005). Recently, however, it was proposed that MSCI is a manifestation of a more general silencing mechanism, known as MSUC, that targets unsynapsed autosomes at the pachytene stage of meiosis in male and female germ cells (Baarends et al., 2005; Burgoyne et al., 2009; Schimenti, 2005; Turner et al., 2005). Our knowledge of the importance of MSUC as a surveillance mechanism in germ cells is, as yet, very limited.

In the present study we have explored the role of MSUC in female mouse germ cells, which given their XX complement do not undergo MSCI, yet have been shown to mount a MSUC response. We took advantage of several mouse models that allowed us to study how different levels of asynapsis affected the ability of oocytes to trigger an MSUC response. We now show that MSUC contributes to the elimination of oocytes that contain asynapsed chromosomes. The MSUC response, however, is impaired if more than two to three pairs of homologous chromosomes are asynapsed, due to a limited pool of BRCA1. In addition, we demonstrate that a structural component of the prophase meiotic chromosome axis, Sye3, is essential for recruitment of BRCA1 to asynapsed chromosomes.
Results

Axial accumulation of BRCA1 on asynapsed chromosomes is impaired when oocytes display extensive asynapsis

A recent study showed that mouse spermatocytes with extensive asynapsis have an impaired MSUC response leading to a failure of MSCI (Mahadevaiah et al., 2008). To find out if the MSUC response in female germ cells is also impaired by extensive asynapsis, we first analyzed BRCA1 localization in Sycp1–/– mouse oocytes. Mouse spermatocytes (de Vries et al., 2005) and oocytes (supplementary material Fig. S1) deficient for Sycp1, an essential component of the central region of the synaptonemal complex, form normal meiotic chromosome axes, but the homologous pachytene chromosomes fail to synapse and to repair DNA double-strand breaks (DSB). Labeling of Sycp1–/– and wild-type zygotene (the stage that precedes pachytene) oocytes with an antibody against BRCA1 identified an axis-associated focal pattern in both cell types (supplementary material Fig. S1), reflecting an ongoing DSB repair activity. In wild-type oocytes, a majority of these foci rapidly disappeared as the cells entered pachytene and the chromosomes synapsed. In a small number of wild-type oocytes derived from females at E18.5 (around 15%, n=126, s.d. 4.3%), however, one or two chromosomes remain asynapsed at the pachytene stage (Alton et al., 2008). We find that the asynapsed chromosomes in the wild-type oocytes have an intense axial BRCA1 labeling pattern (Fig. 1A). By contrast, a fainter and much less uniform BRCA1 pattern remained along the axis of the asynapsed chromosomes in Sycp1–/– pachytene oocytes (Fig. 1A). This result shows that BRCA1 accumulation on asynapsed chromosomes at the pachytene stage is defective when oocytes have extensive asynapsis.

Axial deposition of BRCA1 is greatly reduced when more than 10% of the chromosome axes are asynapsed

To define how much asynapsis is required to limit BRCA1 deposition on asynapsed axes, we used a mouse mutant (Smc1β−/−) that has varying degrees of asynapsis. Smc1β is a meiosis-specific isoform of the cohesion subunit SMC1 in mammals, essential for timely maintenance and release of sister chromatid cohesion during meiosis (Revenkova et al., 2004). The lack of Smc1β does not affect DSB formation and synaptonemal complex assembly, but most Smc1β−/− pachytene oocytes show a variable degree of asynapsis, affecting one to several chromosomes (Novak et al., 2008; Revenkova et al., 2004). We labeled Smc1β−/− pachytene oocytes with an antibody against BRCA1 and observed cells with strong BRCA1 staining on the asynapsed chromosomes (similar to the intense continuous axial staining seen for unsynapsed meiotic chromosomes in wild-type oocytes), as well as cells with weak staining (similar to the fainter and much less uniform pattern observed for unsynapsed chromosomes in Sycp1–/– oocytes; Fig. 1B). Remarkably, the mean intensity of axis-associated BRCA1 staining abruptly declined as the level of asynapsis increased above 10% (Fig. 1C). In order to find out if the different BRCA1 staining patterns in Smc1β−/− oocytes reflected different cellular
concentrations of BRCA1, we measured the total axial BRCA1 intensity per nucleus and found it was similar for all Smc1β−/− oocytes, irrespectively of the level of asynapsis (Fig. 1D). These results show that the amount of BRCA1 available to accumulate on asynapsed chromosomes in pachytene oocytes (the ‘BRCA1 pool’) is limited. When the level of asynapsis increases past a 10% threshold, the local BRCA1 concentration on the asynapsed chromosome axes is greatly reduced.

Chromosome-wide phosphorylation of H2A.X and ATR is restricted to oocytes with a few asynapsed chromosomes. BRCA1 has been shown to be required for domain-wide phosphorylation of H2A.X on unsynapsed chromatin in meiocytes (Turner et al., 2005). We therefore investigated whether the different levels of BRCA1 on the axes of unsynapsed pachytene chromosomes correlated with the degree of H2A.X phosphorylation at these chromosomal sites. We found that Smc1β−/− oocytes with extensive asynapsis show a γH2A.X staining pattern very similar to that observed for Sycp1−/− oocytes, i.e. irregular small patches localizing to only a subset of asynapsed chromosomes, and encompassing only part of the asynapsed chromosomes (Fig. 2A). By contrast, Smc1β−/− oocytes with a few asynapsed chromosomes showed large bright γH2A.X domains on the chromatin of the affected chromosomes, enclosing the entire chromosomes, and similar to the pattern seen at asynapsed chromosomes in wild-type oocytes (Fig. 2A, arrows). Quantification showed that the average intensity of the γH2A.X signal per asynapsed chromosome axis was considerably reduced when more than 10% of the chromosomal axes were asynapsed (Fig. 2B). Furthermore, double labeling of Smc1β+/+ oocytes with BRCA1 and γH2A.X antibodies showed that the mean intensity of BRCA1 and γH2A.X in oocytes were positively correlated (Fig. 2C; supplementary material Fig. S2).

MSUC is impaired in oocytes with more than two to three pairs of asynapsed chromosomes

In spermatocytes, the accumulation of BRCA1 and γH2A.X on unsynapsed chromosomes is associated with chromatin silencing (Mahadevaiah et al., 2008). In order to investigate whether the transcriptional activity of asynapsed chromosomes is also affected
in oocytes, we used RNA polymerase II (RNA pol II) staining as a marker for active transcription (Baarends et al., 2005). We observed a significant drop in RNA pol II intensity around the asynapsed chromosomes axes (in oocytes with one asynapsed chromosome pair), when compared with the synapsed chromosomal regions in the same cell (supplementary material Fig. S4; \( P = 0.024 \), nested ANOVA). Furthermore, the reduced RNA pol II signal at asynapsed chromosomes correlated with an increased \( \gamma H2A.X \) signal (supplementary material Fig. S4). By contrast, no statistical difference in RNA pol II staining was detected between fully synapsed wild-type oocytes and completely asynapsed \( Sycp1^{\sim/\sim} \) oocytes (\( P = 0.50 \), Student’s \( t \)-test). We also labeled oocytes with an antibody against ubiquitinated histone H2A (ubi-H2A), a marker that is preferentially associated with the axis of transcriptionally inactive chromosomes (Baarends et al., 2005). We observed strong axis-associated accumulation of ubi-H2A in wild-type oocytes with a few asynapsed chromosomes and in \( Smc1^{\beta/\beta} \) oocytes in which less than 10% of the chromosome axes were unsynapsed (Fig. 3A,B). Furthermore, the bright ubi-H2A signal correlated with intense axis-associated BRCA1 labeling (Fig. 3C). In summary, our results show that in pachytene oocytes, MSUC fails to occur because of insufficient axial BRCA1 only when more than 10% of the chromosome axes are unsynapsed, corresponding to 2-3 pairs of homologous asynapsed chromosomes.

**The axial element protein Sycp3 is required for BRCA1 accumulation on asynapsed chromosomes**

An obvious difference between synapsed and asynapsed meiotic chromosomes, is the continued ‘open’ configuration of the meiotic chromosome axis in asynapsed pachytene chromosomes. Two major structural constituents of the meiotic chromosome axis are the cohesin complex proteins (Eijpe et al., 2000; Nasmyth and Haering, 2005; Pelttari et al., 2001) and the axial element of the synaptonemal complex (Page and Hawley, 2004; Zickler and Kleckner, 1999). In order to find out if the integrity of the meiotic axes is important for recruitment of BRCA1 and MSUC response, we analyzed mice deficient for the Sycp3 protein, an essential structural component of the axial element (Yuan et al., 2002; Yuan et al., 2000). \( Sycp3^{\sim/\sim} \) pachytene oocytes had an asynapsis level similar to that of wild-type oocytes (Novak et al., 2008). We monitored the distribution of BRCA1 along the meiotic chromosome axis (as defined by the cohesin complex protein STAG3) in \( Sycp3^{\sim/\sim} \) zygote and pachytene oocytes. Importantly, although we observed axis-associated BRCA1 staining in \( Sycp3^{\sim/\sim} \) oocytes, no axial staining of BRCA1 was recorded in \( Sycp3^{\sim/\sim} \) pachytene oocytes (Fig. 4A,B). To further investigate the requirement of Sycp3 for BRCA1 recruitment to the asynapsed meiotic chromosome axis, we examined \( Sycp3^{\sim/\sim} Smc1^{\beta/\beta} \) double mutant oocytes (Novak et al., 2008). In contrast to the situation in \( Smc1^{\beta/\beta} \) pachytene oocytes (Fig. 1B), no axis-associated BRCA1 staining was observed in \( Sycp3^{\sim/\sim} Smc1^{\beta/\beta} \) pachytene oocytes (Fig. 4A,B), even though \( Sycp3^{\sim/\sim} Smc1^{\beta/\beta} \) oocytes show levels of asynapsis at pachytene similar to \( Smc1^{\beta/\beta} \) cells (Novak et al., 2008). A failure to localize BRCA1 to asynapsed chromosomes in the absence of Sycp3 is further supported by the observation that pachytene oocytes derived from \( Sycp3^{\sim/\sim} \) or \( Sycp3^{\sim/\sim} Smc1^{\beta/\beta} \) females (supplementary material Fig. S5) have small irregular \( \gamma H2A.X \) patches and never the large bright \( \gamma H2A.X \) domains observed on wild-type oocytes

Fig. 3. Silencing of asynapsed chromosomes axes is verified only in \( Smc1^{\beta/\beta} \) oocytes displaying limited asynapsis. (A) \( Smc1^{\beta/\beta} \) oocytes, isolated from E18.5 ovaries, were labeled with the marker for silenced chromatin, ubiquitinated H2A (ubi-H2A) (blue), and co-stained with BRCA1 antibody (green) to assess the intensity of BRCA1 signal on asynapsed axes. Chromosomal axes were labeled with Sycp2 antibody (red). ubi-H2A was found on the asynapsed axes of \( Smc1^{\beta/\beta} \) oocytes with a low number of asynapsed chromosomes, when chromosomal axes were labeled with Sycp2 antibody (red). ubi-H2A was found on the asynapsed axes of \( Smc1^{\beta/\beta} \) oocytes with a low number of asynapsed chromosomes, when chromosomal axes are strongly labeled by BRCA1 antibody. Scale bars: 10 \( \mu \)m. (B,C) Quantification of ubi-H2A signal intensity on asynapsed axes. Chromosomal axes were labeled with Sycp2 antibody (red). ubi-H2A was found on the asynapsed axes of \( Smc1^{\beta/\beta} \) oocytes with a low number of asynapsed chromosomes, when chromosomal axes are strongly labeled by BRCA1 antibody. Scale bars: 10 \( \mu \)m. (B,C) Quantification of ubi-H2A signal intensity on asynapsed chromosomal axes drops to background levels (red line) at around 10% asynapsis. (C) ubi-H2A displays a drastic increase in intensity above a certain BRCA1 intensity.
type or Smc1β−/− oocytes with low levels of asynapsis (Fig. 2A). A similar absence of BRCA1 accumulation on asynapsed chromosomal axes and lack of bright γH2A.X domains was observed for spermatocytes derived from Syeap3−/− or Syeap3−/−Smc1β−/− males (data not shown). Our results indicate that Syeap3 is required for recruitment of BRCA1 to asynapsed pachytene chromosome axes and the ensuing chromatin modifications that elicit an MSUC response.

Inactivation of Syeap3 transiently increases oocyte survival. Finally, we investigated if MSUC affects oocyte survival. Taking advantage of the similar levels of asynapsis at pachytene in Smc1β−/− and Syeap3−/−Smc1β−/− mice (Novak et al., 2008), we decided to analyze whether there was a difference in postnatal oocyte survival rates of these two mutant strains. In 33% of the pachytene oocytes derived from Smc1β−/− animals (n=187, s.d. 3.1%), less than 10% of the chromosome axes are asynapsed and would therefore be subjected to a MSUC response, presumably resulting in prenatal or early post-natal elimination of those oocytes. We reasoned that inactivating Syeap3 in an Smc1β−/− background might abrogate the MSUC response in these oocytes during pachytene, allowing them to survive further into postnatal development. Indeed, the number of oocytes per ovary during postnatal days 1 and 2 in the Syeap3−/−Smc1β−/− double mutant was 25% higher than that of the Smc1β−/− single mutant mice (Fig. 4C). Our results indicate that the MSUC response contributes to the elimination of pachytene oocytes suffering limited asynapsis. The increased oocyte survival in Syeap3−/−Smc1β−/− females in comparison to Smc1β−/− animals, however, is reversed by postnatal day 4 (Novak et al., 2008), suggesting the existence of overlapping surveillance mechanisms monitoring oocyte quality.

Discussion

Transcriptional silencing of heteromorphic sex chromosomes in germ cells (MSCI) occurs in most organisms (Turner, 2007), and has been regarded as a male-specific surveillance mechanism. Recently, however, a similar mechanism of transcriptional silencing for asynapsed autosomes (MSUC) was described, suggesting that MSCI represents a special case of a more globally acting asynapsis-silencing mechanism (Mahadevaiah et al., 2008). The primary focus of our study has been to quantify the MSUC response in oocytes. In contrast to a previously published paper studying spermatocytes (Mahadevaiah et al., 2008), our study deals exclusively with MSUC, as MSCI is not active in female germ cell. Furthermore,
our study, for the first time, defines a precise quantitative border for MSUC activation (using Smc1β−/− oocytes as a model), which we find to be remarkably low. In addition, we have observed an apparent requirement of Sycp3 for MSUC activation.

A restricted role for MSUC in quality control in oocytes
We have shown that the MSUC response is restricted to oocytes carrying less than two to three pairs of asynapsed homologous chromosomes. Eliminating the MSUC response (by inactivating the Sycp3 gene) results in an increased postnatal oocyte survival rate, suggesting that MSUC contributes to the elimination of oocytes with limited asynapsis. A number of surveillance mechanisms have been shown to be active in mouse oocytes, including the spindle assembly checkpoint (SAC) (Di Giacomo et al., 2005; Homer et al., 2005). Interestingly, the SAC in oocytes has been shown to be insensitive if there are only a small number of acentric chromosomes (Kouznetsova et al., 2007), and we now show that the opposite is true for MSUC, suggesting that MSUC and SAC constitute complementary quality assurance mechanisms that would eliminate most chromosomally abnormal oocytes. However, the fact that the increased postnatal survival rate observed at day 2 for oocytes in the absence of a MSUC response is eliminated by postnatal day 4, shows that additional quality control mechanisms are operational during postnatal development of oocytes, including DNA damage or recombination checkpoints (Di Giacomo et al., 2005; Homer et al., 2005). Furthermore, it cannot be excluded that inactivation of Sycp3 (and the subsequent loss of the axial element of the synaptonemal complex) abrogates MSUC-independent checkpoints that affect oocyte viability. Interestingly, a MSUC response has been reported to occur in human oocytes trisomic for chromosome 21 (Garcia-Cruz et al., 2009). In this situation, however, MSUC silencing of the extra chromosome 21 in the abnormal oocytes, would restore normal levels of chromosome transcription, potentially rescuing the trisomic oocytes from elimination (Garcia-Cruz et al., 2009).

Oocytes carrying more than three pairs of asynapsed chromosomes have limited axial BRCA1 deposition and cannot mount a MSUC response
BRC1 has been shown to accumulate on the unsynapsed axes of sex chromosomes and autosomes, thus triggering the MSCI and the MSUC responses in male and female mouse germ cells (Burgoyne et al., 2009; Mahadevaiah et al., 2008; Turner et al., 2005). We find, as previously shown for spermatocytes (Mahadevaiah et al., 2008), that MSUC is abrogated by extensive asynapsis and that this impaired response can be linked to a limiting axis-associated fraction of BRC1. Importantly, however, we have quantified the extent of asynapsis that abrogates the MSUC response in oocytes. We find that when the axis-associated BRC1 pool in oocytes is distributed between more than three pairs of asynapsed homologous chromosomes, the BRC1 localization pattern changes and the MSUC response is abrogated.

Sycp3 is required for recruitment of BRC1 to asynapsed chromosomes
The meiotic chromosome axis is composed of cohesin-complex proteins and the axial element proteins Sycp2 and Sycp3 (Revenkova and Jessberger, 2006). We show that loss of the cohesin protein Smc1β does not preclude BRC1 loading on asynapsed pachytene chromosomes. On the contrary, axial element protein Sycp3, a meiosis-specific synaptonemal complex component, is essential for recruitment of BRC1 to the asynapsed chromosome axes. Sycp3 becomes associated with chromosome axes at the leptotene stage and remains associated until the diplotene stage (Eijpe et al., 2003), whereas BRC1 associates with the meiotic chromosome axes in two successive rounds (Mahadevaiah et al., 2008). We find that the first round, involving recruitment of BRC1 to potential DNA DSB sites on the axes of leptotene or zygotene chromosomes, is independent of Sycp3, whereas the second round involving BRC1 recruitment to the axes of asynapsed pachytene chromosomes is Sycp3 dependent. The pattern for the first round of BRC1 accumulation on chromosomes in Sycp3−/− spermatocytes is very similar to what we observe in oocytes. BRC1 is initially incorporated into the chromosome axes of Sycp3−/− zygotene spermatocytes in a dot-like pattern, but fails to accumulate on the asynapsed regions at early pachytene. Analysis of the BRC1 pattern at later stages of pachytene in Sycp3−/− spermatocytes is not possible, as these undergo apoptosis at mid-pachytene (spermatogenic stage IV) (Hamer et al., 2008).

Inactivation of Sycp3 in mouse meiocytes also results in a loss of the second known axial element protein, Sycp2, from the meiotic chromosome axis (Yuan et al., 2002; Yuan et al., 2000), opening up the possibility that recruitment of BRC1 to the axial element could be dependent on either Sycp3 or Sycp2. Mice expressing a truncated Sycp2 protein (Sycp2Δt) do not incorporate Sycp3 protein into the chromosomal axes, do not form axial elements as detected by electron microscopy and phenotypically resemble Sycp3−/− mice (Yang et al., 2006). It has, however, not been reported if BRC1 accumulates on asynapsed pachytene chromosomes in Sycp2Δt-expressing mice. Furthermore, as the axial element is lost in both Sycp3−/− and Sycp2t mutant meiocytes, it is not possible to distinguish whether BRC1 localization to asynapsed pachytene chromosomes is dependent on Sycp3 and Sycp2 function, or more indirectly on some other aspect of axial integrity.

Materials and Methods

Mice
Derivation of Sycpl, Sycp3, Smc1βknockout and Smc1βSycp3 double knockout lines (all have C57BL/6J background) has been described previously (de Vries et al., 2005; Novak et al., 2008; Revenkova et al., 2004; Yuan et al., 2000). To obtain oocytes at meiotic prophase, heterozygous females were mated with heterozygous males and daily examined for the presence of the vaginal plugs. The day the plug was detected was denoted as day 0.5 (E0.5). Oocytes were isolated from E16.5-E18.5 ovaries, depending on the stage. All animal procedures were in accordance with institutional guidelines.

Immunofluorescence microscopy
Slides with oocytes from Sycpl−/−, Sycp3−/−, Smc1β−/− and Smc1β−/− Sycp3−/− ovaries were prepared using a ‘dry-down’ technique (Peters et al., 1997) and immunostained as described previously (Kouznetsova et al., 2005; Novak et al., 2008; Wang and Hoog, 2006). For protein detection and quantification, we used guinea pig anti-SATG3 (Kouznetsova et al., 2005) at 1:100, guinea pig anti-Sycp2 (Kouznetsova et al., 2005) at 1:100, guinea pig anti-Sycp1 (Kouznetsova et al., 2005) at 1:100, rabbit anti-BRC1 (a gift from James Turner, Division of Stem Cell Biology and Developmental Genetics, MRC NIMR, London, UK) at 1:400, human anti-CREST at 1:1000, rabbit anti-γH2AX (Upstate Biotechnology) at 1:100 or mouse anti-γH2AX (Upstate Biotechnology) at 1:200, ubi-H2A (Upstate Biotechnology) at 1:200, rabbit anti-ATR-FRP (Onconeo) at 1:100, RNA Pol II (Abcam) at 1:200, rabbit anti-DMC1/Rad51 [gift from Peter Moens (deceased), York University, Toronto, Canada] at 1:100. Secondary antibodies were swine-anti-rabbit conjugated to FITC (DakoCytomation) at 1:400, goat anti-mouse Alexa Fluor 488 (Invitrogen) at 1:1000, donkey anti-guinea pig conjugated to TRITC (Jackson ImmunoResearch) at 1:400, goat anti-mouse Alexa Fluor 635 (Invitrogen) at 1:1000, goat anti-human Cy5 (Jackson ImmunoResearch) at 1:1000 and donkey anti-rabbit Cy5 (Jackson ImmunoResearch) at 1:1000. Slides were viewed using a Leica DMRA2 microscope with a 100× objective and epifluorescence. Images were captured by a Hamamatsu digital CCD camera C4742-95 and Openlab 3.1.4 software and processed using Openlab 3.1.4 and Adobe Photoshop 9.0.
Staging of the oocytes
In detail, in developmental stages in Smc1β–/–, Smc3β–/–, SMC1β–/– and Smc1β/Smc3β–/– oocytes, we took advantage of the synchronous development of oocytes in embryonic ovaries (Dietrich and Mulder, 1983). Therefore we labeled chromosomal axes and centromeres in oocytes derived from E16.5, E17.5, E18.5 and E19.5 mutant oocytes and matched the patterns found with wild-type littermates. The type found only in E16.5 oocytes was classified as ‘early zygotene’, the one prevalent in E17.5 oocytes as ‘pachytene’, etc.

Oocyte survival
Ovaries from Smc1β–/– females were collected at day 1–2 after birth, fixed in 4% formaldehyde for 4 hours, embedded in paraffin and sectioned at 5 mm. To count the number of oocytes in the ovary, each fifth section was immunostained for GCNA (Enders and May, 1994), as described previously (Wang and Hoog, 2006). The dataset published by Novak et al. (Novak et al., 2008) (n=3 for wild type, n=4 for Smc1β–/– and n=3 for Smc3β–/–Smc1β–/– oocytes) was complemented with new data (n=3 for wild type, n=2 for Smc1β–/– and n=2 for Smc3β–/–Smc1β–/– oocytes), and the total dataset was used for statistical analysis of oocyte survival. Importantly, data for day 1 and day 2 were collected in parallel and analyzed by the same person. The number of oocytes for each mouse is given in supplementary material Table S1.

Quantification was performed using ImageJ 1.40g software. Oocytes derived from mutant animals and their wild-type littermates were spread as described above, stained with the antibodies of interest plus Syp2 antibody to assess axis morphology, and counterstained with DAPI. All slides with one antibody staining were processed simultaneously to minimize variation; images were taken with the same exposure times. Only pachytene oocytes with undamaged morphology (as judged by DAPI staining) and adequate spreading (nucleus diameter between 30 and 50 μm) were processed. The measurements were taken from one image representing the focal plane for the whole cell. To measure the total axial BRCA1 fluorescence in Smc1β–/– oocytes, all axes, as determined by Syp2 staining, were outlined and the integrated signal density of BRCA1 within these areas was measured. To measure the total RNA pol II intensity in Smc1β–/– and wild-type oocytes, the whole nucleus was outlined and the integrated signal density was measured within this outline. To measure BRCA1 and ubi-H2A intensity on the asynapsed axes, the whole asynapsed axis (as judged by Syp2 staining) was carefully marked and the mean intensity of the signal was measured on this line. For measurements of H2A.X, the areas around the asynapsed chromosomes with an intensity above the threshold were outlined, and the mean intensity of the H2A.X signal was measured within this outline. For quantification of RNA pol II signal in Smc1β–/– oocytes, we measured the mean integrated density around all asynapsed axes (as judged by intense H2A.X signal) and the mean integrated density around five randomly selected synapsed euchromatic regions within the same cell; the intensity of the H2A.X signal was also measured in the selected regions. As the RNA pol II signal showed variation between the cells, we applied nested ANOVA tests to probe the difference in RNA pol II intensity. The level of asynapsis was calculated as the proportion of the asynapsed axial element length to the total axial element length (asynapsed axes plus twice the length of the syndapsis regions) for each nucleus. In our quantification analysis we have assumed that the protein concentration is directly proportional to the observed intensity of the immunofluorescent signal.

Statistical analysis
Statistical analyses were performed using Excel 2004 and Statistica 7.0 programs.

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We thank Ivana Novak for help with experiments on Smc1β–/– mice and Mary-Rose Hoja for the comments on the manuscript. This work was supported by grants from the Swedish Cancer Society, the Swedish Research Council, the Novo Nordisk Foundation and Karolinska Institute.

References


Table S1. Number of GCNA-positive oocytes in the sections of wild type, \textit{Sycp3}^{−/−}, \textit{Smc1β}^{−/−} and \textit{Sycp3}^{−/−}\textit{Smc1β}^{−/−} 1-2 days old female mice.

<table>
<thead>
<tr>
<th></th>
<th>Wild type</th>
<th>\textit{Sycp3}^{−/−}</th>
<th>\textit{Smc1β}^{−/−}</th>
<th>\textit{Sycp3}^{−/−}\textit{Smc1β}^{−/−}</th>
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<tr>
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<td>513</td>
<td>250</td>
<td>597</td>
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<tr>
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<td>484</td>
<td>295</td>
<td>371</td>
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