SYCE2 is required for synaptonemal complex assembly, double strand break repair, and homologous recombination

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Synapsis is the process by which paired chromosome homologues closely associate in meiosis before crossover. In the synaptonemal complex (SC), axial elements of each homologue connect through molecules of SYCP1 to the central element, which contains the proteins SYCE1 and -2. We have derived mice lacking SYCE2 protein, producing males and females in which meiotic chromosomes align and axes form but do not synapse. Sex chromosomes are unaligned, not forming a sex body. Additionally, markers of DNA breakage and repair are retained on the axes, and crossover is impaired, culminating in both males and females failing to produce gametes. We show that SC formation can initiate at sites of SYCE1/SYCP1 localization but that these points of initiation cannot be extended in the absence of SYCE2. SC assembly is thus dependent on SYCP1, SYCE1, and SYCE2. We provide a model to explain this based on protein–protein interactions.

Introduction

Meiosis is the process of producing haploid gametes from diploid germ cells by one round of DNA replication followed by two rounds of cell division. Recombination takes place in the prophase of the first of the two cell divisions and is dependent on the formation of double strand breaks (DSBs) by SPO11 (Bergerat et al., 1997; Keeney et al., 1997). After homology search and strand invasion, the homologous chromosomes pair and, in many organisms, form the synaptonemal complex (SC). This is a structure with two axes, each joining together the pair of sister chromatids of each homologue (for review see Page and Hawley, 2004). These axes contain several specific proteins in addition to the cohesins that are generally responsible for the maintenance of chromosome structure (Eijpe et al., 2003). In mammals, this includes the coiled-coil domain proteins SYCP2 and -3. In mice with a deletion of the *Sycp2* gene, which removes the part of the protein interacting with SYCP3, assembly of SYCP3 into the axes is impaired. Males suffer a block in meiosis that leads to apoptosis and infertility, and females have a severely reduced litter size (Yang et al., 2006). Likewise, mice engineered to be null for SYCP3 exhibit a dimorphic phenotype. Although males are infertile, females show a reduced litter size caused by the death of aneuploid embryos produced from aneuploid oocytes (Yuan et al., 2002). The repair of DSBs and recombination is also affected in these mutants (Wang and Hoog, 2006).

Transverse filaments extend from and meet between axes in a structure called the central element (CE). This ultrastructural feature is common to many organisms and, until recently, was thought to consist entirely of the overlap between the N termini of SYCP1 molecules originating from the paired axes (Schmekel et al., 1996). In agreement with this, overexpression of SYCP1 (or ZIP1 in yeast) produces structures termed polycomplexes, which have dimensions similar to SCs (Dong and Roeder, 2000; Ollinger et al., 2005). Targeted mutation of the *Sycp1* gene in mouse results in infertility in both sexes, leading to the failure of synapsis and the absence of completed crossover in males (de Vries et al., 2005). The female cytology in this mutant has not yet been described.

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Abbreviations used in this paper: AE, axial element; CE, central element; DSB, double strand break; SC, synaptonemal complex.

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We have recently defined two proteins, SYCE1 and -2 (previously known as CESC1), with localization confined to the CE of mouse SCs (Costa et al., 2005). Homologous genes exist in other vertebrate genomes, and structural homologues may exist more widely. The role of these proteins is suggested by their location and biochemical interactions. They colocalize with SYCP1 to synapsed axes at the light microscope level and are confined to the CE at electron microscope resolution. They are both capable of interacting with themselves, with each other, and with the N terminus of SYCP1. We have postulated that they provide reinforcement to the N-terminal SYCP1 interactions. A third CE protein, TEX12, which interacts with SYCE2, was recently described (Wang et al., 2001; Hamer et al., 2006). To test the dependence of SC formation on SYCE1 and -2, we are generating mice that lack these proteins. In this paper, we report mice derived from an embryonic stem cell line in which the *Syce2* gene was disrupted by insertion of a gene trap vector (Chen et al., 2004). We have analyzed these mice immunocytochemically to look not only at the structural effect of the mutation but also at its effect on DSB processing and crossing over.

Results and discussion

Generation of gene-trapped *Syce2* mice

Searches of the Sanger Center gene trap database (http://www.sanger.ac.uk/PostGenomics/genetrap/) revealed a gene trap line, S8-7E, in which the *Syce2* gene was disrupted by insertion of a ROSAFARY vector into the locus (Chen and Soriano, 2003; Chen et al., 2004; Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200610027/DC1). Founder mice generated from this cell line, $Syce2^{+}/Syce2^{gtrl}$, were fertile and were intercrossed to give homozygous $Syce2^{gtrl}/Syce2^{gtrl}$ (*SYCE2KO*) animals. The gene-trapped allele was transmitted in Mendelian ratio to offspring. *SYCE2KO* animals of both sexes were infertile when crossed to wild-type animals but showed no other overt phenotype. Ovaries of adult females were minute, and testes weights of adult males averaged 20% of wild-type littermates. Adult testis sections showed a stage IV arrest, with all spermatocytes undergoing apoptosis (Fig. S2).

From the site of insertion and the nature of the gene trap vector, we predicted that the $Syce2^{gtrl}$ allele was likely to represent a null mutation, as only 10 amino acids remain from the original protein (Fig. S1). To confirm that aberrant splicing events had not rescued the expression of the trapped allele, we used Northern blots to check the level of wild-type RNA in *SYCE2KO* testes. Normal *Syce2* mRNA was not detectable using this technique, nor by RT-PCR (Fig. S1). Accordingly, we could not detect the SYCE2 protein in testis cell spreads (unpublished data).

SYCE2KO animals fail to synapse homologous chromosomes

SYCE2KO spermatocytes were analyzed using spread preparations. These were initially stained for axial element (AE) components, showing that mutant spermatocytes have AEs of normal morphology and composition at light microscope level. Cohesins SMC3 and the meiosis-specific REC8 and STAG3 are all present, together with SC proteins SYCP2 (not depicted) and SYCP3 (Fig. S2 A), in both male and female *SYCE2KO* animals. In adult males, the AE appear to align homologously, at least in the majority of the cells, but a minority show alignment of only

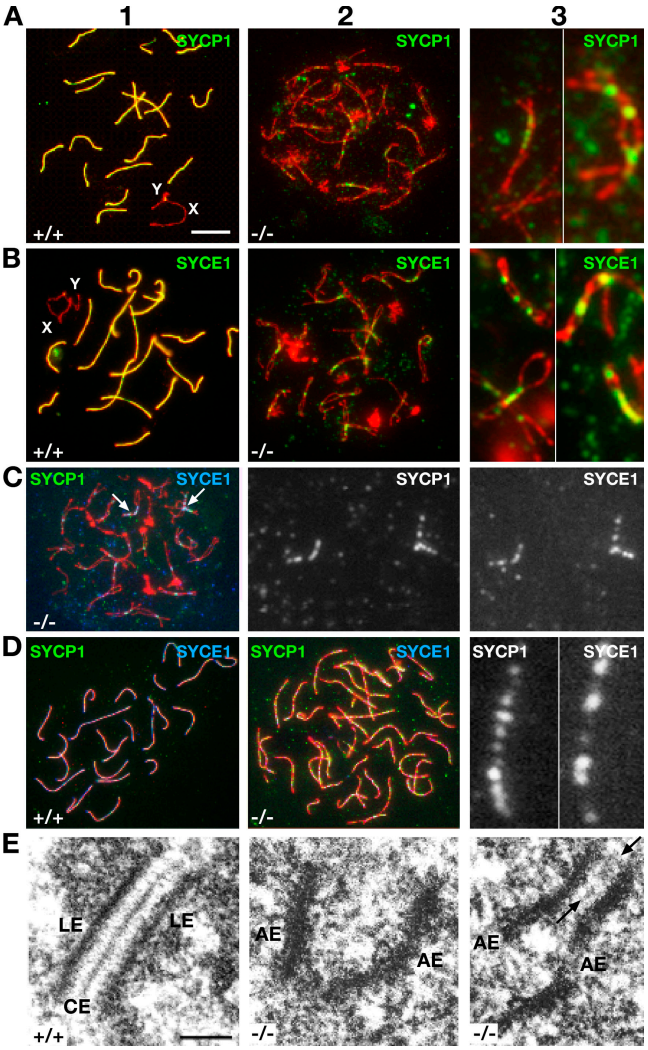

Figure 1. ***Syce2* mutants fail to form a SC between homologues, but small regions of synapsis can be observed.** Wild-type and mutant meiotic spreads were immunostained with anti-SYCP1 or anti-SYCE1 (green) and anti-SYCP3 (red; AE) antibodies (A–D). Although male +/+ pachytene cells show complete synapsis of autosomes (A1 and B1), mutant cells with well-aligned chromosomes show only small foci of synapsis staining for SYCP1 or SYCE1 on some autosomes (A2 and B2, respectively). A3 and B3 display higher magnification of SYCP1 and SYCE1 localization to regions of AE juxtaposition, respectively. SYCP1 (green) colocalizes with SYCE1 (blue) in the small foci of synapsis (arrows) in male −/− cells (C). C2 shows SYCP1, and C3 shows SYCE1 localization at a higher magnification on the chromosomes indicated by arrows in C1. In female +/+ pachytene cells, synapsis is established between homologous chromosomes as observed by SYCP1 (green) and SYCE1 (blue) staining (D1), but −/− female cells with unpaired AEs show SYCP1 and SYCE1 punctate localization (D2). D3 displays a higher magnification of a single univalent with SYCP1 and SYCE1 signals. Electron microscopic characterization of *SYCE2KO* male meiotic cells confirmed the existence of small foci of synapsis (E). E1 shows a wild-type SC, whereas E2 and E3 show AEs aligned but not synapsed and the occasional short region of synapsis in the mutant, respectively. Arrow in E3 points to the CE-like structure found in synapsis foci in the mutant. LE, lateral element; X and Y, sex chromosomes. Bars: (A–D) 5 μm; (E) 200 nm.

some chromosome pairs or no alignment at all (Fig. S2 B). It is possible that the last two classes of cells are entering apoptosis. In females (embryonic days 16.5–18.5), a lower percentage of AEs are in close alignment, suggesting that this stage may be of shorter duration in females than in males.

In wild-type animals, entry into the zygotene stage is characterized by initiation of synapsis between homologous chromosomes. This can be visualized by staining with anti-SYCP1, -SYCE1, or -SYCE2 antibodies (Fig. 1; Costa et al., 2005). In the *SYCE2KO* males, however, synapsis fails to develop between homologues, except for some small regions, varying in number and extent, of closer association that stain for both SYCE1 and SYCP1 (Fig. 1, A2, A3, B2, and B3). In these regions, SYCE1 largely colocalizes with SYCP1 (Fig. 1 C). SYCP1 was not associated with male AEs, suggesting that SYCE2 is necessary for the C terminus of SYCP1 to bind the axes. *SYCE2KO* females, when immunostained with SYCP1/SYCE1 antibodies, showed no signs of synapsis, but SYCP1 and SYCE1 were detected on dispersed univalents as bright foci coating the AE even in the absence of synapsis (Fig. 1 D). These sites were not always coincident. This is not observed when homologous chromosomes are aligned. To test whether the regions of synapsis in *SYCE2KO* males were as short as was observed by immunocytochemistry, or if this was an artifact of the spreading technique, we performed electron microscopy on fixed and sectioned material from adult testes (Fig. 1 E). Again, we found multiple unpaired AEs that were, however, thicker and showed a less regular surface than wild-type AEs/lateral elements (Fig. 1, E2). Occasional regions of synapsis were also found (Fig. 1, E3). Traces of a CE were present where this occurred. The overall width of these regions of synapsis was similar to that of a wild-type SC, although AEs are thicker and the central region thinner than wild type. This data is consistent with the immunocytochemistry, suggesting that the short regions of synapsis are not an artifact of the spreading technique. We propose that synapsis is initiated but is not, or is only minimally, extended.

The dimensions of the polycomplex formed when SYCP1 is overexpressed in mitotic cells, in the absence of other meiotic chromosome components, are very similar to those of the SC (Sym and Roeder, 1995; Dong and Roeder, 2000). SYCP1 has C- and N-terminal globular domains separated by a coiled-coil region. When the length of this coiled-coil region is varied experimentally, the spacing of the arrays in polycomplexes generated is increased or reduced accordingly (Ollinger et al., 2005). This supports the concept that the SYCP1 molecule determines the spacing of the axes. Our new data shows that in vivo the assembly and/or stability of the system are dependent on more than SYCP1 alone.

In the absence of SYCP1, both SYCE1 and -2 are delocalized from the axes of the chromosomes, and at the biochemical level, interactions occur between the N terminus of SYCP1 and SYCE1/2 (Costa et al., 2005). In addition, SYCE1 and -2 can interact with themselves and with each other (Costa et al., 2005). We suggested a model in which the CE proteins provided a structural role, perhaps associated with the postulated need to resist compression forces as a mechanism to produce interference (Kleckner et al., 2004). Here, we revise this model.

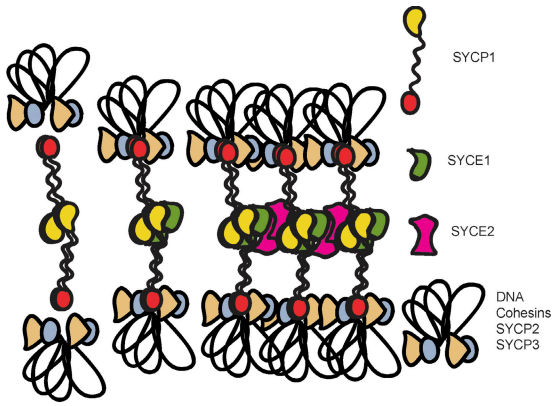

Figure 2. **Model for SC assembly.** Homodimers of SYCP1 form unstable N-terminal self-associations and do not form C-terminal associations with AE components. These associations in A are stabilized by interactions with SYCE1 in the rudiments of the CE. At this point, the C terminus of the SYCP1 molecule is associated with the AE. These short regions of synapsis are not stably extended until interactions between the three proteins, SYCE2, SYCP1, and SYCE1, are established, forming the completed SC.

Based on the observation that short points of synapsis are detectable in the male as sites of colocalization of SYCP1 and SYCE1, we suggest that synapsis can initiate in the absence of SYCE2 but cannot propagate along the AE. One testable prediction is that, in the absence of both SYCE1 and -2, these short stretches of synapsis will not occur. We have not seen regions of synapsis in female meiosis in the *SYCE2KO* animals. This may reflect a real difference in mechanism, but as prophase1 of female meiosis takes place in a compressed time scale compared with male, it is possible that we have not detected limited synapsis because it is more transient (Handel and Eppig, 1998).

The known interactions between these proteins suggest a process of polymerization that would result in the self-assembly of the SC. Dimers of SYCP1 form head-to-head associations via their N termini to set the basic spacing between the lateral elements. This association alone would not be stable, but the interaction with SYCE1, probably in a multimeric form, could cause its stability to increase. The short regions of synapsis we observe would represent such sites of stable association. Extension of this would require the association of a dimer or tetramer of SYCE2 with the SYCE1–SYCP1 complex through a SYCE1–SYCE2 interaction. SYCE2 would then interact with an SYCP1 dimer and, through a repetition of the process, polymerize the SC. This model is represented in Fig. 2. Again, there are testable predictions; for example, the N terminus of SYCP1 should be able to interact with SYCE1 and -2 simultaneously and *Syce1*$^{-/-}$ animals should be phenotypically similar to *SYCE2KO* animals in having stabilized points of axial contact. The sites of limited synapsis we observe in the *SYCE2KO* males could represent a mammalian equivalent of the synaptic initiation complex in yeast (Fung et al., 2004; Tsubouchi et al., 2006). This seems unlikely for two reasons. First, we do not see a preferential association of recombination proteins such as MSH4 with these sites of SYCP1 and SYCE1 localization. Second, the distribution does not match that of recombination events, with many chromosomes lacking these sites of synapsis and some chromosomes having multiple sites.

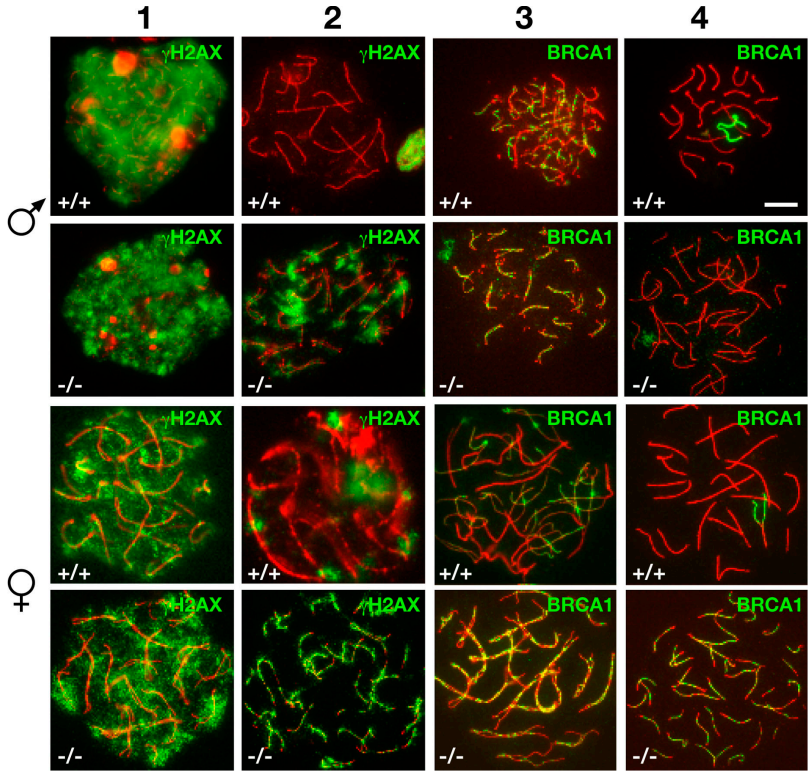

Figure 3. **Altered distribution of γH2AX and BRCA1 in *Syce2* mutant mice.** Surface-spread nuclei were immunostained with anti-γH2AX or BRCA1 (green) and anti-SYCP3 (red). The top row shows male +/+ cells in leptotene (1), pachytene (2 and 4), and zygotene (3). Row 2 shows a *SYCE2KO* male leptotene cell (1) and cells with aligned (2 and 3) and unaligned chromosomes (4). Row 3 shows +/+ female cells in late zygotene (1), pachytene (2 and 4), and zygotene (3). Row 4 shows *SYCE2KO* oocytes with aligned (1 and 3) and unaligned chromosomes (2 and 4). Bar, 5 μm.

DSBs are formed in the *Syce2* mutant but are not efficiently processed

At the leptotene stage of meiosis, DSBs are formed by the topoisomerase-like protein SPO11 and are then resected, leading to invasion of the homologous chromosome. Damage to the genome, including DSBs, is marked by the presence of the phosphorylated form of histone H2AX (γH2AX; Rogakou et al., 1998). The phosphorylation of H2AX is mediated by the kinase ATR, which in turn is recruited by BRCA1 (Turner et al., 2004). γH2AX first appears during premeiotic S-phase, but it is most abundant in leptotene and early zygotene spermatocytes, before synapsis initiation (Mahadevaiah et al., 2001). As synapsis progresses, γH2AX-positive domains decrease and, by late zygotene through to pachytene, only the unpaired sex chromatin shows positive staining. When SYCE2 is absent, γH2AX shows a different dynamic. Although in early stages no difference between wild type and mutant is visible, later stages show only a moderate decrease in γH2AX (Fig. 3). Pachytene-like spermatocytes that show alignment of all the chromosomal complement display a patchy distribution of γH2AX over the aligned AEs. The distribution of γH2AX in mutant females is subtly different from that in males. In females, some cells with unaligned chromosomes display a very close association of the γH2AX-positive domains with the axes (Fig. 3). Analysis of an earlier step of the pathway, namely, BRCA1 distribution, revealed a slightly different picture. In wild-type spermatocytes, BRCA1 staining is first observed in leptotene spermatocytes as a punctate signal on the forming AEs (Turner et al., 2004). By pachytene, the staining becomes continuous, covering the asynapsed axes of the sex chromosomes and rare autosomes that did not synapse (Turner et al., 2004). In the male *SYCE2KO* mutant, however, BRCA1 punctate staining remains strongly associated with the chromosome axes (Fig. 3). Only in cells with little or no chromosome alignment could we see decreased or absent BRCA1 staining (Fig. 3). In female mutant cells, BRCA1 staining shows the same distribution when chromosomes are aligned, but it seems to cover more contiguous regions of the AEs when the alignment is lost. This suggests that DSBs are being formed but do not appear to be processed efficiently, if at all.

To achieve a better understanding of the extent of DSB processing, we studied the distribution of components of meiotic recombination nodules. These included RAD51 and DMC1, two recombinases that are involved in the formation of the nucleoprotein filament and strand invasion; RPA, a single-strand DNA binding protein that localizes to DSBs soon after RAD51; and MSH4, a component of recombination nodules that are found after synapsis is established (for review see Svetlanov and Cohen, 2004). In contrast to our observations on γH2AX and BRCA1, mutant male and female meiosis appear to be very similar with respect to the recombination proteins studied.

Cells that have RAD51, DMC1, RPA, or MSH4 have closely aligned chromosomes (Fig. 4 and not depicted). Although RAD51 and DMC1 seem to disappear from these cells, RPA and MSH4 are unable to follow on the natural progression of meiosis and remain between the AEs until the alignment is lost. This suggests that the recombination process is being halted at some point after RAD51/DMC1 removal and MSH4 loading on to the AE. Also, late recombination foci components whose distribution pattern closely resembles that of chiasmata, MLH1 and -3, were not detected (Fig. 4). Either DSBs are being processed up to the loading of MSH4 but not later or cells do not survive beyond this point.

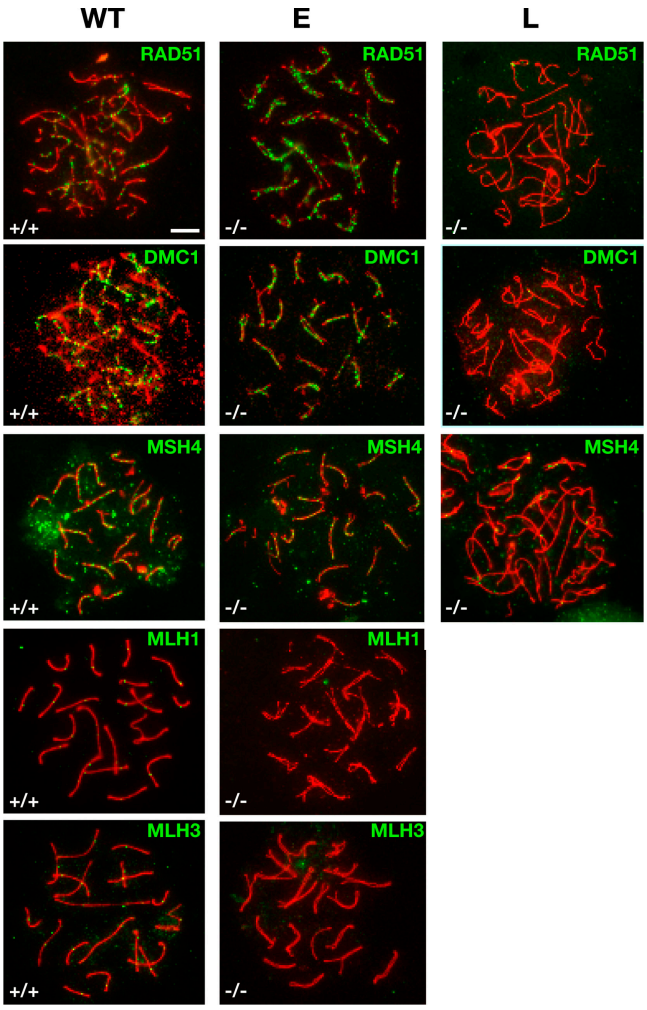

Figure 4. **Distribution of repair and recombination proteins in wild-type and mutant meiosis.** SYCP3 signal is represented in red and recombination and repair proteins in green. Columns show wild type (WT) and early *SYCE2KO* (E) and late *SYCE2KO* (L) stages. Bar, 5 μm.

Syce2 mutants do not form an XY body

As the X and Y are not true homologues, homology is only found along the distal pseudoautosomal region (Ferguson-Smith, 1966). They are the last pair of chromosomes to align and synapse in late zygotene and the first to desynapse from early pachytene. In mutant *Syce2* spermatocytes, contrary to autosomes that show a high degree of alignment, the X and Y chromosomes are only found aligned in ~10% of cells. Like the autosomes, AE composition is normal, except for REC8. REC8 is normally present in reduced levels in the asynapsed regions of the gonosomes (Page et al., 2006), but in mutant spermatocytes the levels of REC8 in the X and Y are comparable to those found in autosomes (Fig. 5). BRCA1 also has an unexpected distribution. When staining is present on the Y, it is limited to a single focus on the tip of the chromosome, which we confirmed to be the pseudoautosomal region (Fig. 5). Similarly, γH2AX shows patchy staining over the autosomes and the X chromosome but is absent from the Y or only present in a small distal region of that chromosome (Fig. 5).

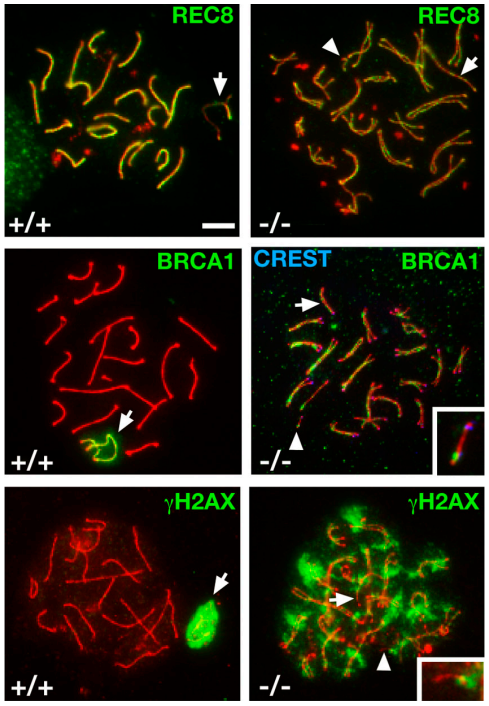

Figure 5. ***SYCE2KO* spermatocytes do not form an XY body.** SYCP3 signal is represented in red; REC8, BRCA1, or γH2AX in green; and CREST staining for centromeres in blue. Arrows in +/+ mark the XY body, whereas −/− unpaired X and Y are marked by arrows and arrowheads, respectively. Insets show higher magnification of the Y chromosome. Bar, 5 μm.

In summary, *SYCE2KO* mice can make DSBs, align homologous chromosomes normally, and initiate recombination processes, but cannot complete SC formation and, in the males, do not form an XY body. A detailed study of the male phenotype of a null mutation in the transverse filament protein SYCP1 (de Vries et al., 2005) describes effects of this mutation that are very similar to those seen in *SYCE2KO* male mice in terms of the localization of recombination and repair proteins. The differences we do see could be due to differences in genetic background or technique, rather than basic biology of the system. The marking of chromosome axes by SYCP1 in zygotene/pachytene cells has been regarded as a hallmark of synapsis (Meuwissen et al., 1992). In *SYCE2KO* males, SYCP1 is not associated with the AE except at the short regions of synapsis, even though this protein is present in the cell. This is the most striking difference we see between the *SYCE2KO* mice and the *Sycp1* knockout animals, as it leads to our model for SC assembly. The nature of the interactions that in wild-type animals result in the C termini of SYCP1 molecules localizing to the AE is not known, although based on protein motifs, this region of the protein has been suggested to have DNA binding activity. Importantly, whatever the molecular basis of these interactions, our observations show that they critically depend on protein–protein interactions at the opposite end of the SYCP1 molecule, which involve SYCE1. The otherwise high degree of similarity is unsurprising, given that both proteins are essential for SC formation. The mutual dependence means that we cannot say whether delocalization of SYCP1 or lack of SYCE2 in the

SYCE2KO mice causes the incomplete DSB repair, lack of crossing over, and failure of XY body formation. Conversely, in the *Sycp1*$^{-/-}$ mice, delocalization of SYCE2 could be responsible for the phenotype. Both proteins, and probably others such as TEX12, are needed to form the functional SC necessary to complete recombination and meiosis.

Materials and methods

Generation of *Syce2*^{gtr1}/*Syce2*^{gtr1} mice

Embryonic stem cell line S8-7E was purchased from the laboratory of P. Soriano (Fred Hutchinson Cancer Research Center, Seattle, WA) as a sequence-verified clone and injected into F1 C57BL6/CBA blastocysts using standard methods. Chimeric males were mated to C57BL6 females and progeny genotyped by PCR (Fig. S1). Animals were intercrossed to generate homozygous *Syce2*^{gtr1}/*Syce2*^{gtr1} mice. Timed mating was used to generate embryonic material, with the plug date set to 0.5 d postcoitum.

Spread chromosomes from males and females were prepared and stained and previously described (Costa et al., 2005, 2006). Images were captured using a system comprising a charge-coupled device camera (Orca-AG; Hamamatsu), a fluorescence microscope (Axioplan II; Carl Zeiss MicroImaging, Inc.) with Plan-neofluar objectives (100× NA 1.3), a 100-W Hg source (Carl Zeiss MicroImaging, Inc.), and quadruple band-pass filter set (model 86000; Chroma Technology Corp.), with the single excitation and emission filters installed in motorized filter wheels (Prior Scientific Instruments). Image capture and analysis were performed using in-house scripts written for IPLab Spectrum (Scanalytics). Images were imported into Photoshop (Adobe), and the curves of individual channels were adjusted for reproduction. Electron microscopy was performed using ultra thin sections of testis tissue fixed in 2.5% glutaraldehyde and 1% OsO_4 as described previously (Liebe et al., 2004).

Staging of mutant testis tubules

In testis sections, the stages of the cycle of the seminiferous epithelium were distinguished as described by Russell et al. (1990). In the absence of spermatids in the *SYCE2KO* mice, epithelial stage IV was identified by the presence of intermediate spermatogonia in late phases of the cell cycle or in mitosis and very early B spermatogonia (Ashley et al., 2004). Images were captured as described in the previous section.

Antibodies used were directed against SYCE1 and -2, SMC3 (Revenkova et al., 2001), and STAG3 (Pelttari et al., 2001). REC8 (Eijpe et al., 2003), SYCP1 (rabbit and guinea pig; Costa et al., 2005), SYCP2 (Offenberg et al., 1998), and SYCP3 antibodies were as described previously (Lammers et al., 1994; Tarsounas et al., 1999; Eaker et al., 2001), and ab12452 was obtained from Abcam. Antibodies directed against DNA damage and recombination proteins were γH2AX (Upstate Biotechnology), BRCA1 (Turner et al., 2004), RAD51 (Abcam), DMC1 (Turner et al., 2004), MSH4 (Her et al., 2001), MLH1 (BD Biosciences), and MLH3 (Lipkin et al., 2002). Antibodies were provided by M.A. Handel (The Jackson Laboratory, Bar Harbor, ME), R. Jessberger (Technische Universität Dresden, Dresden, Germany), C. Heyting (Wageningen Agricultural University, Wageningen, Netherlands), C. Hoog (Karolinska Institutet, Stockholm, Sweden), P. Cohen (Cornell University, Ithaca, NY), and P. Moens (York University, Toronto, Canada).

Online supplemental material

Fig. S1 shows details of gene trap characterization and the histological effect of the knockout in testis. Fig. S2 shows the distribution of cohesins in wild-type and knockout male and female meiotic chromosomes. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200610027/DC1.

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