# Genetic Evidence That Synaptonemal Complex Axial Elements Govern Recombination Pathway Choice in Mice

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**ABSTRACT** Chiasmata resulting from interhomolog recombination are critical for proper chromosome segregation at meiotic metaphase I, thus preventing aneuploidy and consequent deleterious effects. Recombination in meiosis is driven by programmed induction of double strand breaks (DSBs), and the repair of these breaks occurs primarily by recombination between homologous chromosomes, not sister chromatids. Almost nothing is known about the basis for recombination partner choice in mammals. We addressed this problem using a genetic approach. Since meiotic recombination is coupled with synaptonemal complex (SC) morphogenesis, we explored the role of axial elements – precursors to the lateral element in the mature SC - in recombination partner choice, DSB repair pathways, and checkpoint control. Female mice lacking the SC axial element protein SYCP3 produce viable, but often aneuploid, oocytes. We describe genetic studies indicating that while DSB-containing *Sycp3*$^{-/-}$ oocytes can be eliminated efficiently, those that survive have completed repair before the execution of an intact DNA damage checkpoint. We find that the requirement for DMC1 and TRIP13, proteins normally essential for recombination repair of meiotic DSBs, is substantially bypassed in *Sycp3* and *Sycp2* mutants. This bypass requires RAD54, a functionally conserved protein that promotes intersister recombination in yeast meiosis and mammalian mitotic cells. Immunocytological and genetic studies indicated that the bypass in *Sycp3*$^{-/-}$ *Dmc1*$^{-/-}$ oocytes was linked to increased DSB repair. These experiments lead us to hypothesize that axial elements mediate the activities of recombination proteins to favor interhomolog, rather than intersister recombinational repair of genetically programmed DSBs in mice. The elimination of this activity in SYCP3- or SYCP2-deficient oocytes may underlie the aneuploidy in derivative mouse embryos and spontaneous abortions in women.

ANEUPLOIDY is the major cause of birth defects and chromosome abnormalities in humans (Hassold *et al.* 2007). Most are traceable to meiosis I (MI) errors during oogenesis. Erosion of homologous chromosome cohesion is one contributor to age-related increases in oocyte aneuploidy (Chiang *et al.* 2010). Additionally, in most organisms, crossing over is essential to ensure accurate segregation of homologous chromosome pairs at the first meiotic division. Cohesin-stabilized chiasmata physically tether homologous chromosomes, contributing to their eventual congression to, and coalignment at the metaphase plate (Hodges *et al.* 2005; Tachibana-Konwalski *et al.* 2010). There, the pair is held in balance by opposing forces: centromere cohesion and chiasmata maintaining attachment on one hand *vs.* spindle fibers pulling each homolog toward opposite poles. The absence of a crossover (CO) between any chromosome pair can result in random disjunction and aneuploidy, potentially leading to embryonic death or birth defects. How meiocytes create and distribute COs among all chromosomes has been a longstanding subject of research.

In mice, pairing and synapsis of homologs is dependent upon homologous recombination (HR). HR is induced by the genetically programmed creation of >200 double strand breaks (DSBs) in the genome (Plug *et al.* 1996). The distribution of DSBs is not entirely random, and is influenced by DNA sequence, chromatin structure/epigenetic state, and *trans*-acting factors (Getun *et al.* 2010; Parvanov *et al.* 2010; Wu *et al.* 2010). The SPO11-induced DSBs trigger a meiotic DNA damage response, whereby key proteins such as the ATM sensor kinase initate a signaling cascade

Copyright © 2011 by the Genetics Society of America
doi: 10.1534/genetics.111.130674
Manuscript received May 13, 2011; accepted for publication July 1, 2011
Supporting information is available online at http://www.genetics.org/content/suppl/2011/07/12/genetics.111.130674.DC1.
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(including H2AX phosphorylation and p53 activation) that recruits HR proteins to repair the breaks (Burgoyne *et al.* 2007; Lu *et al.* 2010). DSB repair eventually yields noncrossover (NCO) and CO events, with the former being favored in an ~10:1 ratio in mice (Anderson *et al.* 1999; Koehler *et al.* 2002). The CO and NCO pathways are temporally and mechanistically distinct both in yeast (Allers and Lichten 2001; Hunter and Kleckner 2001; Borner *et al.* 2004; Cromie and Smith 2007) and (probably) mice (Guillon *et al.* 2005).

As in mitotic cells, meiocytes have surveillance systems ("checkpoints") to monitor DSB repair (Roeder 1997; Ghabrial and Schupbach 1999; Bhalla and Dernburg 2005). Defects in recombination and/or chromosome synapsis trigger delay or arrest in the pachytene stage of prophase I. This response to meiotic defects is often referred to as the "pachytene checkpoint" (Roeder and Bailis 2000). Persistence of checkpoint-sensed defects can result in gamete apoptosis. These systems are important for preventing the transmission of genetic aberrations to offspring.

The pachytene checkpoint monitors two aspects of meiotic chromosome metabolism in *Saccharomyces cerevisiae* and *Caenorhabditis elegans*: DSB repair and chromosome synapsis (Bhalla and Dernburg 2005; Wu and Burgess 2006). Genetic analyses indicate that mice also have distinct DNA damage and synapsis monitoring/response pathways. Spermatocytes homozygous for a hypomorphic allele (*Trip13$^{Gt}$*) of the yeast Pch2 ortholog exhibit complete homolog synapsis, but undergo pachytene arrest and death due to incomplete DSB repair (Li and Schimenti 2007; Roig *et al.* 2010). SPO11 deficiency, in which meiocytes lack DSBs, also triggers elimination of both oocytes and spermatocytes due to asynapsis (Baudat *et al.* 2000; Romanienko and Camerini-Otero 2000). The distinction in surveillance/checkpoint pathways is also evident by the timing by which mutant oocytes are eliminated. DSB repair-defective mutant oocytes (*e.g.*, *Dmc1*, *Msh5*, and *Trip13$^{Gt}$*) undergo elimination by birth before follicle formation, reflecting a rigorous pachytene DNA damage checkpoint (we will subsequently refer to it as such) identical to that in spermatocytes. To the contrary, strictly asynaptic mutants (*Spo11* and *Mei1*) can form follicles after birth at ~15–20% the WT level, and oocytes contained therein can persist and mature for weeks after birth (Di Giacomo *et al.* 2005; Reinholdt and Schimenti 2005).

Whereas elimination of oocytes with severely defective synaptonemal complexes (SCs) is mediated by the scHop1 ortholog HORMAD1 (Daniel *et al.* 2011), the "synapsis checkpoint" in males may not be a checkpoint *per se*; rather, it appears to be a consequence of disrupted meiotic sex chromosome inactivation (MSCI), which is common in highly asynaptic spermatocytes (Mahadevaiah *et al.* 2008; Royo *et al.* 2010). The mammalian meiotic DNA damage checkpoint pathway components are not well delineated. TRP53 is activated in response to SPO11-induced DSBs in spermatocytes (Lu *et al.* 2010), but it is not yet known whether this pathway is responsible for elimination of spermatocytes with unrepaired DSBs.

Not only must meiocytes repair their DSBs to avoid checkpoint-mediated elimination, but also the template used for homologous recombination repair is crucial. In 4C mitotic cells, there is a strong preference for repairing DSBs using the sister chromatid as template (Kadyk and Hartwell 1992; Stark and Jasin 2003), likely a consequence of their physical association. Since the "purpose" of SPO11-induced DSBs in meiosis is to stimulate recombination between homologous chromosomes, which is ultimately critical for proper disjunction at the first meiotic division, mechanisms have evolved to overcome the predilection for intersister (IS) DSB repair such that recombination between homologs is predominant (Jackson and Fink 1985). Observations in yeast led to the idea that there is a "barrier to sister chromatid recombination" that suppresses IS recombination to favor recombination between homologs (Niu *et al.* 2005). Nevertheless, despite the suppression by such a barrier, intersister meiotic recombination occurs at substantial rates (Schwacha and Kleckner 1997; Goldfarb and Lichten 2010). However, the proportion of DSBs that undergo IS recombination is inhibited so as to enable sufficient amounts of interhomolog (IH) repair (Lao and Hunter 2010).

In *S. cerevisiae*, elements of both the DNA damage checkpoint and the SC influence DSB repair partner choice. The SC is a tripartite structure that defines synapsis and consists of a proteinaceous central element flanked by two chromosome-bound lateral elements. The precursors of lateral elements are called axial elements, and they form before, and as a prerequisite to, mature SC assembly and synapsis. Deletion of yeast genes encoding the axial element proteins Red1 or Hop1 allow repair of DSBs in the absence of the meiosis-specific RecA homolog Dmc1, which is otherwise essential for homologous recombination repair of DSBs and synapsis in yeast and mice (Schwacha and Kleckner 1997; Xu *et al.* 1997; Pittman *et al.* 1998; Yoshida *et al.* 1998; Bishop *et al.* 1999; Carballo *et al.* 2008). Although Red1 or Hop1 deficiency allows bypass of meiotic arrest in *dmc1* yeast, thus resembling the consequences of an ablated checkpoint, the rescue actually occurs because intersister recombination is activated to repair the DSBs (Niu *et al.* 2005). In yeast, the ability to monitor recombination intermediates on 2D gels and to exploit unique mutants that facilitate such analyses (for example, which allow meiosis to occur in haploid cells or which block processing of DSBs) allow direct detection of intersister and interhomolog recombination intermediates.

In mammals, it is assumed but not formally known that a bias to interhomolog repair of meiotic DSBs exists. The complex nature of mammalian gametogenesis greatly hinders the search for genes that might be involved in this process, and strategies such as unbiased genetic screening would be extraordinarily difficult. As an alternative, we drew on the yeast knowledge to hypothesize that axial element proteins in mice might play a role in recombination partner choice or checkpoint function. Here, we report that eliminating either of two such proteins, SYCP2 and SYCP3,

rescues early elimination of recombination-defective oocytes and that this rescue is dependent on RAD54, a protein required for intersister recombination in yeast. We hypothesize that SC axial elements promote interhomolog recombination at the expense of intersister exchange.

## Materials and Methods

### Mice

The mouse alleles were as follows: *Dmc1*, *Dmc1*$^{tm1Jcs}$ (Pittman *et al.* 1998); *Spo11*, *Spo11*$^{tm1Sky}$ (Baudat *et al.* 2000); *Sycp3*, *Sycp3*$^{tm1Hoog}$ (Yuan *et al.* 2000); *Rec8*, *Rec8*$^{mei8}$ (Bannister *et al.* 2004); *Trip13*, *Trip13*$^{Gt(RRB047)Byg}$ (Li and Schimenti 2007); *Rad54*, *Rad54l*$^{tm1Jhjh}$ (Essers *et al.* 1997); *Prkdc*, *Prkdc*$^{scid}$; *Ccnb1ip1*, *Ccnb1ip1*$^{mei4}$ (Ward *et al.* 2007); *Mei1*, *Mei1*$^{m1Jcs}$ (Libby *et al.* 2003); *Sycp2*, *Sycp2*$^{tm1Jw}$ (Yang *et al.* 2006); and *Atm*, *Atm*$^{tm1Led}$ (Elson *et al.* 1996). Genotyping was performed as described in the original publications of these mutations, or in the case of Scid mice, according to The Jackson Laboratory's recommended PCR assay. The diverse origins of the mice necessitated the use of mixed backgrounds. Most of the strains maintained in the colony were bred into the C57BL/6J background, but others contain proportions of FvB, 129, and C3H. Comparisons of compound mutants and controls involving a key mutation (*e.g.*, *Trip13* + *Sycp3*) utilized siblings or pups from the same or related parents.

### Histology and oocyte quantification

Testes or ovaries were fixed in Bouin's, embedded in paraffin, serially sectioned at 5 μm, and stained by hematoxylin and eosin. The ovaries were taken from females that were 3 weeks old, ±2 days. For oocyte quantification, every fifth section was scored for the presence of the following classes of oocytes: primordial, primary, secondary, preantral, and antral, using the criteria of Myers *et al.* (2004). Only those with a visible nucleus were counted. These five categories were grouped into two (primordial and the remaining four categories) for reporting in Figure 2. Note that this method underestimates total oocyte numbers; thus, the oocyte counts reported in Figure 2 are intended for intergenotype comparisons.

### Immunocytochemistry

Immunolabeling of surface-spread spermatocytes and newborn oocytes was performed as described (Bannister *et al.* 2004; Reinholdt *et al.* 2004). To reach conclusions on the pattern of staining for various proteins, 30 (unless otherwise indicated) well-spread nuclei of particular meiotic stages were first identified under the fluorescent microscope on the basis of SYCP3, SYCP1 or STAG3 staining, then imaged at both appropriate wavelengths to determine the pattern of second proteins with focal patterns such as γH2AX, RAD51 or RPA. Unless otherwise indicated, the panels shown in the figures were the exclusive or predominant patterns seen.

Primary antibodies used in this study were as follows: rabbit anti-SYCP1 (1:1,000; a gift from C. Heyting) (Meuwissen *et al.* 1992); mouse anti-γH2AX (1:500, JBW301 Upstate Biotechnology); rabbit anti-STAG3 (1:1,000; a gift from R. Jessberger); guinea pig anti-STAG3 (1:500; a gift from C.Hoog); rabbit anti-RAD51 (1:250; Calbiochem); and rabbit anti-RPA (1:250; a gift from C. Ingles). All secondary antibodies conjugated with either Alexa Fluor 488 or 594 (Molecular Probes) were used at a dilution of 1:1000. All images were taken with a 60X or 100x objective lens (the latter under immersion oil). Graphs and statistical analysis was performed with GraphPad Prism5. Focus number comparisons between genotypes were evaluated using the non-parametric one-tailed Mann-Whitney test.

## Results

### Genetic analyses indicate that *Sycp3*$^{-/-}$ oocytes have an intact DNA damage checkpoint and repair their meiotic DSBs

Spermatocytes lacking SYCP3 undergo arrest and apoptosis in meiotic prophase I as a result of defective chromosome synapsis (Yuan *et al.* 2000). About 2/3 of mutant diplotene oocytes in perinatal *Sycp3*$^{-/-}$ females exhibit markers of DNA damage (RAD51/DMC1, γH2AX, and RPA), and only about 1/3 of *Sycp3*$^{-/-}$ oocytes survive (albeit with elevated aneuploidy) in adult ovaries (Wang and Hoog 2006). While it is not certain if the surviving oocytes are those that have repaired meiotically-induced DSBs [which occur at roughly normal amounts in males as judged by RAD51 focus formation (Yuan *et al.* 2000)], the results suggest that SYCP3 normally promotes conditions for DSB repair and crossing over (Wang and Hoog 2006). Loss of that fraction of *Sycp3*$^{-/-}$ oocytes that do die, particularly those with aneuploidy, occurs by 8 dpp. This prompted Wang and Hoog to propose that the DNA damage checkpoint eliminated them (Wang and Hoog 2006). However, DSB-repair defective mutations like *Dmc1*, *Trip13*, *Rec8* and *Msh5* are subject to the rigorous pachytene DNA damage checkpoint (discussed earlier) that eliminates oocytes shortly after birth before follicle formation (Bannister *et al.* 2004; Di Giacomo *et al.* 2005; Li and Schimenti 2007), thus constituting a temporal inconsistency.

We considered two possible explanations for the substantial survival and fertility of *Sycp3*$^{-/-}$ oocytes in ovaries that also eliminate, in a delayed manner, the fraction of oocytes (2/3) that presumably are those that retained DNA damage at birth. One is that SYCP3 is a component of the DNA damage checkpoint, and its absence allows some *Sycp3*$^{-/-}$ oocytes to escape embryonic meiotic arrest and neonatal apoptosis to allow for subsequent DSB repair. This subset of oocytes would have had extra time to repair DSBs and gain the ability to generate viable offspring. The second possibility is that while meiotic DSBs in the neonatally-surviving oocytes were repaired in an SYCP3-independent manner before checkpoint-mediated elimination, most underwent a degree of repair that was insufficient for viability but sufficient to postpone neonatal death for several days (to 8 dpp).

To distinguish between these possibilities, we adopted a genetic approach that involved epistasis analyses of several

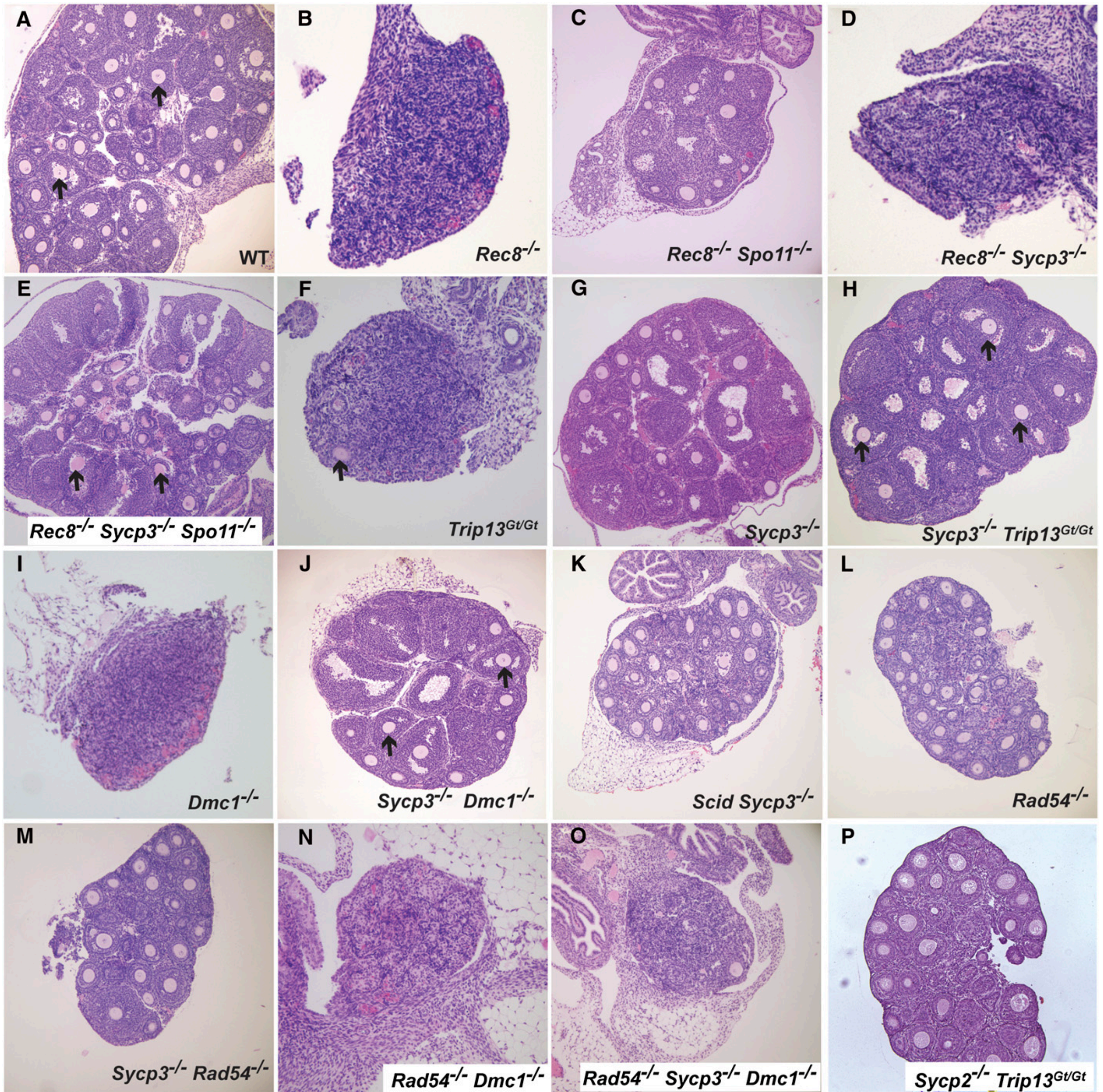


**Figure 1** Ovarian histology of meiotic mutants. Shown are hematoxylin and eosin stained ovaries of 18- to 23-day-old mouse ovaries. Genotypes are indicated in an abbreviated fashion; the actual allele names are given in *Materials and Methods*. Arrows point to oocytes in developing follicles. Note that the oocyte is often out of the section plane in many follicles. At least three animals of each genotype were examined, with consistent qualitative results in all cases.

meiotic mutants. If the DNA damage checkpoint is compromised in *Sycp3*$^{-/-}$ oocytes, then the absence of SYCP3 should rescue other DSB repair defective mutants. REC8 is a meiosis-specific cohesin that is required for fertility in both sexes. *Rec8* mutant spermatocytes show precocious separation of sister chromatids and persistent markers of DSBs (Bannister *et al.* 2004), consistent with reduced homologous recombination (HR) repair as in *S. pombe* (De Veaux *et al.* 1992). Young mutant females have residual ovaries that are devoid of follicles (Figure 1B) (Bannister *et al.* 2004), indicative of DNA damage checkpoint-mediated oocyte elimination (Di Giacomo *et al.* 2005). *Spo11* deletion rescued the oocyte loss, supporting this interpretation (Figure 1C), but *Sycp3* deletion did not (Figure 1D). This was not a consequence of an undefined synthetic interaction between the *Sycp3* and *Rec8* mutations, as *Spo11* deletion restored oocyte survival in *Rec8*$^{-/-}$ *Sycp3*$^{-/-}$

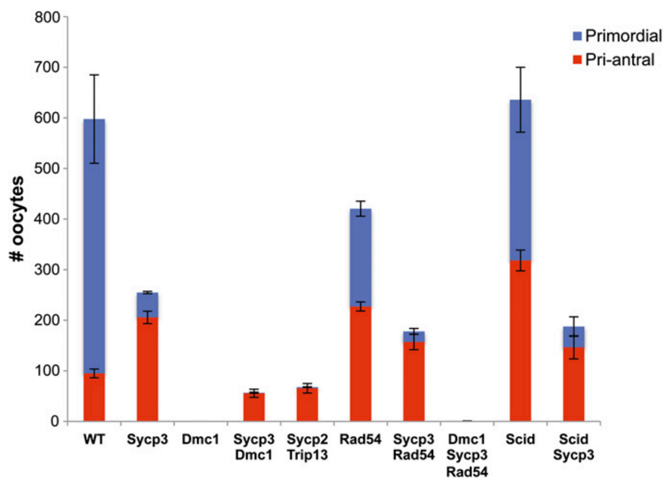


**Figure 2** Oocyte counts from mutant genotypes. For each genotype category listed at the bottom, presence of the gene names refers to homozygosity for the mutant allele. The red-coded class ("pri-antral") represents the sum of primary, secondary, preantral, and antral oocytes. Error bars = SEM. $N \geq 4$ ovaries for all genotypes. The wild type (WT) samples ($N = 6$) included heterozygote littermates of mutants.

*Spo11*$^{-/-}$ triple mutants (Figure 1E), thus indicating that DNA damage triggers oocyte loss in *Rec8*$^{-/-}$ *Sycp3*$^{-/-}$ mutants. Similarly, mutation of *Sycp3* did not rescue *Atm*$^{-/-}$ oocytes (data not shown), which also undergo pachytene checkpoint elimination in a *Spo11*-dependent manner (Di Giacomo *et al.* 2005). The robust oocyte elimination in *Atm*$^{-/-}$ *Sycp3*$^{-/-}$ and *Rec8*$^{-/-}$ *Sycp3*$^{-/-}$ females indicates that the DNA damage checkpoint is not compromised in *Sycp3*$^{-/-}$ oocytes, consistent with what was suggested (Wang and Hoog 2006). Furthermore, this implies that viable oocytes in *Sycp3*$^{-/-}$ adults underwent adequate repair of meiotic DSBs before execution of the DNA damage checkpoint.

### *The meiotic DSB repair proteins TRIP13 and DMC1 become nonessential for oocyte survival in the absence of SYCP3*

To identify the means by which *Sycp3*$^{-/-}$ oocytes conduct DSB repair, we constructed compound mutants between *Sycp3* and other genes that disrupt particular repair pathways. *Trip13* is required for meiotic DSB repair and IH recombination in mice (Li and Schimenti 2007; Roig *et al.* 2010). The *Trip13*$^{Gt(RRB047)Byg}$ allele (abbreviated *Trip13*$^{Gt}$) appears to affect primarily NCO recombination, causing meiotic arrest and infertility in both sexes (Li and Schimenti 2007). *Trip13*$^{Gt/Gt}$ oocytes undergo death around the time of birth, prior to follicle formation, indicative of the pachytene DNA damage checkpoint-mediated elimination (Di Giacomo *et al.* 2005). As reported previously (Li and Schimenti 2007), C57BL/6J-*Trip13*$^{Gt/Gt}$ ovaries (3 weeks old) are severely dysplastic due to complete absence of primordial and primary follicles and complete or nearly complete absence of more developed follicles (Figure 1F; $N = 4$ ovaries from two females had zero follicles; $N = 2$ ovaries from one female had a total of four). This stands in contrast to WT or *Sycp3*$^{-/-}$ ovaries (Figure 1, A and G), which have hundreds (Figure 2). The ovarian and follicular agenesis of *Trip13*$^{Gt/Gt}$ oocytes is dependent upon SPO11 and MEI1, two proteins required for meiotic DSB formation, indicating that oocyte death is triggered by defects in DSB repair (Li and Schimenti 2007). Surprisingly, *Sycp3* deletion rescued the near-complete elimination of *Trip13*$^{Gt/Gt}$ oocytes, as visualized by the presence of numerous developing follicles in the doubly mutant ovaries (Figure 1H). However, primordial follicles were conspicuously absent; these are also depleted severalfold in *Sycp3* single mutants compared to WT (Figure 2). Despite the presence of the growing follicles, *Trip13*$^{Gt/Gt}$, *Sycp3*$^{-/-}$ females were infertile ($N = 3$; they failed to produce any litters after several months mating to fertile males).

Because *Trip13*$^{Gt/Gt}$ mutants allow chromosome synapsis and thus progression to pachynema (in spermatocytes) before being eliminated due to persistent DSBs, we tested whether deletion of *Sycp3* could rescue a more severe recombination mutant, a *Dmc1* null. *Dmc1*$^{-/-}$ meiocytes fail to conduct homologous recombination repair (HRR) of DSBs, leading to zygotene/pachytene elimination that can be alleviated (in oocytes) by genetically abrogating meiotic DSBs (Pittman *et al.* 1998; Di Giacomo *et al.* 2005; Reinholdt and Schimenti 2005). Histology of ~3-week-old postnatal *Dmc1*$^{-/-}$ *Sycp3*$^{-/-}$ ovaries revealed a rescue of the absolute oocyte depletion and ovarian agenesis characteristic of *Dmc1* single mutants (Figure 1, I and J). The rescue was incomplete, as the primordial oocyte pool was essentially depleted, and the more developed pool (primordial–antral stages) was lower than in WT and *Sycp3* single mutants (Figure 2).

If the survival of *Dmc1*$^{-/-}$ *Sycp3*$^{-/-}$ oocytes was enabled by enhanced DSB repair, we would expect a decrease of damage markers compared to *Dmc1* single mutants. To test this, surface spread nuclei from newborn oocytes were immunostained for RAD51 and RPA, recombination proteins that form detectable foci at DSB sites during early and intermediate stages of meiotic DSB repair. Indeed, median numbers of RAD51 and RPA foci were significantly lower in *Sycp3*$^{-/-}$ *Dmc1*$^{-/-}$ oocytes *vs.* *Dmc1*$^{-/-}$, though higher than in *Sycp3*$^{-/-}$ oocytes (Figure 3; supporting information, Figure S3). These data indicate that an alternative, DMC1-independent DSB repair pathway(s) is activated in SYCP3-deficient oocytes, but that the DMC1 pathway remains functional unless disrupted.

One such alternative DMC1-independent pathway could be interhomolog CO recombination which, as indicated earlier, occurs at a much lower rate than NCO recombination in mice. However, mice lacking *Ccnb1ip1*, which is required for CO recombination specifically (Ward *et al.* 2007), did not negatively impact survival of *Sycp3*$^{-/-}$ oocytes (not shown). To test the possibility that nonhomologous end-joining (NHEJ) is hyperactivated to repair meiotic DSBs in *Sycp3*$^{-/-}$ oocytes, double mutants with *Prkdc* (Scid) were constructed.

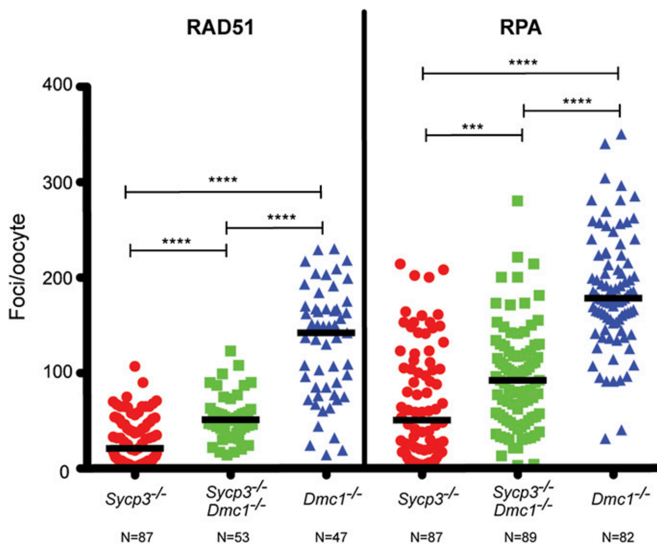


**Figure 3** Immunocytological evidence that SYCP3 deficiency increases DSB repair in *Dmc1* null oocytes. Plotted are numbers of foci/oocyte for the indicated markers (RAD51 or RPA) in surface-spread chromosomes of newborn oocytes. Each represents a single oocyte. The indicated number of oocytes were scored, and these oocytes were obtained from three pups/genotype. Black horizontal lines in the graph indicate median values. Asterisks represent the significance levels (by Mann–Whitney test): $^{***}P = 0.0004$; $^{****}P < 0.0001$.

This genotype did not ablate oocyte survival either (Figure 1K; Figure 2).

In summary, in the absence of an intact SC (SYCP3 deficiency), a state triggering arrest and apoptosis in spermatocytes (Yuan *et al.* 2000), IH recombination is no longer absolutely essential for all or most meiotic DSB repair in oocytes. Additionally, NHEJ does not appear to be responsible for DSB repair and survival of *Sycp3*$^{-/-}$ oocytes. The question then becomes: What DSB repair pathway is adopted by *Sycp3*$^{-/-}$ oocytes?

#### *Genetic evidence that SYCP3 governs recombination partner choice*

In S-G2 mitotic cells, DSBs are repaired preferentially via IS recombination, where proximity and cohesion of sister chromatids predisposes to this outcome (Kadyk and Hartwell 1992; Stark and Jasin 2003; Watrin and Peters 2006). It has been hypothesized that in many species [including budding yeast (Schwacha and Kleckner 1997), but possibly to a lesser degree in fission yeast (Cromie *et al.* 2006)], the barrier to sister chromatid recombination (BSCR) overcomes this preference in order to drive IH recombination and thus ensure proper disjunction at MI. Alternatively or in addition, there may exist an active stimulus of IH repair at the expense of IS recombination, as suggested for *Schizosaccharomyces pombe* (Latypov *et al.* 2010).

Since SYCP3 is required for elimination of oocytes with certain DSB repair defects but does not appear to be a checkpoint protein *per se*, we hypothesized that it might have a role in regulating recombination partner choice. Specifically, if SYCP3 promotes DSB repair via IH recombination and/or functions as a component of the BSCR, then deleting *Sycp3* would permit IS recombination, allowing DSB repair in the context of IH repair deficiency (*e.g.*, *Dmc1*$^{-}$ or *Trip13*$^{Gt}$). This hypothesis can be tested by attempting to override the *Sycp3*$^{-/-}$ rescue of an IH recombination mutant by disabling IS recombination repair.

In *S. cerevisiae*, IH recombination is driven by Dmc1 in concert with Rad51, but Rad51-driven IS recombination can occur efficiently in the absence of the BSCR and Dmc1 (see *Discussion* for elaboration). Intersister recombination is normally repressed in meiotic cells, presumably by downregulation of Rad51. Optimal Rad51 activity in yeast meiosis is dependent upon interaction with Rad54 to enhance strand invasion of a Rad51 presynaptic filament (Raschle *et al.* 2004; Heyer *et al.* 2006; Busygina *et al.* 2008; Niu *et al.* 2009). Rad54 in yeast is required to stimulate IS recombination. It can rescue *dmc1* mutations under certain circumstances, particularly when axial element (AE) structure is disrupted (Arbel *et al.* 1999; Bishop *et al.* 1999). On the basis of analogy to yeast, we reasoned that IS recombination in mice might be suppressed by *Rad54* deletion, which alone does not ablate meiosis in either sex (for oocytes, see Figure 1L) (Essers *et al.* 1997). We therefore constructed triple mutants (*Dmc1*$^{-/-}$ *Sycp3*$^{-/-}$ *Rad54*$^{-/-}$) and examined the effects on oocyte survival. Whereas *Sycp3*$^{-/-}$ *Dmc1*$^{-/-}$ and *Sycp3*$^{-/-}$ *Rad54*$^{-/-}$ oocytes can escape the neonatal DNA damage checkpoint (Figures 1, J and M; unlike *Dmc1*$^{-/-}$ and *Dmc1*$^{-/-}$ *Rad54*$^{-/-}$ animals, Figure 1, I and N; also Figure 2), the triply mutant ovaries were devoid of oocytes (Figure 1O; Figure 2), demonstrating that rescue of *Dmc1* mutants by SYCP3 deficiency requires RAD54. Interestingly, *Rad54*$^{-/-}$ single mutants had significantly fewer total oocytes (~25% less) than WT, attributable to ~60% fewer primordial follicles (Figure 2). Given that RAD54 deficiency causes abnormal persistence of RAD51 foci in pachytene spermatocytes despite apparently normal fertility in these mice (Wesoly *et al.* 2006), this may be a reflection of RAD54 involvement in mammalian IS recombination as it is in yeast (see *Discussion*). Overall, these experiments suggest that activation of IS recombination rescues *Sycp3*$^{-/-}$ *Dmc1*$^{-/-}$ oocytes, and that SYCP3 functions either to inhibit homology-directed IS repair or to promote IH repair at the expense of IS repair. It also raises the possibility that IS recombination may occur in WT oocytes and that loss of IS recombination may have some consequence. These interpretations imply that the escape of *Sycp3*$^{-/-}$ *Rad54*$^{-/-}$ oocytes from the DNA damage checkpoint occurs either by IH repair entirely (presumably DMC1 mediated) or a combination of IS and IH repair conducted by DMC1. In any case, the 60% reduction of primordial oocytes in these mutants compared to *Sycp3* single mutants highlights a role for RAD54 in meiosis, at least in certain non-WT conditions.

SYCP3 is just one component of SC axial/lateral elements, so it is conceivable that the rescue effects with

*Sycp3*$^{-/-}$ may reflect a general role for the SC in partner choice/BSCR function. Supportive of this possibility is that although axial elements form on pachytene chromosomes of SYCP3-deficient oocytes, they are abnormal and have discontinuities in the axis structure (Kouznetsova *et al.* 2005). If the axial element itself is governing partner choice, this would predict that deletion of other axial element components would have similar effects. *Sycp3* deletion prevents SYCP2 loading onto meiotic chromosomes, and conversely, the coiled-coil domain of SYCP2 is required for loading of SYCP3 onto axial elements (Yang *et al.* 2006). Thus, we predicted that deletion of *Sycp2* would also render oocytes to be nondependent on IH recombination for DSB repair. Consistent with this, we found that ovaries from *Sycp2*$^{-/-}$ *Trip13*$^{Gt/Gt}$ females exhibited substantial numbers of surviving oocytes, unlike *Trip13*$^{Gt}$ single mutants (Figure 1P; Figure 2), but like *Sycp3*$^{-/-}$ *Trip13*$^{Gt/Gt}$.

## Discussion

There is now molecular and genetic evidence from multiple systems that although IS recombination occurs during normal meiosis, (Schwacha and Kleckner 1994; Cromie *et al.* 2006) and can be relatively frequent at least at hemizygous loci (Goldfarb and Lichten 2010), it is attenuated by mechanisms that enable sufficient IH recombination for disjunction of chromosomes during the reductional division. Since IS recombination is decreased even in the absence of a homologous chromosome (Callender and Hollingsworth 2010), it appears that the IH preference is mainly or partly due to inhibition of IS interactions. In *S. pombe*, however, an organism lacking SC and which has a higher IS:IH recombination ratio, it is possible that IH recombination is stimulated to enable a sufficient number of crossovers (Latypov *et al.* 2010).

Little is known about the incidence of IS recombination in mammalian meiosis. Sister chromatid exchange (SCE) has been observed in hamster and mouse spermatocytes (Kanda and Kato 1980; Allen and Gwaltney 1984), albeit rarely and with a preference for the sex chromosomes (Allen and Latt 1976). Aside from the small pseudoautosomal region (PAR) that synapses between the X and Y, it is presumed that repair of the remaining DSBs requires IS recombination. However, IS "crossovers" that result in SCEs are estimated to constitute only ~17–25% of all IS recombination events in yeast (Goldfarb and Lichten 2010), suggesting that most IS events will be undetectable by the classical cytological method to visualize SCEs (typically involving differential labeling of chromatids by BrdU). Another implication that IS recombination is rare in normal mammalian meiosis can be inferred by comparison to Dmc1-deficient fission yeast, which have normal spore viability but decreased crossing over (Fukushima *et al.* 2000). *Dmc1*$^{-/-}$ mice, however, undergo complete pachytene arrest and death of meiocytes (Pittman *et al.* 1998) due to the DSB damage checkpoint (Di Giacomo *et al.* 2005). It is possible that the DSB repair in *dmc1* fission yeast is due to eventual Rad51-mediated IS + IH recombination (see below), whereas IS repair in mutant mice is infrequent despite RAD51 focus formation, with the possible exception of the asynapsed regions of the X and Y chromosomes. Notably, if indeed XY DSB repair is essential for male fertility, it must not be absolutely RAD54 dependent (*Rad54*$^{-/-}$ males are fertile). It is possible that DMC1 repairs these DSBs in *Rad54* mutants, similar to the ability of *S. cerevisiae* Dmc1 to conduct intersister repair of DSBs in haploid meiotic cells under certain circumstances (Callender and Hollingsworth 2010).

Intersister recombination, which is efficient in mitotically growing cells, is driven by Rad51 and stimulated by Rad54, a member of the SWI/SNF class of translocases. Rad54 enhances strand invasion of a Rad51 presynaptic filament (Raschle *et al.* 2004; Heyer *et al.* 2006; Sung and Klein 2006). Although Rad51 supports efficient Dmc1-mediated IH recombination in yeast meiosis, the independent activity of Rad51 is inhibited by at least two mechanisms that block Rad54/Rad51 complex formation or synergy: (1) phosphorylation of Rad54 by Mek1 and (2) the action of Hed1 (Tsubouchi and Roeder 2006; Busygina *et al.* 2008; Niu *et al.* 2009). Deletion of BSCR proteins such as Hop1 or Red1 prevents Mek1 activation, thus releasing Rad51 inhibition and allowing IS repair to a degree that can rescue *dmc1* mutant yeast (Sheridan and Bishop 2006; Niu *et al.* 2007). Importantly, not only do mammals have orthologs of these key recombination proteins (DMC1, RAD51, and RAD54), but also the damage signaling molecules involved in determining IH bias, including the sensor kinases (ATM/ATR) and the Hop1 mediator (HORMAD1 and/or HORMAD2) (Wojtasz *et al.* 2009).

In the absence of myriad tools and biological advantages in yeast that permit precise analysis of meiotic recombination events, such as the ability to visualize meiotic recombination intermediates molecularly, we must rely on informed interpretation of phenotypes we observed here. We believe the data best support a model in which the rescue of IH recombination-deficient oocytes by *Sycp3* or *Sycp2* deletion is attributable to altered recombination partner choice. Specifically, we hypothesize that SC axial elements facilitate IH recombination and inhibit IS recombination, possibly as part of the same mechanism.

The following experimental results support this hypothesis. First, rescue of *Dmc1*$^{-/-}$ and *Trip13*$^{Gt/Gt}$ oocytes by *Sycp3* deletion does not appear to be a result of checkpoint ablation, since other DSB-repair defective mutants (*Rec8* and *Atm*) were not rescued by SYCP3 deficiency. This, in conjunction with the observation of reduced levels of DSB markers in *Dmc1*$^{-/-}$ *Sycp3*$^{-/-}$ *vs. Dmc1*$^{-/-}$ surviving oocytes, and that *Scyp3* single mutants appear to have an intact DSB repair checkpoint, suggests that DSBs were repaired via an alternative pathway in the double mutants. Second, the alternative pathway is not NHEJ exclusively because *Prkdc*$^{-/-}$ *Sycp3*$^{-/-}$ contained ample follicles at birth. It also does not appear to be DMC1-independent IH recombination; otherwise, we

might expect restored fertility and synapsis in *Sycp3*$^{-/-}$ *Dmc1*$^{-/-}$ oocytes. Furthermore, *Sycp3* single mutants have reduced levels of crossing over (chiasmata) even in the presence of DMC1 (Wang and Hoog 2006), arguing against stimulation of such a pathway. Nevertheless, in consideration of yeast data, we do not rule out the possibility that RAD51 or RAD51 paralogs, in conjunction with RAD54, conduct some degree of interhomolog noncrossover recombination in *Dmc1*$^{-/-}$ *Sycp3*$^{-/-}$ oocytes, which is insufficient to cause synapsis and crossovers (Bishop *et al.* 1999). Third, deletion of *Rad54*, which is critical for IS recombination in *S. cerevisiae*, prevented rescue of *Sycp3*$^{-/-}$ *Dmc1*$^{-/-}$ oocytes. Importantly, the *in vitro* functions and activities of RAD54 are highly conserved (Mazin *et al.* 2010). Both human and mouse orthologs have branch migration activity that is promoted by Rad51 (Bugreev *et al.* 2006; Rossi and Mazin 2008), and they stimulate the Mus81-Eme1/Mms4 endonuclease that resolves Holliday junctions (Mazina and Mazin 2008; Matulova *et al.* 2009). RAD54 is also important for repair of induced DNA damage by IS repair in mouse cells (Mills *et al.* 2004). Interestingly, we observed a modest (~2-fold) decrease in the primordial oocyte pool in *Rad54* mutants, raising the possibility that IS recombination plays a significant role in normal oocytes. Finally, that *Sycp2* deletion also rescued *Trip13*$^{Gt/Gt}$ oocytes supports the notion that the SC axial element structure itself, rather than any specific components, drives the IH preference for homologous recombination-mediated DSB repair.

Whether the same putative partner choice phenomena apply to male meiosis remains an open question. Indeed, we evaluated males of all the genotypes described for females in this study. However, analyses of spermatocytes are confounded by the fact that the timing of meiotic arrest in mutants is the same regardless of whether the defect is asynapsis (*e.g.*, *Spo11*$^{-}$), DSB repair (*e.g.*, *Trip13*$^{Gt}$), or both (*e.g.*, *Dmc1*$^{-}$) (Barchi *et al.* 2005; Li and Schimenti 2007). This complicates assessment of SYCP3's role as a checkpoint protein *vs.* a recombination choice factor, since *Sycp3* mutants themselves undergo zygotene/pachytene arrest with failed synapsis. For example, if SYCP3 were to have DSB repair checkpoint function exclusively, then its deletion might be expected to allow progression of *Trip13*$^{Gt/Gt}$ spermatocytes through meiosis. However, histological and immunocytological analysis of doubly mutant spermatocyte chromosomes confirmed expectations that *Sycp3* is epistatic to *Trip13* (Figure S1, a–d) (Yuan *et al.* 2000). That is, the double mutants arrested in a state resembling *Sycp3* single mutants, displaying extensive asynapsis marked by γH2AX, which is indicative of meiotic silencing of unsynapsed chromatin (MSUC), which in turn disrupts XY silencing (Turner *et al.* 2005). Neither occurs in *Trip13*$^{Gt/Gt}$ spermatocytes (Figure S1c) (Li and Schimenti 2007). Similarly, *Sycp3* deletion did not ameliorate spermatogenic arrest or the meiotic chromosomal defects in spermatocytes deficient for *Dmc1* or *Rec8* (Figure S2, a and b); in fact, it appeared to disrupt asynaptic homolog pairing that occurs in *Rec8* mutants (Figure S2b) (Bannister *et al.* 2004; Xu *et al.* 2005).

Despite the genetic data supporting a role for SYCP3 in recombination partner choice, we caution that other explanations are conceivable. We concluded that SYCP3 is not strictly a DNA damage checkpoint protein because its deletion failed to rescue *Rec8*$^{-/-}$ or *Atm*$^{-/-}$ oocytes, both of which undergo SPO11-dependent checkpoint elimination. Additionally, a DNA damage checkpoint appears to remain intact in *Sycp3* single mutants. However, we cannot exclude the possibility that SYCP3 and/or SYCP2 are checkpoint proteins solely responsible for detecting lesions left by certain mutants including *Trip13* and *Dmc1*. Still, the case of *Rec8* mutants may be consonant with a role of SYCP3 in blocking IS recombination. Since IH recombination is defective in *Rec8*$^{-/-}$ oocytes, and cohesins are required for efficient repair of DSBs by sister chromatid recombination (Sjogren and Nasmyth 2001; Cortes-Ledesma and Aguilera 2006; Sjogren and Strom 2010), this could explain the lack of rescue by *Sycp3* deletion. The situation with *Atm* mutants is more difficult to interpret. Their chromosomal defects—including chromosome fragmentation and chromosome axis disruption—may be of a nature that cannot be repaired by IS recombination (in *Sycp3* mutants) to a degree that allows bypass of damage and spindle checkpoints (Xu *et al.* 1996; Barchi *et al.* 2008). Alternatively, ATM, which is a key DNA damage response factor in somatic cells and leptotene spermatocytes (Bellani *et al.* 2005), may be required for triggering efficient repair by both IH and IS recombination.

Another caveat is that the reduced rate of DSB repair in *Sycp3*$^{-/-}$ oocytes (Wang and Hoog 2006) is not easily reconciled with our hypothesis that the axial element (AE) promotes IH bias, at least in part by inhibiting IS repair. We suggest two possible explanations. One is that certain proteins involved in IS recombination are limiting in oocytes (for example, RAD51), such that, whereas IS interactions are favored, processing of recombination intermediates is hampered. Another possibility is that the AE not only inhibits IS recombination, but also enhances IH recombination to a greater relative degree. In this scenario, the decreased efficiency of IH recombination slows overall DSB repair, increasing the ratio of IS:IH recombination, leading to the observed reduction in crossovers, elevated aneuploidy, and checkpoint-mediated elimination of many oocytes before they complete DSB repair (Wang and Hoog 2006).

Our data show that the SC proteins SYCP2 and SYCP3 are required for the complete elimination of oocytes that are defective for repair of IH meiotic DSBs by homologous recombination, and genetic evidence suggests that SYCP3 does so by inhibiting IS recombination. Our results, considered in conjunction with data from budding yeast and mice, lead us to propose that intact axial elements, the precursors of the lateral element of the mature SC, constitute the critical organizer of recombination pathway and partner bias in mammalian meiosis. As diagrammed in Figure 4, the basic tenet of this model is that RAD51/DMC1 nucleoprotein filaments that form

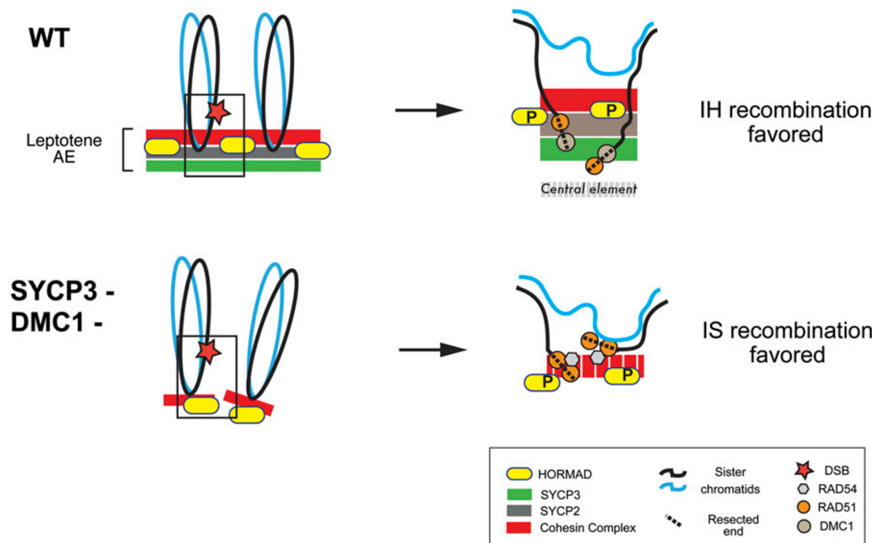


**Figure 4** Model for role of axial element in promoting interhomolog recombination bias. (Top) DSBs occur during leptonema. In yeast it is thought that DSBs are dependent upon AE formation, in part because mutations of AE proteins such as Red1 and Hop1 decrease DSB levels (Zickler and Kleckner 1999). However, the temporal relationship is not clear in mice because RAD51/DMC1 foci (surrogates of DSBs) appear concurrent to and colocalize with AE components. Nevertheless, numerous immunocytological studies show that the DSB ends become localized to the AE cores. RAD51 and DMC1 foci in normal meiosis also colocalize to nascent AEs and HORMADs in leptonema (Barlow *et al.* 1997; Wojtasz *et al.* 2009) and actually directly interact with SYCP3 (Tarsounas *et al.* 1999). (Bottom) Localization of SYCP2 is dependent upon SYCP3, so as indicated, loss of SYCP3 results in aberrant pseudoaxial elements, or cores, consisting only of cohesin proteins such as REC8, SMC1β, and STAG3 (Pelttari *et al.* 2001; Fukuda *et al.* 2010). In our model, the disrupted AE structure caused by SYCP2/SYCP3 absence, which also causes discontinuities in the cohesin complex (dashed red line) allows the RAD51-bound DSB ends (depicted here in *Dmc1* mutants) to have unimpeded access to the sister and will recombine in a RAD54-dependent manner. See text for more details.

at resected ends of DSBs are oriented by, or bound to AE components (possibly SYCP2 and/or SYCP3) in such a way as to inhibit interaction with the sister chromatid, while spatially favoring homologous chromosome interactions.

Yeast-based models to explain IH bias have in common either a physical orientation of DSB ends toward the SC central element (and homologous chromosome), highlighting specific molecules such as the Rec8 cohesin (Kim *et al.* 2010) or Hed1 and Mek1 (Sheridan and Bishop 2006) or emphasizing local chromatin modifications that have a similar consequence (Goldfarb and Lichten 2010). It is not clear whether the mechanisms of homolog bias will be the same in mammals, given our limited state of knowledge. Important similarities are that essential components of the IH bias/barrier to sister chromatid recombination in yeast are axial element proteins (Red1, Mek1, and Hop1), as are SYCP3 and SYCP2 in mice (our data), as well as the cohesin Rec8. Mice have two Hop1 orthologs, HORMAD1 and HORMAD2, that become colocalized to nascent AEs ("cores") during leptonema and are removed upon synapsis, consistent with a role conserved with Hop1 and/or Red1 (Wojtasz *et al.* 2009; Fukuda *et al.* 2010). Additionally, *Hormad1* mutants have decreased DSB levels as do *hop1* yeast (Shin *et al.* 2010), but it is not known whether *Hormad1* has a role in partner choice. On the other hand, there are some differences between the organisms. Notably, SYCP3 is not required for DSB formation unlike yeast AE proteins, and REC8 is not required for AE formation in mice as it is in *S. cerevisiae* (Bannister *et al.* 2004). These differences may have to do with the higher number of axis proteins present in mammalian chromosomes *vs.* yeast, especially cohesins (Revenkova *et al.* 2010). Furthermore, *Rec8* mutation is so severe (*Rec8*$^{-/-}$ oocyte elimination is SPO11 dependent) that it cannot be determined whether it influences initial partner choice as it does in yeast (Kim *et al.* 2010). However, it is possible that as in the Kim *et al.* (2010) model for yeast, SYCP3 may function in part to locally disrupt the bias toward IS recombination that REC8 otherwise promotes early after DSB formation. Alternatively, our results may be consistent with the Kim *et al.* (2010) finding that in later stages of recombination, yeast Rec8 acts to enforce IH bias. Kouznetsova *et al.* (2005) observed that the lateral axis SC structure in *Sycp3*$^{-/-}$ oocytes has discontinuities in staining for cohesins including STAG3 and REC8. These local disruptions of the cohesin complex may compromise IH bias. A final difference with yeast, in which Tel1 and/or Mec1 phosphorylate Hop1 in response to Spo11 DSBs (Carballo *et al.* 2008), is that mouse Hop1 (HORMAD1) appears to act upstream of ATM and ATR (Shin *et al.* 2010). HORMAD1 is indeed phosphorylated, but the kinase remains unknown, as does the potential ortholog of the downstream effector Mek1.

HORMAD1 coimmunoprecipitates with SYCP3, SYCP2, and AE-bound cohesins, but it does not require SYCP3 to colocalize to aberrant core-like structures that are defective due to lack of SYCP3 (Fukuda *et al.* 2010). Therefore, we conclude that HORMAD1 loss is not sufficient for the phenomena (rescue of *Dmc1* or *Trip13* mutants by *Sycp3* deletion) we observe here. Conversely, SYCP3 does not require HORMAD1 to integrate into AEs (Shin *et al.* 2010). Recently, evidence has been presented that HORMAD1 depletion depresses SPO11-induced DSBs and is involved in the oocyte checkpoint that detects asynapsis (Daniel *et al.* 2011). It remains to be seen whether all the AE components (other than REC8, the deletion of which causes early oocyte death) are important for maintaining IH bias. We favor the idea that the overall AE structure is critical, and that loss of certain individual components may phenocopy the effects we have observed here.

The control of meiotic recombination and partner choice is of high relevance to human health. This is indicated by the

phenotype of *Sycp3*$^{-/-}$ mice. They manage to conduct DSB repair but, as a result of decreased crossing over, undergo oocyte loss and produce aneuploid gametes. SYCP3 mutations in human females have been associated with recurrent pregnancy loss, suggestive of fetal chromosome abnormalities (Bolor *et al.* 2009). Nevertheless, obtaining proof that axial elements control recombination partner choice awaits the development of effective methodologies for assaying sister chromatid recombination in meiocytes. This is complicated by the fact that oocytes undergo meiosis *in utero*. Such analyses may be made possible by developments that allow cytological analyses of chromosome exchanges, molecular analysis of individual DSB repair events, or bulk physical studies of recombination intermediates that occur at strong "hotspots."

## Acknowledgments

The authors thank Sarah Zanders and Eric Alani for advice on the manuscript and Michael Lichten, Scott Keeney, and Paula Cohen for helpful discussions. We are indebted to P. Jeremy Wang for providing *Sycp2* mice, Christer Hoog for *Sycp3* mice (via Paula Cohen), and Roland Kanaar for *Rad54* mice. This work was supported by National Institutes of Health grant R01 GM45415 to J.C.S.

## Literature Cited

Allen, J. W., and S. A. Latt, 1976 In vivo BrdU-33258 Hoechst analysis of DNA replication kinetics and sister chromatid exchange formation in mouse somatic and meiotic cells. Chromosoma 58: 325–340.

Allen, J. W., and C. W. Gwaltney, 1984 Sister chromatid exchanges in mammalian meiotic chromosomes. Basic Life Sci 29 Pt B: 629–645.

Allers, T., and M. Lichten, 2001 Differential timing and control of noncrossover and crossover recombination during meiosis. Cell 106: 47–57.

Anderson, L. K., A. Reeves, L. M. Webb, and T. Ashley, 1999 Distribution of crossing over on mouse synaptonemal complexes using immunofluorescent localization of MLH1 protein. Genetics 151: 1569–1579.

Arbel, A., D. Zenvirth, and G. Simchen, 1999 Sister chromatid-based DNA repair is mediated by *RAD54*, not by *DMC1* or *TID1*. EMBO J. 18: 2648–2658.

Bannister, L. A., L. G. Reinholdt, R. J. Munroe, and J. C. Schimenti, 2004 Positional cloning and characterization of mouse *mei8*, a disrupted allele of the meiotic cohesin *Rec8*. Genesis 40: 184–194.

Barchi, M., S. Mahadevaiah, M. Di Giacomo, F. Baudat, D. G. de Rooij *et al.*, 2005 Surveillance of different recombination defects in mouse spermatocytes yields distinct responses despite elimination at an identical developmental stage. Mol. Cell. Biol. 25: 7203–7215.

Barchi, M., I. Roig, M. Di Giacomo, D. G. de Rooij, S. Keeney *et al.*, 2008 ATM promotes the obligate XY crossover and both crossover control and chromosome axis integrity on autosomes. PLoS Genet. 4: e1000076.

Barlow, A., F. Benson, S. West, and M. Hultén, 1997 Distribution of the Rad51 recombinase in human and mouse spermatocytes. EMBO J. 16: 5207–5215.

Baudat, F., K. Manova, J. P. Yuen, M. Jasin, and S. Keeney, 2000 Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking *Spo11*. Mol. Cell 6: 989–998.

Bellani, M. A., P. J. Romanienko, D. A. Cairatti, and R. D. Camerini-Otero, 2005 SPO11 is required for sex-body formation, and *Spo11* heterozygosity rescues the prophase arrest of *Atm*−/− spermatocytes. J. Cell Sci. 118: 3233–3245.

Bhalla, N., and A. F. Dernburg, 2005 A conserved checkpoint monitors meiotic chromosome synapsis in *Caenorhabditis elegans*. Science 310: 1683–1686.

Bishop, D. K., Y. Nikolski, J. Oshiro, J. Chon, M. Shinohara *et al.*, 1999 High copy number suppression of the meiotic arrest caused by a *dmc1* mutation: *REC114* imposes an early recombination block and *RAD54* promotes a *DMC1*-independent DSB repair pathway. Genes Cells 4: 425–444.

Bolor, H., T. Mori, S. Nishiyama, Y. Ito, E. Hosoba *et al.*, 2009 Mutations of the *SYCP3* gene in women with recurrent pregnancy loss. Am. J. Hum. Genet. 84: 14–20.

Borner, G. V., N. Kleckner, and N. Hunter, 2004 Crossover/non-crossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell 117: 29–45.

Bugreev, D. V., O. M. Mazina, and A. V. Mazin, 2006 Rad54 protein promotes branch migration of Holliday junctions. Nature 442: 590–593.

Burgoyne, P. S., S. K. Mahadevaiah, and J. M. Turner, 2007 The management of DNA double-strand breaks in mitotic G2, and in mammalian meiosis viewed from a mitotic G2 perspective. Bioessays 29: 974–986.

Busygina, V., M. G. Sehorn, I. Y. Shi, H. Tsubouchi, G. S. Roeder *et al.*, 2008 Hed1 regulates Rad51-mediated recombination via a novel mechanism. Genes Dev. 22: 786–795.

Callender, T. L., and N. M. Hollingsworth, 2010 Mek1 suppression of meiotic double-strand break repair is specific to sister chromatids, chromosome autonomous and independent of Rec8 cohesin complexes. Genetics 185: 771–782.

Carballo, J., A. Johnson, S. Sedgwick, and R. Cha, 2008 Phosphorylation of the axial element protein Hop1 by Mec1/Tel1 ensures meiotic interhomolog recombination. Cell 132: 758–770.

Chiang, T., F. E. Duncan, K. Schindler, R. M. Schultz, and M. A. Lampson, 2010 Evidence that weakened centromere cohesion is a leading cause of age-related aneuploidy in oocytes. Curr. Biol. 20: 1522–1528.

Cortes-Ledesma, F., and A. Aguilera, 2006 Double-strand breaks arising by replication through a nick are repaired by cohesin-dependent sister-chromatid exchange. EMBO Rep. 7: 919–926.

Cromie, G. A., and G. R. Smith, 2007 Branching out: meiotic recombination and its regulation. Trends Cell Biol. 17: 448–455.

Cromie, G. A., R. W. Hyppa, A. F. Taylor, K. Zakharyevich, N. Hunter *et al.*, 2006 Single Holliday junctions are intermediates of meiotic recombination. Cell 127: 1167–1178.

Daniel, K., J. Lange, K. Hached, J. Fu, K. Anastassiadis *et al.*, 2011 Meiotic homologue alignment and its quality surveillance are controlled by mouse HORMAD1. Nat. Cell Biol. 13: 599–610.

De Veaux, L. C., N. A. Hoagland, and G. R. Smith, 1992 Seventeen complementation groups of mutations decreasing meiotic recombination in *Schizosaccharomyces pombe*. Genetics 130: 251–262.

Di Giacomo, M., M. Barchi, F. Baudat, W. Edelmann, S. Keeney *et al.*, 2005 Distinct DNA-damage-dependent and -independent responses drive the loss of oocytes in recombination-defective mouse mutants. Proc. Natl. Acad. Sci. USA 102: 737–742.

Elson, A., Y. Wang, C. J. Daugherty, C. C. Morton, F. Zhou *et al.*, 1996 Pleiotropic defects in ataxia-telangiectasia protein-deficient mice. Proc. Natl. Acad. Sci. USA 93: 13084–13089.

Essers, J., R. W. Hendriks, S. M. Swagemakers, C. Troelstra, J. de Wit *et al.*, 1997 Disruption of mouse *Rad54* reduces ionizing radiation resistance and homologous recombination. Cell 89: 195–204.
Fukuda, T., K. Daniel, L. Wojtasz, A. Toth, and C. Hoog, 2010 A novel mammalian HORMA domain-containing protein, HORMAD1, preferentially associates with unsynapsed meiotic chromosomes. Exp. Cell Res. 316: 158–171.
Fukushima, K., Y. Tanaka, K. Nabeshima, T. Yoneki, T. Tougan *et al.*, 2000 Dmc1 of *Schizosaccharomyces pombe* plays a role in meiotic recombination. Nucleic Acids Res. 28: 2709–2716.
Getun, I. V., Z. K. Wu, A. M. Khalil, and P. R. Bois, 2010 Nucleosome occupancy landscape and dynamics at mouse recombination hotspots. EMBO Rep. 11: 555–560.
Ghabrial, A., and T. Schupbach, 1999 Activation of a meiotic checkpoint regulates translation of Gurken during *Drosophila* oogenesis. Nat. Cell Biol. 1: 354–357.
Goldfarb, T., and M. Lichten, 2010 Frequent and efficient use of the sister chromatid for DNA double-strand break repair during budding yeast meiosis. PLoS Biol. 8: e1000520.
Guillon, H., F. Baudat, C. Grey, R. M. Liskay, and B. de Massy, 2005 Crossover and noncrossover pathways in mouse meiosis. Mol. Cell 20: 563–573.
Hassold, T., H. Hall and P. Hunt, 2007 The origin of human aneuploidy: where we have been, where we are going. Hum. Mol. Genet. 16 Spec No. 2: R203–208.
Heyer, W. D., X. Li, M. Rolfsmeier, and X. P. Zhang, 2006 Rad54: The Swiss army knife of homologous recombination? Nucleic Acids Res. 34: 4115–4125.
Hodges, C. A., E. Revenkova, R. Jessberger, T. J. Hassold, and P. A. Hunt, 2005 SMC1beta-deficient female mice provide evidence that cohesins are a missing link in age-related nondisjunction. Nat. Genet. 37: 1351–1355.
Hunter, N., and N. Kleckner, 2001 The single-end invasion: an asymmetric intermediate at the double-strand break to double-Holliday junction transition of meiotic recombination. Cell 106: 59–70.
Jackson, J. A., and G. R. Fink, 1985 Meiotic recombination between duplicated genetic elements in *Saccharomyces cerevesiae*. Genetics 109: 303–332.
Kadyk, L. C., and L. H. Hartwell, 1992 Sister chromatids are preferred over homologs as substrates for recombinational repair in *Saccharomyces cerevisiae*. Genetics 132: 387–402.
Kanda, N., and H. Kato, 1980 Analysis of crossing over in mouse meiotic cells by BrdU labelling technique. Chromosoma 78: 113–121.
Kim, K. P., B. M. Weiner, L. Zhang, A. Jordan, J. Dekker *et al.*, 2010 Sister cohesion and structural axis components mediate homolog bias of meiotic recombination. Cell 143: 924–937.
Koehler, K. E., J. P. Cherry, A. Lynn, P. A. Hunt, and T. J. Hassold, 2002 Genetic control of mammalian meiotic recombination. I. Variation in exchange frequencies among males from inbred mouse strains. Genetics 162: 297–306.
Kouznetsova, A., I. Novak, R. Jessberger, and C. Hoog, 2005 SYCP2 and SYCP3 are required for cohesin core integrity at diplotene but not for centromere cohesion at the first meiotic division. J. Cell Sci. 118: 2271–2278.
Lao, J. P., and N. Hunter, 2010 Trying to avoid your sister. PLoS Biol. 8: e1000519.
Latypov, V., M. Rothenberg, A. Lorenz, G. Octobre, O. Csutak *et al.*, 2010 Roles of Hop1 and Mek1 in meiotic chromosome pairing and recombination partner choice in *Schizosaccharomyces pombe*. Mol. Cell. Biol. 30: 1570–1581.
Li, X. C., and J. C. Schimenti, 2007 Mouse pachytene checkpoint 2 (*Trip13*) is required for completing meiotic recombination but not synapsis. PLoS Genet. 3: e130.
Libby, B. J., L. G. Reinholdt, and J. C. Schimenti, 2003 Positional cloning and characterization of *Mei1*, a vertebrate-specific gene required for normal meiotic chromosome synapsis in mice. Proc. Natl. Acad. Sci. USA 100: 15706–15711.
Lu, W. J., J. Chapo, I. Roig, and J. M. Abrams, 2010 Meiotic recombination provokes functional activation of the p53 regulatory network. Science 328: 1278–1281.
Mahadevaiah, S. K., D. Bourc'his, D. G. de Rooij, T. H. Bestor, J. M. Turner *et al.*, 2008 Extensive meiotic asynapsis in mice antagonises meiotic silencing of unsynapsed chromatin and consequently disrupts meiotic sex chromosome inactivation. J. Cell Biol. 182: 263–276.
Matulova, P., V. Marini, R. C. Burgess, A. Sisakova, Y. Kwon *et al.*, 2009 Cooperativity of Mus81.Mms4 with Rad54 in the resolution of recombination and replication intermediates. J. Biol. Chem. 284: 7733–7745.
Mazin, A. V., O. M. Mazina, D. V. Bugreev, and M. J. Rossi, 2010 Rad54, the motor of homologous recombination. DNA Repair (Amst.) 9: 286–302.
Mazina, O. M., and A. V. Mazin, 2008 Human Rad54 protein stimulates human Mus81-Eme1 endonuclease. Proc. Natl. Acad. Sci. USA 105: 18249–18254.
Meuwissen, R. L., H. H. Offenberg, A. J. Dietrich, A. Riesewijk, M. van Iersel *et al.*, 1992 A coiled-coil related protein specific for synapsed regions of meiotic prophase chromosomes. EMBO J. 11: 5091–5100.
Mills, K. D., D. O. Ferguson, J. Essers, M. Eckersdorff, R. Kanaar *et al.*, 2004 Rad54 and DNA Ligase IV cooperate to maintain mammalian chromatid stability. Genes Dev. 18: 1283–1292.
Myers, M., K. L. Britt, N. G. Wreford, F. J. Ebling, and J. B. Kerr, 2004 Methods for quantifying follicular numbers within the mouse ovary. Reproduction 127: 569–580.
Niu, H., X. Li, E. Job, C. Park, D. Moazed *et al.*, 2007 Mek1 kinase is regulated to suppress double-strand break repair between sister chromatids during budding yeast meiosis. Mol. Cell. Biol. 27: 5456–5467.
Niu, H., L. Wan, B. Baumgartner, D. Schaefer, J. Loidl *et al.*, 2005 Partner choice during meiosis is regulated by Hop1-promoted dimerization of Mek1. Mol. Biol. Cell 16: 5804–5818.
Niu, H., L. Wan, V. Busygina, Y. Kwon, J. A. Allen *et al.*, 2009 Regulation of meiotic recombination via Mek1-mediated Rad54 phosphorylation. Mol. Cell 36: 393–404.
Parvanov, E. D., P. M. Petkov, and K. Paigen, 2010 *Prdm9* controls activation of mammalian recombination hotspots. Science 327: 835.
Pelttari, J., M. R. Hoja, L. Yuan, J. G. Liu, E. Brundell *et al.*, 2001 A meiotic chromosomal core consisting of cohesin complex proteins recruits DNA recombination proteins and promotes synapsis in the absence of an axial element in mammalian meiotic cells. Mol. Cell. Biol. 21: 5667–5677.
Pittman, D., J. Cobb, K. Schimenti, L. Wilson, D. Cooper *et al.*, 1998 Meiotic prophase arrest with failure of chromosome pairing and synapsis in mice deficient for *Dmc1*, a germline-specific RecA homolog. Mol. Cell 1: 697–705.
Plug, A. W., J. Xu, G. Reddy, E. I. Golub, and T. Ashley, 1996 Presynaptic association of Rad51 protein with selected sites in meiotic chromatin. Proc. Natl. Acad. Sci. USA 93: 5920–5924.
Raschle, M., S. Van Komen, P. Chi, T. Ellenberger, and P. Sung, 2004 Multiple interactions with the Rad51 recombinase govern the homologous recombination function of Rad54. J. Biol. Chem. 279: 51973–51980.
Reinholdt, L., T. Ashley, J. Schimenti, and N. Shima, 2004 Forward genetic screens for meiotic and mitotic recombination-defective mutants in mice. Methods Mol. Biol. 262: 87–107.
Reinholdt, L. G., and J. C. Schimenti, 2005 *Mei1* is epistatic to *Dmc1* during mouse meiosis. Chromosoma 114: 127–134.
Revenkova, E., C. Adelfalk, and R. Jessberger, 2010 Cohensin in oocytes: Tough enough for mammalian meiosis? Genes 1: 495–504.

Roeder, G. S., 1997 Meiotic chromosomes: it takes two to tango. Genes Dev. 11: 2600–2621.
Roeder, G. S., and J. M. Bailis, 2000 The pachytene checkpoint. Trends Genet. 16: 395–403.
Roig, I., J. A. Dowdle, A. Toth, D. G. de Rooij, M. Jasin *et al.*, 2010 Mouse TRIP13/PCH2 is required for recombination and normal higher-order chromosome structure during meiosis. PLoS Genet. 6: e1001062.
Romanienko, P. J., and R. D. Camerini-Otero, 2000 The mouse *Spo11* gene is required for meiotic chromosome synapsis. Mol. Cell 6: 975–987.
Rossi, M. J., and A. V. Mazin, 2008 Rad51 protein stimulates the branch migration activity of Rad54 protein. J. Biol. Chem. 283: 24698–24706.
Royo, H., G. Polikiewicz, S. K. Mahadevaiah, H. Prosser, M. Mitchell *et al.*, 2010 Evidence that meiotic sex chromosome inactivation is essential for male fertility. Curr. Biol. 20: 2117–2123.
Schwacha, A., and N. Kleckner, 1994 Identification of joint molecules that form frequently between homologs but rarely between sister chromatids during yeast meiosis. Cell 76: 51–63.
Schwacha, A., and N. Kleckner, 1997 Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. Cell 90: 1123–1135.
Sheridan, S., and D. K. Bishop, 2006 Red-Hed regulation: recombinase Rad51, though capable of playing the leading role, may be relegated to supporting Dmc1 in budding yeast meiosis. Genes Dev. 20: 1685–1691.
Shin, Y. H., Y. Choi, S. U. Erdin, S. A. Yatsenko, M. Kloc *et al.*, 2010 *Hormad1* mutation disrupts synaptonemal complex formation, recombination, and chromosome segregation in mammalian meiosis. PLoS Genet. 6: e1001190.
Sjogren, C., and K. Nasmyth, 2001 Sister chromatid cohesion is required for postreplicative double-strand break repair in *Saccharomyces cerevisiae*. Curr. Biol. 11: 991–995.
Sjogren, C., and L. Strom, 2010 S-phase and DNA damage activated establishment of sister chromatid cohesion–importance for DNA repair. Exp. Cell Res. 316: 1445–1453.
Stark, J. M., and M. Jasin, 2003 Extensive loss of heterozygosity is suppressed during homologous repair of chromosomal breaks. Mol. Cell. Biol. 23: 733–743.
Sung, P., and H. Klein, 2006 Mechanism of homologous recombination: mediators and helicases take on regulatory functions. Nat. Rev. Mol. Cell Biol. 7: 739–750.
Tachibana-Konwalski, K., J. Godwin, L. van der Weyden, L. Champion, N. R. Kudo *et al.*, 2010 REC8-containing cohesin maintains bivalents without turnover during the growing phase of mouse oocytes. Genes Dev. 24: 2505–2516.
Tarsounas, M., T. Morita, R. E. Pearlman, and P. B. Moens, 1999 RAD51 and DMC1 form mixed complexes associated with mouse meiotic chromosome cores and synaptonemal complexes. J. Cell Biol. 147: 207–220.
Tsubouchi, H., and G. S. Roeder, 2006 Budding yeast Hed1 down-regulates the mitotic recombination machinery when meiotic recombination is impaired. Genes Dev. 20: 1766–1775.
Turner, J. M., S. K. Mahadevaiah, O. Fernandez-Capetillo, A. Nussenzweig, X. Xu *et al.*, 2005 Silencing of unsynapsed meiotic chromosomes in the mouse. Nat. Genet. 37: 41–47.
Wang, H., and C. Hoog, 2006 Structural damage to meiotic chromosomes impairs DNA recombination and checkpoint control in mammalian oocytes. J. Cell Biol. 173: 485–495.
Ward, J. O., L. G. Reinholdt, W. W. Motley, L. M. Niswander, D. C. Deacon *et al.*, 2007 Mutation in mouse *Hei10*, an e3 ubiquitin ligase, disrupts meiotic crossing over. PLoS Genet. 3: e139.
Watrin, E., and J. M. Peters, 2006 Cohesin and DNA damage repair. Exp. Cell Res. 312: 2687–2693.
Wesoly, J., S. Agarwal, S. Sigurdsson, W. Bussen, S. Van Komen *et al.*, 2006 Differential contributions of mammalian *Rad54* paralogs to recombination, DNA damage repair, and meiosis. Mol. Cell. Biol. 26: 976–989.
Wojtasz, L., K. Daniel, I. Roig, E. Bolcun-Filas, H. Xu *et al.*, 2009 Mouse HORMAD1 and HORMAD2, two conserved meiotic chromosomal proteins, are depleted from synapsed chromosome axes with the help of TRIP13 AAA-ATPase. PLoS Genet. 5: e1000702.
Wu, H. Y., and S. M. Burgess, 2006 Two distinct surveillance mechanisms monitor meiotic chromosome metabolism in budding yeast. Curr. Biol. 16: 2473–2479.
Wu, Z. K., I. V. Getun, and P. R. Bois, 2010 Anatomy of mouse recombination hot spots. Nucleic Acids Res. 38: 2346–2354.
Xu, H., M. D. Beasley, W. D. Warren, G. T. van der Horst, and M. J. McKay, 2005 Absence of mouse REC8 cohesin promotes synapsis of sister chromatids in meiosis. Dev. Cell 8: 949–961.
Xu, L., B. M. Weiner, and N. Kleckner, 1997 Meiotic cells monitor the status of the interhomolog recombination complex. Genes Dev. 11: 106–118.
Xu, Y., T. Ashley, E. E. Brainerd, R. T. Bronson, M. S. Meyn *et al.*, 1996 Targeted disruption of *Atm* leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma. Genes Dev. 10: 2411–2422.
Yang, F., R. De La Fuente, N. A. Leu, C. Baumann, K. J. McLaughlin *et al.*, 2006 Mouse SYCP2 is required for synaptonemal complex assembly and chromosomal synapsis during male meiosis. J. Cell Biol. 173: 497–507.
Yoshida, K., G. Kondoh, Y. Matsuda, T. Habu, Y. Nishimune *et al.*, 1998 The mouse RecA-like gene *Dmc1* is required for homologous chromosome synapsis during meiosis. Mol. Cell 1: 707–718.
Yuan, L., J. G. Liu, J. Zhao, E. Brundell, B. Daneholt *et al.*, 2000 The murine *SCP3* gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. Mol. Cell 5: 73–83.
Zickler, D., and N. Kleckner, 1999 Meiotic chromosomes: integrating structure and function. Annu. Rev. Genet. 33: 603–754.

*Communicating editor: N. M. Hollingsworth*

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**Supporting Information**
http://www.genetics.org/content/suppl/2011/07/12/genetics.111.130674.DC1

# Genetic Evidence That Synaptonemal Complex Axial Elements Govern Recombination Pathway Choice in Mice

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DOI: 10.1534/genetics.111.130674

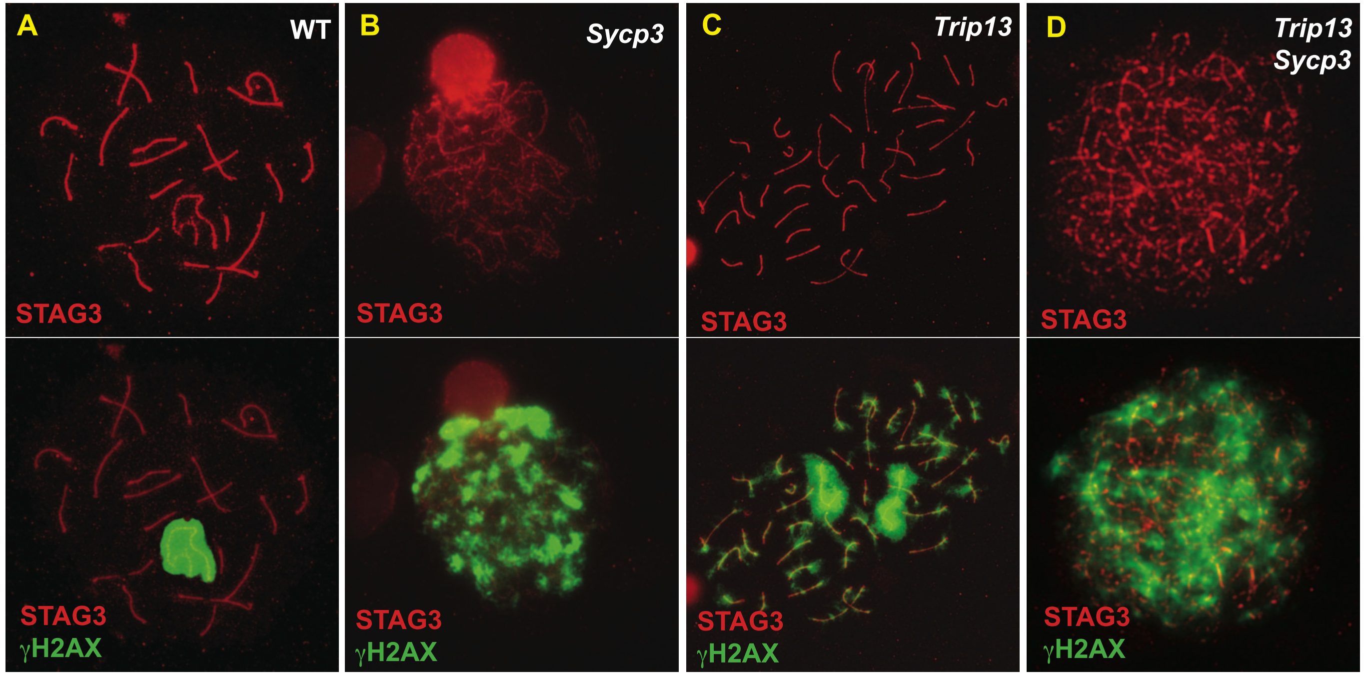
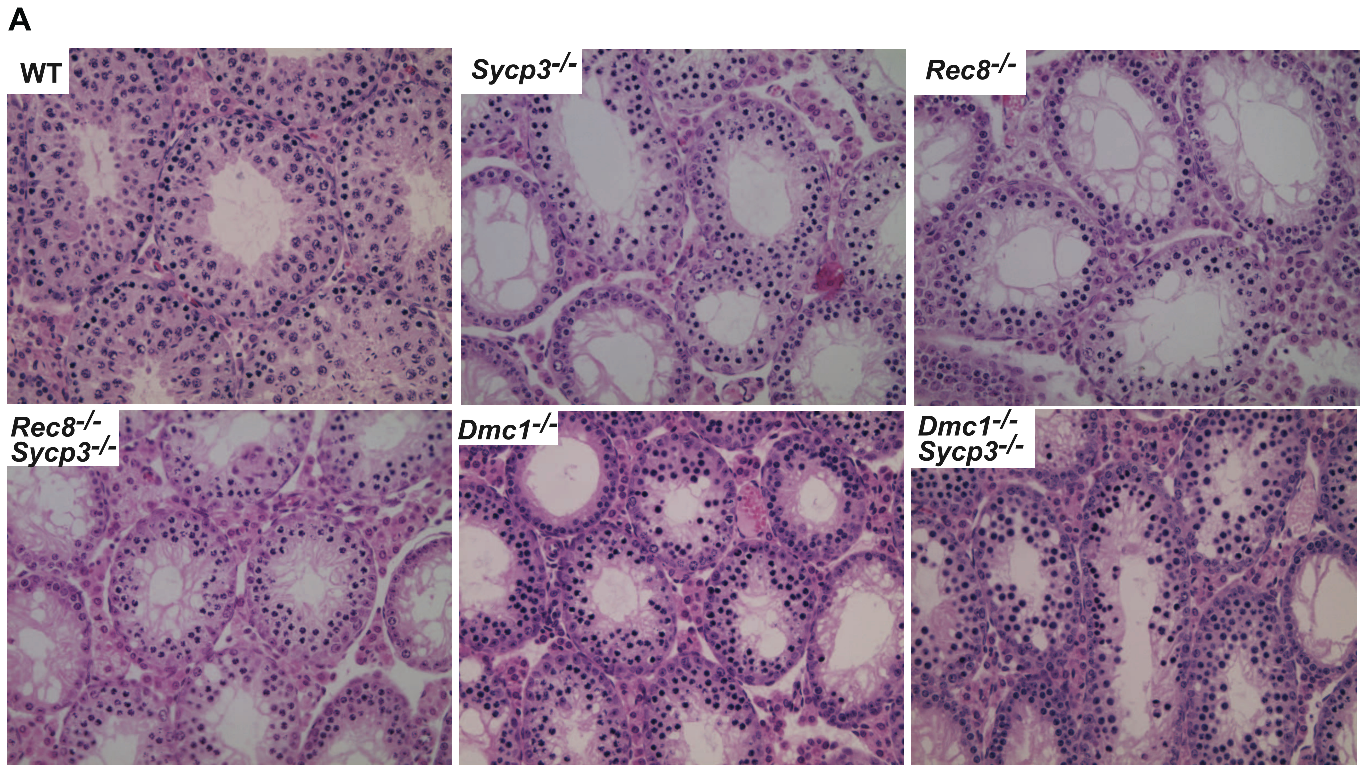



**Figure S1** Immunocytological analysis of spermatocyte chromosomes. A pair of images is shown for each genotype: the topmost shows staining for the SC axial element marker STAG3, and the lower image is a merge betwen STAG3 (in red) and γH2AX (in green). The genotypes of each spermatocyte nucleus are abbreviated in the upper right of each panel pair, in which the gene symbol(s) listed indicates the gene(s) that is mutated in that sample. Each example is either a pachytene nucleus or the most advanced stage of spermatocyte occuring in the particular genotype.



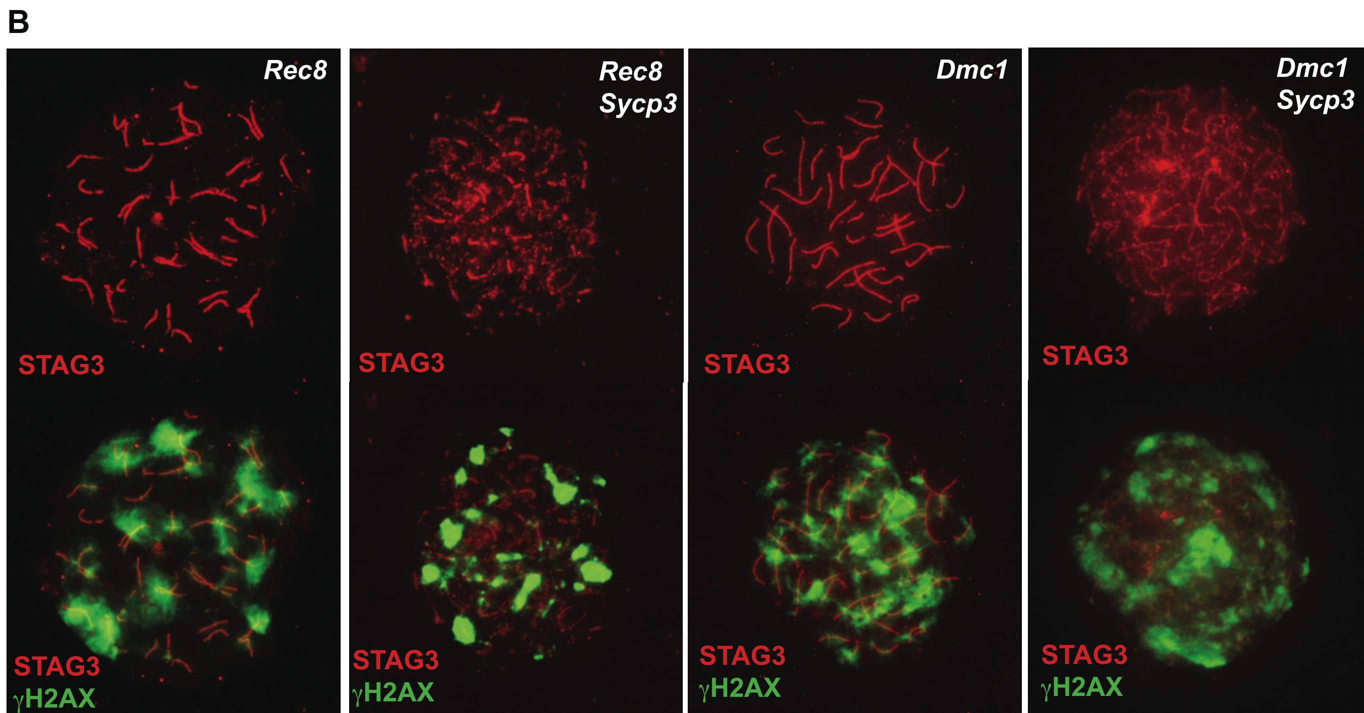




**Figure S2** Epistasis analysis between *Sycp3* and other meiotic mutants. (A) Histological sections of testis showing identical point of meiotic arrest in all mutant genotypes. Genotypes are indicated. (B) Immunostaining of surface spread spermatocyte chromosomes of indicated genotypes (upper right, all are mutant for the listed gene symbols) with indicated antibodies (lower left of each panel, color-coded).

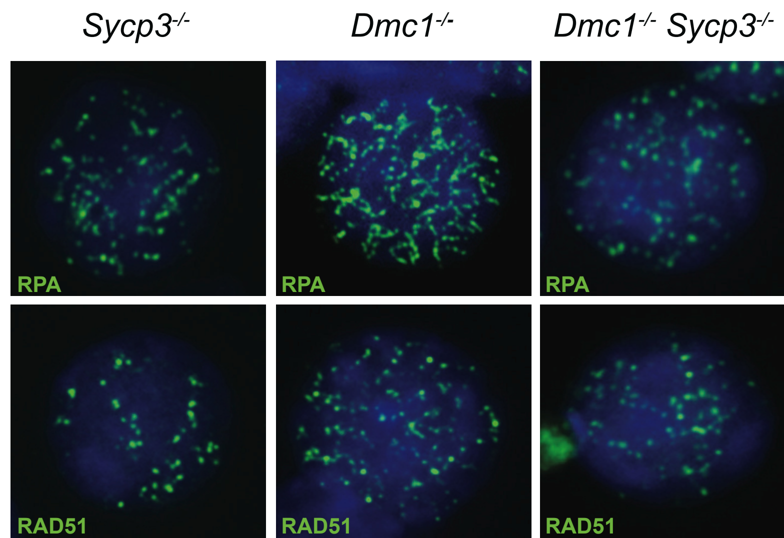



**Figure S3** Representative immunostaining of newborn oocytes. Genotypes and antibodies are indicated. Note lower numbers of RPA and RAD51 foci in double mutants *vs* the *Dmc1* single mutant.