Multiple Barriers to Nonhomologous DNA End Joining During Meiosis in *Drosophila*

Eric F. Joyce,[1] Anshu Paul, Katherine E. Chen, Nikhila Tanneti, and Kim S. McKim[2]

Waksman Institute and Department of Genetics, Rutgers, the State University of New Jersey, Piscataway New Jersey 08854-8020

ABSTRACT Repair of meiotic double-strand breaks (DSBs) uses the homolog and recombination to yield crossovers while alternative pathways such as nonhomologous end joining (NHEJ) are suppressed. Our results indicate that NHEJ is blocked at two steps of DSB repair during meiotic prophase: first by the activity of the MCM-like protein MEI-218, which is required for crossover formation, and, second, by Rad51-related proteins SPN-B (XRCC3) and SPN-D (RAD51C), which physically interact and promote homologous recombination (HR). We further show that the MCM-like proteins also promote the activity of the DSB repair checkpoint pathway, indicating an early requirement for these proteins in DSB processing. We propose that when a meiotic DSB is formed in the absence of both MEI-218 and SPN-B or SPN-D, a DSB substrate is generated that can enter the NHEJ repair pathway. Indeed, due to its high error rate, multiple barriers may have evolved to prevent NHEJ activity during meiosis.

SEXUAL reproduction is facilitated by the halving of the chromosome number during meiosis. This is achieved through the two rounds of meiotic divisions and, while the second division is much like mitosis, it is the unique first division that separates the two homologous chromosomes. Prior to the first division, each pair of homologous chromosomes is linked by a chiasma, which forms once a crossover has occurred between the homologs. The connections established by crossovers/chiasmata, in conjunction with sister chromatid cohesion, hold the homologs together until their segregation at anaphase I (Hawley 1988).

The formation of crossovers during meiotic prophase requires the induction of DNA double-strand breaks (DSBs) that occur at several locations along each chromosome (Keeney 2001). Superimposed on this repair pathway are several types of regulation (Phadnis *et al.* 2011). For example, the number of crossovers is regulated such that there is often one per chromosome arm, which results in a nonrandom distribution of crossovers and an excess of DSBs that lead to noncrossover products. In addition, for a crossover to generate a chiasma, the crossover must occur between homologs and not between sisters. Thus, there appears to be a barrier (or

semipermeable barrier) between the sister chromatids that promotes homolog exchange, possibly involving the activity of chromosome axis proteins (Couteau *et al.* 2004; Webber *et al.* 2004; Niu *et al.* 2005; Kim *et al.* 2010; Li *et al.* 2011). Finally, homologous repair must be promoted while more error-prone mechanisms that could introduce *de novo* mutations in the germline such as nonhomologous end joining (NHEJ) are inhibited. The mechanism that prevents NHEJ during meiosis, however, is poorly understood.

As in most other organisms, meiotic DSBs are repaired in *Drosophila* using DNA repair proteins in the Rad52 epistasis group. Thus, mutation of *Drosophila* Rad51, *spn-A*, and its paralogs *spn-D* and *spn-B*, block meiotic DSB repair and accumulate DSBs into late stages of pachytene, as detected by an antibody to the phosphorylated form of H2AV (Ghabrial *et al.* 1998; Ghabrial and Schupbach 1999; Abdu *et al.* 2003; Jang *et al.* 2003; Staeva-Vieira *et al.* 2003; Mehrotra and McKim 2006). *spn-D* and *spn-B* encode orthologs of RAD51C and XRCC3, respectively, which are believed to function as a heterodimer in mammalian cells to promote DSB repair by homologous recombination (HR) (Kurumizaka *et al.* 2001; Abdu *et al.* 2003; Sung *et al.* 2003; Radford and Sekelsky 2004). Rad51C or XRCC3 plays an important role in mouse and *Arabidopsis* meiotic recombination as well (Bleuyard and White 2004; Abe *et al.* 2005; Li *et al.* 2005; Kuznetsov *et al.* 2007).

In addition to the DSB repair genes, crossover formation requires a second set of genes with mutant phenotypes

consistent with regulatory functions. These include the precondition genes *mei-218* and *rec*. Mutations in these genes reduce the frequency of crossovers without affecting DSB formation or the timing of their repair, indicating a specific defect in the ability to repair DSBs as crossovers (Carpenter 1982, 1984; Blanton *et al.* 2005; Joyce and McKim 2009). As such, precondition gene products have been proposed to establish which DSBs will become crossovers rather than to be directly involved in the repair machinery (Carpenter and Sandler 1974; Bhagat *et al.* 2004). In this article, we describe new genetic interactions between the precondition gene *mei-218* and DSB repair genes *spn-D* and *spn-B*, which are important for the suppression of the NHEJ repair pathway during meiosis. We further found that both *mei-218* and *rec* are required for full DSB repair checkpoint activity. These results reveal two novel functions for precondition genes early in meiotic prophase in both the detection of DSBs and the regulation of repair choices.

Materials and Methods

Fly stocks and genetic techniques

The following mutations were used and have been previously described: *spn-A¹*, *spn-A⁰⁹³*, *spn-Bᴮᵁ*, and *spn-D¹⁵⁰* (Ghabrial *et al.* 1998; Abdu *et al.* 2003; Jang *et al.* 2003; Staeva-Vieira *et al.* 2003), *mei-218¹*, *mei-218⁶* (Carpenter and Sandler 1974; McKim *et al.* 1996), *rec¹* and *rec²* (Blanton *et al.* 2005), and *lig4⁵⁷* (Gorski *et al.* 2003; Romeijn *et al.* 2005). Experiments with *rec* were done with *rec¹/rec²* heterozygotes. The *lig4⁵⁷ mei-218⁶* chromosome was made from a *w lig4⁵/y mei-218⁶ f* heterozygote and selecting recombinants between *w* and *f*. The presence of the *lig4⁵⁷* allele was confirmed with a PCR test for the deletion (Gorski *et al.* 2003), while the *mei-218⁶* allele was confirmed by a test for nondisjunction. All crosses were raised at 25°.

To determine the frequency of dorsal–ventral polarity defects in repair-defective mutants, embryos were scored for abnormal dorsal appendages indicating a ventralized phenotype. As has been described (Klovstad *et al.* 2008), the ventralized phenotype can be weaker in embryos from younger mothers, especially for *spn-Bᴮᵁ* and *spn-D¹⁵⁰* mutants. Therefore, the data are from embryos collected after 4 days of mating and egg laying.

Cytology and immunofluorescence

For immunolocalization experiments, females were aged at room temperature for about 16 hr and ovaries were dissected and fixed using buffer A (Belmont *et al.* 1989; McKim *et al.* 2008). The antibody to γ-H2AV was described by Mehrotra and McKim (2006) and used at a 1:500 dilution and the mouse anti-C(3)G antibody was used at 1:500 (Page and Hawley 2001). The secondary antibodies were Cy3-labeled goat antirabbit (Jackson Labs) used at 1:250, Cy3-labeled goat antirat (Jackson Labs) used at 1:100, and Alexa Fluor 488 goat antimouse (Invitrogen) used at 1:100. Chromosomes were stained with Hoechst 33342 at 1:50,000

(10 mg/ml solution) for 7 min at room temperature. Images were collected using a Leica TCS SP2 confocal microscope with a ×63, N.A. 1.3 lens. In most cases, whole germaria were imaged by collecting optical sections through the entire tissue. These data sets are shown as maximum projections. The analysis of the images, however, was performed by examining one section at a time. The oocytes were observed using an anti-C(3)G antibody. *P*-values were calculated using Fisher's exact test.

The γ-H2AV foci were counted from germaria where the foci were clear and distinct. Foci numbers in wild type were at a maximum in region 2a (early pachytene) and few foci were visible by region 2b (midpachytene). Therefore, to compare foci numbers in different genotypes, we used a method that includes all cysts with γ-H2AV foci, averaging the number in each pair of pro-oocytes. We compared the average number of foci in all the pro-oocytes or oocytes of each germarium, starting with the youngest cysts at the anterior end, by examining a full series of optical sections.

Yeast two-hybrid assay

The two-hybrid experiments were performed as described (James *et al.* 1996). Full-length cDNA clones of *spn-B* and *spn-D* were cloned into pGADT7 (Gal4 activation domain) and pGBKT7 (Gal4 binding domain). Clones were transformed into yeast strain H125 and, for all spotting assays, the cultures were normalized to an OD of 2.0 and then diluted fivefold. Strains expressing both pGADT7 and pGBKT7 plasmids were plated on SD −TRP −LEU in serial dilutions for control. Tests were then plated in serial dilutions on SD −TRP −LEU −HIS medium that also contained 5 mM 3-amino-1,2,4-triazole.

Results

Drosophila orthologs of Rad51C and XRCC3 physically interact

Drosophila SPN-D and SPN-B are putative orthologs of mammalian RAD51C and XRCC3, proposed to function as a heterodimer to promote DSB repair by HR (Ghabrial *et al.* 1998; Kurumizaka *et al.* 2001; Abdu *et al.* 2003; Sung *et al.* 2003). To determine whether SPN-D and SPN-B physically interact, we carried out a yeast two-hybrid assay. Whether or not SPN-D was fused to the activation domain or the DNA binding domain, it specifically interacted with SPN-B (Figure 1). Neither protein interacted with itself. Thus, SPN-D and SPN-B may function as a heterodimer like their mammalian orthologs. This finding is in line with the similar meiotic phenotypes observed in *spn-D* and *spn-B* mutants, including the accumulation of γ-H2AV foci, a DSB marker, into late-stage pachytene oocytes (Abdu *et al.* 2003; Radford and Sekelsky 2004; Mehrotra and McKim 2006). We note, however, that when γ-H2AV foci levels are monitored in later stages of oogenesis, a weaker persistence of γ-H2AV is observed in *spn-B* mutants; that is, the presence of γ-H2AV foci disappears earlier in *spn-B* mutants
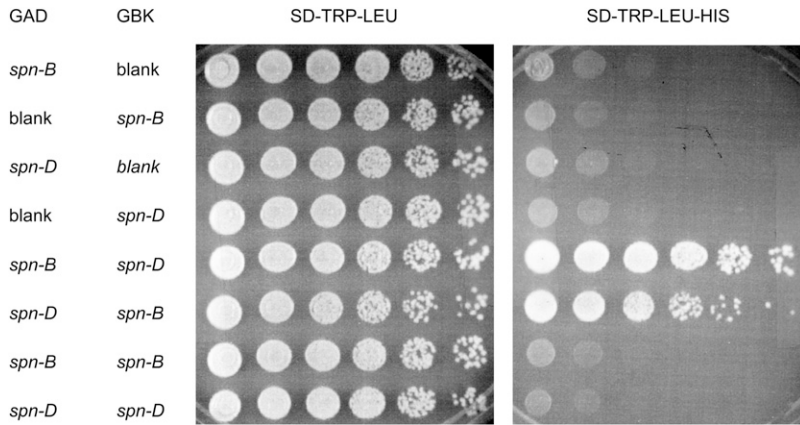
Figure 1 SPN-B and SPN-D physically interact in a yeast two-hybrid assay. Clones in the pGADT7 vector containing the Gal4 activation domain (GAD) are noted in the left column and clones in the pGBKT7 vector containing the Gal4 binding domain (GBD) are noted in the right column. Yeast were also transformed with an empty vector ("blank"). Growth with serial dilutions on the SD −TRP −LEU dropout media indicates both pGBKT7 and pGADT7 vectors were present. Growth with serial dilutions on the SD −TRP −LEU −HIS dropout media indicates a physical interaction between the GBD and GAD fusion proteins.

as compared to *spn-D* or *spn-A*, the *Drosophila* RAD51 homolog (data not shown). This result suggests that *spn-D* has a function in DSB repair that does not depend on *spn-B*, although it is possible the existing *spn-B* mutants are not null.

Persistence of DSBs in spn-D mutants depends on mei-218

In a previous study of the meiotic pachytene checkpoint, we observed that in a *mei-218*; *spn-D* double mutant, both the numbers of γ-H2AV foci in pachytene and the kinetics of foci formation and disappearance closely paralleled those observed in wild type rather than that of *spn-D* (Joyce and McKim 2009). We have repeated this experiment with the construction of a new double mutant stock and observed a similar suppression in γ-H2AV persistence ($P < 0.0001$, Figure 2). In contrast, *mei-218*; *spn-A* double mutants exhibited similar levels of persistent γ-H2AV foci to that of *spn-A* single mutants ($P = 0.2841$), indicating *mei-218* mutants retain the ability to phosphorylate H2AV in late pachytene. These results suggest that DSBs are being repaired in the *mei-218*; *spn-D* double mutant with near-wild-type efficiency, even though repair is not resulting in crossovers and may not involve HR. Since *spn-D* and *spn-B* encode homologs of RAD51C and XRCC3 that physically interact, we predicted that *spn-B* mutants might have similar effects on γ-H2AV persistence as *spn-D* in a *mei-218* mutant background. Indeed, we found that the accumulation of γ-H2AV foci in late stages of pachytene normally observed in *spn-B* mutants was significantly reduced in a *mei-218* mutant background ($P = 0.0016$); however, the reduction was not as prominent as compared to *spn-D* (Figure 2). Below, we describe additional experiments to determine whether the DSBs were being repaired by an alternative to HR in *mei-218*; *spn-D* and *mei-218*; *spn-B* double mutants.

Repair of DSBs in mei-218; spn-D does not result in elevated sister chromatid exchange

During meiotic recombination, several mechanisms are in place to inhibit alternative DSB repair pathways and promote homolog bias. For instance, DSB repair via sister chromatid exchange (SCE) is normally suppressed during meiotic recombination by the cohesion-associated protein ORD (Webber *et al.* 2004). To test whether *mei-218* is also required to inhibit exchange between sisters, we monitored the transmission of a ring X chromosome during meiosis. A single crossover between two ring sister chromatids will create a dicentric chromosome that will not be transmitted efficiently (Webber *et al.* 2004). In contrast, recombination between two normal rod sister chromatids will not impair their transmission. Therefore, an elevation of SCE should reduce the recovery of ring X chromosomes relative to the frequency of rod X chromosomes. We monitored meiotic transmission of the ring X chromosome *R(1)2* in wild type, *mei-218*, and *mei-218*; *spn-D* mutant females as well as in two other precondition mutants *rec* and *mcm5*. The recovery of the *R(1)2* chromosome in all mutants was similar to wild type (Table 1). These data argue that SCE is not elevated and thus not responsible for the repair of DSBs in these double mutant combinations.

Repair of DSBs in mei-218; spn-D depends on ligase4

An alternative explanation for the precocious repair of DSBs in *mei-218*; *spn-D* double mutants is nonhomologous end joining (NHEJ), wherein the broken chromosome ends are sealed together by Ligase4 (LIG4) activity without the use of external homologies (Lieber *et al.* 2003). To examine the contribution of NHEJ to repair in the *mei-218*; *spn-D* and *mei-218*; *spn-B* females, we constructed double and triple mutants with a *lig4* mutation and stained for γ-H2AV. The frequency and dynamics of the γ-H2AV foci in the *lig4* single mutant and the *lig4 mei-218* double mutant followed a similar trend to that observed in wild type, indicating that NHEJ does not play a significant role in normal meiotic DSB repair

Table 1 Ring chromosome transmission assay

Genotype	Ring/rod[a]	Total progeny
R(1)2/+	0.90	4698
R(1)2, mei-218¹/mei-218¹	0.92	424
R(1)2, mei-218¹/mei-218¹; spn-D¹⁵⁰	0.96	450
R(1)2/+; rec¹/rec²	0.92	655
R(1)2/+; mcm5ᴬ⁷	0.86	227

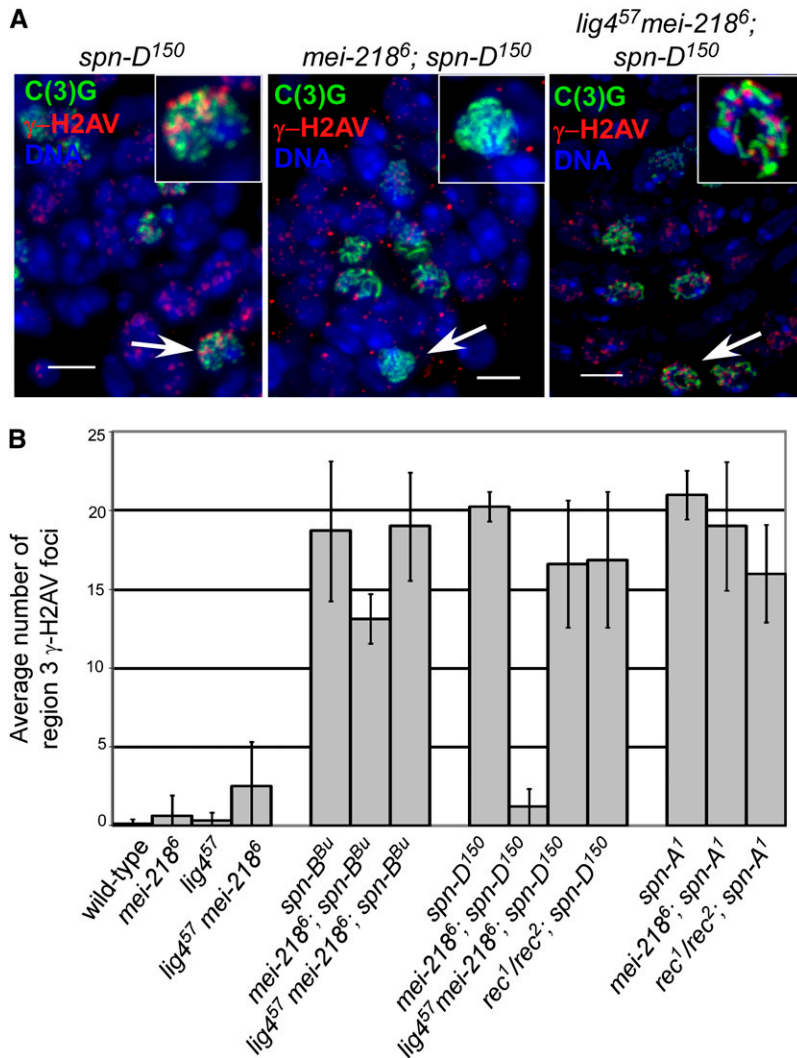[a] Number of ring X chromosomes recovered relative to normal rod X chromosomes.

Figure 2 γ-H2AV foci in germaria from precondition and DSB repair mutant combinations: *spn-D*, *mei-218; spn-D* and *mei-218 lig4; spn-D* females. (A) The γ-H2AV foci (red) persist into late stage (region 3) oocytes (arrows, which are also shown in insets) of *spn-D^150* and *lig4^57-mei-218^6; spn-D^150* but not *mei-218^6; spn-D^150* mutants. DNA is in blue and the oocytes were identified with C(3)G (green). Each image shows a projection of all confocal sections through the oocyte nucleus. Bars, 5 μM. (B) Average number of late-stage γ-H2AV foci in repair-defective backgrounds ±SD. *P* values (in text) were determined by an unpaired *t*-test.

(Figure 2). Indeed, the frequency of γ-H2AV foci in *lig4* and *lig4 mei-218* region 3 oocytes was low and similar to wild type, indicating no defect in DSB repair (Figure 2). The contribution of NHEJ becomes evident, however, when homologous repair is blocked by mutations in either *spn-D* or *spn-B*. In the *lig4 mei-218; spn-D* triple mutant, late pachytene oocytes exhibited robust γ-H2AV labeling, with a similar number of foci to *spn-D* single mutants (Figure 2). A similar result was found in *lig4 mei-218; spn-B* triple mutant females, suggesting that LIG4-dependent NHEJ of meiotic DSBs occurs when both MEI-218 and either SPN-D or SPN-B activity are absent. Additionally, we cannot rule out that SPN-A is required for NHEJ, and thus prohibiting LIG-4–dependent repair in the *mei-218 spn-A* double mutant.

DSB repair checkpoint activity depends on both precondition genes mei-218 and rec

Delays in meiotic DSB repair lead to oocyte dorsal–ventral polarity defects in the developing embryos of females due to the meiotic DSB repair checkpoint (Ghabrial and Schupbach 1999; Abdu *et al.* 2002). We reasoned that if DSB repair occurs by NHEJ in *mei-218; spn-D* and *mei-218; spn-B* females, then the frequency of abnormal embryos (a readout for checkpoint activity) laid by these mothers would be reduced. While the frequency of abnormal embryos laid by *spn-B* females was higher than that of *spn-D* females, there is no evidence that this can be correlated with the severity of their repair defect. Therefore, we only compared the *spn-*single mutants to the double mutants. We found a dramatic reduction in the frequency of abnormal embryos from both *mei-218; spn-D* and *mei-218; spn-B* mothers as compared to the *spn-D* and *spn-B* single mutants, respectively (Table 2). Thus, efficient activation of the DSB repair checkpoint was suppressed in the absence of MEI-218. Interestingly, the double mutants still exhibited reduced fertility compared to *mei-218* single mutants, perhaps due to the production of inviable embryos due to an error-prone repair mechanism (Liu *et al.* 2000, 2002).

If the DSB repair checkpoint was suppressed in the absence of MEI-218 due to repair by NHEJ, then checkpoint activation should be restored in the *lig4 mei-218; spn-D* triple mutants, which exhibit DSB repair defects (Figure 2).

Table 2 Egg shell phenotypes in DSB repair mutants

Genotype	N	Ventralized	% ventralized
spn-D^{150}	347	208	59.9
spn-B^{BU}	707	574	81.2
mei-218^6; spn-D^{150}	522	120	23.0
mei-218^6; spn-B^{BU}	405	53	13.1
lig4^{57}mei-218^6; spn-D^{150}	112	23	20.5
spn-A^1/spn-A^{093}	422	365	86.5
mei-218^6; spn-A^1/spn-A^{093}	2981	911	30.6
rec^1 spn-A^1/spn-A^1	452	363	80.3
rec^1 spn-A^1	125	26	20.8

N = total number of embryos counted

Surprisingly, the triple mutants also produced a low frequency of ventralized embryos, indicating the DSB repair checkpoint is suppressed despite the persistence of unrepaired DSBs (Table 2). Thus, an alternative explanation for the suppression of eggshell phenotypes in the triple mutants is that MEI-218 is required for DSB repair checkpoint activity in addition to its role in suppressing NHEJ. To test this model, we examined a mei-218; spn-A double mutant in which the meiotic DSBs are not repaired by NHEJ. Interestingly, the frequency of ventralized embryos was reduced as compared to the spn-A single mutant. This is probably not due to a failure in responding to DSBs since the appearance of γ-H2AV, which depends on Ataxia-Telangiectasia Mutated (ATM), is not affected (Joyce et al. 2011). We also tested mutations in another precondition gene, rec, and found a similar suppression of checkpoint activity in spn-A mutants (Table 2). These results suggest that the precondition genes are required for full activity of the DSB repair checkpoint. Despite this similarity to mei-218, however, rec mutations had no effect on the γ-H2AV persistence observed in spn-D mutants (Figure 2), suggesting the suppression of NHEJ in meiosis is a precondition function specific to mei-218. To our knowledge, this is the first phenotypic difference found between precondition mutants mei-218 and rec.

Discussion

In this report, we have provided evidence for two new functions of precondition genes during Drosophila meiosis. First, MEI-218 and REC are each required for the full signaling activity of the DSB repair checkpoint. Second, MEI-218, in combination with DSB repair proteins SPN-D and SPN-B, is necessary to suppress a LIG-4–dependent repair pathway, most likely involving NHEJ. There are only a few cases in other organisms where defects in meiotic DSB repair have been shown to result in elevated use of NHEJ. In Caenorhabditis elegans, NHEJ has been shown to participate in meiotic DSB repair in either brc-2 mutants, where homologous recombination is impaired due to misregulation of RAD-51, or in a double mutant combination: one affecting the SC (syp-3) and the other affecting sister chromatid cohesion (rec-8) (Martin et al. 2005; Smolikov et al. 2007). All three studies may be revealing genes with regulatory functions in DSB repair and crossover formation. As discussed below, our findings support the idea that two groups of Drosophila genes may contribute to two independent regulatory mechanisms, which promote crossing over while inhibiting NHEJ.

Blocks to NHEJ: the precondition gene MEI-218

Precondition genes such as mei-218 and rec have generally been defined by two mutant phenotypes: a reduction in the frequency of crossovers and an altered distribution of residual events that is more proportional to the physical map. Recently, precondition genes have also been found to have a DSB-independent role in the Pch2-dependent pachytene checkpoint (Joyce and McKim 2009). Epistasis experiments further suggest that the precondition genes function prior to DSB repair genes (this report and Joyce and McKim 2009). We have shown here that the precondition proteins are also essential for proper DSB repair checkpoint signaling. Thus, the precondition genes are the first example of Drosophila factors that contribute to both checkpoint pathways. Intriguingly, Ho and Burgess (2011) found that yeast Xrs2, which is required for the response to DSBs, interacts with Pch2 and is also required for the checkpoint-mediated delay due to synapsis defects. In this viewpoint, MEI-218 and Xrs2 might have dual roles in both checkpoints due to their involvement in the generation of DNA structures that signal checkpoints. Of note, the structure that signals the pachytene checkpoint is not known. Taken together, these results imply that the precondition genes are required at or prior to the time of DSB formation and regulate the repair of DSBs. Thus, the first block to NHEJ may be associated with the early processing of the DSBs.

One clue as to how precondition gene products perform this function comes from their homology to the eukaryotic MCM family of proteins (MCM2–7), which form a heterohexameric helicase required for replication (Forsburg 2004; Blanton et al. 2005; Lake et al. 2007). Previous studies have also shown that the majority of MEI-218 protein was in the cytoplasm (Manheim et al. 2002). While it is possible that the effects of MEI-218 on recombination are indirect, a more likely explanation given the sequence homology is that nuclear import and export of MEI-218 is regulated, with the majority of protein being retained outside the nucleus.

Given their relation to proteins with helicase activity coupled with their checkpoint mutant phenotypes, we propose that MEI-218 and REC are required during the resection stage of meiotic DSB processing (Joyce and McKim 2009) (Figure 3). This proposal is also consistent with the finding by Blanton et al. (2005) that rec mutants have shorter gene conversion tracts than wild type and our finding that MEI-218 and REC promote checkpoint activity. In the absence of MEI-218, for example, ATM may respond to the DSBs, but subsequent activities, including the generation of long ssDNA substrates, recruitment of RPA, and MEI-41–dependent checkpoint activation could be defective (Zou and Elledge 2003). Longer tracts of ssDNA, dependent on
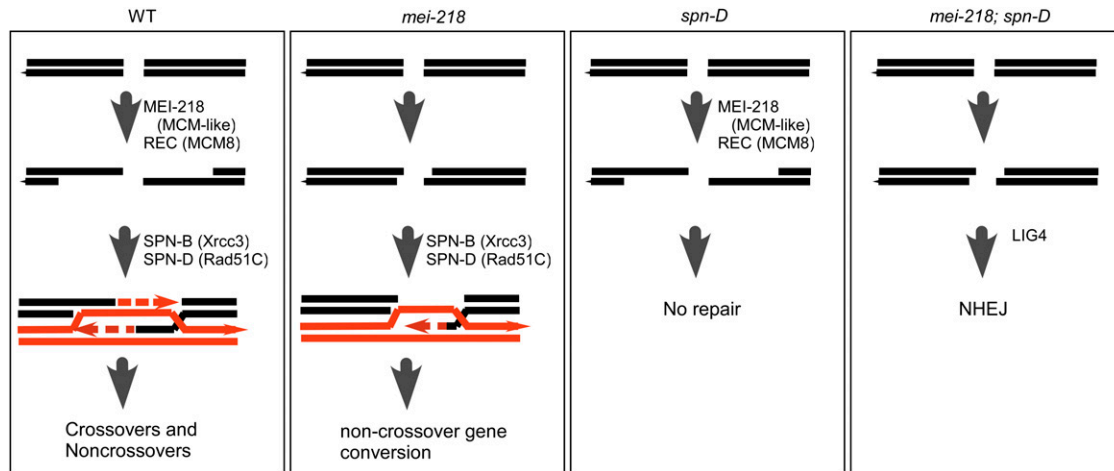
Figure 3 Model for how *mei-218*, *spn-B*, and *spn-D* affect the regulation of DSB repair during meiosis. In wild type, proteins encoded by precondition genes, *mei-218* and *rec*, function in HR by facilitating or extending single-strand resection at the sites of DSBs. SPN-D and SPN-B facilitate the homology search and single-strand invasion, forming repair intermediates that can yield either crossovers or noncrossovers. We propose that, in *mei-218* mutants, resection is defective. The resulting small tracts of ssDNA might be sufficient for the initial stages of recombination such as strand invasion, yet insufficient for crossover formation and checkpoint activation. In *spn-D* mutants, HR is defective and DSB repair is blocked. End joining may be inhibited by the presence of ssDNA or ssDNA-binding proteins such as RPA and MEI-41 (not shown). In *mei-218*; *spn-D* double mutants, however, LIG-4–dependent end-joining repair is permitted due to the loss of both ssDNA and the initiation of HR.

MEI-218, may be incompatible with the NHEJ repair pathway (Figure 3).

Whether the amount of resection influences crossover formation as well is not yet clear (Keelagher *et al.* 2010; Zakharyevich *et al.* 2010); however, in the absence of *mei-218* or *rec*, the frequency of crossovers is dramatically reduced and the residual crossovers exhibit an even distribution, suggesting pericentric suppression of crossovers is also disrupted. One possibility is that the change in distribution is an indirect consequence of the crossover reduction, similar to the interchromosomal effect (Bhagat *et al.* 2004). Alternatively, shorter gene conversion tracts may preferentially result in a noncrossover repair product while also creating an equal, yet low likelihood of crossover formation along the entirety of chromosome arms. Indeed, the amount of resection may even pose a potent mechanism to control and adjust the placement of crossovers across the genome. If precondition protein and thus resection activity were enhanced in particular chromosome regions, DSBs in those regions may experience a higher frequency of crossovers. Of course, we cannot exclude the possibility that precondition gene products also regulate DNA synthesis following resection and strand invasion during meiotic DSB repair (Blanton *et al.* 2005; Radford *et al.* 2007).

Our data along with others also argue that all precondition genes may not share the same function(s). While both MEI-218 and REC have MCM homology and are required for crossing over and full checkpoint activity, we have found that only MEI-218 affects NHEJ. Furthermore, whereas Blanton *et al.* (2005) reported that *rec* mutants have shorter conversion tracts, Curtis and Bender (1991) reported that *mei-218* mutants did not, although this finding conflicts with previous work from Carpenter (1982, 1984).

Thus, MEI-218 and REC may have at least some nonoverlapping functions that have yet to be elucidated.

Blocks to NHEJ: the Rad51 paralogs

The role of the Rad51 paralogs is only slightly less enigmatic than the precondition genes. Mutations in *spn-B* and *spn-D* have phenotypes very similar to mutations in the gene encoding the *Drosophila* Rad51 homolog, *spn-A* (Ghabrial *et al.* 1998; Abdu *et al.* 2003; Staeva-Vieira *et al.* 2003). These mutants fail to repair meiotic DSBs, resulting in checkpoint activity that causes dorsal–ventral polarity defects in the oocytes. However, while *spn-B* and *spn-D* mutants have repair defects, almost identical to that of *spn-A*, the Xrcc3 (SPN-B) and Rad51C (SPN-D) proteins do not have strand exchange activity. Instead, these accessory proteins are thought to mediate the assembly of Rad51 on the ssDNA at DSB sites (Heyer *et al.* 2010). Therefore, the second block to NHEJ may be in regulating important reversible transitions in the DSB repair pathway (Sung and Klein 2006). For example, reversal of Rad51 filament formation by a helicase like SRS2 could lead to NHEJ rather than HR (Symington and Heyer 2006). Loss of SPN-B or SPN-D may allow an SRS2-like activity (there is no true *Drosophila* SRS2 homolog) to dissociate SPN-A/Rad51-ssDNA filaments. Indeed, two budding yeast Rad51 paralogs that form a dimer, Rad55–Rad57, appear to have this function. It has been proposed that Rad55 and Rad57 protect the Rad51-ssDNA filament by blocking the translocation of Srs2 (Liu *et al.* 2011). This activity explains why the sensitivity to ionizing radiation found in yeast *rad55* or *rad57* mutants is suppressed by deletion of *SRS2*. Interestingly, a protein with activities similar to SRS2 has recently been identified in mammalian cells (Moldovan *et al.* 2012). We propose that

SPN-B–SPN-D has a similar effect on a protein with an anti-recombinase activity in *Drosophila*.

Together, these results suggest that the sites of DNA damage in meiotic prophase require a rapid response by early DSB processing enzymes to ensure proper HR while precluding error-prone repair pathways. If both MEI-218 and SPN-B–SPN-D are absent, DSBs form, but the limited resection and DSB processing generates a broken DNA substrate that can enter the NHEJ repair pathway.

Literature Cited

Abdu, U., M. Brodsky, and T. Schupbach, 2002   Activation of a meiotic checkpoint during Drosophila oogenesis regulates the translation of Gurken through Chk2/Mnk. Curr. Biol. 12: 1645.

Abdu, U., A. Gonzalez-Reyes, A. Ghabrial, and T. Schupbach, 2003   The Drosophila *spn-D* gene encodes a RAD51C-like protein that is required exclusively during meiosis. Genetics 165: 197–204.

Abe, K., K. Osakabe, S. Nakayama, M. Endo, A. Tagiri *et al.*, 2005   Arabidopsis *RAD51C* gene is important for homologous recombination in meiosis and mitosis. Plant Physiol. 139: 896–908.

Belmont, A. S., M. B. Braunfeld, J. W. Sedat, and D. A. Agard, 1989   Large-scale chromatin structural domains within mitotic and interphase chromosomes in vivo and in vitro. Chromosoma 98: 129–143.

Bhagat, R., E. A. Manheim, D. E. Sherizen, and K. S. McKim, 2004   Studies on crossover specific mutants and the distribution of crossing over in Drosophila females. Cytogenet. Genome Res. 107: 160–171.

Blanton, H. L., S. J. Radford, S. McMahan, H. M. Kearney, J. G. Ibrahim *et al.*, 2005   REC, Drosophila MCM8, drives formation of meiotic crossovers. PLoS Genet. 1: e40.

Bleuyard, J. Y., and C. I. White, 2004   The Arabidopsis homologue of Xrcc3 plays an essential role in meiosis. EMBO J. 23: 439–449.

Carpenter, A. T. C., 1982   Mismatch repair, gene conversion, and crossing over in two recombination-defective mutants of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 79: 5961–5965.

Carpenter, A. T. C., 1984   Meiotic roles of crossing over and of gene conversion. Cold Spring Harb. Symp. Quant. Biol. 49: 23–29.

Carpenter, A. T. C., and L. Sandler, 1974   On recombination-defective meiotic mutants in *Drosophila melanogaster*. Genetics 76: 453–475.

Couteau, F., K. Nabeshima, A. Villeneuve, and M. Zetka, 2004   A component of *C. elegans* meiotic chromosome axes at the interface of homolog alignment, synapsis, nuclear reorganization, and recombination. Curr. Biol. 14: 585–592.

Curtis, D., and W. Bender, 1991   Gene conversion in Drosophila and the effects of meiotic mutants *mei-9* and *mei-218*. Genetics 127: 739–746.

Forsburg, S. L., 2004   Eukaryotic MCM proteins: beyond replication initiation. Microbiol. Mol. Biol. Rev. 68: 109–131.

Ghabrial, A., and T. Schupbach, 1999   Activation of a meiotic checkpoint regulates translation of Gurken during Drosophila oogenesis. Nat. Cell Biol. 1: 354–357.

Ghabrial, A., R. P. Ray, and T. Schupbach, 1998   *okra* and *spindle-B* encode components of the RAD52 DNA repair pathway and affect meiosis and patterning in Drosophila oogenesis. Genes Dev. 12: 2711–2723.

Gorski, M. M., J. C. Eeken, A. W. de Jong, I. Klink, M. Loos *et al.*, 2003   The *Drosophila melanogaster DNA Ligase IV* gene plays a crucial role in the repair of radiation-induced DNA double-strand breaks and acts synergistically with Rad54. Genetics 165: 1929–1941.

Hawley, R. S., 1988   Exchange and chromosomal segregation in eucaryotes, pp. 497–527 in *Genetic Recombination*, edited by R. Kucherlapati, and G. Smith. American Society of Microbiology, Washington, DC.

Heyer, W. D., K. T. Ehmsen, and J. Liu, 2010   Regulation of homologous recombination in eukaryotes. Annu. Rev. Genet. 44: 113–139.

Ho, H. C., and S. M. Burgess, 2011   Pch2 acts through Xrs2 and Tel1/ATM to modulate interhomolog bias and checkpoint function during meiosis. PLoS Genet. 7: e1002351.

James, P., J. Halladay, and E. A. Craig, 1996   Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. Genetics 144: 1425–1436.

Jang, J. K., D. E. Sherizen, R. Bhagat, E. A. Manheim, and K. S. McKim, 2003   Relationship of DNA double-strand breaks to synapsis in Drosophila. J. Cell Sci. 116: 3069–3077.

Joyce, E. F., and K. S. McKim, 2009   Drosophila PCH2 is required for a pachytene checkpoint that monitors double-strand-break-independent events leading to meiotic crossover formation. Genetics 181: 39–51.

Joyce, E. F., M. Pedersen, S. Tiong, S. K. White-Brown, A. Paul *et al.*, 2011   Drosophila ATM and ATR have distinct activities in the regulation of meiotic DNA damage and repair. J. Cell Biol. 195: 359–367.

Keelagher, R. E., V. E. Cotton, A. S. Goldman, and R. H. Borts, 2010   Separable roles for Exonuclease I in meiotic DNA double-strand break repair. DNA Repair (Amst.) 10: 126–137.

Keeney, S., 2001   Mechanism and control of meiotic recombination initiation. Curr. Top. Dev. Biol. 52: 1–53.

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.

Klovstad, M., U. Abdu, and T. Schupbach, 2008   Drosophila *brca2* is required for mitotic and meiotic DNA repair and efficient activation of the meiotic recombination checkpoint. PLoS Genet. 4: e31.

Kurumizaka, H., S. Ikawa, M. Nakada, K. Eda, W. Kagawa *et al.*, 2001   Homologous-pairing activity of the human DNA-repair proteins Xrcc3.Rad51C. Proc. Natl. Acad. Sci. USA 98: 5538–5543.

Kuznetsov, S., M. Pellegrini, K. Shuda, O. Fernandez-Capetillo, Y. Liu *et al.*, 2007   RAD51C deficiency in mice results in early prophase I arrest in males and sister chromatid separation at metaphase II in females. J. Cell Biol. 176: 581–592.

Lake, C. M., K. Teeter, S. L. Page, R. Nielsen, and R. S. Hawley, 2007   A genetic analysis of the Drosophila mcm5 gene defines a domain specifically required for meiotic recombination. Genetics 176: 2151–2163.

Li, W., X. Yang, Z. Lin, L. Timofejeva, R. Xiao *et al.*, 2005   The *AtRAD51C* gene is required for normal meiotic chromosome

synapsis and double-stranded break repair in Arabidopsis. Plant Physiol. 138: 965–976.

Li, X. C., E. Bolcun-Filas, and J. C. Schimenti, 2011   Genetic evidence that synaptonemal complex axial elements govern recombination pathway choice in mice. Genetics 189: 71–82.

Lieber, M. R., Y. Ma, U. Pannicke, and K. Schwarz, 2003   Mechanism and regulation of human non-homologous DNA end-joining. Nat. Rev. Mol. Cell Biol. 4: 712–720.

Liu, H., J. K. Jang, J. Graham, K. Nycz, and K. S. McKim, 2000   Two genes required for meiotic recombination in Drosophila are expressed from a dicistronic message. Genetics 154: 1735–1746.

Liu, H., J. K. Jang, N. Kato, and K. S. McKim, 2002   mei-P22 encodes a chromosome-associated protein required for the initiation of meiotic recombination in Drosophila melanogaster. Genetics 162: 245–258.

Liu, J., L. Renault, X. Veaute, F. Fabre, H. Stahlberg et al., 2011   Rad51 paralogues Rad55-Rad57 balance the antirecombinase Srs2 in Rad51 filament formation. Nature 479: 245–248.

Manheim, E. A., J. K. Jang, D. Dominic, and K. S. McKim, 2002   Cytoplasmic localization and evolutionary conservation of MEI-218, a protein required for meiotic crossing over in Drosophila. Mol. Biol. Cell 13: 84–95.

Martin, J. S., N. Winkelmann, M. I. Petalcorin, M. J. McIlwraith, and S. J. Boulton, 2005   RAD-51-dependent and -independent roles of a Caenorhabditis elegans BRCA2-related protein during DNA double-strand break repair. Mol. Cell. Biol. 25: 3127–3139.

McKim, K., E. F. Joyce, and J. K. Jang, 2008   Cytological Analysis of Meiosis in Fixed Drosophila Ovaries, Humana Press, Totowa, NJ.

McKim, K. S., J. B. Dahmus, and R. S. Hawley, 1996   Cloning of the Drosophila melanogaster meiotic recombination gene mei-218: a genetic and molecular analysis of interval 15E. Genetics 144: 215–228.

Mehrotra, S., and K. S. McKim, 2006   Temporal analysis of meiotic DNA double-strand break formation and repair in Drosophila females. PLoS Genet. 2: e200.

Moldovan, G. L., D. Dejsuphong, M. I. Petalcorin, K. Hofmann, S. Takeda et al., 2012   Inhibition of homologous recombination by the PCNA-interacting protein PARI. Mol. Cell 45: 75–86.

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.

Page, S. L., and R. S. Hawley, 2001   c(3)G encodes a Drosophila synaptonemal complex protein. Genes Dev. 15: 3130–3143.

Phadnis, N., R. W. Hyppa, and G. R. Smith, 2011   New and old ways to control meiotic recombination. Trends Genet. 27: 411–421.

Radford, S. J., and J. J. Sekelsky, 2004   Taking Drosophila Rad51 for a SPiN. Nat. Struct. Mol. Biol. 11: 9–10.

Radford, S. J., S. McMahan, H. L. Blanton, and J. Sekelsky, 2007   Heteroduplex DNA in Meiotic Recombination in Drosophila mei-9 mutants. Genetics 176: 63–72.

Romeijn, R. J., M. M. Gorski, M. A. van Schie, J. N. Noordermeer, L. H. Mullenders et al., 2005   Lig4 and rad54 are required for repair of DNA double-strand breaks induced by P-element excision in Drosophila. Genetics 169: 795–806.

Smolikov, S., A. Eizinger, A. Hurlburt, E. Rogers, A. M. Villeneuve et al., 2007   Synapsis-defective mutants reveal a correlation between chromosome conformation and the mode of double-strand break repair during Caenorhabditis elegans meiosis. Genetics 176: 2027–2033.

Staeva-Vieira, E., S. Yoo, and R. Lehmann, 2003   An essential role of DmRad51/SpnA in DNA repair and meiotic checkpoint control. EMBO J. 22: 5863–5874.

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.

Sung, P., L. Krejci, S. Van Komen, and M. G. Sehorn, 2003   Rad51 recombinase and recombination mediators. J. Biol. Chem. 278: 42729–42732.

Symington, L. S., and W. D. Heyer, 2006   Some disassembly required: role of DNA translocases in the disruption of recombination intermediates and dead-end complexes. Genes Dev. 20: 2479–2486.

Webber, H. A., L. Howard, and S. E. Bickel, 2004   The cohesion protein ORD is required for homologue bias during meiotic recombination. J. Cell Biol. 164: 819–829.

Zakharyevich, K., Y. Ma, S. Tang, P. Y. Hwang, S. Boiteux et al., 2010   Temporally and biochemically distinct activities of Exo1 during meiosis: double-strand break resection and resolution of double Holliday junctions. Mol. Cell 40: 1001–1015.

Zou, L., and S. J. Elledge, 2003   Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science 300: 1542–1548.

Communicating editor: N. Hollingsworth