***mei-W68* in *Drosophila melanogaster* encodes a Spo11 homolog: evidence that the mechanism for initiating meiotic recombination is conserved**

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Meiotic recombination requires the action of several gene products in both *Saccharomyces cerevisiae* and *Drosophila melanogaster*. Genetic studies in *D. melanogaster* have shown that the *mei-W68* gene is required for all meiotic gene conversion and crossing-over. We cloned *mei-W68* using a new genetic mapping method in which P elements are used to promote crossing-over at their insertion sites. This resulted in the high-resolution mapping of *mei-W68* to a <18-kb region that contains a homolog of the *S. cerevisiae spo11* gene. Molecular analysis of several mutants confirmed that *mei-W68* encodes an *spo11* homolog. Spo11 and MEI-W68 are members of a family of proteins similar to a novel type II topoisomerase. On the basis of this and other lines of evidence, Spo11 has been proposed to be the enzymatic activity that creates the double-strand breaks needed to initiate meiotic recombination. This raises the possibility that recombination in *Drosophila* is also initiated by double-strand breaks. Although these homologous genes are required absolutely for recombination in both species, their roles differ in other respects. In contrast to *spo11*, *mei-W68* is not required for synaptonemal complex formation and does have a mitotic role.

[Key Words: Meiotic recombination; synaptonemal complex; *Drosophila*; meiosis; double-strand break]

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Meiotic recombination events result when a break in the DNA is repaired using the homolog as a template. Part of this reaction occurs between homologs that are paired tightly and held together by a structure known as the synaptonemal complex. In addition, most evidence is consistent with the idea that meiotic recombination is mediated by an organelle associated with the synaptonemal complex known as the recombination nodule (Carpenter 1987). In most organisms, the majority of events resolve as gene conversions, with 20% or less becoming crossovers (Carpenter 1987; Hilliker et al. 1988). Only the small percentage of events that become crossovers mature into chiasmata. As chiasmata direct segregation by linking and orienting homologous chromosomes on the developing spindle so that they segregate to opposite poles at anaphase (Hawley 1988), it is easy to see why crossovers are central to the progression of meiosis. Without crossovers and, therefore, chiasmata, the proper segregation of homologous chromosomes could not be ensured.

With the notable exception of *Saccharomyces cerevi-*

siae, nothing is known about how meiotic recombination is initiated. Two models incorporating the Holliday junction have dominated thinking about meiotic recombination. In the first, Meselson and Radding (1975) proposed that a Holliday junction was initiated with a single-strand break on one homolog. In the second model, Szostak et al. (1983) proposed that a double Holliday junction was initiated with a double-strand break in one homolog. In *S. cerevisiae*, there is abundant physical evidence that double-strand breaks are involved in meiotic recombination. Double-strand breaks are induced early in meiotic prophase and are resolved eventually as recombinants (for review, see Lichten and Goldman 1995). Often these breaks occur at hot spots, which tend to be regions of open chromatin within promoters (Wu and Lichten 1994). Additional support for the double-strand break model came from the physical identification and analysis of joint molecules and double Holliday junctions in meiotic prophase (Schwacha and Kleckner 1995). These results have not, however, been extended to any other organism.

To characterize the initiation of meiotic recombination in *Drosophila melanogaster*, we have been studying mutants in which meiotic gene conversion is reduced or eliminated. Our rationale is that if gene conversion is

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eliminated, it is possible that the defect is a failure to initiate recombination. This assay differentiates these mutants from those that reduce crossing over but have no effect on gene conversion (Carpenter 1984). Previous work described mutants in two genes, *mei-W68* and *mei-P22*, that meet these criteria (McKim et al. 1998). In this paper we report the cloning of *mei-W68* and show that it encodes a homolog of Spo11, known previously to be required for double-strand break formation in *S. cerevisiae* (Cao et al. 1990). The homology to *spo11* gives strong support to our conclusion that *mei-W68* is required for the initiation of meiotic recombination. A surprising aspect of the *mei-W68* mutant phenotype is that it affects somatic as well as meiotic cells. In *S. cerevisiae*, the expression of *spo11* and the mutant phenotypes are meiosis-specific (Atcheson et al. 1987). Conversely, *mei-W68* is transcribed in many somatic cell types and *mei-W68* mutants exhibit defects during the mitotic cell cycle (Baker et al. 1978; Lutken and Baker 1979).

Results

Genetic mapping of *mei-W68* by a new method using P element-mediated crossing-over

mei-W68 was cloned based on its genetic map position. Preliminary mapping experiments placed *mei-W68* between 56C1-2 and 56F5 (Fig. 1 and Materials and Methods). Higher resolution mapping was accomplished using P-element insertion mutations in the 56C-56F region from the *Drosophila* Genome Project (Spradling et al. 1995). This approach facilitated the cloning of the gene for two reasons: First, there were insertions present at a relatively high density, and second, the DNA flanking each P element could be isolated by inverse PCR and then used as a probe to determine the location of the insertion on the physical map.

The major problem in high-resolution mapping of *mei-*

W68 (or any gene) relative to closely linked P elements is the difficulty in generating useful recombinants. We have used two features of P-element biology to collect recombinants efficiently that are informative for the mapping of genes (see Chen et al. 1998). First, crossing-over is stimulated by the simultaneous presence of P elements and transposase (Hiraizumi 1971; Kidwell and Kidwell 1976). Second, by doing the experiment in *Drosophila* males, in which meiotic crossing-over does not occur, most of the crossover events are located at the P element (Preston et al. 1996). By controlling where the crossover event will be, each event is expected to be informative for mapping purposes (Fig. 2). Using this technique, *mei-W68* was mapped relative to six P-element insertions (Table 1) and, all of the crossover events did appear to be at the P element. On the basis of the cytological positions of the six inserts, *mei-W68* was localized to cytological interval 56D7-56D11 (Fig. 1); the closest proximal insert was *P{lacW}l(2)k00705* (to be abbreviated 705) and the closest distal insert was *P{lacW}l(2)k06323* (to be abbreviated 6323).

We constructed *yw/yw; 705 mei-W68^{+/+} + 6323* females in an attempt to measure the genetic distance between the two inserts. No *white⁻* crossovers were recovered in ~13,000 progeny. Thus, these two insertions were estimated to be <.02 cM apart. This result emphasizes the advantage of using transposase-induced male crossing-over to map genes. During female meiosis, a very large number of progeny would have had to have been screened to get a crossover within the small distance between one of the inserts and *mei-W68*.

Localization of *mei-W68* to a region of genomic DNA containing a *spo11* homolog

The region to which *mei-W68* was mapped is covered by a contig of P1 clones, DS00433 (56D7-56E1), from the

Figure 1. Genetic map of the *mei-W68* region. *mei-W68* was mapped relative to the P-element insertions (triangles). The P-element insertions flanking the *mei-W68* locus that were inserted within the overlap of P1 clones DS07982 and DS00571 are shaded. Deficiencies that complemented *mei-W68* are shown with their cytogenetic breakpoints. The *P{PZ}05338* insertion is an allele of *smooth* (Lage et al. 1997). *P{PZ}01103* is inserted at the *hts* locus (Berkeley *Drosophila* Genome Project).

Figure 2. The types of recombinants recovered from P element-mediated male crossing-over. This schematic map shows the markers used in most of the mapping experiments. It is not drawn to scale as the two insertions are much closer together relative to the flanking markers *cn*, *px*, and *sp*. If the P element was to the left (distal) of *mei-W68*, the + *px sp* cross-overs (A) also carried the *mei-W68* mutation. Conversely, if the insertion was to the right (proximal) of *mei-W68*, then the + *px sp* cross-overs (B) did not carry the *mei-W68* mutation. Shown is the direction of only one of the two crossover classes that was recovered from each experiment. Relative to the + *px sp* crossovers, the *cn* ++ crossovers had the opposite linkage arrangement with *mei-W68*.

Drosophila Genome Project (Hartl et al. 1994; Kimmerly et al. 1996). We used inverse PCR to amplify the DNA adjacent to seven P-element insertions near *mei-W68* (Materials and Methods). This DNA was labeled and used to probe Southern blots containing DNA from a series of P1 clones representing DS00433. In combination with the genetic mapping, these data showed that the *mei-W68* coding region was located within the overlap of P1 clones DS07982 and DS00571 (Fig. 1). Our restriction map of the region shows the distance between the distal marker 6323 and the proximal marker 705 to be < 18 kb (Fig. 3). DS07982 has also been sequenced by the *Drosophila* Genome Project (AC004299; Berkeley *Drosophila* Genome Project, unpubl.) and the two maps are in agreement. Subsequent to this work, sequence from the insertion site of each P element was obtained by the genome project, which showed that the distance between 6323 and 705 is 12.3 kb. The sequence also revealed a candidate for *mei-W68*; an open reading frame (ORF) was identified in a BLAST search using the protein sequence of either *spo11* from *S. cerevisiae* or its *Caenorhabditis elegans* homolog (see below).

Isolation and molecular analysis of *mei-W68* mutants

We mobilized P-element insertions to induce new mutations of *mei-W68* that would provide physical markers

identifying the coding region. We hoped to isolate new insertion mutations of *mei-W68* using closely linked P elements, which, in the presence of transposase, tend to move to new locations near the original site, so-called 'local hops' (Tower et al. 1993). Excision events were collected simultaneously, with the hope of isolating deletions that resulted from imprecise excision of the P element. Both types of events were screened for a failure to complement *mei-W68*¹.

Extensive experiments using either 705 or *P{PZ}01103* (Fig. 1) failed to produce new *mei-W68* mutants (see Materials and Methods). From the mobilization of the 6323 insertion we recovered 267 excision chromosomes, seven of which failed to complement *mei-W68*, and 216 local hop chromosomes, 11 of which failed to complement *mei-W68*. The excision events were assumed to be deletions because they failed to complement two loci, *mei-W68* and the lethality of the parental 6323 chromosome. The phenotype of the local hops was surprising for two reasons. First, all 11 of the local hops were homozygous lethal and failed to complement the lethality of the parental 6323 chromosome and, therefore, were imprecise excisions of the 6323 element. Second, they had a strong nondisjunction phenotype in trans to *mei-W68*¹ (data not shown), which is unusual because P elements frequently cause weak mutations because they insert preferentially into promoter regions or introns.

Table 1. Mapping *mei-W68* by P element-mediated male crossing-over

Insertion ^a	<i>cn</i> ⁺	⁺ <i>sp</i>	Total ^b
Cross A			
<i>P{PZ}l(2)01103</i>	7 (7/7 <i>mei-W68</i> ⁺)	10 (10/10 <i>mei-W68</i> ⁺)	3300
<i>P{PZ}l(2)05338</i>	9 (9/9 <i>mei-W68</i> ⁺)	9 (9/9 <i>mei-W68</i> ⁺)	4100
<i>P{PZ}emm</i>	5 (2/2 <i>mei-W68</i> ⁺)	3 (2/2 <i>mei-W68</i> ⁺)	3300
Cross B			
<i>P{lacW}l(2)k06323</i>	1 (1/1 <i>mei-W68</i> ⁺)	3 (3/3 <i>mei-W68</i> ⁺)	N.D.
<i>P{lacW}l(2)k00705</i>	1 (1/1 <i>mei-W68</i> ⁺)	2 (2/2 <i>mei-W68</i> ⁺)	N.D.
<i>P{lacW}l(2)k16914</i>	1 (1/1 <i>mei-W68</i> ⁺)	4 (4/4 <i>mei-W68</i> ⁺)	N.D.

^a(Cross A) *cn P{PZ} px sp/mei-W68* +; Δ2-3, *Sb/ry* males crossed to *cn bw sp/SM6*, *cn sp*; *ry* females. (Cross B) *P{lacW}/cn mei-W68 px sp* +; Δ2-3, *Sb/ry* males crossed to *cn bw sp/SM6*, *cn sp*; *ry* females.

^b(N.D.) Not determined.

Figure 3. Physical map of the *mei-W68* region. (A) The restriction map came from two sources: our own restriction mapping of DS00571, and the Genome Project sequencing of the overlapping P1 DS07982. The molecular structures of three *mei-W68* deletion mutants are shown. The brackets above the genetic map are to indicate that the *LL4*, *LL5*, and *LD3* chromosomes are deletions with one endpoint at the original *P{lacW}l(2)k06323* insertion site. (B) Depiction of the *mei-W68* transcript showing the intron/exon structure and the location of the insertions in *mei-W68^l* and *mei-W68^{k05603}*. To show that the *mei-W68^l* insertion was not in the first or second introns, we probed *HindIII* and *XhoI* digests with labeled *EcoRI* fragment pWE31 (B). The appearance of the larger insertion band in the *HindIII* digest and the wild-type bands in the *XhoI* digest (data not shown) showed that the insertion site was between the first and second introns. The position of the *k05603* insertion was determined from sequence obtained by the genome project off the 5' end of the P element (Berkeley *Drosophila* Genome Project).

Molecular analysis of three local-hop mutations showed that they were associated with deletions (Fig. 3). Inverse PCR was used to isolate the genomic DNA flanking the local-hop insertion sites. Probes were made from the DNA on either the 5' or 3' end of two mutants, *local lighthop 4* (*LL4*) and *LL5*, and used to probe blots containing P1 clone-derived restriction fragments of the region. The probes from the 5' end of each *LL* mutant hybridized to a new fragment, whereas the probes from the 3' end hybridized to the original fragment that contained 6323. It is likely that in each case, the P element made a 'half-jump'. The 5' end (left in Fig. 3) excised and inserted at a new location within or on the other side of *mei-W68*, whereas the 3' end (right in Fig. 3) did not excise and thus remained at the same site as 6323. This event resulted in the deletion of the intervening DNA. A third mutant, *local darkhop 3* (*LD3*), was analyzed by Southern blot and probably is also a deletion. The half-jump model may be a general mechanism for P-element induced deletions because events of this nature have been recovered before (for review, see Preston et al. 1996). The molecular and genetic analysis shows that the local-hop *mei-W68* mutants are deletions, and their lethal phenotype can be explained by mutation of the 6323 locus. The *mei-W68^l/Df* females were as viable and fertile as the *mei-W68^l/mei-W68^l* females. Thus, there is no evidence that the *mei-W68* gene product is required for normal viability.

The above analysis showed that three *mei-W68* deletions also affected the *spo11* homolog, consistent with the idea that it is the *mei-W68* gene (Fig. 3). For direct evidence we analyzed the *spo11*-coding region of the strong *mei-W68^l* mutant. Southern blot and PCR analy-

sis has shown that there is a ~5 kb insertion within the second exon of the *mei-W68* transcript (Fig. 3B). The *Drosophila* Genome Project has also isolated a P-element insertion allele of *mei-W68*, known previously as *P{lacW}l(2)k05603*, that is inserted in the 5' untranslated region (UTR) of *mei-W68* (Fig. 3B). Whereas this mutant was classified originally as a lethal, we found the P-element insertion homozygotes to be viable. We have renamed this mutation *mei-W68^{k05603}* because it fails to complement the X chromosome nondisjunction phenotype of *mei-W68^l* and *Df(2R)LL5*. The analysis of *mei-W68^l* and *mei-W68^{k05603}* demonstrates that *mei-W68* encodes an Spo11 homolog.

cDNA and protein sequence

Several clones were isolated from a germline cDNA library (see Fig. 5, below) using a genomic fragment containing the *spo11* homolog as a probe (see Materials and Methods). The largest clones were 2.3 kb and most likely full length because they had a similar size to the predominant transcript seen on a Northern blot (see below and Fig. 3B). An unusual feature of the transcript is the relatively large first intron (996 bp) just before the predicted initiator methionine codon. The transcript also has a large 5' UTR (861 bp), which suggests that *mei-W68* might be regulated at the translational level. The multiple sequence alignment of the predicted *mei-W68* protein to other *spo11* homologs (Fig. 4) shows that the nematode and fly genes have the least divergence, whereas the *S. cerevisiae* gene is the most diverged. In describing this gene family, Bergerat et al. (1997) defined five homologous domains, and *mei-W68* has high similarity in all of them. In the first domain, *mei-W68* has

Figure 4. *mei-W68* is a homolog of *spo11* and the *topo6A* type II topoisomerase. (A) Protein sequence alignment *spo11* homologs from *S. cerevisiae* Spo11 (Atcheson et al. 1987), *Schizosaccharomyces pombe* Rec12 (Lin and Smith 1994), *Sulfolobus shibatae* topo6A (Bergerat et al. 1997), and the predicted *C. elegans* gene from cosmid T05E11.4 (Wilson et al. 1994; Dernburg et al. 1998). (B) Dendrogram comparing the extent of similarity between the different proteins. The percentages of similar and identical amino acids are relative to MEI-W68.

the tyrosine (position 81) which is believed to make a covalent bond to DNA. All of the homologs except *spo11* have two tyrosines at this position. Nonetheless, the overall similarity of the proteins is quite low (21%–28% identity, 34%–38% similarity; Fig. 4B).

mei-W68 is expressed in a variety of tissues and developmental stages

The *mei-W68* transcript is expressed in a variety of developmental stages, including embryos, larvae, and pupae (Fig. 5A). In all stages two transcripts are visible. There is a predominant 2.3-kb transcript, which is consistent with the size of the cDNA clones, and also a smaller 2.1-kb transcript. There is also a larger 2.4-kb transcript in embryos and pupae. The function and structure of the 2.1- and 2.4-kb transcripts are not known. The presence of the 2.3-kb transcript in most developmental stages suggests that *mei-W68* has a function in somatic cells. The transcript was difficult to detect in males on a Northern blot, but RT-PCR experiments confirmed that *mei-W68* is transcribed in the testis at a low level (data not shown and P.J. Romanienko, B. Oliver, and R.D.

Camerini-Otero, pers. comm.). The difference in *mei-W68* RNA levels between males and females can be attributed to the relatively high level of expression in the ovaries. Thus, there is little or no transcript in adult somatic tissue. For the ovary, we also used in situ hybridization and detected the *mei-W68* transcript at a low level in the germarium, which is where meiotic prophase occurs. It was not detected in later stages of ovary development (Fig. 5B).

Discussion

mei-W68 encodes a topoisomerase II-like protein homologous to Spo11

mei-W68 is required for all meiotic recombination but not for normal synaptonemal complex between homologs (McKim et al. 1998). Whereas the simplest hypothesis is that *mei-W68* is required for initiating meiotic recombination, supporting molecular evidence has been lacking as the gene was not cloned. Here we have shown that the MEI-W68 protein has sequence homology to Spo11 from *S. cerevisiae*. Like *mei-W68*, in *spo11*

Figure 5. Developmental Northern blot using a *mei-W68* antisense RNA probe. The ribosomal protein gene *RP49* was used as a standard for the amount of mRNA loaded on the gel. (A) There are three transcripts of 2.4, 2.3, and 2.1 kb. The 2.3-kb transcript is seen through all developmental stages. The largest transcript (2.4 kb) is detected in the first 12 hr of embryogenesis and in the pupal stage. The 2.1-kb transcript is seen in all stages but is clear at 12–24 hr embryonic stage and larval stages. A transcript was difficult to detect in males but was detected by RT-PCR (see text). The numbers for lanes 2–6 indicate the hours of embryonic development. (7d) The pupal stage at day 7 of development. (B) Two examples of an in situ hybridization to whole ovaries using an RNA probe made from the *mei-W68* full-length cDNA. (Left) The transcript was detectable in the latter half of the germarium, which corresponds to regions 2 and 3, but there was little or no transcript was detectable in later stages. (Right) Another germarium at higher magnification. The transcript was not detected in region 1, where the premeiotic mitotic divisions occur.

mutants both gene conversion and crossing over are eliminated (Cao et al. 1990; McKim et al. 1998). In addition, *spo11* is required for meiotic double-strand break formation in *S. cerevisiae*. In *Drosophila*, therefore, it is likely that double-strand breaks are the event that initiate meiotic recombination, and that *mei-W68* has a vital role in their generation. The connection is further strengthened by two observations that support the hypothesis that Spo11 is the enzymatic factor that makes double-strand breaks. First, the predicted amino acid sequence of Spo11 shows that it is a member of a new family of proteins with sequence similarity to the *topo6A* gene product from the archaea *Sulfolobus shibatae* (Bergerat et al. 1997). In *S. shibatae*, a dimer consisting of *topo6A* and *topo6B* is a novel type II topoisomerase (Bergerat et al. 1994). Second, several authors found that a protein is linked covalently to unprocessed double-strand breaks from meiotic cells of yeast *rad50S* mutants (de Massy et al. 1995; Keeney and Kleckner 1995; Liu et al. 1995; Bergerat et al. 1997). Keeney et al. (1997) identified this protein as Spo11. Whereas the suggestion is that Spo11 is a type II topoisomerase, it should be emphasized that the biochemical activity of Spo11 has yet to be described. If Spo11 makes the meiotic double-strand break, then it does not act strictly as a type II topoisomerase because the double-strand break is not rejoined.

Further experiments will be required to determine whether processing of the double-strand break differs in yeast and flies. The generation of crossovers requires different genes in these two organisms. Two genes in *Drosophila*, *mei-9*, and *mei-218*, that are required for 95% of crossing-over (but not gene conversion) have been cloned. The *mei-9* gene is a homolog of *RAD1* from *S. cerevisiae* (Sekelsky et al. 1995). Surprisingly, *RAD1* is

not required for meiotic crossing over. A homolog of the *Drosophila mei-218* gene is not found in the *S. cerevisiae* genome (McKim et al. 1996).

Meiotic chromosome pairing and synapsis without double-strand breaks

The similar sequence and phenotypes of *spo11* and *mei-W68* suggests that they play analogous roles in the generation of meiotic double-strand breaks. On the other hand, *spo11* and *mei-W68* mutants have different effects on synaptonemal complex formation. Meiotic recombination is eliminated in *mei-W68* homozygotes, but normal synaptonemal complex forms between the homologs (McKim et al. 1998). In *S. cerevisiae*, however, *spo11* and other similar *rec⁻* mutants do not form synaptonemal complex (see Klapholz et al. 1985; Giroux et al. 1989; for review, see Roeder 1997), and analysis of the *zip1* mutant lent support to a model in which synaptonemal complex formation initiates at the sites of meiotic recombination events (Rockmill et al. 1995). The difference in flies, therefore, is that synaptonemal complex can form in the absence of meiotic recombination. With the finding that *mei-W68* is a *spo11* homolog, the most likely scenario for *Drosophila* meiosis is that double-strand breaks initiate meiotic recombination, but they are not needed for the homologs to form synaptonemal complex. These results confirm our earlier conclusion (McKim et al. 1998) that yeast and *Drosophila* have different controls on synaptonemal complex formation. Furthermore, the phenotype of mutants in the *C. elegans spo11* homolog is almost identical to *mei-W68* with respect to meiotic recombination and synaptonemal complex formation (Dernburg et al. 1998). Thus, our conclu-

sions concerning the relationship of double-strand breaks to meiotic recombination and synaptonemal complex formation may apply to a variety of metazoans.

Pairing between homologs prior to the initiation of recombination has been implied in studies involving a variety of organisms. Presynaptic alignment, which is when homologs pair at a distance prior to synaptonemal complex formation, probably occurs before the initiation of meiotic recombination (Giroux 1988; Loidl 1990). In *S. cerevisiae*, there is significant alignment or pairing of homologs prior to double-strand break formation (Weiner and Kleckner 1994), as well as in mutants that fail to make double-strand breaks (Loidl et al. 1994; Nag et al. 1995). The mechanisms for presynaptic alignment of homologs are understood poorly, but there are various models that fall into two major types. First, specialized regions, or pairing sites, could mediate homolog pairing (Hawley 1980; McKim et al. 1993; Zetka and Rose 1995). Second, a homology search could involve unstable interactions between intact DNA molecules (Weiner and Kleckner 1994; Kleckner 1996). The two models are not, however, mutually exclusive, since the pairing sites might be specialized regions of the chromosome that are capable of interacting at the DNA level without breaks. These models can account for the observations of synaptonemal complex formation without double-strand breaks in *Drosophila* and *C. elegans*. Perhaps the main difference between yeast and the metazoans is the extent to which double-strand break independent pairing can stimulate synaptonemal complex between homologs.

MEI-W68 has a somatic function

S. cerevisiae spo11 is expressed only during meiosis (Atcheson et al. 1987; but see Bruschi and Esposito 1983), but *mei-W68* has a role in somatic cells. Baker et al. (1978) found that in *mei-W68* mutants the frequency of somatic clones in the abdomen (but not in the wing) was increased three times over wild type. Most of this increase was attributed to an increase in mitotic recombination. The unusual features of the clones, however, such as their tissue specificity and large size, caused these investigators to suggest that the majority of *mei-W68*-induced clones occurred in nondividing cells. Similarly, Lutken and Baker (1979) showed that mitotic crossing-over in the germ-line of *mei-W68* males was increased 10 times over wild type (there is no meiotic crossing-over in *Drosophila* males). Finally, and as predicted by the genetic studies, we have found that the *mei-W68* transcript is expressed in many somatic cell types. *mei-W68* is even found at a low level in male testis, in which meiotic recombination does not normally occur. Whereas it is possible that *mei-W68* may be required for an aspect of DNA repair, we do not favor this hypothesis because *mei-W68* mutants are not mutagen sensitive (K.S. McKim, unpubl.). Unfortunately, our limited understanding of *mei-W68* function does not allow us to resolve the paradox that during meiosis the mutant eliminates recombination, whereas in mitosis it increases recombination. A similar situation occurs in two

S. cerevisiae genes, *RAD50* and *XRS2*. Mutations in these two genes eliminate all meiotic recombination, yet have a hyper-recombination phenotype in mitotic cells (Malone et al. 1990; Ivanov et al. 1992).

These data have the implication that many eukaryotes may have two type II topoisomerase enzymes. In addition to the MEI-W68 enzyme, *Drosophila* has a typical type II topoisomerase enzyme (Heller et al. 1986; Whalen et al. 1991). The meiotic-specificity of Spo11 suggests that metazoans might have a requirement for these proteins that is not present in yeast. A problem with this interpretation is the uncertainty regarding the biochemical activity of the Spo11-like proteins. During meiosis, Spo11 does not act like a type II topoisomerase since the double-strand breaks are not resealed. Thus, the eukaryotic Spo11 proteins may have lost the second part of the typical type II function. In this case, during mitosis MEI-W68 might behave as an endonuclease, a function consistent with a role in DNA repair (see above). Having only part of the type II enzymatic activity could be caused by the absence in all of the Spo11 homologs of an ATPase domain. Canonical type II enzymes lacking their ATPase domain can still cut DNA, but the other activities are compromised (Berger et al. 1996). In *S. shibatae* the topo6B protein contains the ATPase domain, but so far no subunit that interacts with a eukaryotic Spo11 has been identified.

As topoisomerase activity of *top6A* is dependent on a subunit, the activity of MEI-W68 could be altered in meiosis and mitosis by interacting with different sets of proteins. In *S. cerevisiae* and *Drosophila*, several gene products are required for creating double-strand breaks. For example, nine yeast genes have been identified that are required for double-strand break formation, six of which are meiosis specific, including *spo11* (Roeder 1995). In *Drosophila*, the *mei-P22* gene has the same mutant phenotype as *mei-W68* and is therefore also required for the initiation of meiotic recombination and likely for the generation of double-strand breaks (McKim et al. 1998). During meiosis, several proteins, including MEI-P22, might interact with MEI-W68 to prevent it from sealing the double-strand break. In mitosis, however, a different set of interacting proteins would allow MEI-W68 to have a more typical type II activity.

Materials and methods

Genetic techniques

All crosses were carried out at 25°C. Some stocks used in this study were received from the Bloomington Stock Center or Todd Lavery of the *Drosophila* Genome Project at the University of California at Berkeley. The P-element insertion lines used in this study are described by Spradling et al. (1995). There were two alleles of *mei-W68* isolated prior to this study (Lindsley and Zimm 1992). The first allele is *mei-W68¹*, which is a strong allele showing 38.7% X chromosome nondisjunction (McKim et al. 1998). The second allele is *mei-W68^{L-1}*, which is weaker and has only 3.6% X chromosome nondisjunction (K.S. McKim, un-

publ.). *mei-W68*¹ appeared during a screen for X-linked meiotic mutants (A.T.C. Carpenter and B.S. Baker, pers. comm.; Baker and Carpenter 1972). Because in that screen the mutagenized second chromosomes were eliminated in the crosses to isolate the X-linked mutants, *mei-W68*¹ was not ethyl methane sulfonate (EMS) induced but arose spontaneously. The *mei-W68*^{k05603} allele was identified because sequence off the end of the P element (Berkeley *Drosophila* Genome Project, unpubl.) showed that it was inserted into the 5' UTR.

mei-W68 was mapped originally to genetic position 94, which corresponds to cytological position 57B (Lindsley and Zimm 1992). None of the available deficiencies in the region failed to complement *mei-W68*. These deficiencies covered the entire division 57, making it likely that *mei-W68* was in division 56 or 58. We mapped *mei-W68* to division 56 in a standard three-factor mapping experiment using a P-element insertion at 56C1-2 *P{PZ}l(2)02029* and the *px* mutation at 58F (Fig. 1). From *cn P{PZ}l(2)02029 + px sp/+ + mei-W68 ++; ry* females, 23 *ry px sp* crossovers and 85 *ry⁺ px⁺ sp⁺* crossovers were picked and tested for the *mei-W68* phenotype of elevated X-chromosome nondisjunction: 21 of the *ry px sp* crossovers were *mei-W68* and two were *mei-W68*⁺, 80 of the *ry⁺ px⁺ sp⁺* crossovers were *mei-W68*⁺, and 5 were *mei-W68*. These data placed *mei-W68* close to the right of *P{PZ}l(2)02029*.

The *mei-W68* nondisjunction phenotype was assayed by crossing females to *C(1;Y), v f B; C(4)RM, ey^R ci* males. From this cross, the regular progeny were Bar females and wild-type males. Progeny resulting from X chromosome nondisjunction in the mother appeared as wild-type females and Bar males. Alternatively (i.e., the screen for *mei-W68* mutations; Fig. 6), X-chromosome disjunction was tested in the cross of *y/y* females to *y/y⁺Y* males. The regular progeny were yellow females and wild-type males, whereas the nondisjunctive progeny were wild-type females and yellow males.

When mapping *mei-W68* relative to P-element insertions, the crosses to generate P element-induced crossovers are shown in Table 1. To generate these males, *y w/ y⁺Y; mei-W68/CyO; Δ2-3, Sb/+* males were crossed to *yw; P{lacW}/CyO* or *y; P{PZ}/CyO* females. The recombinant males were crossed to *mei-W68/SM6* females and the recombinant/*mei-W68* female progeny were then tested for nondisjunction by one of the crosses described above.

Insertion mutations by local hopping were isolated by picking second chromosomes carrying a *P{lacW}* insertion that expressed a different eye color than the original chromosome (Fig. 6). The new insertions can be distinguished from the original

element by a change in eye color because expression of *w⁺* is very sensitive to dosage and position effects. Stocks expressing less eye pigment were referred to as *LL*, whereas stocks expressing more eye pigment were referred to as *LD*. *LL* mutants were expected to be cases in which there was both an insertion and precise excision of the original P element. *LD* mutants could also have been from this type of event, but they could also have arisen from a case in which the new insertion and original P element were present. In the same screen we isolated excision events by picking second chromosomes lacking the *P{lacW}* insertion, that is flies with white eye color. Whereas mobilization of 6323 produced new *mei-W68* mutants (see Results), similar attempts with the two elements to the left of *mei-W68* failed to yield mutants. We screened 73 excision and 47 local hop chromosomes of 705 and none failed to complement *mei-W68*. We screened 250 excision chromosomes of *P{PZ}l(2)01103* (Fig. 1), but none failed to complement *mei-W68*. This failure was not caused by a haploinsufficient locus between 705 and *mei-W68*. *Df(2R)LL5* fails to complement both 705 and 6323, and thus spans the region between them, but has excellent viability as a heterozygote. Another possibility is that deletions induced by P-element activity might be directional, and in the case of 705 and 1103 any deletions that were induced went in the wrong direction.

Genomic DNA cloning

Standard molecular techniques were as described previously (Sambrook et al. 1989). The source of the cloned genomic DNA was from a contig of P1 clones, DS00433, mapped previously to the *mei-W68* region (56D7–56E1) (Hartl et al. 1994; Kimmerly et al. 1996). We used inverse PCR to amplify the DNA adjacent to seven P-element insertions in the region. In addition, the genome project had established already that the *P{PZ}l(2)01103* insertion site anchored the left end of DS00433 to the cytological (56D5–6) and genetic maps. Probes from *l(2)01103*, *l(2)k00705*, *l(2)k16914*, and *l(2)k06323* each hybridized to the same two P1 clones, DS07982 and DS00571. Probes from *l(2)k09810*, *emm*, and *l(2)05338* did not hybridize; thus, these insertions were farther away from *mei-W68*. The P1 clone DS00571 was restriction mapped in two steps. First, we subcloned most of the *EcoRI*, *BamHI*, and *PstI* fragments. Second, restriction and Southern blot analysis was used to order the subclones. Southern blots of genomic and clone DNA were probed with random prime labeled DNA using nonradioactive protocols from the Boehringer Mannheim Genius system.

Figure 6. Genetic crosses to generate P element-induced local-hop insertions and deletions of the *mei-W68* gene. *CyO* and *SM6* are second chromosome balancers carrying the dominant *Cy* marker; *TMS* is a third chromosome balancer carrying the source of P-element transposase, *Δ2-3* and the dominant *Sb* marker. In the F₁ generation, the P elements are exposed to transposase, and any mobilization events are detected in the F₂ by a change in eye color. These exceptional flies were picked and mated separately to establish stocks and test for nondisjunction (Materials and Methods).

Genomic DNA flanking a P-element insertion was obtained by inverse PCR. Genomic DNA was isolated from stocks carrying a P-element insertion and digested with either *Hin*PI or *Msp*I. Approximately three flies worth was added to a 100- μ l ligation reaction. This reaction was precipitated and dissolved in 20 μ l of 10 mM Tris (pH 8.0). For the PCR reaction, 2.5 μ l of this was used as a template in a 25- μ l reaction containing 2 mM NTPs, 50 μ M each primer, 5% glycerol, 2.5 mM MgCl₂, 2 units of *Taq*, and 1 $\times$ Perkin-Elmer PCR buffer. The typical PCR conditions were 1 min at 95°C, 1 min at 55°C, and 2 min at 72°C for 30–35 cycles. The products were run on a 1.5% agarose gel and the bands cut out of the gel. In some cases the amplification was poor, so the excised band was amplified again in a second round of PCR. The DNA extracted from the gel slices was labeled for Southern hybridization.

Isolation of cDNA clones and DNA sequencing

We found initially the *Spo11* homolog in a search of the genomic DNA sequence from the Berkely *Drosophila* Genome Project (clone DS07982). To isolate cDNA clones, we used PCR to amplify 0.5-kb and 0.7-kb fragments using the primer pairs AH36–AH38 and AH37–AH39, respectively, and as template a germanium cDNA library in the Lambda ZapII vector (gift of D. Thompson-Stewart, Carnegie Institute of Washington). The PCR products were digested and cloned into Bluescript SK+. The plasmid pAH68 contains 1.0 kb of *mei-W68*-coding region and was made by ligating together the 0.5- and 0.7-kb PCR products at an internal *Bam*HI site. The PCR primers used were 5'-GACTAGTCATGGATGAATTTTCGGAG-3' (AH36), 5'-AG-GCCTTCTACAAAAACAAGTTCCG-3' (AH37), 5'-TCTTCTC-GGGATCCGTGG-3' (AH38), and 5'-TCCCCCTAAGCTTGG-GC-3' (AH39). The 0.7-kb PCR fragment was used to screen the cDNA library. A total of 19 positive clones were isolated from screening 5 $\times$ 10⁵ phage. The cDNA phage clones were converted to plasmids by the Exassist/SOLR excision system (Stratagene) and then three of the clones were sequenced by Research Genetics. pAH69 is one of these clones and contains a putative full-length (2.3 kb) cDNA. The sequence was analyzed using the GCG package (Devereux et al. 1984) or DNA Strider. Genbank database searches were done with the BLAST program (Altschul et al. 1990).

Northern blot hybridization and in situ hybridization to ovaries

RNA was isolated as described previously (Kerrebrock et al. 1995). The poly(A)⁺ RNA from several developmental stages was loaded on an agarose gel and transferred to Hybond-N(A-masham). After UV cross-linking, the membrane was hybridized using an antisense RNA probe generated by digesting pAH68 with *Spe*I and was using it as a template for [³²P]CTP-labeled transcripts synthesized by T7 RNA polymerase. A probe for the ribosomal protein RP49 transcript (O'Connell and Rosbash 1984) was used as loading control.

Ovaries were dissected, fixed, and hybridized as described previously (Tautz and Pfeifle 1989). DIG-labeled RNA probes were made from linearized pAH69 using the Boehringer Mannheim RNA-labeling kit.

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***mei-W68* in *Drosophila melanogaster* encodes a Spo11 homolog: evidence that the mechanism for initiating meiotic recombination is conserved**

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