

# Genetic Studies of *mei-P26* Reveal a Link Between the Processes That Control Germ Cell Proliferation in Both Sexes and Those That Control Meiotic Exchange in *Drosophila*

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## ABSTRACT

We present the cloning and characterization of *mei-P26*, a novel *P*-element-induced exchange-defective female meiotic mutant in *Drosophila melanogaster*. Meiotic exchange in females homozygous for *mei-P26*<sup>l</sup> is reduced in a polar fashion, such that distal chromosomal regions are the most severely affected. Additional alleles generated by duplication of the *P* element reveal that *mei-P26* is also necessary for germline differentiation in both females and males. To further assess the role of *mei-P26* in germline differentiation, we tested double mutant combinations of *mei-P26* and *bag-of-marbles* (*bam*), a gene necessary for the control of germline differentiation and proliferation in both sexes. A null mutation at the *bam* locus was found to act as a dominant enhancer of *mei-P26* in both males and females. Interestingly, meiotic exchange in *mei-P26*<sup>l</sup>; *bam*<sup>Δ86</sup>/+ females is also severely decreased in comparison to *mei-P26*<sup>l</sup> homozygotes, indicating that *bam* affects the meiotic phenotype as well. These data suggest that the pathways controlling germline differentiation and meiotic exchange are related and that factors involved in the mitotic divisions of the germline may regulate meiotic recombination.

**M**EIOSIS is the component of gametogenesis responsible for the segregation of homologous chromosomes into haploid gametes. In most organisms, including humans and *Drosophila melanogaster* females, the proper segregation of homologs during meiosis is facilitated by the formation of reciprocal genetic exchanges along the lengths of the chromosomes. Chromosomes with no exchange or with abnormally positioned exchanges are segregated with decreased fidelity (Koehler *et al.* 1996; Lamb *et al.* 1996). Although organisms have evolved systems to ensure the segregation of nonexchange chromosomes, these systems can usually effectively handle only one or two pairs of nonexchange homologs (Hawley and Theurkauf 1993), so the proper regulation of recombination is essential for reliably producing euploid offspring.

In *Drosophila* oogenesis, meiotic recombination occurs in the context of a cyst of 16 interconnected germline cells. Germline cyst development begins with the asymmetric division of a germline stem cell (GSC) to produce a GSC daughter and a cystoblast (for a review

of germline cyst development, see de Cuevas *et al.* 1997). The cystoblast then undergoes four mitotic divisions with incomplete cytokinesis. The residual connections between the cells, called ring canals, are inherited by one of the daughters after each division, such that after the four mitoses, two cells each retain four ring canals each. As the gonial mitotic divisions progress, a structure called a fusome is constructed within the cyst. Fusome formation begins with the asymmetric inheritance of spectrosome material into the cystoblast (Deng and Lin 1997). The fusome eventually takes on a branched structure, entering each cell of the cyst through the ring canals (de Cuevas and Spradling 1998). Although the process of oocyte selection is unknown, one of the two cells with four ring canals becomes the oocyte and progresses through meiosis. The remaining cells within the cyst take on a nurse cell fate. The result is a cyst containing one oocyte and 15 nurse cells.

A similar process of cyst development occurs in males (reviewed by Lindsley and Tokuyasu 1980), where GSCs divide to produce a daughter GSC and a primary spermatogonial cell. The primary spermatogonial cell then divides four times to form a cyst of 16 interconnected primary spermatocytes. In contrast to oogenesis, no meiotic recombination occurs, and all 16 primary spermatocytes within the cyst go through both meiotic divisions synchronously to produce 64 spermatids, which then differentiate into mature spermatozoa.

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During meiotic prophase in females, chromosomes condense and pair to form bivalents. Synapsis of homologous chromosomes culminates in the formation of synaptonemal complex (SC) along the lengths of the chromosomes (Carpenter 1975a; 1979b). Mutations in the *Drosophila c(3)G* gene cause both a complete absence of SC formation and a lack of exchange (Smith and King 1968; Hall 1972). SC formation appears to be normal in *mei-W68* and *mei-P22*, although meiotic recombination and gene conversion are abolished (McKim *et al.* 1998). Moreover, cloning of the *mei-W68* locus showed that it encodes a homolog of the yeast gene *SP011*, which is responsible for the first step of recombination, double-strand break formation, in budding yeast (McKim and Hayashi-Hagihara 1999). Thus, SC is apparently necessary for, but not dependent upon, the initiation of meiotic recombination in *Drosophila*.

In wild-type *Drosophila* females, meiotic exchanges are distributed in a nonrandom pattern along the euchromatin of each chromosome arm (with the exception of the small fourth chromosome; Lindsley and Sandler 1977). Crossing over is generally reduced in the centromeric and telomeric regions and entirely absent in the heterochromatin. Meiotic exchange can be assayed genetically in crosses with marked chromosomes, or cytologically by the analysis of the timing and number of recombination nodules (Carpenter 1975b; 1979a,b).

Studies of meiotic mutants in *Drosophila* have demonstrated that multiple levels of control of recombination exist. Because *c(3)G*, *mei-W68*, and *mei-P22* completely suppress exchange and gene conversion, they are known as recombination-null mutants (McKim *et al.* 1998). A greater number of *Drosophila* mutants decrease exchange without eliminating it. These mutants were initially classified on the basis of their effects on the distribution of exchanges. Carpenter and Sandler (1974) expected that mutants that affect the process of exchange itself would produce a uniform decrease in the frequency of exchanges without altering their distribution along a chromosome arm. Mutants that affect the distribution of exchanges were also identified. These mutants, when compared to wild type, bear similar patterns of residual exchanges. These patterns are characterized by a preference for proximal exchanges and an increasing suppression of exchange that increases toward the distal tips of the chromosomes. The term "polar" was used to describe this distribution. Mutants that redistribute exchanges in a polar fashion were suggested to correspond to genes necessary for a set of preconditions required for any given chromosomal interval to be competent to form a crossover and thus were called "precondition mutants" (Sandler *et al.* 1968; Carpenter and Sandler 1974). Differential probabilities of certain chromosomal regions for meeting the necessary preconditions for exchange were

thought to lead to the nonrandom pattern of crossover distribution observed for each chromosome arm in wild type. Precondition mutants were expected to be defective for setting up the preconditions for exchange, thus resulting in the abnormal crossover distribution (Carpenter and Sandler 1974).

A new exchange-defective female meiotic mutant, *mei-P26*, has been cloned and characterized. *mei-P26* causes a polar decrease in recombination, similar to many other exchange-defective mutants. The *mei-P26* gene encodes a novel member of the RING finger B-box coiled coil (RBCC) family of proteins. Phenotypic analyses of additional loss-of-function alleles of this locus show that this gene also affects both male and female gametogenesis. Furthermore, a null mutation in *bag-of-marbles* (*bam*), which affects both male and female gametogenesis, acts as a dominant enhancer of *mei-P26* in both males and females. The effects of *mei-P26* mutations on germline differentiation and meiosis, as well as the genetic interaction of *mei-P26* with *bam*, suggest that *mei-P26* either has multiple roles in the germline, or that the proper regulation of germline cyst formation is required for the normal levels of meiotic exchange.

## MATERIALS AND METHODS

***Drosophila* stocks and culture:** The genetic markers and chromosomes used in this study are described in Lindsley and Zimm (1992), FlyBase (1999), and Sekelsky *et al.* (1999). Flies were reared on standard cornmeal-molasses-dextrose medium at 25°. For egg counts, eggs were collected on grape-juice plates (Ashburner 1989) as described by Wieschaus and Nüsslein-Volhard (1986).

**Molecular techniques:** Plasmid rescue was performed as described previously (Ashburner 1989). Library screening was done essentially as described by Sambrook *et al.* (1989) using Church hybridization and wash buffers (Church and Gilbert 1984). An ABI 377XL (Applied Biosystems, Foster City, CA) was used to perform DNA sequencing.

**Genetic analyses:** The assay for measuring *X* and 4th chromosome nondisjunction in females crossed to *YSX · YL, v f B/0; C(4)RM, ci ey<sup>R</sup>/0* males is described by Sekelsky *et al.* (1999). The calculation of *XX ↔ 4A* nonhomologous disjunction frequency is described by Hawley *et al.* (1993).

Recombination along the left arm of chromosome 2 was scored among the progeny of females of the genotype *X/X; net ho dp Sp b pr cn/+ + + + + +* crossed to *+/Y; net ho dp b pr cn/net ho dp b pr cn* males. For most crosses, only female progeny were scored, because the presence of *w* on one or both of the *X* chromosomes would obscure the scoring of *pr* and *cn* in male progeny, because of the epistasis of *w* to these two eye color markers. Exchange rank frequencies, the frequencies of tetrads bearing zero, one, two, or three exchanges, were calculated as described by Weinstein (1936).

**A screen for additional alleles of *mei-P26*:** New alleles of *mei-P26* were generated by exposing the *P{lacW}* element responsible for *mei-P26<sup>l</sup>* to a source of *P*transposase and isolating imprecise excisions and local hops of the *P* element. Non-*Stubble* (*Sb*) female progeny of *mei-P26<sup>l</sup>/y<sup>+</sup>Y; TMS, Sb Δ2-3/+; spa<sup>mol</sup>* crossed to *FM7w; +/+; spa<sup>mol</sup>* were selected on the basis of loss or change in eye color generated by the mini-*white<sup>+</sup>* (*w<sup>+</sup>mC*) gene encoded by *P{lacW}*. For each of the isolated *X* chromosomes, denoted by an asterisk, *y w mei-P26<sup>\*</sup>/FM7w*;

*spa<sup>pol</sup>* females were crossed to *FM7w/y<sup>+</sup>Y*; *spa<sup>pol</sup>* males to establish stocks. Stocks in which the eye color phenotype failed to segregate with the *X* chromosome (*i.e.*, jumps to the autosomes) were discarded. To assay *X* chromosome nondisjunction, homozygous *y w mei-P26\**; *spa<sup>pol</sup>* females were selected from the stocks and crossed to *FM7w/y<sup>+</sup>Y*; *spa<sup>pol</sup>* males. The normal progeny from this cross are *yellow* females and *yellow<sup>+</sup>* males. Exceptional progeny resulting from diplo-*X* or nullo-*X* ova are recovered as *yellow<sup>+</sup>* (*y w mei-P26\*/y w mei-P26\*/y<sup>+</sup>Y*) females and *yellow* (*FM7w/0*) males.

**Germline transformation:** The transformation construct  $P\{w^{+mC}; N1\}$  was constructed by inserting a restriction fragment containing ~13 kb of genomic DNA (see Figure 2) into the vector pW8 (Klemenz *et al.* 1987).  $pP\{w^{+mC}; N1\}$  DNA was purified (QIAGEN, Valencia, CA) and resuspended in injection buffer at a concentration of 1  $\mu\text{g}/\mu\text{l}$  with the transposase source pTurbo $\Delta 2-3$  (FlyBase 1999) at 0.3  $\mu\text{g}/\mu\text{l}$  and microinjected into *w<sup>1118</sup>* embryos (Spradling and Rubin 1982; Spradling 1986).

**Cytology:** Ovaries were dissected in phosphate-buffered saline (PBS) or modified Robb's medium (Theurkauf 1994) and fixed in 4% formaldehyde, 100 mM sodium cacodylate, pH 7.2, 100 mM sucrose, 40 mM sodium acetate, 10 mM EGTA for 5 min. They were then rinsed in PBS containing 0.1% Tween 20 and stained with 0.5  $\mu\text{g}/\text{ml}$  DAPI (4',6-diamidino-2-phenylindole) in PBS for 5 min. After three washes with PBS + 0.1% Tween 20, the ovarioles were teased apart and mounted in 90% glycerol containing *p*-phenylenediamine at a concentration of 1 mg/ml. Testes were dissected in testis buffer (Ashburner 1989) and viewed unfixed using differential interference contrast (DIC) optics. All microscopy and photography was performed on a Zeiss Axioplan microscope equipped for fluorescence and DIC microscopy. Color photographs were scanned into an Apple Power Macintosh 7500 computer using a Nikon LS-1000 35-mm film scanner, converted to grayscale using Adobe Photoshop 5.0.2, and printed using a Tektronix Phaser 440 dye sublimation printer.

## RESULTS

**Molecular characterization of the *mei-P26* interval in region 8C:** The first allele of *mei-P26*, denoted *mei-P26<sup>l</sup>*, was isolated during a *P*-element screen for mutants that affect female meiosis in *D. melanogaster* (Sekelsky *et al.* 1999). *In situ* hybridization of a *P*-element probe to *mei-P26<sup>l</sup>* larval salivary gland polytene chromosomes revealed the presence of a single *P{lacW}* insertion in region 8 of the *Drosophila* polytene map (Sekelsky *et al.* 1999). Three deficiencies in this region, *Df(1)lZ<sup>90h24</sup>*, *Df(1)9a4-5*, and *Df(1)18.1.15*, failed to complement *mei-P26<sup>l</sup>* (see below). A fourth deficiency, *Df(1)lZ<sup>10-70d</sup>*, complemented *mei-P26<sup>l</sup>* and positioned the gene in region 8C10-8D3 (Figure 1).

We have characterized the region surrounding the *P{lacW}* insertion in *mei-P26<sup>l</sup>*. An *EcoRI* fragment containing ~740 base pairs (bp) of DNA flanking the 3' end of the *P{lacW}* insertion was isolated by plasmid rescue. Two overlapping lambda clones representing the *mei-P26* locus were identified by using the rescue fragment as a probe to screen a *Drosophila* genomic library. Portions of these clones were then subcloned into pBluescript and sequenced. Sequencing and re-

striction mapping allowed the construction of a map representing the genomic region surrounding the *mei-P26<sup>l</sup>* insertion (Figure 2A). cDNA library screening using the genomic subclones and BLAST searches (Altschul *et al.* 1997) using their sequences led to the identification of five transcription units in the region. Two were previously cloned *Drosophila* genes; one was found based on homology to a mammalian gene; and two, one being the *mei-P26* gene, were discovered based on homology to *Drosophila* expressed sequence tags (ESTs). We will first describe the neighboring genes and then in the following section we will describe the *mei-P26* transcription unit.

The 5' ends of the genes encoding the oligosaccharyl-transferase 48-kD subunit (*Ost48*) and histone H3.3B (*His3.3B*) are located ~6 and 8 kb from the 3' end of the *P* element, respectively (Figure 2A). These genes were previously cloned and characterized and are known to be tightly linked (Akhmanova *et al.* 1995; Stagljar *et al.* 1995). Two *P{EP}* insertions have been identified by the Berkeley *Drosophila* Genome Project (BDGP) close to the *His3.3B* transcription start site, in the 5' flanking sequence adjacent to the *His3.3B* transcription start site (Rørth *et al.* 1998; E. J. Rehm and G. M. Rubin, unpublished results). These have both been mapped to 8C15-16 by *in situ* hybridization (T. Laverty, personal communication), which agrees well with the deficiency mapping of *mei-P26*.

The *mei-P26<sup>l</sup>* *P* element was found to be 2.6 kb upstream of a region showing strong homology to the mouse and human *ZPR1* genes (Galcheva-Gargova *et al.* 1996, 1998). In mammalian cells, the ZPR1 protein binds intracellularly to the epidermal growth factor receptor (EGFR), and, upon ligand binding to the EGFR, ZPR1 accumulates in the nucleus, where it appears to regulate nucleolar function in proliferating cells by localizing to the nucleolus and binding to translation elongation factor 1 $\alpha$  (Galcheva-Gargova *et al.* 1996, 1998; Gangwani *et al.* 1998). A total of 13 cDNAs for this gene were isolated by screening a *Drosophila* ovary library (Stroumbakis *et al.* 1994) using a genomic fragment containing this region. Sequencing of the longest of these indicates that this gene is expected to encode a protein of 457 amino acids with 49% identity and 67% similarity to human ZPR1. The ZPR1 protein has a bipartite structure that is conserved throughout eukaryotes and consists of two repeats of a zinc finger and an associated sequence (Galcheva-Gargova *et al.* 1996, 1998; Gangwani *et al.* 1998). Comparison of the genomic and cDNA sequences for the *Drosophila* homolog indicates a single intron located approximately in the middle of the protein coding region, separating the two repeats (Figure 2A).

A BLAST search of the BDGP EST database using sequence located between the *Zpr1* and *Ost48* genes revealed a high scoring match to one EST, GH20078 (Berkeley *Drosophila* Genome Project/HHMI EST

## complementation

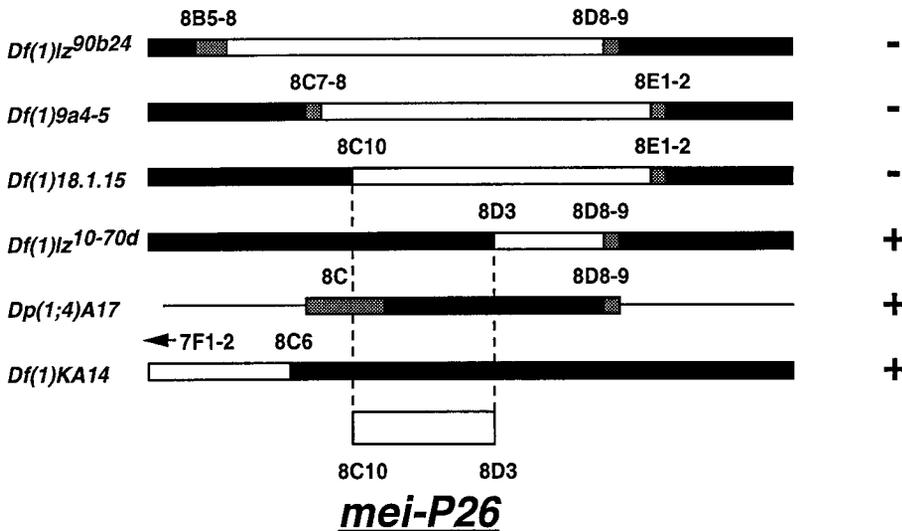


Figure 1.—Deficiency and duplication mapping of *mei-P26* positions the mutation between polytene map positions 8C10 and 8D3 on the X chromosome. Open boxes represent chromosomal regions that are deleted in the deficiencies indicated, solid boxes represent chromosomal regions that are present, and shaded boxes represent ambiguities from the mapping of deficiency and duplication breakpoints.

Project, unpublished results). This 378-bp cDNA appears to represent a spliced transcript oriented in the same direction as *Zpr1* and *Ost48*. The longest open reading frame in this cDNA is 77 amino acids long and is predicted to encode an 8.4-kD polypeptide with no homologies to any known proteins.

**The *mei-P26* transcription unit:** Sequence flanking the 5' end of the *mei-P26<sup>l</sup>* P element was found to match a 5' EST in BLAST searches of the BDGP database. The cDNA corresponding to the EST, LD09982, was sequenced and the genomic structure of the gene was determined (Figure 2A). The P element was found to be located within the 2.4-kb first intron of this gene, and P-element excision data and transgenic rescue experiments (shown below) confirm that this transcription unit does indeed correspond to the *mei-P26* gene.

A total of three probable full-length (GH10646, LD09982, and LD30261) and two partial cDNAs (HL02723 and LD34505) for this gene have been identified among ESTs from the BDGP (Berkeley Drosophila Genome Project/HHMI EST Project, unpublished results). One of these, GH10646, was sequenced as part of the BDGP (GenBank accession no. AF145661), and we sequenced a second, LD09982. The full-length sequencing of these cDNAs revealed two sequence differences between GH10646 and LD09982. These sequence differences can both be explained by alternative splicing. Analysis of the LD09982 sequence predicts a novel 131-kD protein of 1206 amino acids. Interestingly, the GH10646 sequence contains a 51-bp in-frame deletion as the result of one of the alternative splice sites, and is expected to encode a slightly smaller protein of 1189 amino acids and 129.5 kD.

The proteins encoded by both of these cDNAs are expected to contain a RING finger, followed by two B-box motifs and a region predicted to form a coiled coil structure. RING fingers and B-boxes are cysteine-

rich zinc-binding protein motifs thought to be involved in interactions with proteins or nucleic acids (Borden *et al.* 1995, 1996; Cao *et al.* 1997, 1998). Two glutamine-rich stretches reside near the middle of the protein, which may also form coiled coils. The C-terminal region of the protein bears six copies of a repeated motif called an NHL repeat (Slack and Ruvkun 1998). NHL repeats are similar to WD repeats in length and predicted structure and may be involved in protein-protein interactions (Fridell *et al.* 1995; El-Husseini and Vincent 1999). The 17-amino acid region missing from the 1189-amino acid version of the protein is located between the glutamine-rich domains and the NHL repeats and does not overlap with any of the recognizable motifs (Figure 2D). The portion of MEI-P26 containing the RBCC domain is most similar to the TIF1 (transcriptional intermediary factor 1) family of nuclear receptor mediator proteins (TIF1 $\alpha$ , TIF1 $\beta$ , TIF1 $\gamma$ ; Le Douarin *et al.* 1995, 1996; Venturini *et al.* 1999), with ~27% identity and 47% similarity. However, the C-terminal portions of the TIF1 proteins each contain PHD finger and bromodomain motifs, while MEI-P26 contains six NHL repeats.

**Isolation of more severe alleles:** Several lines of evidence indicate that the original *mei-P26<sup>l</sup>* allele is a hypomorph (see below). To isolate stronger alleles of *mei-P26*, the P element was mobilized using  $\Delta 2-3$  transposase and putative excisions or local hops were selected on the basis of eye color. Full or partial excisions, including imprecise excisions, of *P{lacW}* that deleted the mini-*white<sup>+</sup>* gene were expected to result in a total loss of mini-*white<sup>+</sup>* expression (white eyes). Local hops of the P element were expected to produce changes in the mini-*white<sup>+</sup>* expression level, resulting in a change in eye color, due to a change in *P{lacW}* copy number or position effects. For each X chromosome recovered, a stock was established and X chromosome nondisjunction was assayed in females homozygous for the X chromo-

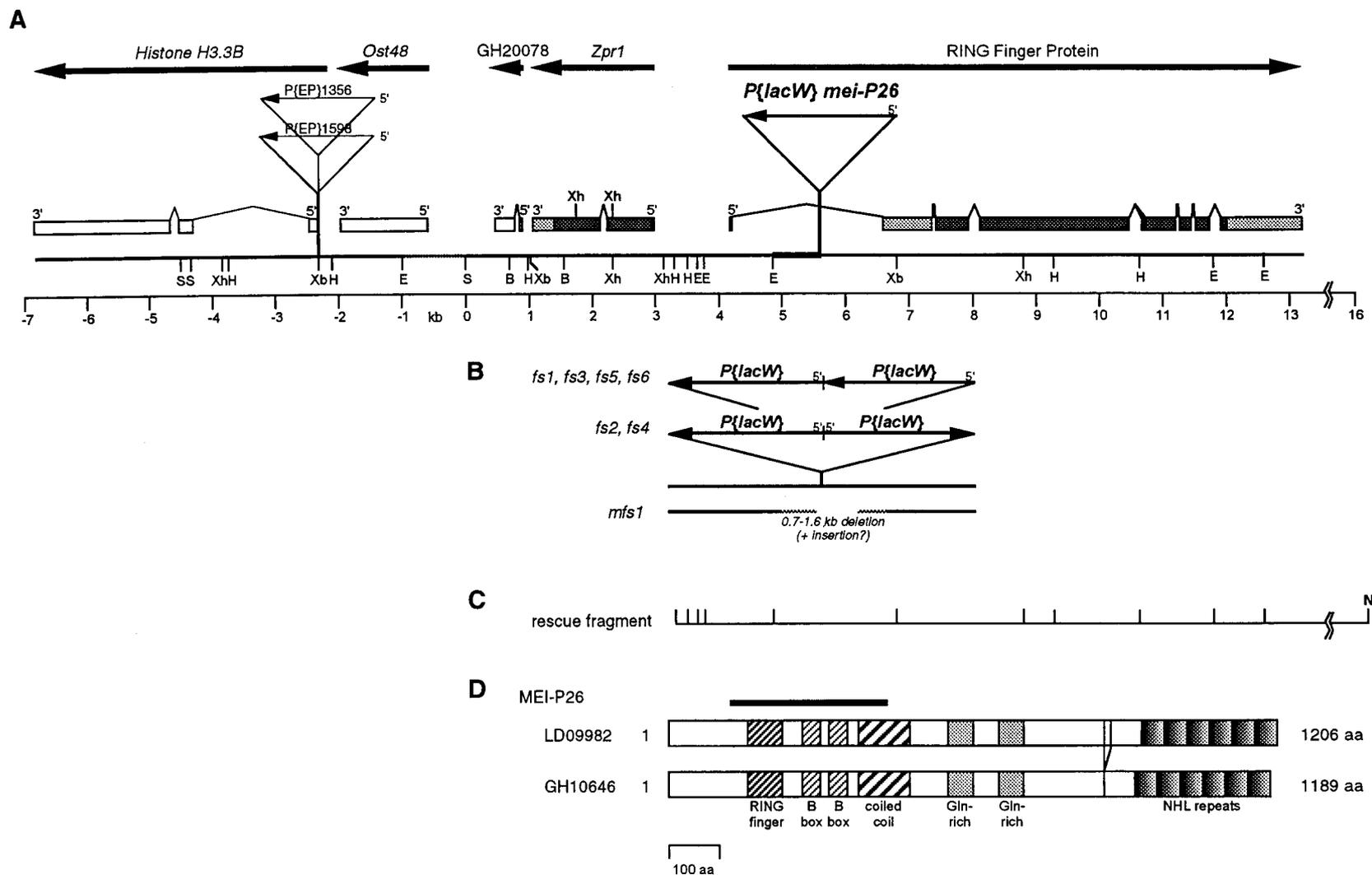


Figure 2.—The *mei-P26* locus. (A) Molecular map of the genomic region near *mei-P26*. The horizontal line shows a partial restriction map of the region surrounding the *mei-P26*' *P{lacW}* insertion. The sequence on the left, including the *His3.3B* and *Ost48* genes, was previously published (accession nos. X81207 and X81999; Akhmanova *et al.* 1995; Stagljar *et al.* 1995). The dashed line signifies ~1 kb of unsequenced genomic DNA. The thickened line indicates the fragment isolated by plasmid rescue. Boxes above the line indicate exons for each gene except *Ost48*, for which the genomic structure was not determined. The positioning of *Ost48* on this map is approximate. For *Zpr1* and the RING finger gene, protein coding sequence is shaded dark gray, and untranslated sequence is shaded light gray. The 5' to 3' orientations of the genes and *P* elements are shown by arrows. B, *Bam*HI; E, *Eco*RI; H, *Hind*III; S, *Sal*I; Xb, *Xba*I; Xh, *Xho*I. (B) Mutations in new *mei-P26* alleles. The mutations in the *mei-P26*<sup>fs1</sup>, *mei-P26*<sup>fs2</sup>, *mei-P26*<sup>fs3</sup>, *mei-P26*<sup>fs4</sup>, *mei-P26*<sup>fs5</sup>, *mei-P26*<sup>fs6</sup>, and *mei-P26*<sup>mfs1</sup> alleles are shown corresponding to the map in A. (C) Restriction fragment used for transformation rescue construct *P{w<sup>+</sup>mC; N1}* is shown corresponding to the map in A. N, *Not*I (site from lambda vector). (D) Schematic representation of the proteins encoded by *mei-P26* cDNAs LD09982 and GH10646, showing the 17-amino acid difference in the C-terminal region resulting from alternative splicing of the transcript. The line above the proteins represents the region of similarity to the TIF1 family.

mosome. Of 44 white-eyed excisions isolated, 37 completely reverted the *mei-P26<sup>l</sup>* phenotype, while 3 retained an *X* nondisjunction frequency of 2–4%, and 3 additional excisions retained a nondisjunction phenotype similar to *mei-P26<sup>l</sup>* (8–17% *X* nondisjunction). PCR analysis of these latter excisions showed small internal deletions of *P{lacW}* sequence, while deletions of most or all of the *P{lacW}* sequence were evident in the revertants (data not shown). These results demonstrate that the *P*-element insertion is indeed responsible for the mutant phenotype. One excision chromosome was a recessive lethal, apparently due to a deletion of the *P{lacW}* insert and flanking DNA on both sides. This chromosome fails to complement *mei-P26<sup>l</sup>*, but its lethality is likely due to the deletion of one or more essential genes near *mei-P26*, rather than *mei-P26* itself, since the lethality is not rescued by a transgenic construct that contains the wild-type *mei-P26* transcription unit (described below).

A total of 20 *X* chromosomes were recovered with a change in eye color, representing putative local hops of the *P* element. Homozygotes for 6 of these produced no progeny in multiple crosses. All heteroallelic combinations of these chromosomes were also sterile in females. While these chromosomes are fertile when placed over *mei-P26<sup>l</sup>*, the fertility of these flies is reduced, and all 6 of these failed to complement the *mei-P26<sup>l</sup>* nondisjunction phenotype. *mei-P26<sup>fs</sup>/mei-P26<sup>l</sup>* females showed higher levels of nondisjunction than *mei-P26<sup>l</sup>* homozygotes assayed contemporaneously (data not shown). The noncomplementation with *mei-P26<sup>l</sup>*, and with each other, suggested that these chromosomes bear novel alleles of *mei-P26*, and they were given the allele designations *fs1*, *fs2*, *fs3*, *fs4*, *fs5*, and *fs6*.

PCR analysis of the *P{lacW}* in these alleles revealed that in each case two partial or full copies of *P{lacW}* were now present in the first intron of the RING finger gene. In four cases, there had been a tandem duplication of the *P* element, and in two of these, an inverted duplication was found (Figure 2B). The insertion of a *P* element into the first intron of this gene may compromise its expression, leading to a meiotic phenotype, as in the original *mei-P26<sup>l</sup>* allele. Addition of a second copy of the *P* element may further compromise expression, leading to the more severe female sterility phenotype.

**A male sterile allele of *mei-P26*:** Unexpectedly, a male and female sterile allele, *mei-P26<sup>ms1</sup>*, was also recovered in the screen for additional *mei-P26* mutants. Crosses using males bearing this chromosome paired with virgin females produced no progeny. This chromosome also failed to complement *mei-P26<sup>l</sup>*, producing ~30% *X* nondisjunction when in heteroallelic combination with *mei-P26<sup>l</sup>*. A duplication of the *mei-P26* region on chromosome 4, *Dp(1;4)A17* (Santamaria and Randsholt 1995), rescued the infertility, suggesting that a wild-type copy of the *mei-P26* gene could complement the male sterility. *mei-P26<sup>ms1</sup>/Y; Dp(1;4)A17/spa<sup>mol</sup>* males were used

to generate females homozygous for the male sterile chromosome. Homozygotes for this chromosome were completely sterile, although homozygote females bearing *Dp(1;4)A17* were fertile.

Although this allele displays an increase in eye color relative to the original *mei-P26<sup>l</sup>* allele, PCR analysis of the *P{lacW}* indicated a deletion of the *P*-element ends and 0.7–1.6 kb of DNA flanking both sides of the original insertion site (Figure 2B). This may be in addition to a more complex rearrangement, however, possibly involving the insertion of sequence, since the deleted region could not be spanned by PCR from primers flanking the deleted sequence. *P{lacW}* sequence may remain, as evidenced by mini-*white<sup>+</sup>* expression, which maps to the same interval of the *X* chromosome as *mei-P26* (*ct-m*). Male sterility was an unexpected phenotype, which is not seen in any of the other *mei-P26* alleles.

**A single rescue construct rescues all three phenotypes:** To confirm that the mutations in the RING finger gene were indeed responsible for the phenotypes observed in the *mei-P26* alleles, a construct containing ~13 kb of genomic DNA, including this gene, 650–850 bp of 5' flanking sequence, and ~3 kb of 3' flanking sequence, in the *P*-element transformation vector pW8 was microinjected into embryos. Four independent transgenic lines bearing this construct were established. In tests for phenotypic rescue, the construct restored levels of nondisjunction and recombination to wild-type levels (Table 1). The slight deviation of the rescued phenotype from wild type may reflect either differences in genetic background, or incomplete rescue by the transgene due to noninclusion of all regulatory sequences or a position effect on expression. Heterozygosity for the rescue construct also rescued the sterility phenotype of *mei-P26<sup>fs1</sup>* and both the male and female sterility of *mei-P26<sup>ms1</sup>*.

**Analysis of nondisjunction in *mei-P26*:** Data for *X* and 4th chromosome segregation in *mei-P26<sup>l</sup>* females are shown in Table 2. Homozygous *mei-P26<sup>l</sup>* females displayed 17.3% *X* chromosome nondisjunction and 8.4% 4th chromosome nondisjunction. These nondisjunction frequencies were compared to those for females hemizygous for *mei-P26<sup>l</sup>* using deficiencies that fail to complement this gene. Females of the genotype *mei-P26<sup>l</sup>/Df(1)18.1.15* show an increase in nondisjunction frequency in comparison to *mei-P26<sup>l</sup>* homozygotes (Table 2) and a concomitant decrease in fertility. *Df(1)9a4-5* and *Df(1)Iz<sup>90h24</sup>* showed an even more profound effect on fertility when heterozygous with *mei-P26<sup>l</sup>*. From these data we may conclude that the original *mei-P26<sup>l</sup>* allele is hypomorphic. Too few progeny are produced by *mei-P26<sup>l</sup>/Df(1)9a4-5* or *mei-P26<sup>l</sup>/Df(1)Iz<sup>90h24</sup>* females to accurately measure nondisjunction or recombination.

Testing of *X* and 4th chromosome nondisjunction in *mei-P26<sup>fs1</sup>/mei-P26<sup>l</sup>* females revealed a more severe phenotype than is seen in *mei-P26<sup>l</sup>* homozygotes. Nondisjunction of both the *X* and 4th chromosome is in-

**TABLE 1**  
**Rescue of *mei-P26* phenotypes with  $P\{w^{+mc}; N1\}$  rescue construct on chromosome 3**

Genotype	Phenotype assayed	- rescue (+/+) (%)	+ rescue ( $P\{w^{+mc}; N1\}/+$ ) (%)
<i>y w mei-P26/y w mei-P26</i>	<i>X</i> nondisjunction	14.1	0.74
	<i>4</i> nondisjunction	7.5	1.11
<i>y w mei-P26 m f/y w mei-P26</i>	<i>m-f</i> map distance	1.7	16.0
<i>y w mei-P26<sup>fs1</sup> m f/y w mei-P26</i>	<i>m-f</i> map distance	1.3	14.1
<i>y w mei-P26<sup>fs1</sup>/y w mei-P26<sup>fs1</sup></i>	Fertility	Sterile	Fertile
	Ovary morphology	Tumors	No tumors
	Fertility	Sterile	Fertile
<i>y w mei-P26<sup>ms1</sup>/y w mei-P26<sup>ms1</sup></i>	Ovary morphology	Tumors	No tumors
	Fertility	Sterile	Fertile
<i>y w mei-P26<sup>ms1</sup>/y<sup>+</sup> Y</i>	Fertility	Sterile	Fertile

creased (Table 2). However, *X* chromosome segregation is more greatly affected in *mei-P26<sup>f</sup>/Df* than in *mei-P26<sup>f</sup>/mei-P26<sup>fs1</sup>*, suggesting that even the *mei-P26<sup>fs1</sup>* allele may not be fully null.

At least the majority of the misbehavior of the *X* chromosome in *mei-P26* mutants appears to be the result of nondisjunction rather than chromosome loss, as shown by comparing the frequencies of nullo-*X* and diplo-*X* exceptions. Chromosome loss would be expected to produce an excess of nullo-*X* exceptions. However, *mei-P26* mutants have approximately equal frequencies of nul-

lo-*X* and diplo-*X* exceptions, showing that nondisjunction is the primary cause of aberrant *X* chromosome segregation.

Two lines of evidence suggest that the *X* chromosome nondisjunction induced by *mei-P26* mutations is due to the failed segregation of nonexchange tetrads. First, as noted by Baker and Hall (1976), the frequency of *X* chromosome nondisjunction in recombination-defective mutants is correlated with the cube of  $E_0$ , the frequency of nonexchange tetrads.  $E_0$  is calculated from recombination data using the method of Weinstein

**TABLE 2**  
***X* and 4th chromosome segregation in *mei-P26* mutants**

Gamete types		Maternal genotype		
Maternal	Paternal	<i>mei-P26<sup>f</sup></i>	<i>mei-P26<sup>f</sup>/mei-P26<sup>fs1</sup></i>	<i>mei-P26<sup>f</sup>/Df(1)18.1.15</i>
Normal				
<i>X; 4</i>	<i>XY; 44</i>	658	124	155
<i>X; 4</i>	<i>0; 44</i>	884	139	173
<i>X</i> nondisjunction				
<i>0; 4</i>	<i>XY; 44</i>	49	14	34
<i>XX; 4</i>	<i>0; 44</i>	63	15	25
<i>4</i> nondisjunction				
<i>X; 0</i>	<i>XY; 44</i>	15	9	0
<i>X; 0</i>	<i>0; 44</i>	14	14	2
<i>X; 44</i>	<i>XY; 0</i>	11	8	10
<i>X; 44</i>	<i>0; 0</i>	11	10	0
<i>X; 4</i> nondisjunction				
<i>0; 0</i>	<i>XY; 44</i>	30	5	9
<i>XX; 44</i>	<i>0; 0</i>	9	4	10
<i>0; 44</i>	<i>XY; 0</i>	7	4	1
<i>XX; 0</i>	<i>0; 44</i>	9	2	0
Total progeny		1760	348	419
Adjusted total		1927	392	498
% nullo- <i>X</i>		8.9	11.7	17.7
% diplo- <i>X</i>		8.4	10.7	14.1
Total % <i>X</i> nondisjunction		17.3	22.4	31.7
% nullo- <i>4</i>		5.6	9.4	4.0
% diplo- <i>4</i>		2.8	8.7	6.4
Total % <i>4</i> nondisjunction		8.4	18.1	10.4
% nonhomologous disjunction		0	0	0

(1936). The cube of  $E_0$ , when plotted against  $X$  chromosome exceptions per 1000 ova, reveals a linear relationship (Baker and Hall 1976). In recombination-defective mutants,  $X$  nondisjunction was found to be increased in oocytes that are also exceptional for one of the major autosomes (Baker and Carpenter 1972; Hall 1972; Parry 1973), which led to the hypothesis that the correlation with  $E_0^3$  reflects a necessity for three chromosome arms (the  $X$  and the two arms of either chromosome 2 or 3) to be nonexchange in oocytes where the  $X$  chromosome fails to segregate properly (Baker and Hall 1976). The nondisjunction presumably results from failures of the achiasmate segregation system, resulting in  $XXA \leftrightarrow A$  segregations (Hawley and Theurkauf 1993). Baker and Hall (1976) demonstrated that the relationship between  $E_0^3$  and  $X$  nondisjunction frequency is evident using recombination data measured on either chromosome  $X$  or 2 to estimate  $E_0$ . We therefore used our recombination data for the left arm of chromosome 2 in *mei-P26* mutant genotypes (Table 3; discussed below) to estimate  $E_0$ , and found that the  $X$  nondisjunction frequency is also proportional to  $E_0^3$  in *mei-P26* mutants (Figure 3).

The second line of evidence that the  $X$  chromosome nondisjunction induced by *mei-P26* mutations is due to the failed segregation of nonexchange tetrads comes from a genetic cross in which *y w mei-P26<sup>l</sup> m f · y<sup>+</sup> / y pn cv mei-P26<sup>l</sup>* females were crossed to *YSX · YL, v f B / 0; C(4)RM, ci ey<sup>R</sup> / 0* males (data not shown). In this cross, recombination can be monitored in the ova leading to diplo- $X$  exceptions, since these will be recovered as progeny resulting from fertilization with nullo- $X$  sperm. If recombination occurs in tetrads leading to the diplo- $X$  exceptions, one-fourth of the progeny would be expected to be homozygous for *pn*, owing to recombination between the centromere and this distal marker. However, 0/74 diplo- $X$  exceptions recovered from this cross were homozygous for *pn*, indicating that most, if not all, of the nondisjunction in *mei-P26* results from the missegregation of nonexchange tetrads.

The levels of chromosome 4 nondisjunction increase as the frequency of  $X$  nondisjunction increases (Table 2). In the genotypes tested, from 26.7 to 62.5% of 4th chromosome exceptions in *mei-P26* mutant females occurred in oocytes that were simultaneously exceptional for the  $X$  chromosome. Moreover, the number of simultaneous  $X$ ; 4 exceptions is about two to four times greater than would be expected if  $X$  and 4th chromosome segregation were independent. Missegregation of the always achiasmate 4th chromosome would be expected from an achiasmate segregation mutant, but in such mutants (*e.g.*, *Axs*), the chromosome pairs predominantly segregate to opposite poles, a process known as nonhomologous disjunction (*e.g.*,  $XX \leftrightarrow 44$ ). In no genotype mutant for *mei-P26* did we observe an excess of nonhomologous disjunction ( $XX \leftrightarrow 44$ ; Table 2). The frequency of  $XX$  and  $44$  segregation to the same pole

was equal to or greater than the frequency of  $XX$  and  $44$  segregation to opposite poles in *mei-P26* mutants. Thus, when simultaneously nondisjunctive, the  $X$  and 4th chromosome segregate randomly to the poles.

Most recombination-defective meiotic mutants in *Drosophila* also show an excess of simultaneous  $X$  and 4th chromosome nondisjunction with no preference for nonhomologous disjunction. These observations led to the hypothesis that nonexchange  $X$  chromosomes disrupt 4th chromosome segregation through physical association of the chromosomes (Baker and Carpenter 1972; Hall 1972). Alternatively, association of the chromosome pairs in the presence of the narrow meiosis I spindle may disrupt the segregation of other chromosomes, resulting in nondisjunction at random with respect to the spindle poles (Hawley and Theurkauf 1993).

**Mutants in the *mei-P26* gene reduce meiotic exchange in a polar manner:** Analyses of recombination along the  $X$  chromosome and the left arm of chromosome 2 revealed a polar recombination defect similar to many other previously identified recombination-defective meiotic mutants in *Drosophila* (Baker and Hall 1976; Sekelsky *et al.* 1999). As shown in Table 3, testing of chromosome 2 recombination in *mei-P26<sup>l</sup>* homozygotes reveals a reduction in recombination frequency and an abnormal distribution of exchanges. Recombination is strongly decreased in distal 2L, but shows frequencies greater than wild type in the proximal region of the chromosome, near the centromeric heterochromatin. The recombination analysis of *mei-P26<sup>ts1</sup> / mei-P26<sup>l</sup>* females agrees with the nondisjunction data in that the decrease in recombination is more severe in these females than in *mei-P26<sup>l</sup>* homozygotes (Table 3). As expected, the largest decrease in recombination is observed in *mei-P26<sup>l</sup> / Df(1)18.1.15* females, in which the genetic map distance is reduced to one-third of that observed in *mei-P26<sup>l</sup>* homozygotes and one-fifth of wild type (Table 3). The recombination distribution in *mei-P26<sup>ts1</sup> / mei-P26<sup>l</sup>* and *mei-P26<sup>l</sup> / Df(1)18.1.15* females is polar, as in *mei-P26<sup>l</sup>* homozygotes, but is more exaggerated.

Comparison of overall map distances shows that the frequency of recovered crossovers is reduced by 30 to 80%, depending on the *mei-P26* mutant genotype. However, analysis of recombination frequencies allows us to assess only one of the four products of a meiotic tetrad. Using the methods of Weinstein (1936), exchange rank frequencies, the frequencies of meiotic tetrads with zero, one, two, or more crossovers, can be calculated from recombination data. This demonstrates that *mei-P26* increases  $E_0$ , the frequency of tetrads in which no exchange occurred (Table 3).  $E_0$  increases to  $\sim 0.5$  in *mei-P26<sup>l</sup>* homozygotes and to  $\sim 0.59$  in *mei-P26<sup>ts1</sup> / mei-P26<sup>l</sup>*, compared to  $\sim 0.2$  in wild type. Similarly,  $E_0$  in *mei-P26<sup>l</sup> / Df(1)18.1.15* females is increased to  $\sim 0.8$ , indicating an elevated frequency of tetrads in which no exchange has occurred, while  $E_2$  and  $E_3$  tetrads are not

TABLE 3

Results of crosses of females of the genotype *X/X; net ho dp Sp b pr cn/+ + + + + +* carrying the indicated *X* chromosomes by *+ / Y; net ho dp b pr cn / net ho dp b pr cn* males<sup>a</sup>

Progeny type <sup>b</sup>	Maternal genotype							
	<i>+ / +<sup>c</sup></i>	<i>y w / +<sup>c</sup></i>	<i>mei-P26<sup>l</sup></i>	<i>mei-P26<sup>l</sup> / +<sup>d</sup></i>	<i>mei-P26<sup>l</sup> / mei-P26<sup>fs1</sup></i>	<i>mei-P26<sup>fs1</sup> / +</i>	<i>mei-P26<sup>l</sup> / Df<sup>e</sup></i>	<i>mei-P26<sup>l</sup>; ban<sup>Δ86</sup> / +<sup>f</sup></i>
Noncrossover	995	1512	773	819	363	2188	169	96
Single crossover								
1	228	299	48	158	13	450	1	1
2	369	598	122	386	36	982	9	4
3	56	54	70	77	21	140	2	1
4	7	15	24	15	26	26	5	1
Double crossover								
1,2	20	7	2	9	1	27	—	—
1,3	3	1	4	4	—	22	—	—
1,4	1	3	2	1	—	4	—	—
2,3	7	6	6	8	2	25	—	—
2,4	2	3	5	7	1	4	—	—
3,4	—	—	2	1	1	—	—	—
Triple crossover	—	—	1	1	—	2	—	—
Quadruple crossover	—	—	—	—	—	—	—	—
Total progeny	1712	2500	1061	1486	464	3891	186	103
Map distances								
1 ( <i>net-dp</i> )	14.7	12.4	5.4	11.6	3.0	13.0	0.5	1.0
2 ( <i>dp-b</i> )	24.2	24.6	12.8	27.7	8.6	27.2	4.8	3.9
3 ( <i>b-pr</i> )	3.9	2.4	7.9	6.1	5.2	4.9	1.1	1.0
4 ( <i>pr-cn</i> )	0.6	0.8	3.1	1.6	6.0	0.9	2.7	1.0
Total	43.4	40.3	29.2	47.0	22.8	46.0	9.1	6.8
Map relative to control								
1 ( <i>net-dp</i> )	1	0.844	0.367	0.789	0.204	0.884	0.034	0.068
2 ( <i>dp-b</i> )	1	1.017	0.529	1.145	0.355	1.124	0.198	0.161
3 ( <i>b-pr</i> )	1	0.615	2.026	1.564	1.333	1.256	0.282	0.462
4 ( <i>pr-cn</i> )	1	1.333	5.167	2.667	10.000	1.500	4.500	3.000
Total	1	0.929	0.673	1.083	0.525	1.060	0.210	0.196
Exchange rank frequencies								
E <sub>0</sub>	0.209	0.226	0.499	0.143	0.586	0.167	0.817	0.864
E <sub>1</sub>	0.714	0.742	0.426	0.779	0.371	0.751	0.183	0.136
E <sub>2</sub>	0.077	0.032	0.068	0.073	0.043	0.078	0	0
E <sub>3</sub>	0	0	0.008	0.005	0	0.004	0	0
E <sub>4</sub>	0	0	0	0	0	0	0	0

<sup>a</sup> Female progeny were scored in the indicated crosses (see materials and methods) except for the *+ / +* control and *y w mei-P26<sup>l</sup>; ban<sup>Δ86</sup> ry e / + + +* crosses, in which all progeny were scored. In the *y w mei-P26<sup>l</sup>; ban<sup>Δ86</sup> ry e / + + +* cross, eye color phenotype was not scored in male progeny, with the assumption that few *b-pr* or *pr-cn* recombinants were present. For this reason, the map distances for these intervals may have been underestimated in this cross.

<sup>b</sup> Region 1 is *net-dp*; region 2 is *dp-b*; region 3 is *b-pr*; region 4 is *pr-cn*.

<sup>c</sup> It should be noted that the control recombination levels differ from the standard *Drosophila* genetic map (Lindsley and Zimm 1992), although they are similar to previously published control values (Baker and Carpenter 1972).

<sup>d</sup> A possible semidominant effect on recombination was suggested by Sekelsky *et al.* (1999) using a comparison to standard map distances. However, comparison of recombination in *mei-P26<sup>l</sup> / +* females vs. wild-type controls indicates no such effect.

<sup>e</sup> *Df(1)18.1.15* was used in this experiment.

<sup>f</sup> *ban<sup>Δ86</sup>* is carried on chromosome 3.

observed at all. These data are consistent with *mei-P26<sup>l</sup>* and *mei-P26<sup>fs1</sup>* being hypomorphic alleles of differing severity.

Exchange in females carrying mutations in *mei-P26* is decreased overall and altered in a polar fashion, where distal and medial exchanges are more strongly sup-

pressed than those near the centromere. In more severe alleles of *mei-P26*, recombination is more severely affected, and the calculated frequency of nonexchange tetrads is increased. The alleles of *mei-P26* form an allelic series as follows: *mei-P26<sup>l</sup> < mei-P26<sup>fs1</sup>* (and other *fs* alleles) *< mei-P26<sup>ms1</sup>*, in which the effects on male and

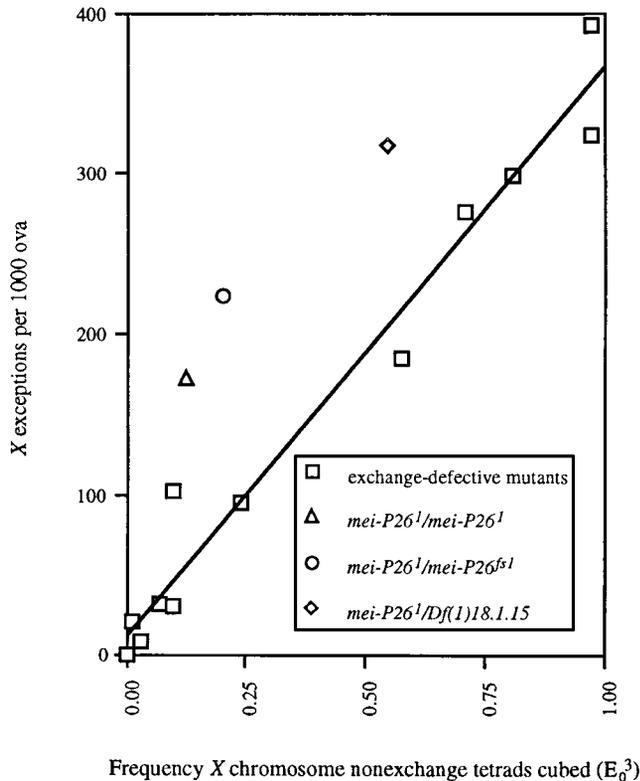


Figure 3.—A linear correlation exists between  $X$  nondisjunction frequency and  $E_0^3$  in exchange-defective mutants and *mei-P26*. This graph is based on Figure 4 of Baker and Hall (1976). Data for the exchange-defective mutants are from Baker and Hall (1976) and references therein. Data for *mei-P26* are from Tables 2 and 3 (this study). While there is some deviation in the frequency of  $X$  nondisjunction in *mei-P26* mutants from that expected based on the other exchange-defective mutants, we believe that this is attributable to differences in genetic background, since the slope of the linear relationship between  $E_0^3$  and  $X$  nondisjunction differs very little between *mei-P26* (slope = 320.2) and the exchange-defective mutants (slope = 348.6).

female germline differentiation (see below) and meiotic recombination increase in severity.

**Cytological studies of female sterility:** To investigate the cause of the sterility in the female sterile alleles, ovaries from homozygous *mei-P26*<sup>fs1</sup> females were analyzed using fluorescence microscopy after DAPI staining. In wild-type ovaries, the ovarioles contain a series of developing egg chambers, each comprised of 15 nurse cells and an oocyte (Figure 4A). In contrast, ovarioles from *mei-P26*<sup>fs1</sup> ovaries often contained chamberscysts filled with large numbers of small, apparently undifferentiated cells (Figure 4D). This phenotype resembled the “tumorous ovary” phenotype observed in a group of female sterile mutants including *bam*, *bgen*, *otu*, etc. (Gateff 1981; Schüpbach 1985; King *et al.* 1986; Perrimon *et al.* 1986; Salz *et al.* 1987; McKearin and Spradling 1990; Oliver *et al.* 1990). A total of 84.4% of the chambers ( $n = 495$ ) in *mei-P26*<sup>fs1</sup> females

were tumorous. The remaining chambers more closely resembled wild type, in that nurse cells were present. Although some of these appeared to be normal, many contained abnormal numbers of nurse cells (Figure 4). In agreement with these findings, *mei-P26*<sup>fs1</sup> females do lay a reduced number of eggs (0.07 eggs per female per hour, compared to 1.87 eggs per female per hour for wild type).

For comparison, ovaries from *mei-P26*<sup>1</sup> homozygotes were also analyzed. While tumorous chambers were very rare (0.7%,  $n = 305$ ), the other abnormalities in oogenesis observed for *mei-P26*<sup>fs1</sup> were also present in *mei-P26*<sup>1</sup> ovaries (Figure 4B). The most prominent of these were the defects in nurse cell number, with chambers often observed to have either too many or too few nurse cells. In *mei-P26*<sup>1</sup>/*Df* females, a more severe phenotype was observed, with a greater frequency of tumorous egg chambers (data not shown).

Ovaries from homozygotes for *mei-P26*<sup>ms1</sup>, the male and female sterile allele of *mei-P26*, were completely tumorous (Figure 4E). No normal-looking egg chambers were seen in ovaries homozygous for this allele. Instead, 100% of the egg chambers present ( $n = 384$ ) resembled the tumorous chambers observed at a lesser frequency in *mei-P26*<sup>fs1</sup> homozygotes, and *mei-P26*<sup>ms1</sup> homozygotes do not lay eggs. The ovarian defects in *mei-P26* mutants appear to affect the differentiation and proliferation of female germline cells, which leads to sterility. The severity of these defects correlates with the severity of the meiotic phenotype and infertility in *mei-P26*<sup>1</sup> homozygotes and *mei-P26*<sup>fs1</sup>/*mei-P26*<sup>1</sup> heterozygotes.

**Effects of *mei-P26*<sup>ms1</sup> on male fertility:** Testes from *mei-P26*<sup>ms1</sup>/*y*<sup>+</sup> *Y* males were examined to determine the cause of the male sterility. In contrast to testes from *mei-P26*<sup>1</sup>/*y*<sup>+</sup> *Y* or *mei-P26*<sup>fs1</sup>/*y*<sup>+</sup> *Y* males, no mature motile spermatozoa were present, although somewhat disorganized immature elongated spermatid bundles are produced (Figure 5C). In addition, the testes contained highly refractile cysts that most likely represent degenerating cysts of spermatogonial cells (Figure 5F). Although *mei-P26*<sup>1</sup>/*y*<sup>+</sup> *Y* males produce motile spermatozoa and are fertile, these refractile cysts are also often observed in testes of this genotype (Figure 5B). Cysts of this morphology are often seen in testes mutant for *bam*, another gene that affects male and female gametogenesis (Figure 5, D and G). *mei-P26*<sup>ms1</sup> represents a more severe class of allele than *mei-P26*<sup>fs1</sup>, since it affects both male and female gametogenesis more drastically than any other *mei-P26* allele.

***mei-P26* shows multiple genetic interactions with a null mutation at the *bam* locus:** The product of the *bam* gene is important in both male and female germlines for both the specification of cystoblast fate and the cessation of the germline mitotic divisions before entry into the meiotic cell cycle (McKearin and Spradling 1990;

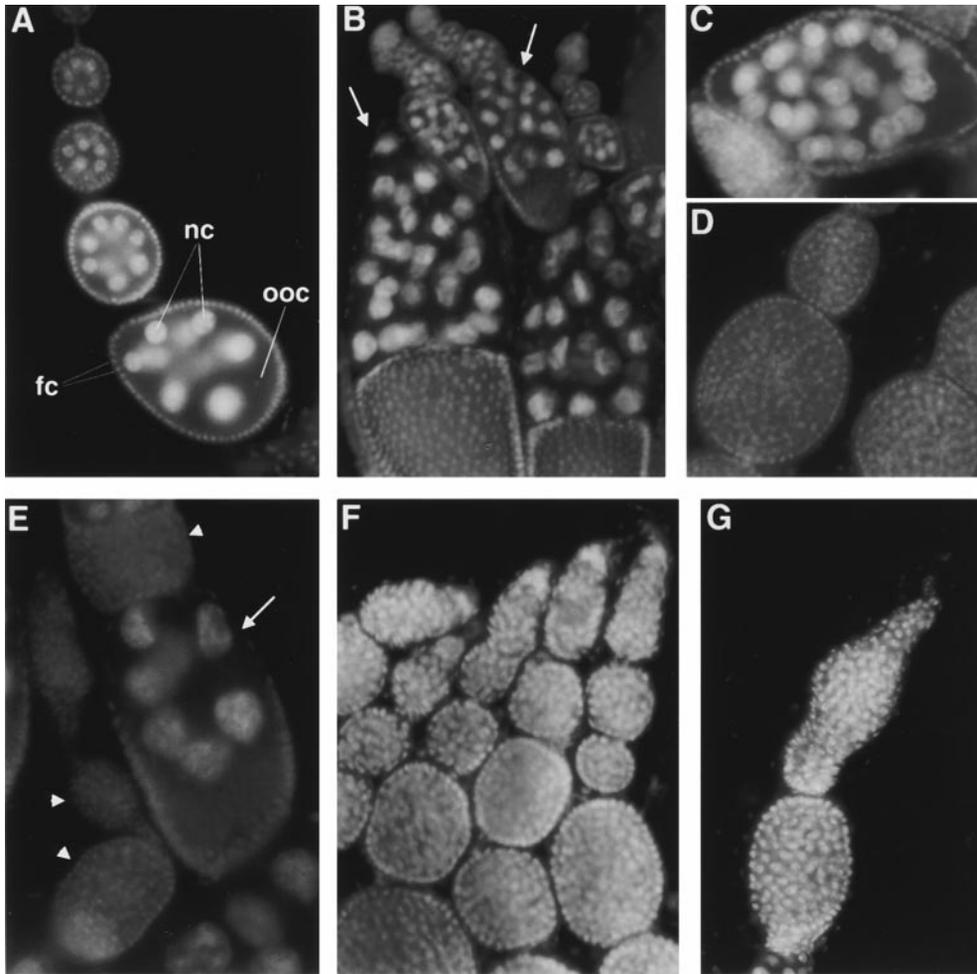


Figure 4.—Ovarian phenotypes in *mei-P26* mutants. Nuclei were visualized by DAPI staining. (A) Wild-type ovarioles are composed of a series of egg chambers that each contain 15 nurse cells (nc) and an oocyte (ooc), surrounded by a layer of follicle cells (fc). (B) Homozygous *mei-P26<sup>l</sup>* ovarioles also show defects, most notably in nurse cell number. Arrows indicate egg chambers with >15 nurse cells. (C) Abnormal egg chamber from a *mei-P26<sup>ls1</sup>* homozygote, with 31 nurse cells. (D) Tumorous egg chambers in ovarioles from *mei-P26<sup>ls1</sup>* homozygote females contain large numbers of undifferentiated cells. (E) *mei-P26<sup>l</sup>; bam<sup>Δ86</sup>/+* ovarioles show a higher frequency of tumorous chambers (arrowheads) than do *mei-P26<sup>l</sup>* homozygotes and egg chambers with abnormal numbers of nurse cells (arrow indicates a chamber with 7 nurse cells). (F) Ovarioles from *mei-P26<sup>ms1</sup>* homozygotes show only tumorous chambers. (G) Ovariole from a *bam<sup>Δ86</sup>* homozygote, showing tumorous phenotype.

McKearin and Ohlstein 1995; Gönczy *et al.* 1997; Ohlstein and McKearin 1997). Since mutations in the *mei-P26* gene produce an ovary phenotype with similarities to that of *bam* and similarities exist in the effect of *mei-P26* in testes, we tested double mutant combinations of *mei-P26<sup>l</sup>* and *bam<sup>Δ86</sup>*, a null allele (McKearin and Ohlstein 1995), to determine whether these mutants could interact genetically.

Although heterozygotes for the *bam<sup>Δ86</sup>* allele are fertile, males of the genotype *mei-P26<sup>l</sup>/Y; bam<sup>Δ86</sup>/+* were found to be completely sterile. Analysis of testes from males of this genotype showed a phenotype almost identical to *bam<sup>Δ86</sup>* homozygote males (Figure 5, E and H). Similarly, *mei-P26<sup>l</sup>; bam<sup>Δ86</sup>/+* females also showed a severe decrease in fertility compared to *mei-P26<sup>l</sup>* homozygotes, due to an increase in the formation of ovarian tumors and other egg chamber defects (Figure 4D). In *mei-P26<sup>l</sup>; bam<sup>Δ86</sup>/+* females, 28.9% of chambers were tumorous, compared to 0.7% in *mei-P26<sup>l</sup>; +/+* females. A normal level of fertility was observed for *mei-P26<sup>l</sup>/+; bam<sup>Δ86</sup>/+* females. Finally, recombination, as measured in the few progeny produced by *mei-P26<sup>l</sup>; bam<sup>Δ86</sup>/+* females, was also severely decreased (Table 3). These data show that *mei-P26* interacts with *bam* in the regulation

of germline differentiation and that defects in proper differentiation of the germline cyst may disturb the proper distribution of meiotic recombination events.

## DISCUSSION

Our genetic analysis of *mei-P26* shows it to be a novel exchange-defective female meiotic mutant in *Drosophila*, which is also essential for proper germline differentiation in both males and females. Furthermore, the effects of *mei-P26* on meiotic recombination and germline differentiation in both sexes are exacerbated by heterozygosity for *bam*, a gene already known to function in the germline to control the differentiation and proliferation of germline cells (McKearin and Spradling 1990; McKearin and Ohlstein 1995; Gönczy *et al.* 1997; Ohlstein and McKearin 1997). These results indicate that some of the processes that underlie proper germline cyst development may also be required for the normal levels and distribution of meiotic crossovers.

**The role of *mei-P26* in female germline development:** *mei-P26*-induced defects in female germline differentiation appear to occur during the early mitotic divisions in cyst formation, and hypomorphic alleles of *mei-P26*,

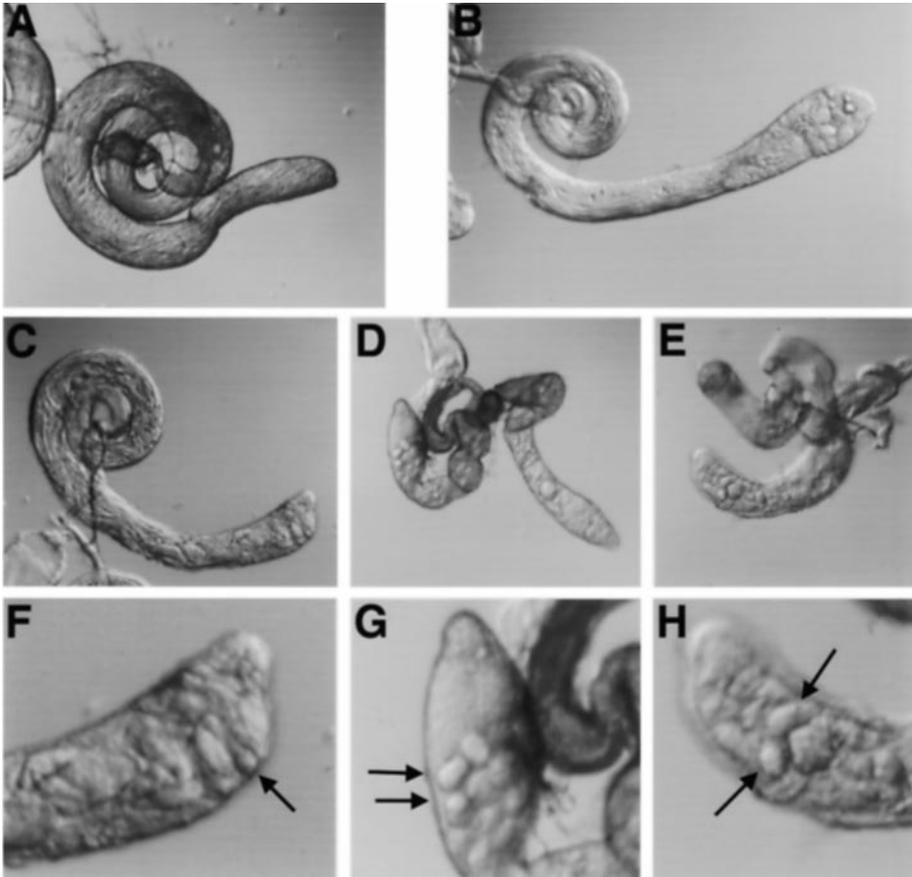


Figure 5.—Testis morphology in *mei-P26* mutants as visualized by DIC microscopy. (A) Wild-type testis. (B) *mei-P26*<sup>1</sup>/*y*<sup>+</sup> Y testis contains highly refractile degenerating cysts (arrow). (C) *mei-P26*<sup>ms1</sup>/*y*<sup>+</sup> Y testes support the production of elongated spermatid bundles, but not motile spermatids. (D) No elongated spermatids are present in testes from *bam*<sup>Δ86</sup> homozygotes. (E) Spermatid differentiation also fails to progress in *mei-P26*<sup>1</sup>/*y*<sup>+</sup> Y; *bam*<sup>Δ86</sup>/+ testes (note morphological similarity to D). (F) Magnified view of anterior tip of the *mei-P26*<sup>ms1</sup>/*y*<sup>+</sup> Y testes from C (above). Arrow, degenerating cyst. (G) Magnified view of anterior tip of the *bam*<sup>Δ86</sup> homozygote testes from D (above). Arrows, degenerating cysts. (H) Magnified view of anterior tip of the *mei-P26*<sup>1</sup>/*y*<sup>+</sup> Y; *bam*<sup>Δ86</sup>/+ testes from E (above). Arrows, degenerating cysts.

such as *mei-P26*<sup>1</sup>, produce egg chambers with abnormal numbers of nurse cells. Cysts with an increased number of nurse cells may be due to an additional round of mitosis occurring in some or all of the cystocytes (Hawkins *et al.* 1996). Alternatively, a defect in follicle cell packaging of normal cysts may result in chambers containing an excess of cells (Ruhola *et al.* 1991; Goode *et al.* 1992; Siegel *et al.* 1993). However, we did not observe egg chambers containing two oocytes, which would be expected if two 16-cell cysts were packaged together. Similarly, cysts with too few nurse cells could also result from packaging of cysts that have abnormally broken their intercellular connections to form smaller clusters of cells (Yue and Spradling 1992). Cysts containing 7 nurse cells and an oocyte may also result from the cystocytes undergoing only three divisions (Lilly and Spradling 1996; de Cuevas *et al.* 1997).

Allelic combinations of *mei-P26* that alter the number of nurse cells also reduce the number of normal eggs produced, but they do not cause complete sterility. In addition to the defects in nurse cell number, a tumorous ovary phenotype is more frequent in severe alleles. In females homo- or hemizygous for these mutations, the egg chambers become filled with hundreds of small cells, and no oocyte develops. This phenotype is similar to that exhibited by mutants in the *bam* and *benign gonial cell neoplasm* (*bgcn*) genes (Gateff 1981; McKearin and Spradling 1990).

The most severe *mei-P26* allele affects germline differentiation in both males and females. In *mei-P26*<sup>ms1</sup>, ovaries consist entirely of tumorous egg chambers and, in males carrying this allele, spermatid differentiation progresses only to the point of producing elongated spermatid bundles, and mature spermatozoa are not produced. The *mei-P26*<sup>ms1</sup> allele thus bears some similarities to mutants in the *bam* and *bgcn* genes, both of which cause a tumorous phenotype in ovaries and arrest of spermatogenesis (Gateff 1981; McKearin and Spradling 1990). However, spermatid differentiation is arrested at an earlier stage in *bam* and *bgcn* mutants. Ovary morphology differs somewhat, in that *mei-P26* mutant ovarioles consist of a series of defined chambers, while *bam* and *bgcn* ovarioles often appear as distended germaria with few distinct chambers. Nevertheless, the interaction of *mei-P26* with *bam* suggests that the similarities in the phenotypes in these mutants are not coincidental (see below).

Certain mutations in the *Sex-lethal* (*Sxl*), *ovarian tumor* (*otu*), and *ovo* genes also cause sterility in females due to the formation of tumorous egg chambers (Schüpbach 1985; King *et al.* 1986; Perrimon *et al.* 1986; Salz *et al.* 1987; Oliver *et al.* 1990). Interestingly, mild to severe defects in meiotic recombination frequencies are observed in females bearing heteroallelic combinations of female sterile alleles for these loci (Cook 1993) and in females in which the effects of *Sxl* mutations have been

partially suppressed using genetic modifiers (Bopp *et al.* 1999). These results further suggest that the processes that control exchange position and determine germline cyst formation may be coordinately controlled.

***mei-P26* encodes a RING finger protein:** The structure of the MEI-P26 protein suggests a few possibilities for the role of *mei-P26*. *mei-P26* is predicted to encode a member of the RBCC family of proteins, which contain, in their N-terminal regions, a RING finger motif followed by one or two copies of a second cysteine-rich motif called the B-box and a coiled coil region (Reddy *et al.* 1992; Saurin *et al.* 1996). The RING finger and B-box motifs are believed to mediate physical interactions with other proteins (Borden *et al.* 1995, 1996; Cao *et al.* 1997, 1998). RBCC proteins are only a subset of the large number of known RING finger proteins, which have diverse roles in oncogenesis, transcriptional regulation, signal transduction, and development (reviewed by Saurin *et al.* 1996).

Several RBCC proteins, such as PML and the TIF1 family, are known to regulate transcription by binding to nuclear hormone receptors as coactivators or corepressors (Le Douarin *et al.* 1995; vom Baur *et al.* 1996; Zhong *et al.* 1999). While assembling factors for transcriptional regulation is one role for RBCC proteins, certain other RBCC proteins appear to function in capacities such as signal transduction (Rpt1, Patarca *et al.* 1988; Staf50, Tissot and Mechtli 1995), or by forming ribonucleoprotein complexes (SS-A/Ro, Keech *et al.* 1995). Although a growing number of RBCC proteins have been identified, only a handful also contain NHL repeats, named after the proteins NCL-1, HT2A, and LIN-41 (Slack and Ruvkun 1998). The NHL repeat has been shown to be involved in protein-protein interactions in the RBCC proteins HT2A and BERP (Fridell *et al.* 1995; El-Husseini and Vincent 1999).

The RBCC-NHL proteins are mostly of unknown function (the predicted proteins KIAA0517, Nagase *et al.* 1998; F54G8.4 and F26F4.7, Wilson *et al.* 1994), or have not been extensively characterized at a molecular level (NCL-1, Frank and Roth 1998; HT2A, Fridell *et al.* 1995; LIN-41, Slack and Ruvkun 1998; BERP, El-Husseini and Vincent 1999; and MEI-P26, this study). RBCC-NHL proteins, which contain both RBCC and NHL domains, include at least two potential protein-protein interaction motifs, so a strong possibility is that these also may participate in the formation of multiprotein complexes. One possible candidate for a MEI-P26 partner protein is Bam.

**The interaction of *mei-P26* and *bam*:** We have demonstrated a genetic interaction between *mei-P26* and *bam*. Heterozygosity for a null mutant in *bam* enhances the phenotype of *mei-P26*, causing sterility in males, an increase in tumor formation in females, and a decrease in meiotic exchange. On the basis of the characterization of the *bam* and *mei-P26* gene products, we can specu-

late on the nature of the interaction between these two genes.

First, MEI-P26 may act as a transcriptional or translational regulator that controls *bam* expression. A variety of ovarian defects like those observed in *mei-P26*, including tumorous chambers and cysts with abnormal numbers of nurse cells, are also seen in mutants for the *Drosophila Rbp9* gene (Kim-Ha *et al.* 1999). The *Rbp9* gene was shown to encode an RNA binding protein that binds specifically to the *bam* transcript and may act to regulate Bam expression in the germarium. Similarly, misregulation of *bam* expression in the *encore (enc)* mutant may underlie the effect of *enc* on nurse cell number (Hawkins *et al.* 1996). However, since heterozygosity for *bam* exacerbates the meiotic phenotype of *mei-P26*, this model predicts that the meiotic defects are due to the misregulation of *bam*, rather than through other genes possibly regulated by *mei-P26*.

Second, *mei-P26* could be required for the proper localization or function of Bam. The product of the *bam* gene is expressed in the cytoplasm of cystoblasts and early germline cysts in females, where it is required for cystoblast differentiation. Bam protein also associates with the fusome, a large organelle mostly comprised of cytoskeletal and vacuolar components, which is present in early germline cysts (McKearin and Ohlstein 1995). According to this model, MEI-P26 may physically interact with Bam, possibly through the RBCC, NHL, or other motifs in the MEI-P26 protein. Alternatively, this regulation may be indirect, requiring other proteins. For example, MEI-P26 may regulate the *bgn* gene product, which is required for Bam function (Lavoie *et al.* 1999). Again, this suggests that the effects on meiotic exchange are mediated by *bam*.

The evidence presented here does not allow us to determine the relative positions of *bam* and *mei-P26* in a pathway. Therefore, *bam* may be required for *mei-P26* function, which in turn would be required for proper germline cyst development and meiotic recombination. In this third model, MEI-P26 may physically interact with Bam and/or other proteins in the cytoplasm, possibly as a component of the fusome, from which MEI-P26 may facilitate normal germline development and meiotic exchange. Alternatively, MEI-P26 might be indirectly controlled by Bam as a downstream effector. While the relationship between *mei-P26* and *bam* has not been fully elucidated, these models are intriguing, as they all suggest a role for *bam* in a pathway ensuring proper meiotic exchange.

**A speculative model for precondition mutants:** *mei-P26* appears to behave as expected for a female meiotic precondition mutant. This group of *Drosophila* mutants presents a phenotype in which the total frequency of meiotic exchange is often reduced, although to differing levels, and residual exchanges are abnormally distributed in a polar fashion, with reduced frequencies in the distal parts of the chromosome arms (for a review,

see Lindsley and Sandler 1977). In *mei-P26*, exchange is decreased overall and the distribution is polar. In more severe alleles, recombination is more severely affected, and  $E_0$  is increased. To provide a first step toward explaining the various components of the *mei-P26* phenotype and the connection with germline differentiation, we propose the following rather speculative model for precondition mutants in general.

In many organisms, telomeres have been proposed as sites responsible for initiating at least part of the pairing interactions between homologous chromosomes. Evidence for the clustering of telomeres during meiotic prophase has been gathered through cytological studies in many species (reviewed by Dernburg *et al.* 1995). In these studies, chromosomes in meiotic prophase were observed to form what has been described as a "bouquet" configuration, where the telomeres are positioned together in a small portion of the nuclear volume. The telomeres are often clustered near a region of the nuclear envelope adjacent to the position of the cytoplasmic centrosome. It is thought that the clustering of telomeres may facilitate homolog pairing in meiosis.

The existence of a bouquet configuration has been demonstrated recently in *Saccharomyces cerevisiae* (Trelles-Sticken *et al.* 1999), and telomeres are responsible for a delay in meiotic progression observed in haploid yeast strains that are disomic for a single chromosome pair (Rockmill and Roeder 1998). Furthermore, the delay associated with the presence of telomeres required an intact *NDJ1* gene (Chua and Roeder 1997; Conrad *et al.* 1997; Rockmill and Roeder 1998). In a wild-type background, deletions of *NDJ1* interfere with synapsis and alter the distribution of recombination events. Ndj1p localizes to telomeres and is believed to be necessary for telomeric pairing (Chua and Roeder 1997; Conrad *et al.* 1997; Rockmill and Roeder 1998). Defects in synapsis and a decrease in the frequency of recombination are observed in the presence of mutations in yeast *KAR3*, which encodes a kinesin-like protein (Bascom-Slack and Dawson 1997). This observation suggests that Kar3p may be involved in chromosome movement that is necessary for proper homolog alignment during meiotic prophase.

This clustering of telomeres has not been demonstrated in *Drosophila* oocytes, but the observations by electron microscopy may not have detected transient telomere clustering at the leptotene or zygotene stage of meiosis (Carpenter 1975a,b, 1979a,b; Dernburg *et al.* 1995). Thus, perhaps the association of telomeres is important for homologous chromosome pairing in *Drosophila*. The mechanism by which telomere clustering occurs may be similar to that in other organisms. In particular, the movement of the chromosomes may require associations with the cytoskeleton, resulting in the clustering of telomeres near centrosomes. Within germline cysts, the fusome acts to orient the spindles

in the cystocyte divisions by interacting with one centrosome at each mitosis (Deng and Lin 1997). Although the fusome begins to break down after the cessation of the mitotic divisions, remnants of this structure may remain in the oocyte during meiotic prophase (germarium regions 2 and 3; Lin *et al.* 1994; de Cuevas and Spradling 1998). Therefore, the fusome may position a determinant, possibly in the form of a cytoskeletal element, necessary for telomere clustering at a point on or near the nuclear membrane. Perhaps Bam, as a component of the fusome, is indirectly necessary for telomere clustering by marking the site of clustering on the nuclear membrane. The polar recombination defect observed in *mei-P26* could therefore be the indirect result of abnormal telomeric clustering due to a disruption of Bam function.

Since proximal euchromatic regions may rely on the pairing of centric heterochromatin, and perhaps not require telomeric clustering, disruption of telomeric clustering may primarily impact the frequency of exchange in the distal regions of the chromosomes. Thus, the specific disruption of telomeric interactions may result in a distribution of residual exchanges like that seen in recombination precondition mutants. Further work will be necessary to determine whether the meiotic defects in precondition mutants are the result of abnormalities in telomeric clustering.

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