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Two Genes Required for Meiotic Recombination in Drosophila Are Expressed From a Dicistronic Message

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ABSTRACT

We have isolated two alleles of a previously unidentified meiotic recombination gene, *mei-217*. Genetic analysis of these mutants shows that *mei-217* is a typical "precondition" gene. The phenotypes of the mutants are meiosis specific. The strongest allele has <10% of the normal level of crossing over, and the residual events are distributed abnormally. We have used double mutant analysis to position *mei-217* in the meiotic recombination pathway. In general, mutations causing defects in the initiation of meiotic recombination are epistatic to mutations in *mei-41* and *spnB*. These two mutations, however, are epistatic to *mei-217*, suggesting that recombination is initiated normally in *mei-217* mutants. It is likely that *mei-217* mutants are able to make Holliday junction intermediates but are defective in the production of crossovers. These phenotypes are most similar to mutants of the *mei-218* gene. This is striking because *mei-217* and *mei-218* are part of the same transcription unit and are most likely produced from a dicistronic message.

CROSSING over is an important mechanism for segregating homologs at meiosis I. Each crossover matures into a chiasma, which links and orients the homologs on the meiosis I spindle (Hawley 1988). Crossing over is one outcome of the meiotic recombination pathway(s); the other is gene conversion. One model explaining these events is that meiotic recombination is initiated by a DNA break, which is repaired via a Holliday junction, and then resolved as either a gene conversion or crossover. In *Saccharomyces cerevisiae*, a double-strand break is responsible for the initiation of meiotic recombination (de Massy *et al.* 1995; Liu *et al.* 1995). This mechanism appears to be conserved in other organisms because the candidate for the enzyme that makes the double-strand break in *S. cerevisiae*, Spo11 (Keeney *et al.* 1997), has homologs required for meiotic recombination in *Drosophila melanogaster* (McKim and Hayashi-Hagihara 1998) and *Caenorhabditis elegans* (Dernburg *et al.* 1998).

While several genes in yeast and Drosophila have been suggested to function either in the induction or repair of double-strand breaks, less is known about the genes and mechanisms that control the resolution of the Holliday junction into a crossover or a gene conversion. In yeast and mammals, only members of the mismatch repair protein family have been suggested to be components of late recombination nodules and required for crossover production (Hollingsworth *et al.* 1995; Ross-Macdonald and Roeder 1995; Baker *et al.* 1996; Hunter and Borts 1997). In *D. melanogaster*, several genes required specifically for crossing over have been identified. In mutants of these genes, such as *mei-9* and *mei-218*, crossing over is reduced but the frequency of gene conversion is normal (Carpenter 1982, 1984).

Crossover-defective mutants in Drosophila have been divided into two classes, the precondition defective and the exchange defective. Precondition-defective mutants have reductions in crossing over that are nonuniform along each chromosome. Thus, these genes are thought to control both the frequency and location of crossover events (Sandler *et al.* 1968; Baker and Carpenter 1972). Exchange-defective mutants do not alter the distribution of exchanges as shown by uniform reductions in crossing over along the chromosome. Genetic studies suggest that exchange genes are required later than the precondition genes (Baker and Carpenter 1972; Carpenter 1982, 1984; Sekelsky *et al.* 1995), most likely in the actual resolution reaction. In support of this hypothesis, the *mei-9* encodes a protein likely to have an enzymatic role in recombination; it is homologous to the Rad1/XPF family of endonucleases (Sekelsky *et al.* 1995).

While the evidence from *mei-9* suggests that the exchange genes function directly in the resolution reaction, the event(s) in the recombination pathway affected by the precondition genes is not known. In a one-pathway model, precondition genes cause a divergence in the recombination pathway after initiation, one leading to gene conversion and the other leading to crossing over. Alternatively, in a two-pathway model, the precondition genes could function at or before recombination initiation, resulting in two distinct pathways for gene conversion and crossing over. Since the *mei-218* gene is

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not conserved, we are characterizing other genes with a similar phenotype to investigate the nature of the precondition function. We have isolated two alleles of a new precondition gene, *mei-217*. Surprisingly, this gene maps very close to another precondition gene, *mei-218*. We have cloned *mei-217* and have shown that it is part of the same transcription unit as *mei-218*. The most likely explanation is that *mei-217* and *mei-218* are two proteins made from the same transcript.

MATERIALS AND METHODS

Screen for X-linked meiotic mutations: Males of the genotype y/y^+Y; spa^{pol} were fed with 25 mm ethyl methane sulfonate for 18 hr and crossed to $l(1)15Eb^{692\text{-}19}/FM7c$ females. Individual $y/FM7$ females were crossed to $FM7/y^+Y$ males to generate X chromosome mutagnized lines. To generate homozygotes, in the next generation the y/y^+Y males were crossed to $y/FM7$ sisters. In the next generation, y/y females were crossed to their y/y^+Y brothers to test for nondisjunction. Normally this cross produces yellow females and wild-type males. Nondisjunction in the female germline produces diplo-X and nullo-X eggs, resulting in yellow males and wild-type females. If at least one exceptional progeny was observed, the line was restested either by repeating the brother-to-sister cross or by crossing virgin females to *C(1;Y), v f B/O; C(4)RM,* ey^R *ci/0* males. This latter cross precluded the isolation of mutants that were specific to the male germline, which are often associated with mutations of the rDNA (McKee and Karpen 1990). All mutations were tested for allelism to *mei-9*, *mei-38*, *mei-41*, and *mei-218* by standard complementation and nondisjunction tests.

Genetic mapping of *mei-217*: Low resolution recombination mapping of *mei-217* was done relative to *pn*, *cv*, *m*, and *f*. Both *g10* and *r1* mapped close to *f*, which is where *mei-218* also maps. To confirm this, both mutants were crossed to deficiencies, duplications, and transgenes in the region (McKim *et al.* 1996). $mei\text{-}217^{g10}$ failed to complement deficiencies *Df(1)BK8* (15C1-4; 16C2-7) and *Df(1)815-6* (15E1-2; 15E6-7) but complemented *Df(1)BK10* (16A2; 16C7-10). For complementation testing with duplications, the fourth chromosome marker spa^{pol} was used in the crossing scheme. To make $mei\text{-}217^{g10}/mei\text{-}217^{g10;}$ *Dp(1;4)* females, $mei\text{-}217^{g10}/FM7$; spa^{pol}/spa^{pol} females were crossed to *r f B; Dp(1;4)*/spa^{pol} males. The $mei\text{-}217^{g10}/Y$; *Dp(1;4)*/spa^{pol} males were crossed back to $mei\text{-}217^{r1}/FM7$; spa^{pol}/spa^{pol} females to make $mei\text{-}217^{r1}/mei\text{-}217^{r1}$ *Dp(1;4)*/spa^{pol} females. The nondisjunction phenotype of $mei\text{-}217^{g10}$ was rescued by duplications *Dp(1;4)fK7* (14A1-2; 15E6-7) and *Dp(1;4)rK20* (14A1-2; 14A4-B1; 15D5-E4; 16A7;B1). This positioned *mei-217* between the distal breakpoint of *Dp(1;4)fK7* and the proximal breakpoint of *Df(1)815-6*, which is also the interval that contains *mei-218*.

Isolation of RNA, RT-PCR analysis, and *in situ* hybridization: Total RNA was collected from dissected ovaries or testis by grinding the tissue in 50% RNA lysis buffer (0.3 m sodium acetate, 5 mm EDTA, 50 mm Tris-HCl pH 9.0, 1% SDS)/50% acid phenol followed by two extractions in acid phenol. For Northern blotting, 50–80 μg of total RNA (from 20 ovaries dissected from 10 females) was loaded into each lane and transferred to a nylon membrane. mRNA was prepared from ovaries using the Ambion (Austin, TX) Poly(A)Pure isolation kit. Reverse transcriptase (RT)-PCR was carried out using the single tube methodology and using reagents from Life Technologies or Boehringer Mannheim (Indianapolis). Digoxygenin (DIG)-labeled RNA probes for *in situ* hybridization were made from linearized *mei-218* cDNA clone pH2-15 using the Boehringer Mannheim RNA labeling kit and hybridized as described by Tautz and Pfeifle (1989).

Sequencing and analysis: DNA clones for sequencing were prepared by alkaline lysis minipreps followed by polyethylene glycol (PEG) precipitation. PCR products were either directly sequenced following isolation from an agarose gel or first cloned using the Perfectly Blunt cloning system (Novagen). Sequencing was done by the University of Medicine and Dentistry of New Jersey sequencing facility. Sequence analysis was done using the Wisconsin Package Version 9.1 [Genetics Computer Group (GCG), Madison, WI]. Sequences from mutant DNA and another strain of the same genetic background were compared to identify the nucleotide change.

Confocal microscopy: Stage 14 oocytes were collected from 3–7-day-old females and fixed as described previously (Theurkauf and Hawley 1992; McKim *et al.* 1993). Oocytes were stained for DNA with propidium iodide or Yo-Pro and for spindles with anti-tubulin (clone DM1A; Sigma, St. Louis) conjugated to either FITC or rhodamine.

RESULTS

Screen for X-linked meiotic mutations: We screened 2106 chromosomes (materials and methods) and confirmed 13 mutants with an elevated frequency of X chromosome nondisjunction in females. The rationale is that proper segregation requires chiasmata, which leads to a correlation between nondisjunction rates and defects in crossing over. Mutants with higher nondisjunction frequencies usually have the greatest reductions in meiotic crossing over. Five alleles of *mei-218* (*j1*, *j2*, *g1*, *g4*, and *g9*) and one allele of *mei-9* (*j3*) were recovered as well as seven mutations in other genes. No alleles of the highly mutable *mei-41* gene were recovered. Two possible explanations are (i) we would not expect to isolate strong alleles because they are sterile and (ii) weaker alleles such as the two recovered by Baker and Carpenter (1972) have low nondisjunction rates, which may have been at the limit of our screen's sensitivity. Based on genetic mapping and complementation testing, five mutations are alleles of two new genes, *gav* (three alleles) and *mei-217* (two alleles). In both genes the mutants are of the precondition type because they alter the distribution of crossovers (see below and data not shown). *mei-217* mutants have a similar effect on crossing over as *mei-218*, which reduces crossing over to 5–10% of wild type (McKim *et al.* 1996). Less severe are the *gav* mutants that reduce crossing over to ~50% of wild type (data not shown). The last two mutations (*g5* and *g11*) have been characterized less, although *g5* had no effect on the frequency of crossing over. Like some other precondition mutants, *gav* and *mei-217* are not DNA repair defective as indicated by their lack of sensitivity to methyl methanesulfonate (MMS; data not shown). Both mutants are also female specific; in hemizygous mutant males there is normal segregation of the X and Y chromosomes (data not shown). The remainder of this article describes the *mei-217* gene. A detailed analysis of *gav* will be published elsewhere.

***mei-217*, a new X-linked meiotic recombination gene:**

TABLE 1

Crossing over on the X chromosome in *mei-217* mutants

	Map interval				X chromosome nondisjunction			
	1	2	3	4	diplo-X	nullo-X	Total	%
r1 map (cM)[a]	2.0	2.6	3.0	1.3	534	531	4738	34.4
% control[b]	12.6	11.2	24.6	16.5				
g10 map(cM)[c]	0.6	1.1	3.1		1354	1453	11018	34.5
% control[b]	3.8	4.7	15.4					

X chromosome nondisjunction was assayed by crossing females to *C(1;Y), v f B / 0; C(4)RM, ey^R ci / 0* males. The X chromosome nondisjunction frequency was calculated as 2(exceptional progeny) / 2(exceptional progeny) + (regular progeny).

[a] Parent was *y pn cv m mei-217^{r1} ++ / y +++ mei-217^{r1} fy$^+$*. Intervals for *r1* are the following: 1, pn–cv; 2, cv–m; 3, m–f; 4, f y$^+$.

[b] Control values in centimorgans are the following: 1, 15.9; 2, 23.2; 3, 12.2; 4, 7.9. For the *g10* experiment, intervals 3 and 4 are combined.

[c] *y pn cv m mei-217^{g10} ++ / y +++ mei-217^{g10} y$^+$*. Intervals for *g10* are the following: 1, pn–cv; 2, cv–m; 3, m–y$^+$.

The primary defect in *mei-217* mutants is a reduction in the frequency of crossing over. Crossing over is reduced by a similar degree on both the X chromosome (Table 1) and the left arm of the second chromosome (Table 2). The severity of the crossover reductions is consistent with the observed frequency of nondisjunction, but it is formally possible that nondisjunction also occurs due to the failure of the chiasmata to ensure segregation at meiosis I, or if there is nondisjunction of sister chromatids at meiosis II. We investigated whether crossover bivalents nondisjoin by simultaneously measuring X chromosome crossing over and disjunction (Table 1). A failure of chiasmata to direct homolog segregation would have been detected by the recovery of females homozygous for X-linked recessive markers. Since there were no such cases observed (*r1*, $n = 534$; *g10*, $n = 1354$), most or all of the nondisjunction events in *mei-217* mutants involved achiasmate chromosomes at the first meiotic division. These data also show an equal number of diplo-X (B$^+$ females) and nullo-X (B males) progeny, showing that meiotic chromosome loss was not a significant factor. There is consistently a higher frequency of crossing over in *r1* than *g10*, which suggests that *g10* is the stronger allele. While this is not reflected in the X chromosome nondisjunction data of Table 1, this reflects the variation found in nondisjunction tests, since in several other experiments *r1 / r1* females had significantly less X chromosome nondisjunction (20–25%). The frequency of X chromosome nondisjunction was similar in *mei-217^{g10} / mei-217^{g10}* and *mei-217^{g10} / Df(1)815-6* females, suggesting that *g10* represents a null allele.

TABLE 2

Second chromosome crossing over in *mei-217* mutants

Allele	*al–dp*	*dp–b*	*b–pr*	*pr–cn*	Total map	Total flies
g10						
Map (cM)	1.05	2.11	1.27	0.87	5.3	2752
% control[a]	6.7	9.6	24.4	62.1	12.0	
r1						
Map (cM)	3.09	3.23	1.28	1.43	9.03	1329
% control[a]	19.8	14.7	24.6	102.1	20.5	

[a] "% of control" based on the following crossover frequencies observed in a separate experiment: *al–dp*, 15.6; *dp–b*, 21.9; *b–pr*, 5.2; *pr–cn*, 1.4 (total = 44.1)

Crossing over was substantially reduced in *mei-217* mutants, but the reductions were not uniform along each chromosome arm. This is most easily seen for crossing over on the second chromosome (Table 2). In both *mei-217* mutants, crossing over was reduced more in distal regions than in proximal regions. For example, in the *dp–b* region, crossing over was reduced to 9.6% of wild type in *mei-217^{g10}* and to 14.7% of wild type in *mei-217^{r1}*. In contrast, in the *pr–cn* region crossing over was reduced to 62.1% of wild type in *mei-217^{g10}* and increased to 102.1% of wild type in *mei-217^{r1}*. A reduction in crossing over that is more severe in distal than proximal regions is the defining feature of the precondition mutants in *D. melanogaster*, such as *mei-218*.

The high frequency of X chromosome nondisjunction is consistent with the low level of meiotic crossing over observed in the two *mei-217* mutants. We also tested for autosomal nondisjunction by crossing *mei-217* mutant females to males carrying second chromosome compound chromosomes. As predicted from the low level of crossing over, second chromosome nondisjunction was elevated (Table 3). Since in these crosses only second chromosome nondisjunction events produce progeny, absolute nondisjunction frequencies cannot be measured. Simultaneous nondisjunction of two chromosome pairs is another characteristic of meiotic mutants. This happens in part because there is a backup system that can direct the segregation of achiasmate chromosomes (Hawley *et al.* 1992). While this system

TABLE 3

Autosomal nondisjunction in *mei-217* mutants

	Phenotype of progeny and the segregation pattern in the oocyte										
	y ♀ XX;22⇔	b px ♂ ⇔0,0	+ ♀ X;22⇔	y b px ♂ ⇔X,0	b px ♀ X,0⇔	y ♂ ⇔X;22	+ ♂ 0,22⇔	y b px ♀ ⇔XX,0	Parents	prog/♀	% X-ND	% XX⇔22
r1/r1	91	192	209	207	224	241	306	134	284	5.65	62.1	60.9
g10/g10	34	126	99	14	152	48	90	6	136	4.18	62.1	37.5[a]
g10/FM7	1	0	1	1	2	0	0	0	88	0.057		

Four *y*/*y* females of the indicated *mei-217* genotype were crossed to +/*Y*; *C(2L),b*; *C(2R),px* males in each vial for two 7-day broods. The normal progeny from this cross die due to aneuploidy. Nondisjunction in the female germline produces diplo-2 and nullo-2 eggs, resulting in wild-type and black plexus progeny.

[a] This number is unusually low (the *r1*% is more typical), probably because the yellow progeny were underrepresented as a result of reduced viability of flies with the *g10* chromosome.

operates efficiently when only a single pair of achiasmate chromosomes is present, multiple pairs of large chromosomes cannot be distinguished. In this situation, the chromosomes are evenly segregated regardless of homology, leading to a high frequency of two X chromosomes segregating from two autosomes. The gametic frequency of simultaneous X and second chromosome nondisjunction was >62% for both mutants. Part of this high nondisjunction can be attributed to meioses where the two X chromosomes segregated from the two second chromosomes. These results show that the effects of the *mei-217* mutants are specific to chiasmate segregation.

In *mei-217* mutants meiotic recombination is initiated but the recombination intermediates are not resolved as crossovers: Evidence from the following two experiments supports the conclusion that *mei-217* mutants initiate meiotic recombination normally, thus making the expected number of chromosomal breakage events (presumably double stranded; McKim and Hayashi-Hagihara 1998). This conclusion is based on the similar behavior of *mei-217* and *mei-218* in double-mutant analysis. In *mei-218* mutants meiotic recombination is presumed to initiate normally [as judged by the wild-type frequency of gene conversion events (Carpenter 1982, 1984) and normal number of early recombination nodules (Carpenter 1989) seen in these mutants].

Double mutants with mei-41: In wild-type mature oocytes (stage 14), meiosis arrests at metaphase I (Theurkauf and Hawley 1992). We have previously observed that in the absence of chiasmata, such as in recombination-defective mutants, this arrest does not occur and meiosis proceeds through the two divisions (McKim *et al.* 1993). Depending on the recombination-defective mutant, precocious anaphase requires the *mei-41* gene product (McKim *et al.* 2000). *mei-41* encodes a homolog of the ataxia telangiectasia mutated (ATM) family of DNA-dependent protein kinases (Hari *et al.* 1995) and has a significant role in regulating the mitotic cell cycle in the presence of double-strand breaks. In double mutants of crossover-defective mutants, such as *mei-218*, and strong *mei-41* alleles, such as *D3* or *D18*, metaphase arrest occurs. In double mutants that fail to initiate meiotic recombination, such as *mei-P22* (McKim *et al.* 1998) and strong *mei-41* alleles, precocious anaphase occurs. It is not known why *mei-41* affects precocious anaphase in mutants that can initiate meiotic recombination, but this effect can be used to determine where in the meiotic recombination pathway a gene is required. As expected for mutants that drastically reduce crossing over, in *mei-217^{g10}* and *mei-217^{r1}* mutants precocious anaphase was observed (Figure 1 and Table 4). In *mei-41^{D18} mei-217^{g10}* and *mei-41^{D18} mei-217^{r1}* double mutants, however, a metaphase arrest was almost always observed, a result that contrasts with that seen in the *mei-41^{D18}*; *mei-P22* double mutant. Our interpretation of these results is that *mei-217* mutants are likely to be proficient at initiating meiotic recombination at normal levels.

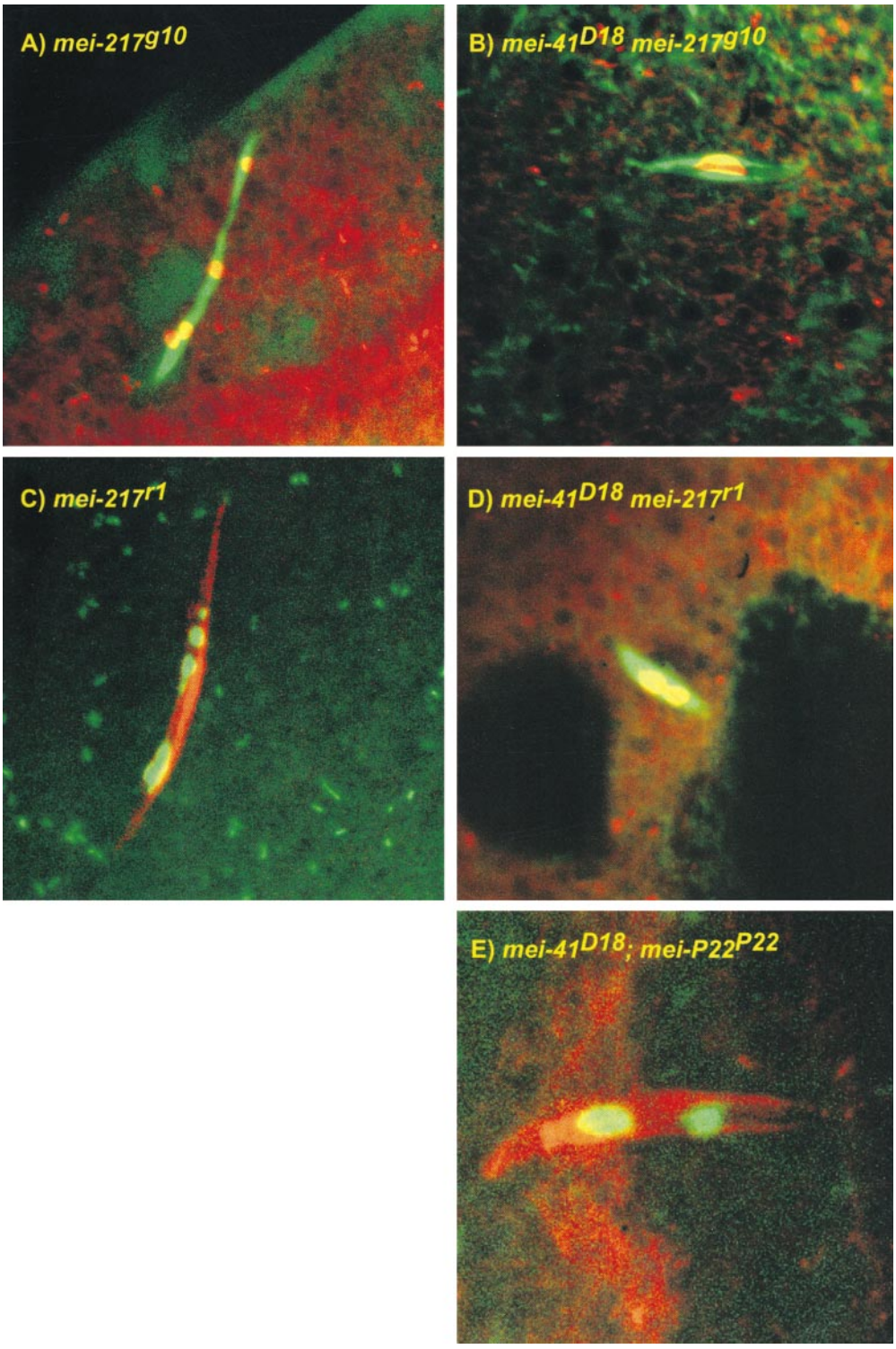

Figure 1.—*mei-217* mutations cause a precocious anaphase but are not epistatic to *mei-41* mutations. Confocal images of stage 14 oocytes with the chromosomes stained red and the spindle stained green (except $mei\text{-}41^{D18}$ $mei\text{-}P22^{P22}$, which is the reverse). The genotypes of representative oocytes are shown. A and B show the precocious anaphase seen in *g10* and *r1* mutants. C and D show typical nuclei in double mutants with $mei\text{-}41^{D18}$. For comparison, E is a $mei\text{-}41^{D18}$; $mei\text{-}P22^{P22}$ oocyte.

Double mutants with spnB: spnB encodes a homolog of the RAD51/DMC1 family of double-strand break repair proteins (Ghabrial *et al.* 1998). Mutations in this gene and others in the same class cause female sterility, most likely a result of the persistence of unrepaired double-strand breaks. Only a few progeny are produced in the first 2 or 3 days of mating (Table 5). Mutations that prevent any genetic recombination and presumably the formation of double-strand breaks, such as *mei-W68*, suppress the sterility of $spnB^{BU}$ (Table 5 and Ghabrial and Schupbach 1999). $spnB^{BU}$ mutants are fertile in the first 2–3 days of mating, so the suppression effects are not readily apparent until after the second day of mating (Table 5). In contrast, in *mei-218* mutants where meiotic recombination is initiated normally the sterility of $spnB^{BU}$ is not suppressed (Table 5). Similar to *mei-218*, we found

TABLE 4

***mei-217* mutants require *mei-41*$^{+}$ for precocious anaphase**

	Cytology	
Female genotype	Metaphase	Anaphase
mei-217^{g10}	5	17[a]
mei-41^{D18} *mei-217*^{g10}	60	0
mei-217^{r1}	6	13
mei-41^{D18} *mei-217*^{r1}	13	1

[a] Some *mei-217*^{g10} anaphase figures (5/17) had defects in spindle assembly, but this could be caused by another mutation on the same chromosome.

that *spnB*^{BU} is not suppressed by *mei-217*^{g10} or *mei-217*^{r1}. In fact, like *mei-218*, the phenotype is actually enhanced (Table 5).

Both of these experiments are consistent with the hypothesis that in *mei-217* mutants meiotic recombination is initiated normally but crossovers are not produced.

***mei-217* and *mei-218* are part of the same transcription unit:** Genetic mapping relative to several X-linked loci placed *mei-217* close to *forked* at position 57 of the X chromosome (materials and methods). This was confirmed by showing that both genes mapped to the same genetic interval defined by *Df(1)815-6* and *Dp(1;4)fK7*. A transgenic fragment containing 15 kb of genomic DNA from this genetic interval was able to rescue mutations in both genes (Figure 2D). These results showed that *mei-217* and *mei-218* are physically very close and raised the possibility that *mei-217* mutations were special alleles of *mei-218*. Since our original complementation tests were done with the *mei-218*^{6} allele, the tests were repeated by crossing both *mei-217* alleles to 10 existing *mei-218* alleles. In all cases the mutations complemented each other. Consistent with these results, a construct containing the *mei-218* coding region driven by the *hsp83* promoter (Figure 2, C and D) rescued *mei-218* mutants but not *mei-217* mutants. These results confirmed the genetic data that *mei-217* and *mei-218* are physically close but remain separable genetic units.

Previous work in this region of the genome showed that the *mei-218* locus produces a 6–7 kb transcript (McKim *et al.* 1996; Figure 3B). This is significantly longer than the largest cDNA, which at 4.2 kb still contains the entire *mei-218* coding region (Figure 2, C and D). Considering that the 5′ end of all three of the longest cDNA clones are at similar positions (Figure 2C), it is possible that the long length combined with strong secondary structure in the region resulted in a failure to recover any full-length cDNA clones. In the region where the cDNA clones begin, there is a direct repeat of the sequence GCCCAC that is located in the loop of a potential hairpin structure (Figure 4A).

To investigate the region upstream of the cDNA clones, we sequenced a 4-kb region that covers the interval between *mei-218* and the next upstream gene, *rpS5*. In addition, a 1.6-kb *Sac*I fragment, which covers the region upstream of the *mei-218* cDNA clones (Figure 2), was used to probe two cDNA libraries for longer cDNA clones. No clones were identified, but the existence of a transcript in the region was confirmed by RT-PCR analysis. Using a primer within the *mei-218* coding region and primers in the upstream region (g10-5 or g10-6, Figure 2B), a single species of transcript was amplified from ovarian RNA. When spliced, this transcript begins at least 830 bp upstream of the *mei-218* AUG. This was shown with the amplification of an ~1400-bp product using the most upstream primer, g10-6, and a *mei-218* primer 4339, or a slightly smaller product with g10-5 (Figure 3A). The extensive overlap of this transcript with the 4.2 kb of *mei-218* cDNA, combined with the large size of the transcript on Northern blots, suggest that the mature transcript has the entire *mei-218* coding region in addition to the upstream sequences. It is likely that the transcript has 5′ untranslated region (UTR) sequences extending upstream of the g10-6 primer site.

TABLE 5

***mei-217* mutations do not suppress the sterility of *spnB*^{BU}**

	Progeny/female/day[a]		
Female genotype	1–3	4–6	No. of females
spn^{BU}/*spn*^{BU}	0.81	0	64
mei-218^{1}/*mei-218*^{1}; *spn*^{BU}/*spn*^{BU}	0.02	0	45
mei-217^{g10}/*mei-217*^{g10}, *spn*^{BU}/*spn*^{BU}	0	0	20
mei-217^{g10}/*FM7*, *spn*^{BU}/*spn*^{BU}	0.56	0	25
mei-217^{r1}/*mei-217*^{r1}, *spn*^{BU}/*spn*^{BU}	0.30	0.075	40
mei-217^{g10}/*mei-217*^{g10}	4.85	3.625	80
mei-W68^{1}/*mei-W68*^{1}	7.26	4.47	54
mei-W68^{1}/*mei-W68*^{1}, *spn*^{BU}/*spn*^{BU}	2.04	1.46	50

[a] Four to eight females were mated to an equivalent number of males in each vial and transferred daily. The number of progeny was determined by counting the eclosed adults.

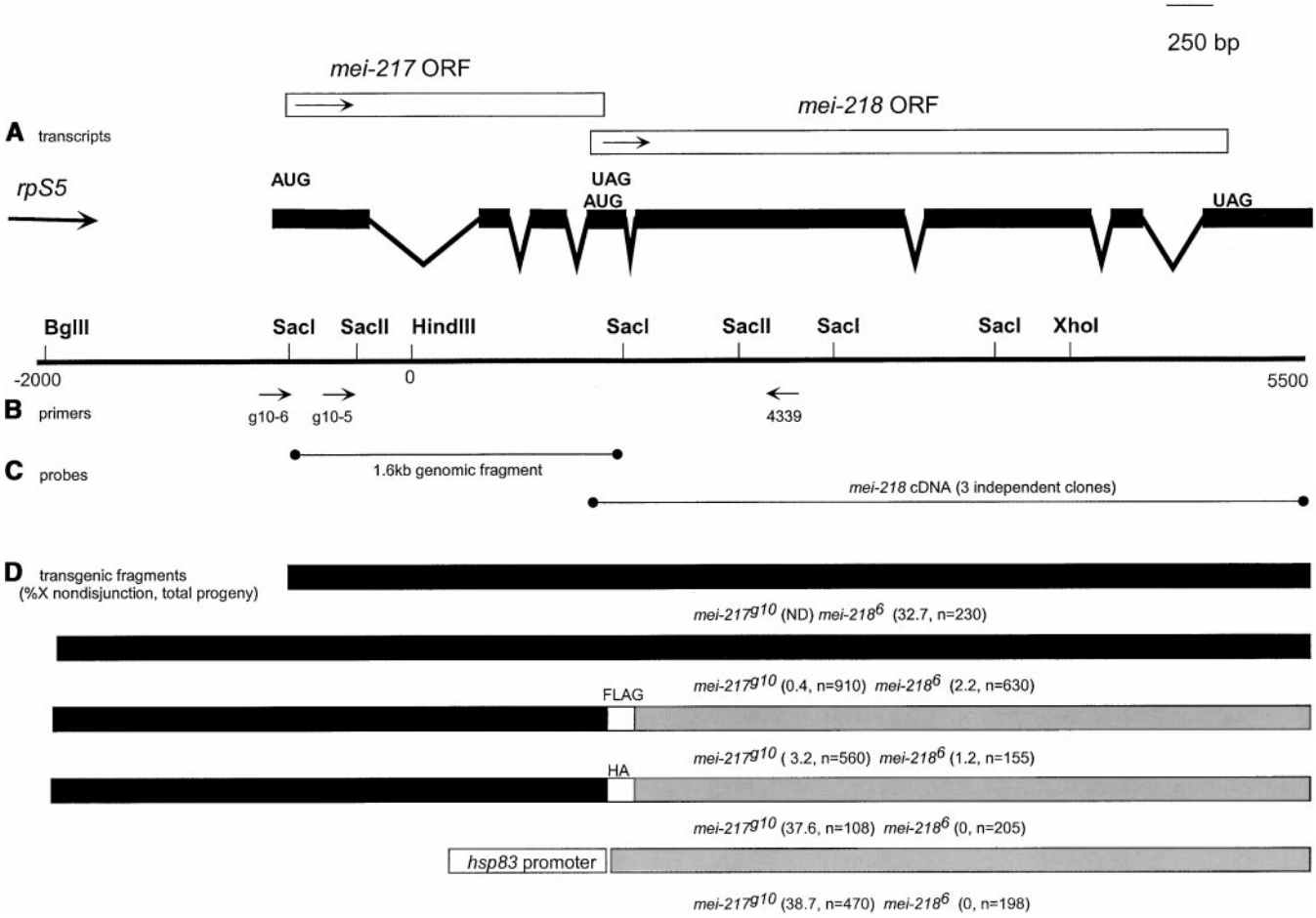

Figure 2.—Restriction map of the *mei-217*/*mei-218* region. (A) The structure of the *mei-217*/*mei-218* transcript and the position of the two ORFs are shown. (B) The primers used in the RT-PCR experiments. (C) The probes used for Northern blots and *in situ* hybridization. The 1.6-kb fragment is a genomic subclone and the cDNA clone contains the entire *mei-218* coding region. Three clones with almost identical start sites were originally isolated (McKim *et al.* 1996). (D) The transgenic fragments show the DNA contained in each construct. Solid segments are derived from genomic DNA and gray segments are derived from a cDNA clone. Below each construct is shown the frequency of nondisjunction observed in a strain homozygous for the indicated mutation and carrying one copy of the transgene. The location of the FLAG or HA tag is also shown by a white box. See Figure 4 for amino acid sequence at the junction of the FLAG and HA clones.

From this site to the 3′ end of the cDNA is only ~5 kb, which is at least 1 kb shorter than the transcript observed on Northern blots. Sequence analysis of the RT-PCR products and comparison to the genomic sequence showed the presence of three introns upstream of the *mei-218* AUG (Figure 2).

Three lines of evidence support the idea that the region upstream of the *mei-218* AUG encodes the MEI-217 protein. First, the upstream splicing pattern maintains an open reading frame (ORF) that begins 662 bp upstream of the *mei-218* AUG. This ORF does not end until 19 bp after the *mei-218* AUG. Second, this ORF shows codon bias typical of *D. melanogaster* proteins. Third, the sequence changes in the two *mei-217* mutations affect this ORF (Figure 4). The $mei\text{-}217^{g10}$ mutation is a G > A change causing a glutamate-to-lysine change (GAA to AAA). The $mei\text{-}217^{r1}$ mutation is an A > T change 42 bp upstream of the AUG. This change does not affect the coding potential of *mei-217*, and since this chromosome contains a normal *mei-218* gene, this mutation may affect translation of MEI-217. The presence of an open reading frame upstream of *mei-218* but within the same transcript and the presence of mutations that affect this ORF support the conclusion that *mei-217* and *mei-218* encode distinct gene products that are made from a dicistronic message.

There are several other possibilities we can eliminate. A fusion protein could be made if the ribosome is able to bypass the stop codon, as occurs in the *D. melanogaster kelch* gene where a single stop codon separates two long ORFs (Robinson and Cooley 1997). This is not the case with the *mei-217* ORF because there are several stop codons immediately following the first one. A variant of this concept is that the stop codon is bypassed by ribosome frameshifting. In this case, and also the previous one, we would not expect the *mei-217* and *mei-218*

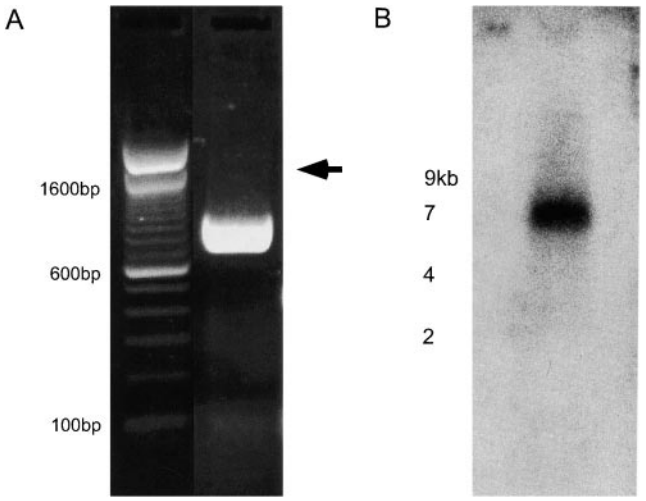

Figure 3.—The *mei-217*/*mei-218* transcript. (A) RT-PCR using primers g10-5 and 4339 (see Figure 2). The spliced product and the expected genomic size (arrow) are shown next to the size markers from the gel. (B) Northern blot of total ovary RNA probed with ^{32}P-labeled *mei-218* cDNA. Molecular weight size markers are shown to the left.

mutants to complement. Finally, we have not ruled out that the two gene products are made from different transcripts generated by either alternative splicing or initiation sites. For example, the 4.2-kb cDNA clones might represent a *mei-218*-specific transcript produced by an alternative transcription initiation site. Evidence supporting this hypothesis is lacking, however, since upon probing Northern blots with the *mei-218* cDNA or the 1.6-kb *Sac*I genomic fragment upstream of the *mei-218* coding region (Figure 2), only one large band at ~6.5 kb was observed (Figure 3B).

The predicted MEI-217 protein is 227 amino acids and 25.8 kD. It has no homologs in the nucleic acid and protein databases, including no similarities to MEI-218, but it has a potential leucine zipper, several phosphorylation sites, and a zinc carboxypeptidase zinc-binding region (Figure 4B). The significance of these motifs has yet to be determined.

The 5′ adjacent gene to *mei-217*/*mei-218* is *M(1)15D*, which encodes a ribosomal protein (*rpS5*). Comparison of the genomic and cDNA sequences (McKim *et al.* 1996, and data not shown) shows that the end of the *M(1)15D* transcript is 960 bp upstream of the *mei-217* coding region (Figure 2). This region probably contains the *mei-217*/*mei-218* promoter. We have shown that this region is required for *mei-218* expression because a transgene containing the *mei-217*/*mei-218* coding region but lacking the upstream promoter sequences failed to rescue *mei-218* mutants (Figure 2). This promoter is probably responsible for the induction of *mei217*/*mei-218* transcription in regions 2 and 3 of the germarium (Figure 5).

A *mei-217-mei-218* fusion protein is functional: In the process of making epitope-tagged *mei-218* genes, the

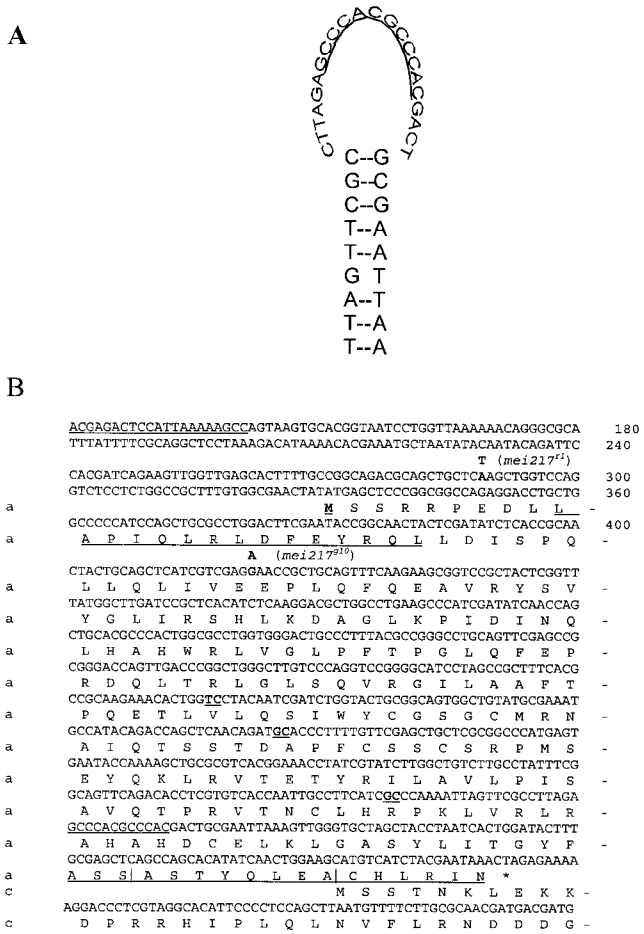

Figure 4.—(A) Predicted stem loop structure found upstream of the *mei-218* AUG. (B) The *mei-217* cDNA sequence and predicted translation product. The underlined sequences are the primer 6, the predicted leucine zipper, and the repeat sequence that is part of the stem loop structure. Also indicated are the nucleotide changes in *mei-217*^{r1} and *mei-217*^{g10}, and the two base pairs on either side of an intron are shown with bold underline. At the end the *mei-217* coding region is the overlap with the *mei-218* coding region. The underlined portion of the Mei-217 protein is altered in the FLAG (pHKF218) and HA (pHK3X218) fusion constructs. In pHKF218 this region is ASSASTYQLEA*WHASIDLDM* while in pHK3X218 this region is ASS*LEVDM*. In each case the FLAG or HA tag follows the methionine.

mei-217 and *mei-218* coding regions were fused. Two constructs were made, resulting in a FLAG (pHKF218) or triple-HA tag (pHK3X218) positioned between the two coding regions, but also resulting in the deletion of MEI-217 amino acids. The HA-tag protein is missing the last 14 amino acids while the FLAG-tag protein is missing only the last 6 amino acids of MEI-217 (Figure 4). Despite these deletions, both constructs rescued *mei-218* mutants, but the FLAG-tag construct rescued *mei-217*^{g10} significantly better than the HA-tag construct (Figure 2). We do not know if the reason for the failure of the HA tag construct to rescue is because of the 14-amino-acid deletion or the presence of the HA tag.

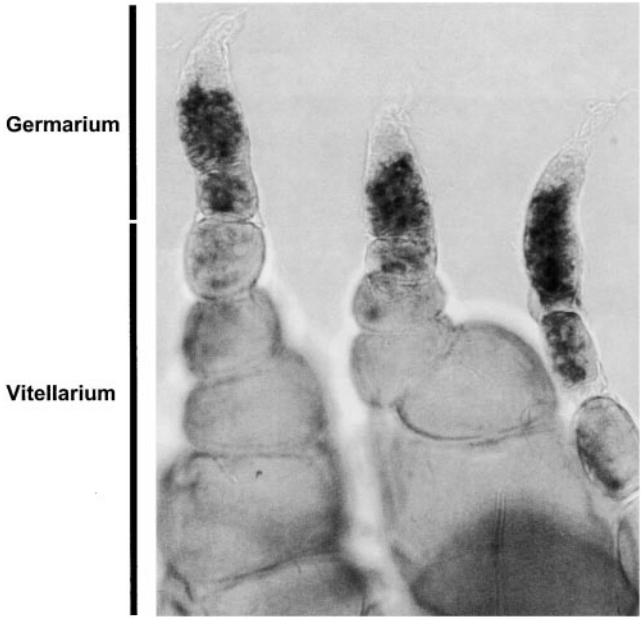

Figure 5.—*In situ* hybridization using an RNA antisense probe made from *mei-218* cDNA showing expression of the *mei-217/mei-218* mRNA in the germarium.

DISCUSSION

We have conducted a screen for X-linked mutants that increase X chromosome nondisjunction in female meiosis. In a similar screen by Baker and Carpenter (1972), 189 mutagenized chromosomes were screened and approximately six mutants (excluding very weak ones) similar to ours were found, including two *mei-9* alleles and one *mei-218* allele. We have screened 10 times the number of chromosomes but only recovered mutations in four new genes. The two strongest new mutations, with levels of crossing over and nondisjunction similar to two of the previously identified genes, *mei-9* and *mei-218*, are members of a new complementation group we have named *mei-217*. The small number of new mutants suggests that there are only a small number of genes on the X chromosome required for meiotic recombination when mutants are viable and fertile.

***mei-217* and *mei-218* are part of the same transcription unit:** Although genetic mapping experiments found them to be adjacent, *mei-217* and *mei-218* are separate genes based on four criteria. First, the *mei-217* alleles *r1* and *g10* complement all alleles of *mei-218*. Second, genomic and cDNA sequences show another open reading frame upstream of the *mei-218* coding region. Third, we have identified the sequence changes in the two *mei-217* mutations and found that they are located in the upstream open reading frame. Finally, one of these mutations, *r1*, is upstream of the *mei-217* coding region. If a single protein was made from the transcript, perhaps involving a ribosome frameshift, then *r1* should fail to complement *mei-218* mutations. Thus, by all standard criteria *mei-217* and *mei-218* are separate genes. Underlying the genetics, however, is the observation that a single transcript encodes both proteins. As in bacterial operons, this organization provides an efficient mechanism to regulate the transcription or translation of genes with related functions. This makes sense in our case because the similarity in the *mei-217* and *mei-218* mutant phenotypes suggests that these two genes function in the same pathway toward the generation of crossovers. Unlike prokaryotes, however, we suspect the importance of this organization is at the post-transcriptional level, since *mei-217*/*mei-218* expression is not limited to meiotic cells (see below).

Eukaryotic polycistronic messages are not unusual. Many *C. elegans* transcripts are polycistronic but are later processed to be monocistronic prior to translation (Blumenthal 1998). In mammals, two proteins, *Snurf* and *Snrpn*, are made from a dicistronic transcript (Gray *et al.* 1999). Two dicistronic messages have been reported in *D. melanogaster*, one at the *stoned* locus (Andrews *et al.* 1996) and another at the *Adh* locus (Brogna and Ashburner 1997). In both of these cases, it appears that the two proteins encoded by the messages have related functions. Our results showing the similar phenotypes of mutations in each coding region are perhaps the clearest demonstration of a dicistronic message where the two genes are required for the same process.

Considering that the conventional mechanism for ribosome binding to the mRNA involves initial contacts with the 5′ m7G CAP followed by scanning for the AUG (Kozak 1989), how is *mei-218* translated? The second coding region could be translated by reinitiation of the ribosome following the first coding region. This mechanism is unlikely in our case because the *mei-217* ORF overlaps the beginning of the *mei-218* ORF, and in other cases there is usually a short intercistronic region (15–78 bp; Kozak 1987; Levine *et al.* 1991). Another possibility is that translation is initiated at an internal ribosome binding site (IRES; reviewed in Sachs *et al.* 1997). Interestingly, we have identified a direct repeat sequence associated with a hairpin loop in a region that could be an internal entry point for ribosome binding (Figures 2 and 4). Internal ribosome binding sites are associated with hairpin loops in the RNA (Sachs *et al.* 1997).

A mechanism for translating dicistronic messages may exist in Drosophila because it is used in monocistronic messages as well. Oh *et al.* (1992) have argued that a significant fraction of *D. melanogaster* mRNAs are translated using internal initiation at IRES sites. This assertion was based on the large number of mRNAs with long (>250 bp) 5′UTRs containing upstream AUGs. They showed that the *Antp* mRNA is translated using an IRES and cited additional evidence that *Ubx* is as well. We suspect that internal initiation may be used by other meiotic recombination gene messages such as the

mei-W68 mRNA, which has a long 5′UTR (860 bp) containing five AUGs (McKim and Hayashi-Hagihara 1998). The *mei-217* coding region is preceded by at least one AUG and may also use an IRES. The *r1* mutation is upstream of the coding region and may affect the initiation of translation.

Promoters for meiotic recombination genes: Meiosis is one of the first tasks undertaken by the oocyte. In region 2 of the germarium pachytene occurs 36–48 hr after the last mitotic division and the zygotene-to-pachytene transition takes <12 hr (Carpenter 1975). Region 2 is also where high levels of *mei-218*/*mei-217* mRNA are first observed. Thus, it might be expected that a common regulatory element might be found for meiotic recombination genes in Drosophila similar to that for *S. cerevisiae* meiosis genes (Mitchell 1994; Gailus-Durner *et al.* 1996). The promoter for *mei-217*/*mei-218* is most likely contained within the 960-bp region defined by the 3′ end of the *rpS5* gene and the start of *mei-217*. We have compared the upstream region of *mei-217*/*mei-218* to several other meiotic recombination genes in *D. melanogaster* [*mei-9* (Sekelsky *et al.* 1995), *spnB* (Ghabrial *et al.* 1998), *mei-W68* (McKim and Hayashi-Hagihara 1998), and *mei-P22* (H. Liu and K. S. McKim, unpublished results)] but have not found sequence similarities suggestive of common promoter response elements. The major similarity between these promoter regions is that they are all small (<1 kb) and probably have a simple structure. We suspect that further analyses will reveal that transcription is not the primary mode of regulating meiotic recombination genes. This idea is based on the observation that *mei-217*/*mei-218* appears to be required only for meiosis (Baker *et al.* 1978), but its transcription can be detected in embryos, larvae, and in male testis (J. K. Jang and K. S. McKim, unpublished data). In addition, RNA levels of numerous other genes increase in germarium region 2, similar to the expression pattern of *mei-217*/*mei-218* (for example, *hsp83*; Ding *et al.* 1993). Perhaps the uniqueness of meiosis is imparted by translational regulation, as is important for many germline differentiation genes (*e.g.*, Lantz *et al.* 1994; Kelley *et al.* 1997; Webster *et al.* 1997).

***mei-217* and *mei-218* are members of a unique class of meiotic recombination genes:** Based mostly on gene conversion analysis, it has been proposed that *mei-P22* and *mei-W68* are required for initiating meiotic recombination (McKim *et al.* 1998), while *mei-9* and *mei-218* are not required to initiate recombination but are required for crossing over (Carpenter 1982, 1984). We have used double mutant analysis with *mei-41* and *spnB* to infer the same information. This analysis is based on the hypothesis that *mei-41* and *spnB* are required during the repair of the DNA breaks that initiate meiotic recombination (Hawley and Friend 1996; Ghabrial *et al.* 1998; Sekelsky *et al.* 1998). Mutations in *mei-P22* and *mei-W68* are epistatic to *mei-41* and *spnB*, which is consistent with the essential role of these genes in making the initiating DNA breaks. In contrast, we found that *mei-41* and *spnB* are epistatic to *mei-217* and *mei-218* mutations. These results place *mei-217* into the same class of genes as *mei-9* and *mei-218*. Like *mei-218*, we propose that *mei-217* is not required for the initiation of recombination but is required for recombination intermediates to be resolved as crossovers.

Differences in the phenotypes of *mei-9* and *mei-218* show that they have different roles in the generation of crossovers. *mei-218* may not be required for the exchange process *per se* but instead is required to determine which recombination sites will be resolved as crossovers (the "precondition" genes). In *mei-218* mutants, the crossover frequency is reduced, and the distribution of the remaining events is altered (Carpenter and Sandler 1974). Even the normally achiasmate fourth chromosome experiences crossing over in a *mei-218* mutant (Sandler and Szauter 1978). In contrast, the function of *mei*-9 seems to be specific to crossover resolution (the "exchange" genes). In *mei-9* mutants crossing over is reduced uniformly along each chromosome (Carpenter and Sandler 1974), there is a defect in the repair of mismatches (Carpenter 1982), and fourth chromosome crossing over does not occur (Sandler and Szauter 1978). The conclusion from these data is that *mei-218* is required earlier than *mei-9*. Two observations support this two-step model. First, *mei-218* mutations are epistatic to *mei-9* mutations (Sekelsky *et al.* 1995). Second, these two mutants have different effects on recombination nodules, which are the organelles at which meiotic recombination occurs. In *mei-218* mutants the nodules are greatly reduced in frequency and some are abnormal, while in *mei-9* mutants the nodules occur at a normal frequency and look normal (Carpenter 1979). Based on the fact that *mei-217* mutants alter the distribution of crossovers, *mei-217* and *mei-218* probably function in the same process.

There is another important difference between these two groups of genes. Both *mei-217* and *mei-218* mutants are not sensitive to MMS and therefore are not DNA repair defective. Similar results have been found with mutants in three other precondition genes (E. Manheim, R. Patel and K. S. McKim, unpublished results). In contrast, the two known genes in the exchange class, *mei-9* (Boyd *et al.* 1976) and *mus-312* (J. Sekelsky, personal communication), are required for DNA repair. Thus, it comes as no surprise that *mei-9* encodes a homolog of the DNA repair enzyme Rad1/XPF (Sekelsky *et al.* 1995). The sequences of *mei-217* and *mei-218* are not as informative because they lack amino acid sequence homology to known genes. Extrapolating from the existing members of each class, genes required specifically for exchanges encode proteins involved in DNA metabolism, whereas the precondition genes encode a new and as-yet-undescribed group of proteins.

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LITERATURE CITED

Andrews, J., M. Smith, J. Merakovsky, M. Coulson, F. Hannan *et al.*, 1996 The stoned locus of Drosophila melanogaster produces a dicistronic transcript and encodes two distinct polypeptides. Genetics **143:** 1699–1711.

Baker, B. S., and A. T. C. Carpenter, 1972 Genetic analysis of sex chromosomal meiotic mutants in *Drosophila melanogaster*. Genetics **71:** 255–286.

Baker, B. S., A. T. C. Carpenter and P. Ripoll, 1978 The utilization during mitotic cell division of loci controlling meiotic recombination in *Drosophila melanogaster*. Genetics **90:** 531–578.

Baker, S. M., A. W. Plug, T. A. Prolla, C. E. Bronner, A. C. Harris *et al.*, 1996 Involvement of mouse *Mlh1* in DNA mismatch repair and meiotic crossing over. Nat. Genet. **13:** 336–342.

Blumenthal, T., 1998 Gene clusters and polycistronic transcription in eukaryotes. Bioessays **20:** 480–487.

Boyd, J. B., M. D. Golino and R. B. Setlow, 1976 The *mei-9*^{a} mutant of *Drosophila melanogaster* increases mutagen sensitivity and decreases excision repair. Genetics **84:** 527–544.

Brogna, S., and M. Ashburner, 1997 The Adh-related gene of Drosophila melanogaster is expressed as a functional dicistronic messenger RNA: multigenic transcription in higher organisms. EMBO J. **16:** 2023–2031.

Carpenter, A. T. C., 1975 Electron microscopy of meiosis in *Drosophila melanogaster* females. I. Structure, arrangement, and temporal change of the synaptonemal complex in wild-type. Chromosoma **51:** 157–182.

Carpenter, A. T. C., 1979 Recombination nodules and synaptonemal complex in recombination-defective females of *Drosophila melanogaster*. Chromosoma **75:** 259–292.

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

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

Carpenter, A. T., 1989 Are there morphologically abnormal early recombination nodules in the *Drosophila melanogaster* meiotic mutant *mei-218*? Genome **31:** 74–80.

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

de Massy, B., V. Rocco and A. Nicolas, 1995 The nucleotide mapping of DNA double-strand breaks at the *CYS3* initiation site of meiotic recombination in *Saccharomyces cerevisiae*. EMBO J. **14:** 4589–4598.

Dernburg, A. F., K. McDonald, G. Moulder, R. Barstead, M. Dresser *et al.*, 1998 Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. Cell **94:** 387–398.

Ding, D., S. M. Parkhurst, S. R. Halsell and H. D. Lipshitz, 1993 Dynamic *Hsp83* RNA localization during *Drosophila* oogenesis and embryogenesis. Mol. Cell. Biol. **13:** 3773–3781.

Gailus-Durner, V., J. Xie, C. Chintamaneni and A. K. Vershon, 1996 Participation of the yeast activator Abf1 in meiosis-specific expression of the *HOP1* gene. Mol. Cell. Biol. **16:** 2777–2786.

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

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

Gray, T. A., S. Saitoh and R. D. Nicholls, 1999 An imprinted, mammalian bicistronic transcript encodes two independent proteins. Proc. Natl. Acad. Sci. USA **96:** 5616–5621.

Hari, K. L., A. Santerre, J. J. Sekelsky, K. S. McKim, J. B. Boyd *et al.*, 1995 The *mei-41* gene of *D. melanogaster* is a structural and function homolog of the human ataxia telangiectasia gene. Cell **82:** 815–821.

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

Hawley, R. S., and S. Friend, 1996 Strange bedfellows in even stranger places: the role of ATM in meiotic cells, lymphocytes,tumors, and its functional links to p53. Genes Dev. **10:** 2383–2388.

Hawley, R. S., H. A. Irick, A. E. Zitron, D. A. Haddox, A. R. Lohe *et al.*, 1992 There are two mechanisms of achiasmate segregation in *Drosophila* females, one of which requires heterochromatic homology. Dev. Genet. **13:** 440–467.

Hollingsworth, N. M., L. Ponte and C. Halsey, 1995 MSH5, a novel MutS homolog, facilitates meiotic reciprocal recombination between homologs in *Saccharomyces cerevisiae* but not mismatch repair. Genes Dev. **9:** 1728–1739.

Hunter, N., and R. H. Borts, 1997 Mlh1 is unique among mismatch repair proteins in its ability to promote crossing-over during meiosis. Genes Dev. **11:** 1573–1582.

Keeney, S., C. N. Giroux and N. Kleckner, 1997 Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell **88:** 375–384.

Kelley, R. L., J. Wang, L. Bell and M. I. Kuroda, 1997 *Sex lethal* controls dosage compensation in Drosophila by a non-splicing mechanism. Nature **387:** 195–199.

Kozak, M., 1987 Effects of intercistronic length on the efficiency of reinitiation by eucaryotic ribosomes. Mol. Cell. Biol. **7:** 3438–3445.

Kozak, M., 1989 The scanning model for translation: an update. J. Cell Biol. **108:** 229–241.

Lantz, V., J. S. Chang, J. I. Horabin, D. Bopp and P. Schedl, 1994 The Drosophila *orb* RNA-binding protein is required for the formation of the egg chamber and establishment of polarity. Genes Dev. **8:** 598–613.

Levine, F., J. K. Yee and T. Friedmann, 1991 Efficient gene expression in mammalian cells from a dicistronic transcriptional unit in an improved retroviral vector. Gene **108:** 167–174.

Liu, J., T. Wu and M. Lichten, 1995 The location and structure of double-strand DNA breaks induced during yeast meiosis: evidence for a covalently linked DNA-protein intermediate. EMBO J. **14:** 4599–4608.

McKee, B. D., and G. H. Karpen, 1990 Drosophila ribosomal RNA genes function as an *X-Y* pairing site during male meiosis. Cell **61:** 61–72.

McKim, K. S., and A. Hayashi-Hagihara, 1998 *mei-W68* in *Drosophila melanogaster* encodes a Spo11 homolog: evidence that the mechanism for initiating meiotic recombination is conserved. Genes Dev. **12:** 2932–2942.

McKim, K. S., J. K. Jang, W. E. Theurkauf and R. S. Hawley, 1993 Mechanical basis of meiotic metaphase arrest. Nature **362:** 364–366.

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

McKim, K. S., B. L. Green-Marroquin, J. J. Sekelsky, G. Chin, C. Steinberg *et al.*, 1998 Meiotic synapsis in the absence of recombination. Science **279:** 876–878.

McKim, K. S., J. K. Jang, J. J. Sekelsky, A. Laurencon and R. S. Hawley, 2000 *mei-41* is required for precocious anaphase in Drosophila females. Chromosoma (in press).

Mitchell, A. P., 1994 Control of meiotic gene expression in Saccharomyces cerevisiae. Microbiol. Rev. **58:** 56–70.

Oh, S. K., M. P. Scott and P. Sarnow, 1992 Homeotic gene *Antennapedia* mRNA contains 5′-noncoding sequences that confer translational initiation by internal ribosome binding. Genes Dev. **6:** 1643–1653.

Robinson, D. N., and L. Cooley, 1997 Examination of the function of two kelch proteins generated by stop codon suppression. Development **124:** 1405–1417.

Ross-Macdonald, P., and G. S. Roeder, 1995 Mutation of a meiosis-specific MutS homolog decreases crossing over but not mismatch correction. Cell **79:** 1069–1080.

Sachs, A. B., P. Sarnow and M. W. Hentze, 1997 Starting at the beginning, middle, and end: translation initiation in eukaryotes. Cell **89:** 831–838.

Sandler, L., and P. Szauter, 1978 The effect of recombination-defective meiotic mutants on fourth-chromosome crossing over in *Drosophila melanogaster*. Genetics **90:** 699–712.

Sandler, L., D. L. Lindsley, B. Nicoletti and G. Trippa, 1968 Mutants affecting meiosis in natural populations of *Drosophila melanogaster*. Genetics **60:** 525–558.

Sekelsky, J. J., K. S. McKim, G. M. Chin and R. S. Hawley, 1995 The Drosophila meiotic recombination gene *mei-9* encodes a homologue of the yeast excision repair protein Rad1. Genetics **141:** 619–627.

Sekelsky, J. J., K. C. Burtis and R. S. Hawley, 1998 Damage control: the pleiotropy of DNA repair genes in *Drosophila melanogaster*. Genetics **148:** 1587–1598.

Tautz, D., and C. Pfeifle, 1989 A non-radioactive *in situ* hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. Chromosoma **98:** 81–85.

Theurkauf, W. E., and R. S. Hawley, 1992 Meiotic spindle assembly in *Drosophila* females: behavior of nonexchange chromosomes and the effects of mutations in the *nod* kinesin-like protein. J. Cell Biol. **116:** 1167–1180.

Webster, P. J., L. Liang, C. A. Berg, P. Lasko and P. M. Macdonald, 1997 Translational repressor *bruno* plays multiple roles in development and is widely conserved. Genes Dev. **11:** 2510–2521.

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