Cloning of the *Drosophila melanogaster* Meiotic Recombination Gene *mei-218*: A Genetic and Molecular Analysis of Interval 15E

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ABSTRACT

The *mei-218* gene product is required for both meiotic crossing over and for the production of recombination nodules, suggesting that these organelles are required for meiotic exchange. In this study the null phenotype of *mei-218* was defined through the analysis of three preexisting and five new alleles. Consistent with previous studies, in homozygous *mei-218* mutants meiotic crossing over is reduced to <10% of normal levels. A molecular analysis of *mei-218* was initiated with the isolation and mapping of lethal mutations and genome rearrangements in the region containing *mei-218*, polytene interval 15E on the X chromosome. This high resolution genetic map was aligned with a physical map constructed from cosmid and P1 clones by genetically mapping restriction fragment length polymorphisms and localizing rearrangement breakpoints. Within a region of 65 kb, we have identified seven transcription units, including *mei-218* and the *Minute(1)15D* gene, which encodes ribosomal protein S5. The *mei-218* mutant phenotype has been rescued by germline transformation with both a genomic fragment and a cDNA under the control of the *hsp83* promoter. The *mei-218* gene is predicted to produce an 1186-amino acid protein that has no significant similarities to any known proteins.

MUTATIONS of the *mei-218* gene reduce meiotic crossing over by >90% and the residual exchanges are abnormally distributed, suggesting a defect in both the completion of genetic recombination and in the mechanism by which the sites for crossing over are chosen (SANDLER *et al.* 1968; BAKER and CARPENTER 1972; CARPENTER and SANDLER 1974). Electron microscopic analysis of *mei-218* oocytes revealed the presence of normal synaptonemal complexes between the chromosome arms, indicating that the chromosomes are able to pair (CARPENTER 1979a). Thus the *mei-218* mutant defect is intimately associated with recombination itself and not a secondary consequence of the failure of homologues to pair. A clue to the cellular defect in *mei-218* mutants was the observation that the recombination nodules, organelles believed to facilitate crossing over, are reduced in number and often have abnormal morphology (CARPENTER 1979a). Analysis of intragenic recombination at the *rosy* locus has shown that gene conversion events occur at least as frequently as in wild type (CARPENTER 1982, 1984). These observations suggest that recombination events initiate in *mei-218* oocytes but are not resolved into crossovers. Since *mei-218* mutants have no defects in somatic cells (BAKER *et al.* 1978), it appears that the *mei-218* gene product is a meiosis-specific component required for correct assembly and function of the recombination nodule.

Here we report the isolation of five new *mei-218* al-

leles isolated in an unbiased screen that did not select against lethal or sterile mutants. Although all *mei-218* alleles exhibit strong reductions in the frequency of meiotic recombination, none display a discernible effect on viability, fertility, or mitotic chromosome segregation. By analyzing the phenotypes of these mutants both as homozygotes and as *Df/mei-218* heterozygotes, we were able to assess the null phenotype of the *mei-218* gene. To precisely map *mei-218* and facilitate cloning, we constructed a genetic and molecular map based on chromosome rearrangements and restriction fragment length polymorphisms (RFLPs). The *mei-218* gene was found to produce a transcript predicted to encode a 1186-amino acid, 134-kD protein. The protein sequence is not similar to any in the protein or translated nucleotide databases.

We also characterized the region flanking *mei-218*. Within this region is *M(1)15D*, a member of a class of genes (*Minutes*) that are recessive lethal but have dominant effects on both growth rate and female fertility (LINDSLEY and ZIMM 1992). Consistent with the recent analysis of two other *Minute* genes, we found that *M(1)15D* encodes a *Drosophila* homologue of ribosomal protein S5.

MATERIALS and METHODS

***Drosophila* stocks:** All cultures were raised at 23–25° on corn meal-molasses yeast media. With the exception of new mutations and aberrations reported here, all mutants are described in LINDSLEY and ZIMM (1992). The *mei-218*¹ mutation was referred to in MCKIM *et al.*

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(1993) as *mei-218^{ad}*. *FM7* is a multiply inverted X chromosome that does not crossover with the normal X chromosome. *FM7a* is homozygous fertile, while *FM7c* is homozygous female sterile. The duplication *Dp(1;4)r⁺* (Figure 1) was isolated by GREEN (1963) as a derivative of *T(1;4)B^S*. *Dp(1;3)f⁺71b* is the duplication element of *Tp(1;3)f⁺71b* separated from the deficiency by making *Dp(1;3)f⁺71b/+*, *Df(1)f⁺71b/FM7a*, *f* females and then recovering the *f(+)* *B* progeny from these females (i.e., *+ /Dp(1;3)f⁺71b; FM7, f/Y*).

Screen for *mei-218* alleles: *y cv v f car/y⁺Y* males were fed 0.025 M EMS and then crossed to *y mei-218¹/FM7a* females. Individual *y cv v f car/y mei-218¹* virgin females were collected and tested by crossing to *Y^SX^{Y^L}, v f B/0; C(4)RM, ci ey^R/0* males. If a new *mei-218* mutation was present on the mutagenized chromosome, then diplo-*X* (*y cv v f car/y mei-218¹*) and null-*X* (*Y^SX^{Y^L}, v f B/0*) exceptions were recovered. The putative new mutants were confirmed by crossing the diplo-*X* female exceptions to the same *Y^SX^{Y^L}, v f B/0* males. The new mutations were recovered linked to the *forked* marker by crossing the *y cv v f car/y mei-218¹* females to *FM7a, f/y⁺Y* males.

In a screen of 7010 EMS-treated chromosomes, 25 were recovered, which upon retesting, gave high levels of nondisjunction *in trans* to *mei-218¹*. Twenty of these, however, were dominant and caused nondisjunction *in trans* to a *mei-218⁺* chromosome. Although not proven, most of these mutations were likely translocations, as many of them were either male sterile or male lethal. In addition, the dominant mutants did not increase the frequency of fourth chromosome nondisjunction, as occurs in *mei-218* mutants. The remaining five chromosomes carried new *mei-218* mutations.

Measuring nondisjunction: In most crosses, the X chromosome nondisjunction frequency was calculated as 2 (exceptional progeny) / 2 (exceptional progeny) + (regular progeny). For *mei-218/Df(1)BK8* females, the nondisjunction frequency was calculated as (exceptional males) / (exceptional males + regular females). The female progeny heterozygous for the deficiency, which is the exceptional females and half of the regular progeny, were not included in the calculations because they were Minute, slow developing, and sporadically represented.

Bar reversion and deletions of *Dp(1;4)r⁺*: We screened for deletions among the progeny of irradiated males carrying both a normal X chromosome marked with the dominant *Bar* mutation and the *Dp(1;4)r⁺* duplication. The presence of *Dp(1;4)r⁺* did not affect the expression of the *Bar* phenotype, thus reversion of *Bar* could be scored in the presence of the duplication in males or females. *r f B; Dp(1;4)r⁺* males were treated with 4000 rad of X-irradiation and crossed to *r f B/r f B; Dp(1;4)r⁺* females (Figure 2). Deletions of *B* could be recognized because *B/B* females have a more severe eye phenotype than *B/+* or *B/Df* females. All *Bar* reversions were initially recovered as *r f B/r f Df(1)B; Dp(1;4)r⁺/+* females. If the deficiency deleted a *Minute*

locus, the dominant slow-growth phenotype was rescued by the duplication. The deficiencies were subsequently balanced as *r f Df(1)B/FM7c; Dp(1;4)r⁺/+*; with the duplication being retained if the deficiency was Minute.

In the same cross, new deletions of *Dp(1;4)r⁺* could be recognized by either a *rudimentary* or *forked* phenotype. The *r⁺ f⁻* duplications were stable as *r f B/r f B; Dp(1;4)r⁺ f⁻/+* stocks because *r* females are sterile. The *r⁻ f⁺* duplications were kept in stock with a lethal mutation covered by the duplication (i.e., *y cv v l(1) f/y⁺Y; Dp(1;4)rK20/+*).

Isolation of new lethal mutations: In a screen of 5000 25 mM EMS-treated *y cv v f* chromosomes, 80 new lethal mutations covered by *Dp(1;4)r⁺* were isolated (R. FRENCH, D. HADDOX and R. S. HAWLEY, unpublished data). The same screen was also done with 4000 rad of X-rays; 5000 chromosomes were screened and 30 mutations were recovered. The stocks were maintained either with *C(1)DX* (and *y cv v f/Y; Dp(1;4)r⁺/+* males) or balanced by *FM7c*. All of the X-ray-induced mutations and the EMS mutations not covered by *Dp(1;4)fK4* (Figure 1) were mapped by complementation crosses to duplications and deficiencies. An additional collection of 16 EMS-induced lethal mutations mapping within the 15A-16A region (Figure 1) were generously provided by S. RUTHERFORD (personal communication).

Complementation test crosses: Each new derivative of *Dp(1;4)r⁺* that was either *r⁺ f⁻* or *r⁻ f⁺* could have carried either a new point mutation in *forked* or *rudimentary* or a deletion including either locus. To determine if the new derivatives carried deletions, they were tested for the ability to rescue a deletion covered by the original *Dp(1;4)r⁺* chromosome. The *r⁻ f⁺* duplications were crossed to *Df(1)rD1, v f/FM7c; Dp(1;4)r⁺/spa^{bol}* females and the *r⁺ f⁻* duplications were crossed to *Df(1)BK16/FM7c; Dp(1;4)r⁺/spa^{bol}* females. If the resulting *r⁻* or *f⁻* mutant males (i.e., *Df(1)/Y; Dp/spa^{bol}*) were lethal, the duplication carried a deletion. One of the *r⁻* duplications (*Dp(1;4)rK20*) and four of the *f⁻* duplications were found to be deletions of *Dp(1;4)r⁺* (Table 3). The breakpoints of the new duplications were determined by complementation crosses to lethal mutations. *y cv v l(1) f/FM7c* females were crossed to *r f B/Y; Dp(1;4)r⁺ f⁻/+* males. The presence of *y cv v l(1) f; Dp(1;4)r⁺ f⁻/+* male progeny indicated that the duplication carried the wild-type allele of the essential gene.

Complementation tests between lethal mutations [designated *l(1)-x* and *l(1)-y*] were done by crossing *y cv v l(1)-x f/Y; Dp(1;4)r⁺/+* males to *y cv v l(1)-y f/FM7c* females. If the two mutations complemented, *forked* females [*y cv v l(1)-x f/y cv v l(1)-y f; +/+*] were observed. Complementation tests between *Minute* loci were based on their dominant phenotype, since in some cases the double *Minute* heterozygotes were subviable. A typical experiment was *Df(1)BK16/FM7; Dp(1;4)r⁺/+*

TABLE 1
Nondisjunction and crossing over in *mei-218* homozygous females

	<i>mei-218</i> ¹	<i>mei-218</i> ⁶⁻⁷	<i>mei-218</i> ^{hfn}	<i>mei-218</i> ⁴	<i>mei-218</i> ⁵	<i>mei-218</i> ⁶	<i>mei-218</i> ⁷	<i>mei-218</i> ⁸
Expt. I: <i>mei-218/mei-218</i>								
X-NDJ (%)	29.0	33.8	37.0	17.6	26.1	32.9	28.4	28.8
4NDJ (%)	17.0	28.3	17.7	7.1	7.3	17.1	7.2	12.5
Total X map (cM)	2.6	ND		11.6		2.2	6.3	2.9
X map relative to <i>mei-218/+</i> (%)	4.3			16.3		3.2	8.8	4.1
Total progeny ^a	6733 ^c	1608	1594	1783	2267	2204	2510	2590
Expt. II: <i>mei-218/Df(1)BK8</i>								
X-NDJ (%)	29.0 ^d	25.4	27.6	18.9	31.7	30.6	23.7	25.8
4NDJ (%)	6.6	23.0	12.0	5.5	12.9	8.4	9.9	9.9
Total X-map (cM)	1.9	ND	2.0	5.9	6.0	3.47	2.7	4.2
X-map relative to <i>+/Df(1)BK8</i> (%)	3.2		3.4	10.2	10.4	6.0	4.6	7.2
Total progeny ^b	731	1003	1112	549	356	715	746	685
Expt. III: <i>mei-218; al dp b pr cn/+</i>								
Total chromosome 2 map (cM)	3.8	ND	1.9	12.0	1.8	ND	ND	3.6
Map relative to <i>mei-218/+</i> (%)	7.5		4.0	23.9	3.6			7.2
Total progeny	15205 ^c		2334	3618	3428			2368

ND, not determined. Experiment I: females of the genotype *y cv v mei-218 f +/+ + + mei-218 f car* were crossed to *Y^s X^s Y^L, v f B/0; C(4)RM, ci ey^R/0* males. Experiment II: Females of the genotype *y cv v mei-218 f car/Df(1)BK8, r* were crossed to *Y^s X^s Y^L, v f B/0; C(4)RM, ci ey^R/0* males or *Y^s X^s Y^L, y B/0; C(4)RM, ci ey^R/0* males. Experiment III: Females of the genotype *al dp b pr cn/+ + + + + +; mei-218/mei-218* were crossed to *+/Y; al dp b pr cn/al dp b pr cn* males.

^aTotal progeny = regular progeny + 2(exceptional progeny), see MATERIALS AND METHODS.

^bTotal progeny = exceptional males + non-Minute regular females, see MATERIALS AND METHODS.

^cData from CARPENTER and SANDLER (1974).

^dA similar result was obtained when *mei-218 f B/Df(1)BK28* females were tested for nondisjunction. The X chromosome nondisjunction frequency was 32.8% and fourth chromosome nondisjunction was 19.8% (total progeny = 554).

females crossed to *M(1)15D/Y; Dp(1;4)r⁺/+* males. The resulting B⁺ females [i.e., *Df(1)BK16/M(1)15D; Dp(1;4)r⁺/+*] were Minute if both the deficiency and *M(1)15D* affected the same Minute gene. Complementation crosses between a lethal mutation and a Minute deficiency were often done with a duplication that covered the Minute locus but not all of the deleted region of the deficiency.

Division 14 Minute mutations: Eighteen X-ray-induced lethal mutations that mapped to division 14 were studied, based on mapping outside *Dp(1;3)f⁺71b* and inside *Dp(1;Y)fK24*. Two of the lethal complementation groups in division 14 defined genetically separable Minute loci based on the following. First, *M(1)14C/M(1)14E; Dp(1;4)r⁺* females were not Minute, showing that in these females there were two wild-type copies of each gene. Second, these two loci have been separated by a deficiency breakpoint. *M(1)14C/Df(1)19* females were not recovered, and *M(1)14C/Df(1)19; Dp(1;4)r⁺/+* females were Minute. In contrast, *M(1)14E/Df(1)19; +/+* females were viable and Minute while *M(1)14C/Df(1)19; Dp(1;4)r⁺/+* females were not Minute. *M(1)14C* may correspond to Minute loci mapped to this region by SCHALET (1986) and STANEWSKY *et al.* (1993).

SCHULTZ (1929) concluded that the dominant effects of the Minute loci were not additive. The generality of this statement is questioned by the following results. We found that the Minute phenotypes associated with *M(1)14E* and *M(1)15D* point mutants were not additive, that is, double mutant heterozygous females with the

genotype *M(1)14E +/+ M(1)15D* were viable. While this result was consistent with SCHULTZ's conclusion, we also had the conflicting result that heterozygosity for either a single deficiency affecting both *M(1)15D* and *M(1)14E* (*Df(1)BK14/+*), or two deficiencies affecting them separately (*Df(1)19 +/+ Df(1)BK28*) were lethal. In this case the survival of the *M(1)14E +/+ M(1)15D* heterozygotes could have occurred because one of the Minute alleles was a hypomorph. The opposite relationship was found with the division 14 Minutes. We found that the double heterozygote *M(1)14C +/+ M(1)14E* was inviable, but the *Df(1)19/M(1)14E* heterozygote was viable. Thus the lethality of the *M(1)14C +/+ M(1)14E* females may result from a specific interaction involving one or both Minute mutations. In addition to characters related to the types of mutations at each Minute locus, these differences in the interactions between Minute mutants could be affected by the dosage of nearby genes. There is a previous finding of Minute mutants acting additively; DEL PRADO and RIPOLL (1983) found that the *M(1)65F +/+ M(1)69E* double heterozygote was lethal.

Molecular techniques: Standard molecular techniques were as described by SAMBROOK *et al.* (1989). The source of the genomic DNA was cosmids (KAFATOS *et al.* 1991) and a P1 clone (SMOLLER *et al.* 1991) previously mapped to the *mei-218* region. The cosmid clones were restriction mapped in two steps. First, each cosmid was subcloned with *HindIII*, *Clal*, and *XbaI* digests. Second, the subclones were ordered by restriction and Southern blot

TABLE 2
Crossing over on chromosome 2 in *mei-218* homozygous females

	<i>mei-218^{hfd}/+</i>	<i>mei-218^{hfd}</i>	<i>mei-218⁴/+</i>	<i>mei-218⁴</i>	<i>mei-218⁵</i>	<i>mei-218⁸</i>
Crossing over (%)						
<i>al-dp</i>	12.5	0.1	16.1	3.3	0.5	0.8
<i>dp-b</i>	28.6	1.2	29.0	6.1	0.8	1.7
<i>b-pr</i>	4.8	0.5	4.4	2.2	0.4	0.7
<i>pr-cn</i>	1.4	0.1	0.8	0.4	0.1	0.3
Total	47.3	1.9	50.2	12.0	1.8	3.6
Total progeny	2092	2334	1975	3618	3428	2368
Relative to control (%)						
<i>al-dp</i>		1.0		20.6	5.2	5.0
<i>dp-b</i>		4.2		21.1	3.0	5.9
<i>b-pr</i>		9.8		50.3	8.5	16.3
<i>pr-cn</i>		9.3		53.1	16.2	37.5
Total		4.0		23.9	3.6	7.2

Female of the genotype *al dp b pr cn/++++*; *mei-218/mei-218* were crossed to *+ / Y*; *al dp b pr cn/al dp b pr cn* males.

analysis. The cDNA clones were obtained either from a mixed male/female library in lambdaZap (Stratagene) or from an ovary specific library in lambda gt22 (STROUMBAKIS and TOLLAS 1994). Southern blots of genomic and cloned DNA were probed with random prime labeled DNA using nonradioactive protocols from either Amersham (ECL) or Boehringer Mannheim (Genius). Northern blots were prepared from total RNA collected from whole males or females, dissected ovaries, or ovariectomized females. Total RNA was prepared by grinding adult flies or ovaries 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. RNA from 20 ovaries (from 10 females), which was usually 50–80 µg of total RNA, was loaded into each lane.

The sequence presented here is a composite of genomic and cDNA clones. Sequencing was done on double-stranded DNA templates using Sequenase 2.0 (USB). Sequence was obtained from the ends of convenient restriction fragments, *ExoIII*-deletion clones made with the Erase-a-base system (Promega) or using custom oligonucleotides. The sequence was analyzed using the GCG package (DEVEREUX *et al.* 1984) and Genbank database searches were done with the BLAST program (ALTSCHUL *et al.* 1990).

in-situ hybridization: *in-situ* hybridization to polytene chromosomes was done using nick translation biotinylated probes as described in ASHBURNER (1989). The following cosmids, which were previously mapped to the 15D–15F region (KAFATOS *et al.* 1991; FLYBASE 1994), were mapped according to their hybridization to rearrangement chromosomes (Figure 1). Cosmids within *Dp(1;4)fK4*: 105A12, 26F5, 189B1, 14H10; cosmids proximal to *Dp(1;4)fK4* but within *Dp(1;4)fK7*: 102G12, 57A3, 20H11, 40C1; cosmids proximal to *Dp(1;4)fK7*: 88C7, 189F7; cosmids proximal to *Dp(1;4)r⁺*: 96E8.

P-element transformation: A 14-kb *EcoRI*-*NotI* genomic DNA fragment from cosmid 102G12, which carries

mei-218, was cloned into pCasPeR4 (THUMMEL and PIRROTTA 1992). The *NotI* site was located in the cosmid (Lorist6) vector adjacent to the cloning site, ~4 kb upstream of the *mei-218* transcription start. A cDNA construct was made under the control of the *hsp83* promoter. First, the *hsp83* promoter was cloned into the *Sall* site of Bluescript. Second, an *EcoRI*-*NotI* fragment containing the full length cDNA was inserted downstream of the *hsp83* promoter. The *hsp83-me-218* construct was then excised from Bluescript with *KpnI* and *NotI* and cloned into pCasPeR4. Germline transformation (RUBIN and SPRADLING 1982) of these constructs was done by coinjecting the helper plasmid puch $\pi\Delta$ 2-3 (ROCHE *et al.* 1995) into *white* embryos. Injection DNA was prepared using the Qiagen midi-prep procedure. *G1 white⁺* progeny were crossed to *y w mei-218⁶ f car/y⁺ Y* males or *y w mei-218⁶ f car/FM7* females, and tested for rescue in *y w mei-218⁶ f car/y w mei-218⁶ f car*; *P[w⁺, mei-218⁺]/+* females.

RESULTS

Genetic analysis of *mei-218*; new alleles and the null phenotype: To define the null phenotype of *mei-218*, we isolated five new alleles (*mei-218⁴*, *mei-218⁵*, *mei-218⁶*, *mei-218⁷*, *mei-218⁸*) and characterized their effects on crossing over and nondisjunction. The new *mei-218* alleles were isolated in a screen of 7010 EMS-mutagenized chromosomes tested *in trans* to *mei-218¹* for high levels of X and fourth chromosome nondisjunction (MATERIALS AND METHODS). Despite the fact that our screen would have isolated lethal or sterile alleles of *mei-218*, all of the new mutations were viable and fertile and, as detailed below, they were phenotypically similar to the existing three alleles of *mei-218* (*mei-218¹*, *mei-218⁶⁻⁷* and *mei-218^{hfd}*). In addition, viability and fertility were not significantly affected when these mutations were heterozygous with a deficiency (see below).

FIGURE 1.—Genetic and schematic cytological map of division 15. The cytological positions of *rudimentary* and *Bar* are shown. Deficiencies (single lines) and duplications (double lines) are shown below the cytological map. Uncertainty in the genetic position of a breakpoint is shown with a dashed line. The duplications with an arrow on their distal end extend to 14A.

Effects on recombination: Table 1 shows the effects of each mutant on X and second chromosome crossing over. The stronger alleles isolated in this study were no more severe than that reported for the canonical allele *mei-218*¹ (CARPENTER and SANDLER 1974). Our consistently most severe alleles, such as *mei-218*⁶ and *mei-218*⁸, reduced crossing over to a frequency (3–7% of wild type) similar to that reported for *mei-218*¹. Similarly, in *mei-218*¹/*mei-218*⁶ or *mei-218*¹/*mei-218*⁸ females, crossing over was reduced to ~4% of the wild-type frequency (data not shown). One allele, *mei-218*⁴, was classified as a hypomorph because the homozygotes had a relatively high frequency of crossing over (see below). The amount of residual crossing over in *mei-218*⁴ females strikingly parallels that observed in females homozygous for a hypomorphic allele of *mei-9* (*mei-9*^b; CARPENTER and SANDLER 1974).

Previous studies on *mei-218*¹ also revealed an unusual distribution of the residual exchanges (BAKER and CARPENTER 1972; CARPENTER and SANDLER 1974). The same effect was observed here (Table 2). The reductions in crossing over observed in *mei-218* mutants were twofold less in the centromere proximal regions (*b* – *pr* and *pr* – *cn*) compared to the distal regions (*al* – *dp* and *dp* – *b*). As it was previously suggested that *mei-218* has a dominant effect on crossover distribution (CARPENTER and SANDLER 1974), the magnitude of the effects on distribution shown in Table 2 may have been underestimated.

The meiotic phenotype of each allele was also tested *in trans* to a deficiency (Table 1). Although the only deficiencies available also deleted *M(1)15D* (see below), in most of the alleles the phenotype was unchanged in the hemizygous condition. Where crossing over was reduced to <10% of wild-type frequencies in

homozygotes, the phenotype was not made more severe *in trans* to *Df(1)BK8*. In the case of the hypomorph *mei-218*⁴, which as a homozygote had a relatively high frequency of crossing over (16.3 and 23.9% of wild type on the X and second chromosomes, respectively), the phenotype was more severe in *Df/mei-218*⁴ heterozygous females where crossing over was reduced to a similar frequency (5.9%) as observed in the other mutants. Given that in homozygotes for the seven severe mutants a low level of crossing over was observed, and that similar frequencies of crossing over were observed in *mei-218/Df* females, we conclude that in the total absence of *mei-218* gene product, functional crossovers still occur at a frequency of ~5% of the normal level. That is, a complete absence of the *mei-218* gene product reduces, but does not eliminate, meiotic crossing over.

Effects on segregation: Table 1 also shows the effects of each mutant on the frequency of X and fourth chromosome nondisjunction. Most of the alleles tested generated levels of X chromosome nondisjunction in the range of 26–37%. Characterization of the preexisting *mei-218* alleles had demonstrated that all of the nondisjunctional events observed in *mei-218* oocytes were the result of heterologous achiasmate segregations involving nonexchange X chromosomes and a nonexchange autosome, *i.e.*, *XX* ↔ *A*, with the remaining autosome segregating at random (CARPENTER and SANDLER 1974). As evidenced by our failure to observe yellow exceptional daughters among the progeny of the crosses reported in Table 1, we conclude that most of the nondisjunctional events in *mei-218* females were the result of improper segregation of nonexchange X chromosomes and not the result of a direct effect of *mei-218* on the segregation process.

F₀ Males treated with 4000 r X-rays:

$$\frac{r \ f \ B}{Y}; \frac{Dp(1;4)r^+}{+} \sigma \quad \times \quad \frac{r \ f \ B}{r \ f \ B}; \frac{Dp(1;4)r^+}{+} \varnothing$$

F₁ Regular progeny with the duplication:

$$\begin{array}{cc} \frac{r \ f \ B}{Y}; \frac{Dp(1;4)r^+}{+} & \frac{r \ f \ B}{r \ f \ B}; \frac{Dp(1;4)r^+}{+} \\ \text{Bar male} & \text{Bar female} \end{array}$$

Exceptional duplication bearing progeny:

i. Deficiency of the duplication (each recovered in males and females):

$$\begin{array}{cc} \frac{r \ f \ B}{Y}; \frac{Dp(1;4)r^- f^+}{+} & \frac{r \ f \ B}{r \ f \ B}; \frac{Dp(1;4)r^+ f^-}{+} \\ \text{rudimentary} & \text{forked} \end{array}$$

ii. Bar reversion

$$\begin{array}{cc} \frac{r \ f \ Df(1)B}{Y}; \frac{Dp(1;4)r^+}{+} & \frac{r \ f \ Df(1)B}{r \ f \ B}; \frac{Dp(1;4)r^+}{+} \\ \text{wild-type male} & \text{weak Bar female} \end{array}$$

Although the fourth chromosomes do not normally undergo crossing over, they do nondisjoin in recombination defective mutants, perhaps as a result of a gen-

FIGURE 2.—Screen to isolate deficiencies and duplications in the *mei-218* region. The progeny from this cross were expected to be wild type if they carried the duplication and r f if they did not. The expression of the Bar phenotype was not affected by the presence of the duplication. Exceptional progeny of irradiated males are indicated along with their phenotypes.

eral disruption of the achiasmate segregation system that normally is responsible for segregating the fourth chromosomes (BAKER and HALL 1976). As expected,

TABLE 3
Cytology of rearrangements used in this study

Name	Breakpoints	Reference
<i>Dp(1;4)r⁺</i>	14A1-2; 16A7-B1	GREEN (1963)
<i>Tp(1;3)f⁺71b</i>	15A4; 16C2-3	GANETZKY and WU (1982)
<i>Df(1)B</i>	15F9-16A1; 16A6-7	SUTTON (1943); HIGASHIJIMA <i>et al.</i> (1992)
<i>Df(1)BK8</i>	15C1-4; 16C2-7	This paper
<i>Df(1)BK10</i>	16A2; 16C7-10	This paper
<i>Df(1)BK16</i>	15A2-4; 16A1-7	This paper
<i>Df(1)BK17^a</i>	15D3-6; 16A1-7	This paper
<i>Df(1)BK18</i>		This paper
<i>Df(1)BK28</i>	15C1-5; 16B8-12	This paper
<i>Df(1)BK46</i>		This paper
<i>Df(1)815-6^b</i>	15E1-2; 15E6-7	This paper
<i>Dp(1;4)fK4</i>	14A1-2; 15D2-4; 102D2	This paper
<i>Dp(1;4)fK7^c</i>	14A1-2; 15E6-7	This paper
<i>Dp(1;4)fK24^d</i>	14A1-2; 15A1-4	This paper
<i>Dp(1;4)fK27</i>	14A1-2; 15E1-7; 15F2-F7	This paper
<i>Dp(1;4)rK20</i>	14A1-2; 14A4-B1; 15D5-E4	This paper
<i>Df(1)rD1</i>	14D1-2; 15D1-2	GANETZKY (1984)
<i>Dp(1;Y)W73^e</i>	16A; 16F	EBERL <i>et al.</i> (1992)
<i>Df(1)19</i>	13F; 14E	STELLER <i>et al.</i> (1987)
<i>Df(1)E50</i>	14B3-4; 14E	STELLER <i>et al.</i> (1987)

^a Associated with a *T(1;3)* translocation. Based on genetic and cytological data.

^b Deficiency was recovered inside an inversion with one breakpoint in 13E-F and the other in the proximal X chromosome heterochromatin.

^c Associated with a *T(3;4)* translocation. Based on genetic and cytological data.

^d Associated with the Y chromosome, plus some fourth chromosome sequences.

^e Derived from an *FM7* chromosome, so it may share the 15D breakpoint. This is consistent with the genetic mapping data (Figure 1).

we observed high levels of fourth chromosome nondisjunction in *mei-218* mutants. Moreover, fourth chromosome exceptions were more frequent among X chromosome exceptions, and they were rarely recovered among gametes containing crossover X chromosomes (data not shown). CARPENTER and SANDLER (1974) suggested that these fourth chromosome nondisjunctional events may result from nonhomologous associations with nonexchange nonhomologues.

Curiously, hemizyosity for *mei-218* increased the frequency of diplo-X, nullo-4 and nullo-X, diplo-4 exceptions. Mutant *mei-218* females, and all other recombination defective mutants, show increased frequency of fourth chromosome nondisjunction among X chromosome exceptions, but there is no preference for the chromosomes to segregate from each other (BAKER and CARPENTER 1972; CARPENTER and SANDLER 1974; and data not shown). A different situation was found in *mei-218/Df(1)BK8* females. For example, in one experiment (*y cv mei-218⁷ f car/Df(1)BK8*), where cases of simultaneous X and fourth chromosomes nondisjunction were scored, 80% resulted from a meiosis where the two fourth chromosomes segregated from the two X chromosomes. One possible explanation for these results is that *Df(1)BK8* has a dominant effect on the segregation of achiasmate chromosomes. This might explain why fourth chromosome nondisjunction was often less in *mei-218/Df(1)BK8* compared to *mei-218/mei-218* females (Table 1). For example, we observed only 8.4% fourth chromosome nondisjunction in *mei-218⁶/Df(1)BK8* females compared to 17.1% in *mei-218⁶/mei-218⁶* females. The effect on achiasmate segregation may be specific to *Df(1)BK8*. While we observed only 6.6% fourth chromosome nondisjunction in *mei-218¹/Df(1)BK8* females, a much higher frequency (19.8%) was observed in *mei-218¹/Df(1)BK28* females (Table 1).

The canonical allele *mei-218¹* had no effect on chromosome segregation in male meiosis (BAKER and CARPENTER 1972). We tested each of our new mutants for nondisjunction during male meiosis by crossing *y mei-218 f/y⁺ Y* males to *y w/y w; C(4)RM, ci ey^R* females. As with the original allele, these mutations had no effect on disjunction of the X and Y chromosome in males (<0.1% nondisjunction, data not shown).

Genetic mapping in the *mei-218* region with duplications and deficiencies: CARPENTER and SANDLER (1974) mapped *mei-218* to genetic map position 57, which is close to *forked* (*f*) at polytene position 15F2 on the standard polytene map of the X chromosome (Figure 1). Here we describe the precise alignment of the genetic and physical maps that allowed us to localize *mei-218* to a small region of DNA.

The major obstacle to this work was the close linkage of *mei-218* to *M(1)15D* (DEMEREK *et al.* 1942), a member of the *Minute* class of genes in *Drosophila*. *Minute* mutations cause recessive lethality, and the heterozygotes display a variety of defects associated with slow

TABLE 4

Mutations in essential genes characterized in this study

Gene name	Alleles ^a
<i>inflated</i>	815-17 ^b
<i>l(1)15Bb</i>	R5, R31
<i>l(1)15Bc</i>	R15
<i>l(1)15Da</i>	R27
<i>l(1)15Db</i>	815-14
<i>l(1)15Dc</i>	R3, R20
<i>l(1)15Dd</i>	R12
<i>l(1)15De</i>	692-47, R10, R11
<i>l(1)15Eb</i>	692-19, 692-66, 815-15, 815-37
<i>l(1)15Ea</i>	692-58, 815-10, ^c R1, R6, R18ts, R19
<i>M(1)15D</i>	1, 2, R28ts
<i>bazooka</i>	692-4, 692-44, 692-59, 815-7, 815-8, R7, R21
<i>l(1)15Fa</i>	815-33
<i>M(1)14C</i>	815-29
<i>M(1)14E</i>	815-13

^a Alleles with a number beginning "692" were induced with EMS; those beginning with a number beginning "815" were induced with X-rays. Alleles with the "R" prefix were isolated using EMS by SUZANNE RUTHERFORD (personal communication).

^b T. ARBEL (personal communication).

^c This chromosome has an inversion with one breakpoint close to the site of the lethal mutation (15E) and the second in proximal X chromosome heterochromatin.

growth, including a slow developmental rate, sterility and small bristles from which they derive their name (FERRUS 1975; LINDSLEY and ZIMM 1992). While *M(1)15D/+* flies are fertile, their slow developmental rate and overall sick disposition causes a difficulty in isolating deficiencies in the region. To circumvent this difficulty, a screen was designed in which deletions of the *Minute* gene could be rescued by the presence of a duplication (Figure 2 and MATERIALS AND METHODS).

We screened for deletions in the 15A–16A region by selecting for radiation-induced reversions of the dominant *Bar* (*B:16A1-3*) in the presence of *Dp(1;4)r⁺* that carries wild-type alleles of *M(1)15D*, *forked* and *rudimentary* (*r:15A1-2*) (Figure 2). This method allowed the recovery of deletions extending distally from *Bar* to include *M(1)15D*. Among ~72,000 progeny, we recovered >20 reversions of the *Bar* mutation. For further analysis we retained the *Bar* revertants that were male lethal as these were likely to be deficiencies. The cytological breakpoints for most of the deficiencies characterized in this study were determined by the analysis of larval salivary gland polytene chromosomes (Table 3). Most of the male-lethal *Bar* revertants had a *Minute* phenotype in females or were lethal when heterozygous with a normal chromosome. That is, *rf B^{rev}/+++* but not *rf B^{rev}/+++*; *Dp(1;4)r⁺* females were either *Minute* (deleted one *Minute* locus) or lethal (deleted more than one *Minute* locus). The deletions that were so large as to delete more than one *Minute* locus were discarded. In addition to the deficiencies isolated as *Bar* revertants, four of the X-ray-induced lethal mutations

FIGURE 3.—Restriction map of the *mei-218* region. At the top of the figure are shown the cosmids used in the mapping of the region. The cosmids are aligned with the *Hind*III (H) and *Xba*I (X) restriction map. Also indicated with large capital letters are the locations of three RFLPs used to position *mei-218* on this map. In the two alleles of RFLP A, a *Hind*III fragment was either 2000 or 2100 kb. In the two alleles of RFLP B, a *Kpn*I fragment was either 1300 or 1600 kb (see Figure 7). It is most likely that RFLP B is within an intron of *M(1)15D* (data not shown). RFLP C was represented by a polymorphic *Hind*III site. A 3-kb *Hind*III fragment of one allele became two fragments, 1400 and 1600 bp, in the other allele. RFLPs A and C were polymorphic in a variety of stocks. In contrast, the 1300-kb allele of RFLP B was found only on the *mei-218*^{hnd} chromosome. Below the restriction map is shown the region deleted on the *Df(1)815-6* chromosome and the duplicated region in *Dp(1;4)fK7*. An insertion into a 10-kb *Hind*III fragment is associated with the *M(1)15D*^l mutation, the details of which are shown in Figure 7. On the bottom of the figure is shown the region expanded in Figure 4.

(MATERIALS AND METHODS) were deletions (*Df(1)815-6*, *Df(1)815-16*, *Df(1)815-32*, *Df(1)815-36*). The latter three were large and deleted at least two Minute loci and as with the large *Bar* revertant deletions, they could only be kept in females that contain a duplication for the Minute loci in addition to the balancer chromosome. In males, a single copy of the duplication was sufficient to rescue the lethality and *Minute* phenotypes of these deletions.

This scheme also allowed us to recover deletions of *Dp(1;4)r*⁺ chromosome that uncovered the *r* or *f* mutations on the normal X chromosome. We recovered two duplications with a *r* mutation and seven with a *f* mutation. One of the *r* duplications and four of the *f* duplications did not rescue the male lethality associated with deficiencies in the region (see MATERIALS AND METHODS), and thus were deletions of *Dp(1;4)r*⁺. The cyto-

logical breakpoints of these deficiencies are listed in Table 3.

Deficiency and duplication genetic breakpoints were determined by complementation tests using lethal mutations in the region (MATERIALS AND METHODS). In these crosses, the lethal mutations were assigned to complementation groups and mapped to specific intervals in the 15A–16A region (Table 4). The complementation analysis divided the 15A–16A region into eight intervals, and 13 complementation groups were identified (Figure 1).

Our further analysis concentrated on the genetic interval containing *mei-218*. The distal limit of *mei-218* was defined by *Dp(1;4)rK20* and *Df(1)815-6*, while the proximal limit was defined by *Dp(1;4)fK7* (Figure 1). Two essential genes were identified in the region; *l(1)15Ea*, represented by four alleles identified in this

TABLE 5
Three factor mapping of *mei-218*

Genotype	Non crossover males	Crossover males	<i>mei-218</i> ⁻ crossovers	m.u. ^a
<i>l(1)15Ea f/mei-218</i> ¹	7428	4 <i>f</i>	4/4	0.054
<i>l(1)15Ea f/mei-218</i> ^{hnd}	12118	20 <i>f</i>	20/20	0.165
<i>M(1)15D</i> ² / <i>mei-218</i> ⁶ or <i>mei-218</i> ⁸ <i>f</i>	33636	23 <i>f</i> ⁺	23/23	0.068
<i>l(1)15Eb f/mei-218</i> ^{hnd}	9307	33 <i>f</i>	19/33	0.353

^a The distance between the lethal and *forked*.

FIGURE 4.—Transcriptional activity in the *mei-218* region. The region shown in this figure is indicated in Figure 3. The restriction map is shown with *Hind*III (*H*) and *Xba*I (*X*), and the genomic restriction fragment used for transformation rescue of *mei-218* is shown with a double-thick line. The transcripts were detected by a combination of Northern blot analysis and screening of two cDNA libraries (see MATERIALS AND METHODS). Northern blots contained total RNA from both adult males and females and dissected ovaries. Transcript *a* was enriched in ovariectomized females, while transcripts *b* and *c* (= *mei-218*) were enriched in ovary RNA. Transcript *g* was enriched in total male RNA. The direction of transcription is shown where it is known. Transcript sizes are based on the Northern blots (except *mei-218*, see RESULTS).

study, and *M(1)15D*, represented by two alleles isolated prior to this study and one allele characterized in this study (*R28ts*).

Genomic DNA in the 15E region and the cloning of *mei-218*: Physical access into the *mei-218* region was provided by cosmid and P1 clones previously shown by *in situ* hybridization to map to the 15D–15F region of the X chromosome (MATERIALS AND METHODS). To determine which cosmid clones were closest to *mei-218*, we conducted *in situ* hybridization experiments against the rearrangements described above. Because *Dp(1;4)fK7* and *Dp(1;4)rK20* carry a functional *mei-218* gene (Figure 1 and see above), we were interested in clones that hybridized to both of these rearrangements, and cosmid clones 102G12, 57A3, 20H11 and 40C1 were found to fulfill this criteria.

Southern analysis showed that these cosmids and P1 clone DS02713 formed a contig of overlapping clones (Figure 3). At the proximal end of the contig, the cosmids 88C7 and 110H3 overlap with 40C1. This links the *mei-218* region to a walk generated by U. KUCHINKE and E. KNUST (personal communication) that covers the *bazooka*–*forked* region. The distance between 110H3 and *forked* is 30 kb. Using restriction fragments from the contig as probes, the breakpoints for both *Dp(1;4)fK7* and *Df(1)815-6* were found on Southern blots. As *Df(1)815-6* does not complement *mei-218*, the region between the two breakpoints, ~65 kb, must contain at least part of the *mei-218* gene. We were not able to find the *Dp(1;4)rK20* breakpoint, suggesting it is distal to the DNA within 102G12, a hypothesis consistent with the fact that *Dp(1;4)rK20* rescues the lethality of *Df(1)815-6*.

Also within the 65-kb region known to contain *mei-218* are the genetically defined genes *l(1)15Ea* and *M(1)15D* [i.e., within *Df(1)815-6*, proximal to *Dp(1;4)rK20* and distal to *Dp(1;4)fK7* (Figure 1)].

We attempted to order these genes with three-factor mapping crosses in which crossovers between either *l(1)15Ea* or *M(1)15D* and *forked* were collected (Table 5; rows 1–3). Nonlethal bearing recombinant chromosomes from either *l(1)15Ea f/mei-218* or *M(1)15D²/mei-218* females were isolated in males and then tested to see if they carried the *mei-218* mutation. None of the 47 recombinants isolated separated *mei-218* from *l(1)15Ea* or *M(1)15D*, demonstrating that these three genes are very closely linked.

A more precise mapping was achieved by selecting viable *forked* males from *l(1)15Eb f/mei-218^{hnd}* heterozygotes. The recombinant chromosomes were then tested for the presence of *mei-218^{hnd}* by failure to complement the nondisjunction phenotype (Table 5; line 4). These data showed that *mei-218* was approximately midway between *l(1)15Eb* and *f*. Higher resolution mapping was obtained by following the segregation of RFLPs. Genomic DNA was prepared from each recombinant stock and the RFLPs were typed by Southern blot analysis. Of the 33 recombinants tested, two placed *mei-218* between RFLPs A and B, which are 15 kb apart (Figure 3). One crossover was between *mei-218* and RFLP A, and the second was between *mei-218* and RFLP B. In addition, one crossover between RFLPs B and C was recovered. The remaining 30 crossovers were either proximal to C or distal to A.

Transcription analysis of the *mei-218* region: To identify potential coding regions for *mei-218*, the region shown by rearrangement breakpoints (Figure 3) to contain *mei-218* was scanned for transcription units by Northern blot analysis and the screening of two cDNA libraries (MATERIALS AND METHODS). Restriction fragments representing the region shown in Figure 4 detected seven different transcripts (labeled *a*–*g*). Transcript *a* on Northern blots was >9 kb (the largest marker on the gel) and was the only transcript in the region to be enriched in ovariectomized

TABLE 6
Transformation rescue of *mei-218* females

	Transformation construct				
	Genomic constructs			cDNA constructs	
	<i>none</i>	<i>43</i>	<i>59</i>	<i>33c1</i>	<i>33c2</i>
X-nondisjunction (%)	40.5	2.2	0.2	<0.4	<0.8
4 nondisjunction (%)	19.8	<0.5	<0.2	<0.4	<0.8
X map (cM) (<i>y-f</i>)	3.2	ND	53.7	ND	ND
Total progeny	1565	623	1257	827	373

ND, not determined. All crosses were $\gamma cv v mei-218^6 f/+ + + + mei-218^1; P[w+, mei-218+]/+$ females crossed to $Y^+ X \cdot Y^l, v f B/0; C(4)RM, ci ey^R/0$ males. Constructs *43* and *59* are insertions on chromosome 3 of a 15-kb genomic fragment (Figure 5). Constructs *33c1* and *33c2* are cDNA constructs inserted on the X and third chromosomes, respectively.

carcasses. Transcript *b* is 6 kb and, based on the analysis of cDNA clones, is alternatively spliced and uses different polyadenylation sites. Partial sequencing of cDNA clones shows that the predicted protein product from transcript *b* is similar to an unknown protein from rat pituitary (data not shown; LEVI *et al.* 1993). Transcript *c* is from *mei-218* (see below). Transcript *d* is abundant and ~200 bp on Northern blots but was not recovered from either of the cDNA libraries screened, suggesting it is not polyadenylated. Transcript *e* (*M(1)15D*) is described in detail below. Sequence from cDNA clones of transcript *f* did not reveal any sequence similarities in the database. Transcript *g* probably corresponds to *l(1)15Ea* [also known as *xmas* (E. Xu, personal communication and see below)].

Identification of the *mei-218* gene: A genomic fragment was chosen for transformation rescue based on the mapping of *mei-218* between RFLPs A and B, and on the identification of transcripts in this interval. A 15-kb restriction fragment from cosmid 102G12 carrying both of these transcripts (Figures 3 and 4) was cloned into the pCaSpeR4 transformation vector (see MATERIALS AND METHODS). Two independent third chromosome transformants were recovered, both of which in a single copy completely rescued both the recombination and nondisjunction phenotypes of *mei-218*⁶ (Table 6). We also tested the effect of *mei-218* overexpression on recombination and nondisjunction in females with four copies of *mei-218* (the two normal copies and two from a transformant) and found it to be normal.

The genomic region used for transformation rescue of *mei-218* contained two transcripts (Figure 4). The larger transcript was shown to be the product of the *mei-218* locus with a transformation rescue experiment using a full length cDNA clone. To achieve expression in the germarium, a 4.3-kb cDNA (see below) was cloned downstream of the *hsp83* promoter (see MATERIAL AND METHODS). Previous studies had shown that the *hsp83* gene is expressed without heat shock induction in the ovaries (ZIMMERMAN *et al.* 1983; DING *et al.* 1993) including the germarium, the region of the ovary where meiotic prophase occurs. Four independent transformants were isolated, and all completely rescued

the *mei-218* nondisjunction phenotype (Table 6 and data not shown).

Sequence of *mei-218*: The transformation experiments showed that a 4.3-kb transcript was sufficient to rescue the *mei-218* phenotype. The rescuing cDNA clone was among five isolated from a library made from ovary RNA (STROUMBAKIS and TOLLAS 1994). For two reasons, three of the cDNA clones are likely to contain all the coding information of *mei-218*. First, they were each the same length, beginning within 10 nucleotides of each other. Second, there is an open reading frame that begins 92 nucleotides from the beginning of these cDNAs. Preceding the start codon, all three reading frames contain stop codons. Thus, if the transcript is longer in the 5' end, it does not include any additional coding sequences. As shown in Figure 5, however, the transcript detected using the cDNA as a probe was larger than the 4.3 kb predicted. Thus it is possible that additional 5' UTR sequences normally associated with the *mei-218* transcript were not represented in the three

FIGURE 5.—Transcription of *mei-218*. (A) Northern blot using *mei-218* cDNA as a probe against total RNA from ovaries (lanes 1–4) and from adult males and females. Approximately 80 μ g of RNA was loaded in each lane. (B) A loading control using a *M(1)15D* cDNA as a probe for the first four lanes of A. These results suggest that in *mei-218*¹ and *mei-218*^{hfnf} the level of transcript is reduced.

A)

B)

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1  MSSTNKLEKK DPRRHIPLQL NVFLRNDDDG EVREVTQAN TSSIGTIPSN
51  GLVESDVTP I MTPTPKAGVY KPNVNVWRLS KEVTKPDRSF VVSAAEYPDL
101 QQRPKRFQR TPRVRNQDDG GTVSCLASRF SRVKDPPVLG NFLPDFMLPK
151 SSNSNHQSVT TWLSSVRPSD SQKSLSDQAR CSSSTNITLL SKDSVQPLTP
201 KSSAQSSPGE KNIQQEQNTQ AKSQQEETSP ANSVEHQQKT PNSPKQQKS
251 SVGLTNMQQK ASANSLQQQN SSASSLQQQN SSVHSLQKRRK SWVNSSQQQR
301 SSVNFIQQR SWVNSSHQK SSASSLQQKS SENSPSKFQQ QQQKSPANNE
351 RSQNNQKSPG IQHSWKQQQK QKPKILQNCP KTSVQQQMNP VNHTQSQSER
401 PTELIYLSHR SPAKRASVTN NSSAPIRIAP RQQTQSSGP SVVHNEDEDV
451 MPPTPNQSER RQRRFPRRNH NPRMVRSLQA VSREFTESLL EGSSGKTPEK
501 EVVPYQQQEK PRQVKSSPEI HASSHMRKLA MGSVLNSNAK EFHPTGESH
551 THKVSRLSQR RCRQRFTRSL HAKKNPREQ LPNPSAPEP ILEYTSEIDD
601 QPCPPRQTV D TPIEAIVPPA MIEVSATGNL PPVFYDQQS GIWSNSSPQ
651 IPVEIQNSV SQSVFRLASL PCTGVGTINM DIPTSSPPPI QHMSSSLPS
701 SISGPTQLSH TSSPPVENQ HQQHLWESCI HQDEDLMYR PLLDMDQEET
751 QPEQQDQEWQ WESEIEPPSF QSENQVVADE HGEENAYLAK DAGPPLERHM
801 IFRAPSGHQG EYKPKDLNVC PSSIKKLHLL ISSQYSDYSF VYALSAQISQ
851 DCPVPMDCFVY LKMILLASIV SIESDEVRAPI ISLCIATDS LMANRLLNKV
901 GQLAPRFLGP HEYGLQPTFN ALPTFRFNWIV ASPLLLAQQG VYYAGDWNRL
951 SKDQCQLEK CIENGAVPVP QLHIDQPLKA AVWTYQPNP STNQTLALAK
1001 LCPIFGLPIY MGAQASNSLW NSIIQKHNAE GQIVVNDGLS IPEDDMRMLI
1051 HLLHQKRTTL TDGAQHMLQK YVVISRKERP NVFSSKTYIV LKQLAECFAN
1101 VALRLVLES DVCVAIFHCE HFVQGIFGAK ENQAPPVFN FNVISCIDPY
1151 MNKFTRWLLQ YLNSYEDEL GMHADKRRRT DSWDMP

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FIGURE 6.—Organization of the *mei-218* gene. (A) Schematic of the *mei-218* transcript. (B) Amino acid sequence based on the conceptual translation of the full length cDNA. The MEI-218 protein is predicted to have 1186 amino acids and have a molecular weight of 134 kD. The full genomic sequence can be obtained with accession number U35631.

largest cDNA clones. The *mei-218* transcript was detected in total RNA from ovaries but not from the ovariectomized carcasses. We also probed a Northern blot with a *Hind*III restriction fragment that extends from 1 to 4 kb upstream of the beginning of the cDNA clones. Because the *mei-218* transcript was not detected by this probe, the *mei-218* transcript can be no more than 1 kb longer than the cDNA clones. A *mei-218* transcript was also detected in total RNA from males, and it may be shorter than the female transcript (Figure 5). This latter expression was surprising considering *mei-218* mutants have no effect on chromosome segregation in males.

Sequence was obtained from both cDNA clones and genomic DNA (Figure 6). The coding region of the transcript is interrupted by four introns. Three of these are small, at 72, 73 and 66 nucleotides. The last intron, however, is 291 nucleotides. The predicted protein is 1186 amino acids, and in searches with this sequence, no significant similarities in the nonredundant and STS sequence databases at National Center for Biotechnology Information were detected. There are notable sequence features that may be related to the function of *mei-218*. The amino half of the protein is basic (isoelectric point = 10.58) and contains several glutamine repeats and is also serine and threonine rich. In contrast, the carboxy half of the protein is slightly acidic (isoelectric point = 4.99).

l(1)15Ea: Transcript *g* (Figure 4) was detected on total RNA blots from males but not females. A cDNA

clone corresponding to this transcript was isolated, and sequence obtained from the ends of the clone was not similar to any in the database. E. XU (personal communication) has isolated a *P*-element insertion-mutation of this gene (*xmas*). The *xmas* mutant is male sterile, consistent with the expression pattern of the transcript. Surprisingly, females heterozygous for *xmas* and alleles of *l(1)15Ea* are sterile. This suggests that *xmas* is a hypomorphic allele of *l(1)15Ea* and that transcript *g* is expressed in females at a low level. Consistent with transcript *g* corresponding to *l(1)15Ea* is our observation that an X-ray-induced allele, *815-10*, is associated with an RFLP in the same *Hind*III fragment that contains transcript *g* (data not shown).

Identification of the M(1)15D gene product: Two alleles of *M(1)15D* were associated with RFLPs on Southern blots (Figure 7). *M(1)15D¹* is associated with an insertion of ~6 kb into a 1.6-kb *Kpn*I fragment. *M(1)15D²* is also associated with an unknown chromosome rearrangement that alters the size of the adjacent 2.0-kb *Kpn*I-*Clal* fragment. Both aberrations effect the genomic region that produces a 800-bp transcript (data not shown). A full length cDNA and several truncations corresponding to this transcript were cloned and sequenced. The predicted protein is homologous to the eukaryotic ribosomal protein S5 (Figure 8). The conservation is remarkably high, with 86% sequence identity to the rat protein. The human and rat proteins differ at only three amino acids, including a single amino acid deletion in the human protein. The second half of

FIGURE 7.—The *M(1)15D* region. A restriction map of the 10-kb *Hind*III fragment shown in Figure 3 to contain the *M(1)15D*¹ gene. The *M(1)15D*¹ mutation is associated with a 6-kb insertion of unknown DNA. A rearrangement breakpoint is detected in *M(1)15D*² by the adjacent *Kpn*I-*Cl*aI fragment, but the nature of this mutation has not been determined. The restriction enzyme sites shown are *Hind*III (H), *Cl*aI (C), and *Kpn*I (K). The *M(1)15D* transcript (solid box) is 800 bp, and the adjacent transcript (shaded box) is 1 kb. Sequence was obtained from the ends of cDNA clones of this transcript and no homologies to other proteins were found in database searches.

M(1)15D is the most highly conserved; the final 89/90 amino acids of *M(1)15D* are identical in the rat protein. This family of proteins also includes rpS7 from archaeobacteria. These data show to be false the proposal by BURNS *et al.* (1984) that *M(1)15D* is ribosomal protein S18, which was mapped by *in-situ* hybridization to 15B.

DISCUSSION

We have reported the cloning and sequence of the *mei-218* gene. In the process, we have characterized the molecular genetics of the region surrounding *mei-218*. In addition to *mei-218*, we have cloned and sequenced *M(1)15D*, which encodes the *Drosophila* homologue

of ribosomal protein S5. Including these two genes, we have identified seven transcripts in the 65-kb region around *mei-218*. The density of genes, one every 9 kb, is consistent with what is known of the *Drosophila* genome size and genome content.

The analysis of *mei-218*: The results of several studies indicate that *mei-218* is required for the resolution of meiotic recombination intermediates into crossovers after the initiation of recombination and pairing. The facts that in *mei-218* mutants normal synaptonemal complex (= sc) forms (CARPENTER 1979a) and gene conversion occurs at normal frequency (CARPENTER 1982, 1984) show that recombination is initiated and can progress to the point of generating heteroduplex DNA. The importance of our conclusion that in the absence of *mei-218* function a low level of crossing over still occurs is that it suggests that once recombination is initiated, a *mei-218*-dependent pathway is just one of multiple ways that crossovers can be generated. Interestingly, a similar level of crossing over (between 5 and 10% of wild type) is observed in *mei-9* (CARPENTER and SANDLER 1974) and *mus-312* (GREEN 1981) mutants. We have speculated previously that these residual exchanges may well reflect the existence of a second pathway for meiotic exchange that is independent of both *mei-218* and *mei-9* (SEKELSKY *et al.* 1995). This is supported by the observation that the frequency of crossing over in *mei-9 mei-218* double mutants is similar to the single mutants (SEKELSKY *et al.* 1995).

We propose that *mei-9*, *mei-218* and *mus-312* form one of two classes of severe meiotic mutants in *Drosophila*. While this group of genes is required to resolve these intermediates into crossovers, the second group of genes is required earlier for recombination intermediates to be created. In mutants of this class of genes, *c(3)G* (HALL 1972), *mei-W68* (B. BAKER, personal com-

	1				50
rat S5					
Human S5					
<i>M(1)15D</i>	MAEVAENVVE	TFEPPAAPME	AEVAETILET	NVVSTTELEPE	IKLFGKWS
consensus			T ET	E P	IKLFG WS D
	51				100
rat S5	DVQINDISLQ	DYIAVKEKYA	KYLPHSAGRY	AANGFRKAQC	PIVERLTNSM
Human S5	DVQINDISLQ	DYIAVKEKYA	KYLPHSAGRY	AANAFRKAQC	PIVERLTNSM
<i>M(1)15D</i>	DVTVNDISLQ	DYISVKEKFA	RYLPHSAGRY	AAKFRKAQC	PIVERLTCSL
consensus	DV NDISLQ	DYI VKEK A	YLPHSAGRY	AA FRKAQC	PIVERLT S
	101				150
rat S5	MMHGRNNGKK	LMTVRIVKHA	FEIIHLLTGE	NPLQVLVNAI	INSGPREDST
Human S5	MMHGRNNGKK	LMTVRIVKHA	FEIIHLLTGE	NPLQVLVNAI	INSGPREDST
<i>M(1)15D</i>	MMKGRNNGKK	LMACRIVKHS	FEIIHLLTGE	NPLQILVSAI	INSGPREDST
consensus	MM GRNNGKK	LM RIVKH	FEIIHLLTGE	NPLQ LV AI	INSGPREDST
	151				200
rat S5	RIGRAGTVRR	QAVDVSPLRR	VNQAIWLLCT	GAREAAFRNI	KTIAECLADE
Human S5	RIGRAGTVRR	QAVDVSPLRR	VNQAIWLLCT	GAR.AAFRNI	KTIAECLADE
<i>M(1)15D</i>	RIGRAGTVRR	QAVDVSPLRR	VNQAIWLLCT	GAREAAFRNI	KTIAECLADE
consensus	RIGRAGTVRR	QAVDVSPLRR	VNQAIWLLCT	GAREAAFRNI	KTIAECLADE
	201				229
rat S5	LINARKGSSN	SYAIKKKDEL	ERVAKSNR		
Human S5	LINAARKGSSN	SYAIKKKDEL	ERVAKSNR		
<i>M(1)15D</i>	LINAARKGSSN	SYAIKKKDEL	ERVAKSNR		
consensus	LINA KGSSN	SYAIKKKDEL	ERVAKSNR		

FIGURE 8.—Amino acid sequence of *M(1)15D* and comparison to rat rpS5 (KUWANO *et al.* 1992) and human rpS5 (FRIGERIO *et al.* 1995). The *M(1)15D* protein is either 211 or 229 amino acids, depending on which initiator ATG codon is used. Shown in bold below the alignment is a consensus sequence in which the amino acids present in all three proteins are shown. The full cDNA sequence of *M(1)15D* can be obtained with accession number U48394.

munication) and *mei-P22* (K. S. MCKIM, T. ARBEL and R. S. HAWLEY, unpublished data), crossovers are 10-fold less frequent than in mutants like *mei-218*. The crossing over that is observed in these mutants (<0.5% of wild type) is likely to be of mitotic origin. The conclusion that these genes are required to initiate recombination comes from the observation that in *c(3)G* (CARLSON 1972) and *mei-W68* (K. S. MCKIM, unpublished data) mutants, the frequency of gene conversion is reduced, and in *c(3)G* mutants, no synaptonemal complex forms between the homologues (MEYER 1963; RASMUSSEN 1975). Thus in our current view of meiosis in female *Drosophila*, the initiation of meiotic recombination requires the *c(3)G*, *mei-W68*, and *mei-P22* gene products. The first two of these genes have also been shown to have a role in mitotic cells (BAKER *et al.* 1978; K. S. MCKIM, unpublished data). These gene products create recombination intermediates that can be processed into crossovers, a process that is proposed to require the *mei-9*, *mei-218* and *mus-312* genes. This second class of genes may be defective in recombination *per se*. The fact that the MEI-9 protein is homologous to a family of endonucleases is also consistent with a direct role in maturation of recombination intermediates (SEKELSKY *et al.* 1995).

The sequence of *mei-218* does not reveal any obvious clues to its function. While *mei-218* and *mei-9* have similar mutant phenotypes, gene conversion events initiate normally but are not resolved as crossovers. The unique aspect of the *mei-218* phenotype is its effect on recombination nodules. The recombination nodule is large enough to be composed of many proteins, and *mei-218* is the only mutant known to affect recombination nodule morphology (CARPENTER 1979a). Thus it may not be surprising that there are no homologues yet identified. To investigate the significant features of the MEI-218 protein sequence, the homologous gene from *D. virilis* has been isolated and is being sequenced. Future experiments will be directed at determining if *mei-218* is a component of the recombination nodule, and if so, does it have a direct role in crossover resolution, or does it have a more structural role in the maturation of the recombination nodule and its relationship with the synaptonemal complex.

Minute genes in *Drosophila*: *M(1)15D* mutants are typical of *Drosophila* Minute genes; they are recessive lethals and dominantly affect growth and fertility. The sequence of *M(1)15D* has shown it to be ribosomal protein S5. The two previously cloned Minute genes are also ribosomal proteins; *M(3)95A* encodes rpS1a (ANDERSON *et al.* 1994), and *M(3)95A* encodes rp49 (KONGSUWAN *et al.* 1985). Several other ribosomal proteins have been cloned out of *Drosophila*, but the high level of sequence identity between *M(1)15D* and the mammalian homologues is exceptional. Most ribosomal proteins in *Drosophila* do not show such high sequence conservation (reviewed in ANDERSON *et al.* 1994), suggesting that there are tight functional constraints on

rpS5 homologues. In *D. melanogaster* there are 46 Minute loci listed by LINDSLEY and ZIMM (1992), but the gene products for most of these are not known. Given the results with the first three cloned Minute genes, it seems likely that most of the Minutes will encode ribosomal proteins. The situation is more interesting, however, as the genetic interactions between Minute loci are complex (SCHULTZ 1929; DEL PRADO and RIPOLL 1983), and some ribosomal proteins do not have a Minute phenotype when mutant (*rpS6* = *air8*; WATSON *et al.* 1992).

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