A new type of *mei-9* allele in *Drosophila melanogaster* which is recombination-competent but repair-deficient

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ABSTRACT

An X-linked meiotic gene *mei-9* controls both meiotic recombination in *Drosophila* females, and somatic excision repair in both sexes. Homozygous females of two of three *mei-9* alleles, induced through hybrid dysgenesis and screened for the sensitivity to MMS, showed no defect in recombination on the second chromosome and had normal X chromosome segregation. The MMS sensitivity of the two recombination-competent alleles was mapped to the close proximity to the *mei-9* locus. It supports the idea that the two mutations are alleles of *mei-9* and that the meiotic and somatic functions of the gene are distinguishable.

1. INTRODUCTION

The *mei-9* gene is one of the most extensively investigated meiotic genes in *Drosophila melanogaster*. It controls the process of recombination in female meiosis (Baker and Carpenter, 1972) as well as the process for an excision type of DNA repair (Boyd et al., 1976). All of the mutants for the locus that have been examined to date are defective in both recombination and excision repair (reviewed by Boyd et al., 1987). We have induced three new alleles of the *mei-9* locus through hybrid dysgenesis which were identified based on sensitivity to methyl methanesulfonate (MMS) (Yamamoto et al., 1990). The frequency of X chromosome nondisjunction in female meiosis was as high as 18% for one of the three but only 0.2 and 0.6% for the other. The low frequencies of nondisjunction suggest that these alleles may not drastically reduce meiotic recombination. We tested this possibility by examining crossing-over along one arm of the second chromosome. We report here that these two alleles of *mei-9* actually support normal frequencies of recombination. A possible molecular nature of the alleles will also be discussed.

2. MATERIALS AND METHODS

Drosophila strains

Drosophila cultures were maintained on a standard medium, containing yeast, cornmeal, glucose and agar, at 23°C. The *mei-9* stocks used in these experiments

were $y\ mei-9^a/FM7a$ and $y\ mei-9^b/FM7a$ (Baker and Carpenter, 1972), $y\ pn\ mei-9^{AT1}/FM7a$ (Smith, 1976), and $mei-9^{RT1,2f}/C(1)DX$, $y\ f$ and $mei-9^{RT4}sd/C(1)DX$, $y\ f$ (Yamamoto et al., 1990). A description of other mutants used is given by Lindsley and Grell (1968).

Nondisjunction frequency

Ten newly hatched females of each genotype of $mei-9$, or 5 females of wild type were crossed to 5 $y\ v/y^+ YB^S$ males in each vial. The parents were transferred to new vials every 2 to 3 days to produce 3 to 4 broods. The progeny were scored up to day 17 in each vial and 5 to 10 vials were examined for each genotype.

Recombination frequency

Recombination on the second chromosome was examined for the regions $al(0.0)-ap(13.0)-b(48.5)-pr(54.5)-centromere-cn(57.5)$ in females of homozygous or heterozygous $mei-9$ alleles. The number in parentheses indicates the standard map position given by Lindsley and Grell (1968). Ten newly hatched females were crossed to 5 males in each vial according to the mating scheme in Fig. 1. The parents were transferred to new vials every 2 to 3 days to produce 3 to 4 broods. The progeny were scored up to day 17 for each vial and 5 to 10 vials were examined for each genotype.

Fig. 1. Mating scheme for recombination on the second chromosome. The X chromosome of $mei-9^{RT4}$ was marked with sd rather than f .

Mapping of MMS sensitivity

MMS sensitivities of $mei-9^{RT1}$, $mei-9^{RT4}$ and $mei-9^a$ were mapped by testing the MMS sensitivity of recombinants using a multiply marked X-chromosome. Recombinant males from $mei-9/ec\ rb\ cv\ ct^6\ sn^3$ females were crossed individually

to *C(1)DX*, *y f/Y* females to establish strains which were tested for MMS sensitivity as described by Yamamoto et al. (1990).

3. RESULTS

Nondisjunction frequency of heteroallelic females

Yamamoto et al. (1990) reported that two of the three alleles of hybrid dysgenic origin, *mei-9^{RT1}* and *mei-9^{RT4}*, were distinctive from known alleles of the locus in frequency of nondisjunction of homoallelic females. We investigated nondisjunction frequency of heteroallelic females of the two categories. Results shown in Table 1 demonstrate complete dominance of *mei-9^{RT1}* and *mei-9^{RT4}* over the three EMS induced alleles, *mei-9^a*, *mei-9^b* and *mei-9^{AT1}*. The frequencies of nondisjunction were from 0.4 to 3.9% which were as low as the control's.

Table 1. Nondisjunction frequency in homoallelic and heteroallelic *mei-9* females crossed to *y v/v⁺ YB^S* males

Genotype of mother ¹⁾	Chromosome configuration					ND ²⁾ ($\times 10^{-3}$)
	XX	XY	XXY	XO	Total	
Controls						
+/+ (Oregon-R)	940	867	1	0	1808	1.1
Homoallelic combination						
<i>RT1/RT1</i>	2521	2313	3	5	4842	3.3
<i>RT4/RT4</i>	1376	888	3	7	2274	8.8
<i>a/a</i>	458	273	60	107	898	314.
<i>b/b</i>	1239	903	28	113	2283	116.
<i>AT1/AT1</i>	987	828	78	192	2085	229.
Heteroallelic combination						
<i>RT1/a</i>	1646	1419	6	0	3071	3.9
<i>RT1/b</i>	2464	2100	1	0	4565	0.4
<i>RT1/AT1</i>	1836	1605	2	3	3446	2.9
<i>RT1/RT4</i>	2403	1758	2	0	4163	1.0
<i>RT4/a</i>	2651	2114	1	4	4770	2.1
<i>RT4/b</i>	2428	1686	3	0	4117	1.5
<i>RT4/AT1</i>	1593	1180	2	1	2776	2.2

1) Alleles of *mei-9* are shown by their superscripts. For flanking markers, see Materials and methods.

2) ND=nondisjunction frequency in ova; calculated as $2 \times (XXY + XO) / [XX + XY + 2 \times (XXY + XO)]$.

Recombination on the second chromosome

The recombination frequencies on the second chromosome were examined in

females homozygous or heterozygous for *mei-9^{RT1}*, *mei-9^{RT2}*, or *mei-9^{RT4}*. The distributions of the recombinants from these females is shown in Tables 2 and 3. Analyses of the data are given in Table 4. The total map distances in controls, 50.3 and 47.6, were slightly shorter than the standard map value, 57.4, of Lindsley and Grell (1968) mainly because of the inability to detect double recombination between *dp* and *b*.

In females homozygous for *mei-9^{RT2}* recombination is dramatically decreased (Table 4); the total map distance between *al* and *cn* being 9.7 centimorgans, one fifth of the control. Crossing-over in these females was uniformly decreased between *al* and *cn*, as seen for other mutant alleles of the locus (Baker and Carpenter, 1972).

In *mei-9^{RT1}* and *mei-9^{RT4}*, however, the recombination frequency between *al* and *cn* were not decreased compared with the controls, but slightly increased (Table 4) especially in regions *b-pr* and *pr-cn* for *mei-9^{RT1}* and *b-pr* for *mei-9^{RT4}*.

Table 2. Number of progeny from crosses of *mei-9^{RTf1}/mei-9^{RTf1}*; *al dp b pr cn/+ + + +* females by *+ /Y: al dp b pr cn/al dp b pr cn* males

Class	<i>mei-9</i> allele		
	<i>RT1</i>	<i>RT2</i>	<i>RT4</i>
Non-recombinant	837	1835	172
Recombinant ²⁾			
1	208	49	45
2	471	80	98
3	105	11	13
4	39 (4/35) ³⁾	3	2
1,2	22	22	14
1,3	23		5
1,4	9 (6/3)		
2,3	33	2	4
2,4	17 (9/8)	2	2
3,4	1 (1/0)		
1,2,3	1		2
1,2,4	2 (0/2)		
1,3,4	1 (1/0)		
2,3,4	19 (2/17)		
1,2,3,4	3 (0/3)		
Total	1791	2004	357

1) *sd* for *mei-9^{RT4}*.

2) Region 1=*al-dp*, region 2=*dp-b*, region 3=*b-pr*, region 4=*pr-cn*.

3) The numbers of progeny of reciprocal recombinants concerning region 4 are shown in a form of *pr/cn*.

Table 3. The number of progeny from crosses of *mei-9^{RTf1}/++: al dp b pr cn/++++* females by *+/Y: al dp b pr cn/al dp b pr cn* males

Class	<i>mei-9</i> allele			Control	
	<i>RT1</i>	<i>RT2</i>	<i>RT4</i>	<i>f</i>	<i>sd</i>
Non-recombinant	217	1750	1288	1494	1468
Recombinant ²⁾					
1	58	438	356	408	353
2	124	840	670	663	677
3	23	90	95	93	62
4	3	25	19	9	18
1,2	3	59	74	47	39
1,3	8	9	9	10	11
1,4	1	6	7	12	2
2,3	4	18	15	13	9
2,4		6	9	7	6
3,4	1	2	1	1	
1,2,3		2	3	10	1
1,2,4		2		1	
1,3,4		1			3
2,3,4		1	2	3	2
1,2,3,4					
Total	442	3349	2548	2771	2651

1) *sd* for *mei-9^{RT4}*.

2) Region 1=*al-dp*, region 2=*dp-b*, region 3=*b-pr*, region 4=*pr-cn*.

In the case of *mei-9^{RT1}* females, we had difficulty distinguishing *cn* from *cn⁺* flies, which probably resulted in an excess number of *pr⁺ cn* recombinants over *pr cn⁺* (Table 2). If we double the number of *pr cn⁺* recombinants to estimate the number of the recombinants in region 4 (*pr-cn*), the recombination frequency goes down to 2.6 from 4.25. For the *b-pr* region, the recombination frequencies in homozygous *mei-9^{RT1}* and *mei-9^{RT4}* females were more than twice of those the controls. Higher frequencies for this region, however, were also observed in *mei-9^{RT1}/+* and *mei-9^{RT4}/+* females (Table 4). Even after revising the estimate of the recombination frequency in region 4 for *mei-9^{RT1}*, total map distance in *mei-9^{RT1}* and *mei-9^{RT4}* are about 20% longer than the control values.

Mapping of MMS sensitivity

The distinctive nature of *mei-9^{RT1}* and *mei-9^{RT4}* raised a possibility that their failure of complementation of *mei-9* in MMS sensitivity came from an epistatic effect of the other gene(s) rather than defects at the locus itself. Therefore, we

Table 4. Analyses of the recombination data in Tables 2 and 3

X chromosome configuration ¹⁾	Map distance (relative length) ²⁾					Tetrad distribution frequency (%)				
	Total <i>al-cn</i>	1 <i>al-dp</i>	2 <i>dp-b</i>	3 <i>b-pr</i>	4 <i>pr-cn</i>	E ₀ ³⁾	E ₁	E ₂	E ₃	E ₄
Homozygote										
<i>RT1 flRT1 fl</i>	62.2 (1.24)	15.0 (0.85)	31.7 (1.18)	10.4 (2.21)	5.1 (4.25)	46.7	46.0	5.9	1.3	0.2
<i>RT2 flRT2 fl</i>	9.7 (0.19)	3.5 (0.20)	5.3 (0.20)	0.6 (0.14)	0.2 (0.21)	91.6	7.1	1.3	0	0
<i>RT4 sd/RT4 sd</i>	59.9 (1.26)	18.5 (1.20)	33.6 (1.21)	6.7 (2.03)	1.1 (0.92)	48.2	44.3	7.0	0.6	0
Heterozygote										
<i>RT1 fl/+</i>	54.8 (1.09)	15.8 (0.90)	29.6 (1.10)	8.1 (1.72)	1.1 (0.92)	49.1	47.1	3.8	0	0
<i>RT2 fl/+</i>	48.1 (0.96)	15.4 (0.88)	27.7 (1.03)	3.7 (0.78)	1.3 (1.08)	55.2	41.6	3.0	0.2	0
<i>RT4 sd/+</i>	54.4 (1.14)	17.6 (1.14)	30.3 (1.09)	4.9 (1.48)	1.5 (1.25)	50.5	44.7	4.5	0.2	0
Control										
<i>fl/+</i>	50.3	17.6	26.8	4.7	1.2	53.9	42.3	3.2	0.5	0
<i>sd/+</i>	47.6	15.4	27.7	3.3	1.2	55.4	41.9	2.5	0.2	0

1) Alleles of *mei-9* are shown by their superscripts.2) The relative value to controls; controls are *sd/+* for *mei-9^{RT4}* and *fl/+* for the other.3) E_n is the percentage of tetrads with n exchanges.Table 5. Mapping of the MMS sensitivity of *mei-9^{RT1}*, *mei-9^{RT4}* and *mei-9^a*

Recombinant genotype	<i>mei-9</i> allele					
	<i>RT1</i>		<i>RT4</i>		<i>a</i>	
	MMS		MMS		MMS	
	+	-	+	-	+	-
+ + + + +	0	30	0	42		
<i>ec rb cv ct sn</i>	21	0	22	0		
+ + + <i>ct sn</i>	0	16	0	11		
<i>ec rb cv</i> + +	4	0	4	0		
+ + <i>cv ct sn</i>	0	16	0	5		
<i>ec rb</i> + + +	6	0	7	0		
+ <i>rb cv ct sn</i>	1	1	2	2	1	0
<i>ec</i> + + + +	6	1	5	5	2	3

Number of examined strains which were established from a single recombinant male was shown.

mapped lesions responsible for their MMS sensitivities. Table 5 shows that the map positions of the sensitivities of *mei-9^{RT1}* and *mei-9^{RT4}* were between *ec* and *rb*; 5.9 for *mei-9^{RT1}* and 6.5 for *mei-9^{RT4}*. They were the proximity to the reported position of *mei-9* (6.8 from Table 5 in this study; 5 by Carpenter and Sandler, 1974; 7 by Boyd et al., 1987; distal but close to *rb* by Banga et al., 1986). Therefore, we concluded that the impair of DNA repair of *mei-9^{RT1}* and *mei-9^{RT4}* resulted from the defects at *mei-9* locus itself.

4. DISCUSSION

Two of three *mei-9* mutants induced by hybrid dysgenesis have a phenotype distinct from alleles induced with EMS. Females homozygous for *mei-9^{RT1}* or *mei-9^{RT4}* are deficient in somatic DNA repair but proficient in meiotic recombination. Mapping data support the idea that they were alleles of *mei-9* rather than the other genes which had an epistatic effect with the locus. If it is the case, the *mei-9* gene have at least two distinguishable functions.

Seven alleles of *mei-9* have been induced using EMS (Bake and Carpenter, 1972; Smith, 1976; Mason et al., 1981); all of them were defective both in DNA repair and meiotic recombination. In the present study we showed that it was the case only for one of the three alleles newly induced by hybrid dysgenesis. EMS usually causes base substitutions, whereas P elements, which are the main factor in the hybrid dysgenic system, induce insertions and deletions. If insertions and/or deletions are responsible for the lesions in *mei-9^{RT1}* and *mei-9^{RT4}*, they may be in a control region of the gene rather than in a coding region, since these alleles are still competent for recombination.

Defects in *mei-9^{RT1}* and *mei-9^{RT4}* might be in a control region responsible for the somatic but not meiotic expression of the gene. The other explanation for the existence of alleles which are competent for meiotic recombination but are defective in repair is that a low level of expression of *mei-9* may be sufficient to support recombination in meiosis but insufficient to repair damages on DNA in somatic cells. It is consistent with this possibility that males of *mei-9^{RT1}* and *mei-9^{RT4}* were less sensitive to MMS when compared to those of *mei-9^{RT2}*. It is noteworthy that a few alleles of *mei-41* are defective in somatic repair, but are able to bear normal meiosis (Mason et al., 1989), although most alleles of the locus are deficient for both somatic DNA repair and meiotic recombination. The MMS sensitivity of the recombination-competent *mei-41* mutants is not as extreme as that of the recombination-defective *mei-41* mutants. Therefore, genes involved in somatic repair may have to be expressed extensively when compelled.

An unexpected observation was the increase of recombination in homozygous females of recombination-proficient alleles, *mei-9^{RT1}* and *mei-9^{RT4}*. It may indicate hyperactivity of these alleles in meiosis, even though they are incompetent in DNA repair. Map distances from the heterozygotes of the two alleles were

larger than those from controls. A hypomorphic manner in DNA repair and a hypermorphic one in meiotic recombination shown here for the two alleles support the idea mentioned above that their lesions are in a control region. Their defect appears to be causing a constitutive expression of the gene.

By means of inductions of hybrid dysgenic mutants, our primary goal has been set to clone *mei-9* and *mei-41* by the P element-tagging method (Boyd et al., 1987). If lesions in *mei-9^{RT1}* and *mei-9^{RT4}* and other alleles of the locus are determined at a molecular level, we will have a clearer understanding of the two types of *mei-9* mutations.

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