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Genetic controls of meiotic recombination and somatic DNA metabolism in *Drosophila melanogaster*

(recombination-defective meiotic mutants/mutagen-sensitive mutants/DNA repair processes/somatic crossing-over)

BRUCE S. BAKER*, JAMES B. BOYD†, ADELAIDE T. C. CARPENTER*, M. M. GREEN†, TRONG D. NGUYEN†, PEDRO RIPOLL‡, AND P. DENNIS SMITH§

*Department of Biology, University of California, San Diego, La Jolla, Calif. 92093; †Department of Genetics, University of California, Davis, Calif. 95616; ‡Centro de Biologia Molecular, Universidad Autonoma de Madrid, Facultad de Ciencias, Canto Blanco, Madrid-34, Spain; and §Biology Department, Emory University, Atlanta, Georgia 30322

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ABSTRACT **Recombination-defective meiotic mutants and mutagen-sensitive mutants of *D. melanogaster* have been examined for their effects on meiotic chromosome behavior, sensitivity to killing by mutagens, somatic chromosome integrity, and DNA repair processes. Several loci have been identified that specify functions that are necessary for both meiotic recombination and DNA repair processes, whereas mutants at other loci appear to be defective in only one pathway of DNA processing.**

Mutational analyses of prokaryotic systems have established that DNA replication, repair, and recombination are, in part, under common genic control (1–3). Although there are strong theoretical grounds for extending this conclusion to eukaryotes in general (4–7), direct experimental evidence for an interrelation of the various pathways of DNA metabolism in organisms that undergo meiosis comes primarily from studies of fungi (8, 9). Recombination-defective and repair-defective mutants have been reported in other eukaryotes (9, 10), although in most instances it is not known whether these mutants affect more than one pathway of DNA processing.

In *Drosophila melanogaster* two classes of mutants with potential effects on DNA metabolism have been reported. First, substantial numbers of meiotic mutants are available, which were recognized because they produce genetically detectable abnormal meiotic chromosome behavior (for reviews see refs. 9, 11–14). Mutants at 12 loci are defective in processes essential for normal exchange (frequency and/or distribution along the chromosome) and are thus recombination-defective mutants. Watson (15, 16) presented evidence that one of these recombination-defective mutants, $c(3)G^{17}$, is more susceptible than wild type to the induction of mutants and rearrangements during meiosis, and on this basis has suggested that the product of $c(3)G^{+}$ functions in both repair and recombination. Second, mutagen-sensitive mutants have been isolated on the basis of hypersensitivity to killing by methylmethanesulfonate (MMS) (17, 18,¶). The characterization of meiotic mutants‖ and mutagen-sensitive mutants (18, 19,¶) by three separate groups using a variety of approaches has led to the realization that overlapping sets of mutants are being examined. In this preliminary communication we coordinate the results of the three groups and outline the conclusions concerning the relation between the genic controls of DNA repair processes and meiotic recombination in *Drosophila*. We focus here primarily on studies of several selected X-linked mutagen-sensitive and meiotic mutants. The detailed data on the arrays of mutants from which our conclusions have been derived will be published elsewhere.

Known X-linked recombination-defective and/or mutagen-sensitive loci

Recombination-defective mutants are known at 5 X chromosomal loci; *mei-9*, *mei-218*, *mei-41*, *mei-352*, and *mei-251* (20, 21). In addition to decreasing the frequency and/or altering the distribution of exchanges along the chromosomes during female meiosis, these mutants all produce elevated frequencies of nondisjunction of all chromosome pairs (20). Several lines of evidence suggest that the nondisjunction in recombination-defective mutants is a secondary consequence of the increased frequency of nonexchange tetrads produced by these mutants (12, 20–22).

Screening procedures similar to that reported by Smith (17) were used to isolate 28 X-linked mutants hypersensitive to killing by MMS (Fig. 1) in Atlanta¶, and 15 mutants have been isolated in Davis (18,**) as hypersensitive to killing by MMS or, γ-rays. Complementation tests for MMS sensitivity define approximately 11 X-linked loci. Mutants in one complementation group are allelic with mutants at the *mei-41* locus and are designated $mei\text{-}41^{A1}$ through $mei\text{-}41^{A17}$¶ and $mei\text{-}41^{D1}$ through $mei\text{-}41^{D5}$ (18). The MMS-sensitive mutant previously reported by Smith (17, 23) and designated mut^{s} is a member of this complementation group and has been renamed $mei\text{-}41^{A1}$. Mutants at the remaining mutagen-sensitive complementation groups are not allelic with known meiotic mutants; these new loci are designated mutagen-sensitive [*mus(1)101* through *mus(1)110*] (18, ¶, **).

That *mei-41* mutants have been initially detected on the basis of either hypersensitivity to killing by mutagens or abnormal meiotic chromosome behavior suggests that at least this gene function is required for both meiotic recombination and the repair of mutagen-induced damage in somatic cells.

Meiotic effects of mutagen-sensitive mutants

Examination of the effects of mutagen-sensitive mutants on sex chromosome disjunction in female meiosis indicates that several of these mutants identify new loci affecting meiotic processes (¶, 18). Of those mutagen-sensitive mutants that are female fertile, all tested alleles of *mei-41*, *mus(1)101*, and *mus(1)102* increase sex chromosome nondisjunction (120- to 300-fold, 65- to 100-fold, and 6- to 13-fold, respectively), whereas mutant alleles at the *mus(1)103*, *mus(1)104*, and *mus(1)105* loci do not affect disjunction. As a consequence of virtual or complete

Abbreviation: MMS, methylmethanesulfonate.
¶ P. D. Smith, manuscript submitted.
‖ B. S. Baker, A. T. C. Carpenter, and P. Ripoll, unpublished work.
** T. D. Nguyen, M. M. Green, and J. B. Boyd, manuscript submitted.

Table 1. Frequency of spontaneous somatic spots expressing the third chromosome somatic cell markers *y*, *jv*, and/or *mwh** in abdominal tergites of *y/y*; $Dp(1;3)sc^{J4}$, y^+ *mwh/jv* females homozygous for the indicated recombination-defective meiotic mutants

		Single spots†				Twin spots					
		y		*y jv*		*y jv/mwh*		*y/mwh*			
Mutant	No. of flies	1	≥2	1	≥2	1	≥2	1	≥2	Events/fly	Twins/fly
+	300	33	3	19	3	2	5	3	1	0.24	0.036
mei-218	155	7	6	13	3	1	6	0	5	0.27	0.077
mei-41	75	129	37	133	25	1	1	1	1	4.61	0.053
mei-9	128	29	6	40	7	5	4	3	1	0.82	0.102

* *y* (yellow) affects bristle color; *jv* (javelin), bristle morphology; *mwh* (multiple wing hair), hair arrangement and morphology. For further descriptions of these and other mutants see ref. 28.

† Single *mwh* spots were not scored; numbers 1 and ≥2 refer to number of bristles in a clone.

sterility of females homozygous for the X chromosomes carrying the remaining mutagen-sensitive mutants [*mus(1)106*, *mus(1)107*, *mus(1)109*, and *mus(1)110*], disjunction has not been examined in these cases.

Since the characterizations of meiotic mutants have shown that a decreased frequency of meiotic recombination is sufficient (but not necessary) to produce nondisjunction in *Drosophila* (9, 12), these results suggest that the *mus(1)101* and *mus(1)102* loci may also specify functions required for both meiotic recombination and the repair of mutagen damage in somatic cells. Conversely, the lack of effect of the *mus(1)103*, *mus(1)104*, and *mus(1)105* alleles on disjunction suggests that these mutants identify a second class of repair functions, those not used in meiotic recombination. Finally, although the female sterility associated with chromosomes bearing *mus(1)106*, *mus(1)107*, *mus(1)109*, or *mus(1)110* may indicate a meiotic involvement of these loci, it is at present not known whether the female sterility and mutagen sensitivity are due to the same mutation in these cases.

That female sterility can be produced by mutations in loci controlling mutagen sensitivity is demonstrated by the effects on female fertility of *mei-41* alleles, all of which produce a much more severe reduction in fecundity than can be accounted for by the observed frequencies of aneuploid gametes (20). The cause of this sterility resides at the *mei-41* locus, since (*i*) the female sterility, meiotic nondisjunction, mutagen sensitivity, and somatic chromosome breakage (see below) phenes are inseparable by recombination (¶, ||) and (*ii*) the *mei-41* alleles fail to complement one another for female fertility (18, ¶). Although the sterility of *mei-41* alleles is manifest as a failure of most eggs laid by homozygous *mei-41* females to hatch, it is not yet known whether this results from a defective meiosis or the dependence of normal embryonic development on the maternal provision of the $mei\text{-}41^+$ product.

Mitotic chromosome stability

The loci identified by some recombination-defective mutants appear to specify functions requisite for normal DNA metabolism during development, since mutant alleles of these loci affect chromosome integrity during mitotic cell divisions||. In flies heterozygous for appropriate recessive somatic cell (cuticle) markers, somatic spots expressing the markers can arise spontaneously from mitotic recombination, mutation, or chromosome breakage that results in the loss of a fragment carrying the wild-type allele of a cell marker, rendering the marker hemizygous (24). Moreover, the somatic spots produced by each of these mechanisms have distinctive characteristics. For example, if two cell markers on one chromosome arm are heterozygous in a *trans* configuration (*a* +/+ *b*), then mitotic recombination can produce twin spots (*a* +/*a* + and + *b*/+ *b*), whereas deletions arising from chromosome breakage do not produce twin spots and the single spots [*a* +/( ) *b* or *a*( )/+ *b*] that are produced should have features characteristic of aneuploidy. Such features include (*i*) short, thin bristles resulting from deletion of one (or more) of the many *Minute* loci scattered throughout the genome [two doses of the wild-type alleles of all *Minute* loci are required for a normal bristle phenotype (25, 26)]; (*ii*) more severely abnormal bristles in instances of extensive aneuploidy (24); and (*iii*) a small clone size due to both the cell autonomous lengthening of the mitotic cell cycle produced by *Minutes* (27) and the fact that aneuploid cells produced late in development (when few cell divisions remain) have a higher probability of surviving than do cells possessing the same degree of aneuploidy generated earlier in development (P. Ripoll, unpublished observation). Thus, by examining the effects of recombination-defective meiotic mutants on the frequency and size of spontaneous somatic spots, as well as the morphology of bristles contained within these spots, it is possible to inquire whether the wild-type alleles of these loci (*i*) are required for mitotic as well as meiotic recombination and (*ii*) function to maintain the integrity of chromosomes in somatic cells. An examination of the frequency of spontaneous somatic spots in *mei-218*, $mei\text{-}9^a$, and *mei-41* flies (Table 1) shows that both *mei-41* and *mei-9* substantially increase the frequency of somatic spots, whereas *mei-218* does not differ from the control in either the frequency or type of somatic spots produced. That the particular pattern of effects evoked by a mutant is a characteristic of its locus is shown by the similar effects of two or more alleles for all three of these loci||.

mei-41 produces a 19-fold increase in the frequency of spontaneous somatic spots in the abdomen. This increase appears to be attributable to the production of deficiencies that delete the dominant alleles of the heterozygous cell markers used since (*i*) the increase in spots comes primarily from the production of single spots, and (*ii*) in approximately 20% of the single spots the bristles were morphologically abnormal, ranging from short and thin to poorly formed nubbins. In *mei-9*, the frequency of both single and twin spots is elevated. Some of the single spots in *mei-9* may result from chromosome breakage (rather than from failure of one member of a potential twin spot to give rise to a detectable clone) since approximately 20% of them encompassed morphologically abnormal bristles. The increase in frequency of twin spots in *mei-9* suggests that this allele increases mitotic recombination as well as chromosome breakage.

These results demonstrate that the wild-type alleles of *mei-9*

and *mei-41* are necessary for normal chromosome stability in somatic cells. If discontinuities in DNA structure can either be repaired or induce chromosome breakage or mitotic exchange, then disruption of a step in a repair or replication pathway by a recombination-defective mutant will increase the probability that such discontinuities in DNA structure will be diverted into pathways leading to mitotic recombination or chromosome breakage. The different phenotypes of the mutants suggest that they are defective either in the repair of different types of lesions or in different aspects of the repair of the same type(s) of lesions. Thus, the abnormality in DNA structure metabolized by the $mei\text{-}41^+$ gene product can initiate chromosome breakage but not mitotic exchange, whereas the abnormality metabolized by $mei\text{-}9^+$ is capable of inducing both mitotic recombination and chromosome breakage.

The occurrence of spontaneous mitotic recombination in *mei-9* homozygotes poses a problem, since it would be expected *a priori* that a locus involved in both meiotic recombination and somatic DNA metabolism would also be involved in mitotic recombination. However, if there be more than one pathway of mitotic recombination in *Drosophila*, then mutants such as *mei-9* could in fact be defective in one such pathway if this defect is obscured by the functioning of alternative pathways. Indeed, evidence for several components of x-ray-induced mitotic exchange in wild type has been presented by Haendle (29–31).

Mutagen sensitivity of meiotic mutants

Additional data on the somatic roles of loci identified by recombination-defective meiotic mutants come from an examination of the sensitivities of these mutants to killing by a variety of mutagens. Larvae bearing alleles of *mei-41* or *mei-9* are hypersensitive to killing by x-rays, UV, MMS, nitrogen mustard, and 2-acetylaminofluorene (Fig. 1; refs. 18 and 32, and footnotes ¶,‖, and **). The recombination deficiency and broad mutagen sensitivity of *mei-41* and *mei-9* alleles suggest that these loci specify functions essential for both meiotic recombination and the repair of damage caused by mutagens in somatic cells. However, neither *mei-218* nor its allele $mei\text{-}218^{6\text{-}7}$ (A. T. C. Carpenter and B. S. Baker, unpublished work) affects sensitivity to either x-rays or UV (Fig. 1; ‖); nor does *mei-218* alter sensitivity to killing with MMS, nitrogen mustard, or 2-acetylaminofluorene (32). Since meiotic recombination is reduced as severely by *mei-218* (8% of wild-type frequencies) as it is by the most defective *mei-9* allele, it may well be that the *mei-218* function is restricted to meiotic recombination.

DNA repair

Assays for excision repair, photorepair, postreplication repair, and repair of single-strand breaks have been developed for *Drosophila* (19, 32–34). Application of these tests to mutagen-sensitive and recombination-defective mutants provides a powerful approach for delineating the defects in such mutants.

Repair replication induced by mutagen treatment of larvae has been investigated with a technique that permits independent analysis of repair replication and semi-conservative DNA synthesis (34). In this system, the substitution of bromodeoxyuridine for thymidine during semi-conservative DNA synthesis results in a sufficient increase in DNA density to permit the separation of newly replicated DNA from unreplicated DNA by isopycnic centrifugation. Equivalent levels of substitution fail to produce a detectable density shift when only short stretches of DNA are replaced during repair replication. Thus, precursors (e.g., bromodeoxyuridine and [^{3}H]thymidine) which contribute both increased density and radioactivity to the product permit the identification of repair replication as that synthesis which produces a labeled product of normal density. Assays of repair replication following UV treatment of $mei\text{-}9^b$ and control first instar larvae are presented in Fig. 2a††.

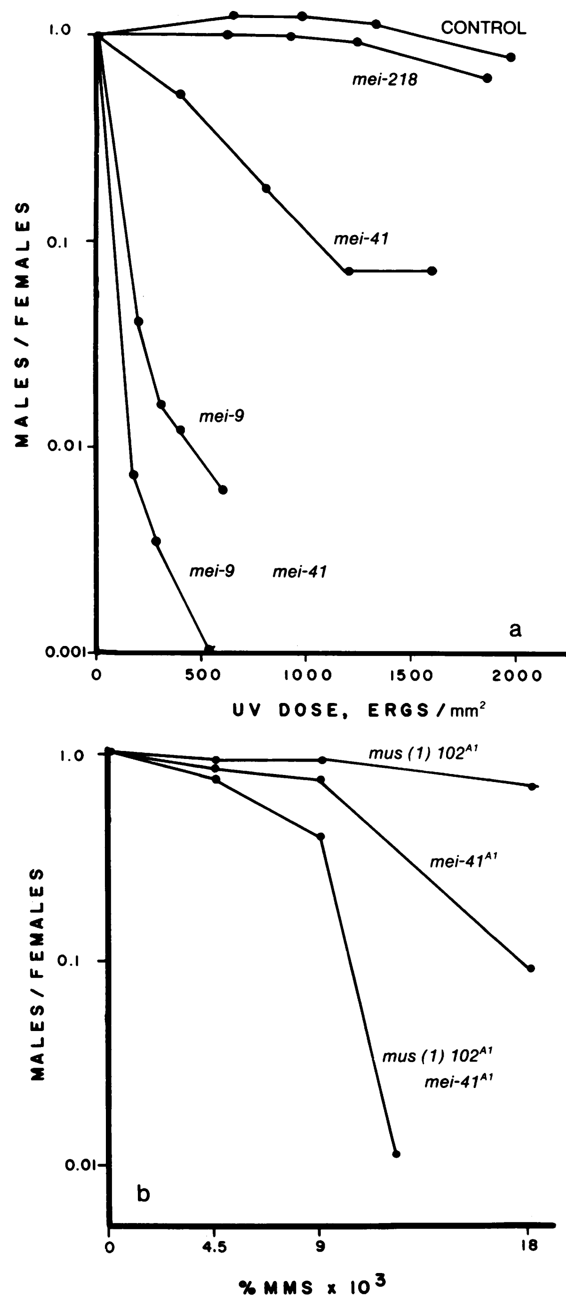

FIG. 1. Survival of males bearing the indicated X-linked meiotic mutant relative to female sibs having an attached X chromosome bearing the wild-type allele of the meiotic mutant. (a) Crosses are: y $mei\text{-}/y^+Y; spa^{pol}$ ♂ × $C(1)DX, y\, f\, bb^-/y^+Y; spa^{pol}$ ♀. Third instar larvae (83 ± 6 hr post oviposition) were collected and irradiated with a germicidal UV light. Care was taken to prevent photoreactivation. The numbers of emerging adults were recorded through day 17 post oviposition. (b) Crosses are $mei\text{-}/Y$ ♂ × $C(1)DX, y\, f\, bb^-/Y$ ♀. Parents were allowed to oviposit for 3 days and discarded, then 1 ml solutions of MMS of the various concentrations (vol/vol) were added to the developing cultures. The numbers of emerging adults were recorded through day 17 post oviposition.

†† T. D. Nguyen and J. B. Boyd, unpublished work.

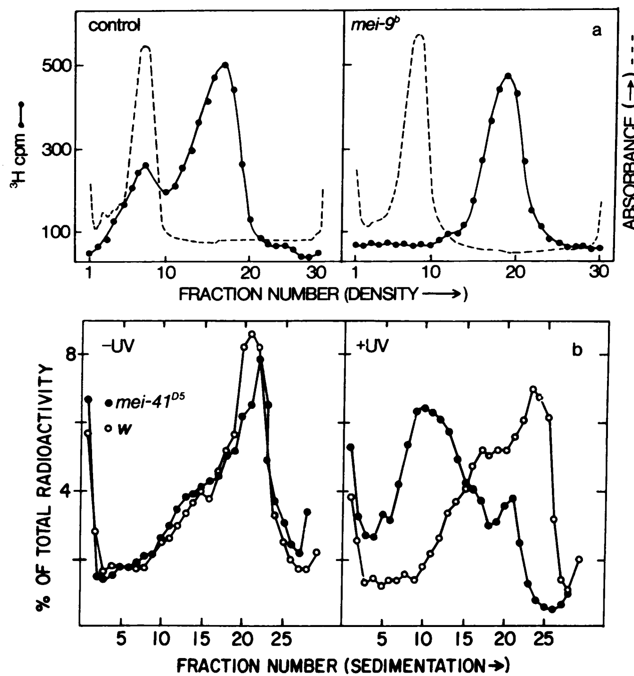

FIG. 2. (a) Repair replication in first instar larvae after UV treatment. First instar larvae were fed a solution containing streptomycin, penicillin, Fungizone, 5-fluorodeoxyuridine, and 5-bromodeoxyuridine for 3.5 hr at 25°. After irradiation with 20 J/m^2, the larvae were fed 4 additional hours with [^{3}H]thymidine added to the feeding solution. DNA was isolated from whole larvae and subjected to neutral isopycnic centrifugation. The broken line represents absorbance. For further experimental details, see ref. 34. The crosses are: *y cv mei-9^b; spa^{pol}* ♀ × *y cv mei-9^b/y$^+$Y; spa^{pol}* ♂ (right) and *y; spa^{pol}* ♀ × *y/y$^+$Y; spa^{pol}* ♂ (left). (b) Postreplication repair in primary cell cultures of *mei-41^{D5}*. Primary cell cultures were established from embryos of the homozygous stocks *w* and *w mei-41^{D5}*. After 20 hr at 25°, cultures were either irradiated with 10 J/m^2 from a germicidal UV lamp or left unirradiated. One half hour after irradiation, all cultures were exposed to 12 μCi/ml of [^{3}H]thymidine for 30 min. The cells were then incubated for 6 hr in nonradioactive medium before the labeled DNA was analyzed by sedimentation in alkaline sucrose gradients. All profiles were generated in separate gradients. For further experimental details, see ref. 19.

Whereas repair replication is readily detected as ^{3}H-labeled DNA co-banding with DNA of normal density (absorbance peak) in control animals, the absence of this fraction in *mei-9^b* animals suggests that repair replication is greatly reduced. The repair defect in *mei-9* mutants is also manifested as a reduction in the capacity to excise pyrimidine dimers (32). These results demonstrate that the *mei-9* locus functions in the repair of UV-induced damage.

Postreplication repair, an alternate form of dark repair, is defined operationally as a process that permits the formation of high molecular weight DNA strands in the presence of damaged templates. Analysis of postreplication repair in short-term cultures of embryonic cells has revealed that a *mei-41* allele dramatically reduces postreplication repair following UV treatment (19), whereas a *mei-9* allele has no effect on this process (32). In the experiment depicted in Fig. 2b (from ref. 19), alkaline sucrose gradients were employed to assay the molecular weight increase of newly synthesized DNA in UV-treated cells from *mei-41^{D5}* and control embryos. In unirradiated cells, there is no detectable difference between mutant and control molecular weight distributions. However, the molecular weight of pulse-labeled DNA from irradiated *mei-41^{D5}* cells is much lower than that of control cells after 6 hr of incubation. The *mei-41* locus thus functions in postreplication repair of somatic DNA as well as in meiotic recombination. The coincidence of these two phenotypes in the *mei-41* mutants suggests that postreplication repair in *Drosophila* may involve recombination of DNA molecules as it does in bacteria (35).

Interactions of mutants

The rationale for determining whether mutagen-sensitive loci affect the same or different pathways of DNA repair has been developed from the characterizations of multiple mutant strains of yeast (36, 37). Briefly, if there exist amorphic mutagen-sensitive mutants at two loci, then the double mutant should be as sensitive as the more sensitive of the two single mutants if the loci control steps in the same pathway. If, on the other hand, the loci control steps in different pathways, then the double mutant should be more sensitive than the most sensitive single mutant. In the latter case, if the genes compete with one another in the repair of a particular type of lesion, then mutants at these loci should interact synergistically, whereas if they act on different types of lesions caused by a single agent, mutant alleles would be expected to have a multiplicative interaction ("additive" on logarithmic plots of survival). (Whereas interactions of hypomorphic mutants are potentially more complex, the most straightforward interpretation of synergistic interactions of hypomorphic mutants is also that the loci control steps in different pathways.)

The sensitivity of the *mus(1)102 mei-41* double mutant to killing by MMS has been compared to that of these mutants singly (Fig. 1b; ¶). The double mutant is much more sensitive than is either of the single mutants and their interaction appears to be synergistic. This implies that these loci identify steps in two different pathways that provide *Drosophila* with alternative modes of repairing MMS damage in somatic cells.

Similarly, *mei-9^a* and *mei-41* appear to interact synergistically in increasing UV sensitivity (Fig. 1a; ‖), suggesting that these loci function in alternative pathways for the repair of UV-induced damage. *mei-41* and *mei-9* are known to be defective in meiotic recombination and the increase in nondisjunction caused by *mus(1)102* is consistent with its being recombination-defective also. Whether the functions specified by *mei-9* and *mei-41* [and similarly *mei-41* and *mus(1)102*] are used in different pathways during meiosis, as they seem to be in somatic cells, is not yet known, although *mei-9* and *mei-41* at least appear to affect different aspects of the meiotic recombinational process (20).

Conclusion

Of the approximately 16 X-chromosome loci so far identified as being involved in the processes of meiotic recombination and/or repair of mutagen damage, three have been most extensively examined by a variety of techniques. Mutants at all three loci (*mei-9, mei-218,* and *mei-41*) decrease the frequency of meiotic recombination; *mei-218* and *mei-41* also affect the chromosomal positions of exchange events. Mutations at the *mei-9* locus confer sensitivity to x-rays, UV, MMS, nitrogen mustard, and 2-acetylaminofluorene; increase the frequency of spontaneous somatic recombination and somatic chromosome breakage; do not increase the frequency of spontaneous mutation (A. T. C. Carpenter, unpublished observation); and reduce both repair replication and the rate of pyrimidine dimer excision. Mutations at the *mei-41* locus confer sensitivity to x-rays, UV, MMS, nitrogen mustard, and 2-acetylaminofluorene; increase the frequency of spontaneous somatic chromosome breakage; increase the frequency of both spontaneous and induced mutation (23); and are deficient in a form of postre-

plication repair. Mutations at the *mei-218* locus do not differ from controls in these parameters. Of the less extensively studied mutants, *mus(1)101* and *mus(1)102* increase meiotic nondisjunction (and hence may affect meiotic recombination), whereas *mus(1)103, mus(1)105,* and *mus(1)104* do not affect meiotic disjunction (and thus probably do not affect meiotic recombination). Thus, each of the three possible combinations of phenes is represented by at least one locus: meiotic-specific functions by *mei-218*, repair-specific functions by *mus(1)104* and possibly *mus(1)103* and *mus(1)105,* and functions used during both repair and meiosis by *mei-41, mei-9, mus(1)101,* and *mus(1)102*. It should be noted with respect to the latter mutants that repair defects are expressed in the somatic cells of both sexes (18,20,[¶,‖]); meiotic defects, on the other hand, are expressed in females in all instances but not in males in the two cases (*mei-9* and *mei-41*) for which this has been examined (20). The absence of a male meiotic effect is consistent with the conclusion that these loci specify functions common to repair processes and meiotic recombination, since meiosis in *Drosophila* males is achiasmate.

Moreover, the existence of at least two and possibly more independent pathways for repair of mutagen damage is suggested not only by the different phenotypes of the various mutants, but also by the synergistic effects of the two tested double mutant combinations.

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