THE *mei-9*^{a} MUTANT OF *DROSOPHILA MELANOGASTER* INCREASES MUTAGEN SENSITIVITY AND DECREASES EXCISION REPAIR[1]

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Manuscript received May 24, 1976
Revised copy received July 24, 1976

ABSTRACT

The *mei-9*^{a} mutant of *Drosophila melanogaster*, which reduces meiotic recombination in females (BAKER and CARPENTER 1972), is deficient in the excision of UV-induced pyrimidine dimers in both sexes. Assays were performed in primary cultures and established cell lines derived from embryos. An endonuclease preparation from *M. luteus*, which is specific for pyrimidine dimers, was employed to monitor UV-induced dimers in cellular DNA. The rate of disappearance of endonuclease-sensitive sites from DNA of control cells is 10–20 times faster than that from *mei-9*^{a} cells. The mutant *mei-218*, which is also deficient in meiotic recombination, removes nuclease-sensitive sites at control rates. The *mei-9*^{a} cells exhibit control levels of photorepair, postreplication repair and repair of single strand breaks. In *mei-9* cells DNA synthesis and possibly postreplication repair are weakly sensitive to caffeine. Larvae which are hemizygous for either of the two mutants that define the *mei-9* locus are hypersensitive to killing by the mutagens methyl methanesulfonate, nitrogen mustard and 2-acetylaminofluorene. Larvae hemizygous for the *mei-218* mutant are insensitive to each of these reagents. These data demonstrate that the *mei-9* locus is active in DNA repair of somatic cells. Thus functions involved in meiotic recombination are also active in DNA repair in this higher eukaryote. The results are consistent with the earlier suggestions (BAKER and CARPENTER 1972; CARPENTER and SANDLER 1974) that the *mei-9* locus functions in the exchange events of meiosis. The *mei-218* mutation behaves differently in genetic tests and our data suggest its function may be restricted to meiosis. These studies demonstrate that currently recognized modes of DNA repair can be efficiently detected in primary cell cultures derived from Drosophila embryos.

EARLY suggestions that certain cellular functions might participate in both genetic recombination and DNA repair (see review by HOWARD-FLANDERS 1975) received strong support from observations of radiation sensitivity in recombination deficient strains of *E. coli* (CLARK and MARGULIES 1965). The molecular mechanism of a repair process which requires recombination functions was subsequently identified in that same organism (RUPP and HOWARD-FLANDERS

[1] ABBREVIATIONS: AAF—2-acetylaminofluorene; MMS—methyl methanesulfonate; xp—xeroderma pigmentosum.

1968). More recently WILDENBERG and MESELSON (1975) have detected an excision-like repair function which participates in recombination of bacteriophage lambda. Studies in fungi have extended the correlation between radiation sensitivity and recombination deficiency to eukaryotic organisms (see review by CATCHESIDE 1974). Genetic studies in fungi have recently been extended at the molecular level by the observation that an endonuclease of Ustilago, which is implicated in recombination, preferentially attacks DNA containing either UV photoproducts or mismatched base pairs (AHMAD, HOLLOMAN and HOLLIDAY 1975). Suggestive evidence for the existence of a similar relationship between repair and recombination in higher eukaryotes is also available in Drosophila (WATSON 1969; WATSON 1972; DONINI 1972; BOYD *et al.* 1976; BAKER, CARPENTER and RIPOLL 1976; SMITH 1976).

The first systematic search for mutants in Drosophila which disrupt genetic recombination was performed by SANDLER *et al.* (1968) with flies recovered from natural populations. This approach was extended by BAKER and CARPENTER (1972) who screened stocks bearing mutagenized (EMS-treated) *X* chromosomes. Ten mutants which affect meiotic recombination and produce elevated levels of nondisjunction in females were recovered from their screen. Genetic analysis of these, and other, recombination-defective mutants has suggested that most of these loci (including *mei-218*) specify functions that affect preconditions for meiotic exchange (SANDLER *et al.* 1968; PARRY 1973; BAKER and CARPENTER 1972; CARPENTER and SANDLER 1974; HALL 1972). However, two recombination-defective mutants, which define the *mei-9* locus, have unique genetic effects and were suggested to be defective in the exchange process. Further genetic analysis generated strong support for this hypothesis (CARPENTER and SANDLER 1974).

Application of a procedure for measuring DNA repair in Drosophila (BOYD and PRESLEY 1974) has demonstrated that the *mei-9* mutants are deficient in repair replication (NGUYEN and BOYD, in preparation). The investigation of this locus is extended in this report to include studies of mutagen sensitivity of larvae, and assays of the capacity of somatic cells to repair damage induced by X-rays and ultraviolet radiation. It is shown that *mei-9* larvae are hypersensitive to chemical mutagens and that cultured cells of *mei-9* embryos have a reduced capacity to excise pyrimidine dimers from DNA. These observations demonstrate that the *mei-9* locus is involved in DNA repair in somatic cells and suggest that this locus may function in the DNA exchange event of meiotic recombination.

MATERIALS AND METHODS

The procedures employed for establishing primary tissue cultures together with their analysis for repair of single-strand breaks and postreplication repair have been described (BOYD and SETLOW 1976).

Stocks. The mutant white (*w*) is employed as a control, because it exhibits normal recombination and radiation sensitivity. The stocks *y mei-9^a/Basc*; *spa^{pol}/spa^{pol}*, *y mei-9^b cv/Basc*; *spa^{pol}/spa^{pol}* and *y mei-218/Basc*; *spa^{pol}/spa^{pol}* together with the related control stock (*y/y*; *spa^{pol}/spa^{pol}*) were a gift from A. T. C. CARPENTER. Homozygous stocks were established and maintained without selection. In the text these stocks are referred to as *mei-9^a*, *mei-9^b*, *mei-218* and *y*, respectively. Males of these stocks all carry *y*+*Y*.

Analysis of mutagen sensitivity. Larval sensitivity to methyl methanesulfonate (MMS) was assayed as described previously (BOYD *et al.* 1976). Males from homozygous mutant stocks were crossed with *y f* compound *X* females [*C(1)DX, y f*]. For each determination three larval cultures were established with 15 pairs per bottle. After a two-day egg collection the parents were discarded and the cultures were held for an additional 24 hr to permit complete hatching of the embryos. At that time 1.25 ml of solution containing the mutagen was added. Cultures were incubated at 23° and the sex ratios of the emerging flies were determined up to day 15.

In testing for sensitivity to nitrogen mustard (mechlorethamine hydrochloride) and 2-acetylaminofluorene (AAF) embryos were collected over a period of 12 hr from population cages containing double X stocks. Isolated embryos were suspended in water and aliquots containing 1,000–1,200 embryos were distributed to ½ pint bottles for the nitrogen mustard tests. The vials employed in the tests for AAF sensitivity received 500–600 embryos each. At 72 hr after the initiation of egg collection the mutagen solution was added. Cultures were incubated at 25° with a relative humidity of 70%. Emerging adults were scored regularly to avoid loss of flies in the medium up to day 18.

Generation of permanent tissue culture. An established cell line has been obtained from the *w* stock with the aid of a procedure suggested by R. L. SEECOF (personal communication). Embryos (0.22 g, 3–19 hr postfertilization) were sterilized and homogenized in Schneider's Revised Medium containing 18% (V/V) heated fetal calf serum, 0.01% phenol red, 0.5% bacto-peptone (Difco), 4 mU/ml insulin, 100 units/ml penicillin, and 0.1 mg/ml streptomycin. The cells were pelleted without filtration and placed in a polystyrene bottle (25 cm^2, Corning). The culture was trypsinized on the third day and began to expand after three weeks. This line, which has been designated *w-1*, was initiated Oct. 1, 1974 and currently multiplies rapidly as single attached cells. Although this basic procedure has also produced a line of cells from the *mei-9^a* stock (*mei-9^a-2*), it has generally failed in the majority of cases due to the development of unidentified black centers which invade and destroy the cultures after 2–3 weeks. The two cell lines derived from stocks of *mei-9^a* and *mei-218* employed in this study were obtained with a procedure of I. SCHNEIDER (1972, and personal communication). This method involves the initial culture of large tissue fragments derived from embryos. Both cultures were initiated Jan. 30, 1975, and are designated *mei-9^a-1* and *mei-218*.

Established cell lines are transferred every 7–14 days in Medium B (Schneider's Revised Medium, 14% heated fetal calf serum, 0.5% bacteriological bacto-peptone (Difco), 2 mM glutamine, 0.005% phenol red, and 4 mU/ml insulin.

Assay for repair of endonuclease-sensitive sites. A modification of the procedure of BUHL, SETLOW and REGAN (1974) was employed with both primary cultures and established cell lines. Technical details, including the preparation of primary cultures, have been described (BOYD and SETLOW 1976). DNA was labeled in Medium A during a 20 hr incubation period. Two cultures of each cell line were exposed to separate isotopes at concentrations of 1.0 μCi/ml thymidine-^{3}H and 0.5 μCi/ml thymidine-^{14}C. These precursor concentrations have been shown to have equivalent effects, if any, on the observed molecular weight of the labeled DNA (BOYD and SETLOW 1976). The medium was replaced with unlabeled medium one hour or more prior to UV treatment. The ^{3}H-labeled culture of each pair was irradiated with 5 J/m^2 (fluence rate, 0.36 J/m^2/sec) after the medium had been removed.

Cells that were to be incubated further were returned to fresh medium. Immediately after irradiation cells which were to be tested for photorepair were covered with Puck's balanced salt solution (for medium N-16 without $NaHCO_3$, Microbiological Assoc., Bethesda). These cultures were then exposed to visible light produced by two fluorescent tubes (Westinghouse, 15W, LLX, Warm White, F15T8/WWX) at a distance of 18 cm for 30 min. During this period the culture dishes were covered with polystyrene lids and rested on white filter paper at about 23°. In some control experiments parallel cultures were held under identical conditions in the dark.

After the appropriate incubation, cells of parallel cultures were isolated in saline-EDTA and mixed to achieve an equal proportion of detectable ^{14}C and ^{3}H. The combined cells were collected by centrifugation, resuspended in 10% sucrose buffered with 0.05 M Tris at pH 7.6, and

held at 0° for 5 min. Cells obtained from primary cultures were then diluted 10-fold with cold water and suspensions of cells from established lines were diluted 20-fold. After a further 5 min at 0° the cell suspensions were spun at 2,000 rpm at 4° for 3 min. The supernatent was replaced with sufficient incubation buffer (0.03 M Tris, pH 8.0, 0.04 M NaCl, 0.01 M EDTA, 5 μg/ml calf thymus DNA) to produce a concentration of 10^4 cpm of each isotope/100 μl. A 100 μl aliquot of the cell suspension was incubated with 10 μl of an extract from *M. luteus* (fraction II_{AS}, CARRIER and SETLOW 1970) for 5 min (primary cultures) or 10 min (established cultures) at 37°. The reaction was terminated by the addition of 10 μl of 1N NaOH. 50 μl was subjected to sedimentation through alkaline sucrose after allowing one hour for cell lysis on the gradients.

RESULTS

Sensitivity of meiotic mutants to chemical mutagens. Mutagen sensitivity was evaluated by analyzing stocks in which only the male *X* chromosome carried a meiotic mutant. Larval cultures were exposed to the mutagen and the ratio of emerging male to female flies was scored. The females thus serve as an internal control in each culture against which the relative sensitivity of the mutation-bearing male is measured. Data presented in Figure 1 reveal that males carrying the *mei-218* mutation are no more sensitive to methyl methanesulfonate (MMS) or nitrogen mustard than are the control females or males carrying the chromosome from which the meiotic mutants were selected (*y*). Males bearing either

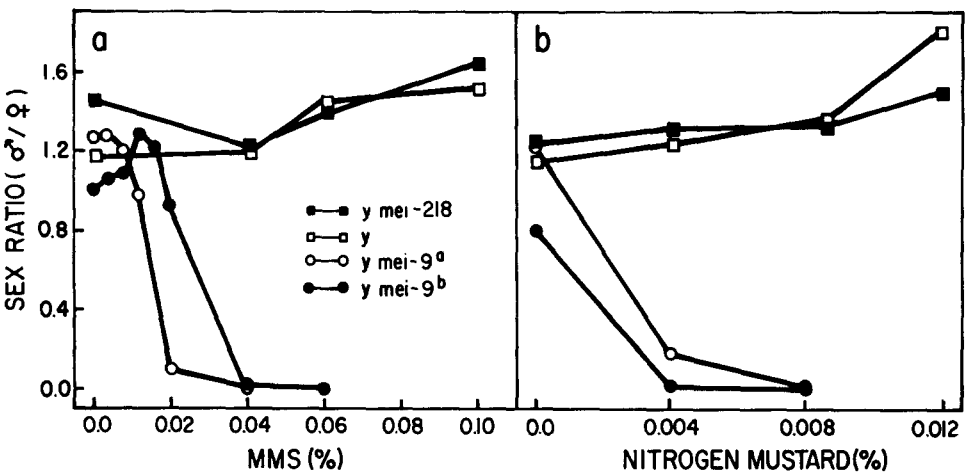

FIGURE 1.—Sensitivity of meiotic mutants to methyl methanesulfonate and nitrogen mustard. Mutagen solution (1.25 ml at the stated conc.) was added to larval cultures growing in 50 ml of standard media. The marker of interest was carried on the *X* chromosome of the males *y mei-9ª/y+Y*; spa^{pol}/spa^{pol}). Females employed in tests for MMS sensitivity carried the compound *X* chromosome *C(1)DX,y f*. In tests for nitrogen mustard sensitivity the female genotype was *C(1)DX, y f/y+Y*; spa^{pol}/spa^{pol}. The sex ratio of emerging flies is plotted against the concentration of mutagen solution added to the cultures. The average number of females per culture dropped by the following percentages within the stated mutagen range: 0–0.06% (V/V) MMS, 20% drop; 0–0.008% (W/V) nitrogen mustard, 37% drop. Experimental details are found under MATERIALS AND METHODS. The total number of embryos employed in the test with nitrogen mustard varied regularly from approximately 2,000 to 5,000 with the higher numbers applying to the higher mutagen concentrations.

of the *mei-9* alleles, however, are extremely sensitive to these mutagens. The relative sensitivity of the two alleles to MMS is correlated with their relative effects on meiotic recombination (BAKER and CARPENTER 1972) in that the *mei-9^a* allele is associated with the stronger mutant phenotype in both tests. The relative sensitivity of these two alleles to nitrogen mustard appears to be the reverse of that observed with MMS. The difference may, however, be related to the lack of isogenicity between mutant stocks. Data presented in Table 1 reveal a similar pattern of sensitivity among these mutants to the carcinogen 2-acetylaminofluorene (AAF). Whereas males carrying the control or *mei-218* chromosomes are relatively insensitive to this compound, both *mei-9* alleles exhibit an elevated sensitivity. In addition, we have detected an extreme sensitivity of *mei-9* cells to killing by UV (unpublished observations).

Repair of single-strand breaks. The capacity to repair single-strand breaks induced by X-rays has been investigated in primary cultures of *mei-9^a*, *mei-218* and *w*. In these experiments the molecular weight of cellular DNA was reduced by X-ray treatment. The subsequent increase in molecular weight was measured by sedimentation in alkaline sucrose gradients. Control experiments performed with unincubated cultures that had either been irradiated or left unirradiated are reported elsewhere (BOYD and SETLOW 1976). Mutant and control strains were labeled with different isotopes under conditions which produce no specific isotope effects (BOYD and SETLOW 1976). The data in Figure 2 demonstrate that uniformly labeled DNA recovers its molecular weight after X-ray treatment at the same rates in both *mei-9^a* and *w* cells. This same result has also been obtained in a comparison of *mei-9^a* and *mei-218* cells. Thus, *mei-218* cells also repair this type of damage at a normal rate.

Influence of caffeine on postreplication repair and DNA synthesis. The rate with which newly synthesized DNA increases in molecular weight has been determined as a function of exposure to caffeine and UV radiation (Figures 3, 4).

TABLE 1

Sensitivity of meiotic mutants to 2-acetylaminofluorene (AAF)

X chromosome carried by male	♂/♀ ratio among emerging flies		
	H_2O	70% ethanol	0.8% (W/V) AAF in 70% ethanol
y	1.43 (1,152)	1.03 (659)	1.62 (816)
mei-218	1.56 (1,565)	1.14 (1,378)	1.07 (1,659)
mei-9^a	1.44 (1,272)	1.59 (743)	0.03 (353)
mei-9^b	1.12 (1,386)	1.13 (1,649)	0.04 (532)

Chemical sensitivity was determined as described in Figure 1 with the exception that the tested compound was dissolved in 70% ethanol. One-half ml was added to larval cultures growing in 20 ml of standard medium in plastic vials. The total number of flies scored is included in brackets. The total number of embryos tested was 2,500 for the controls and 3,500 for the carcinogen tests. The average number of females per culture was depressed to 39% of control values by the addition of AAF. Further experimental details are found under MATERIALS AND METHODS.

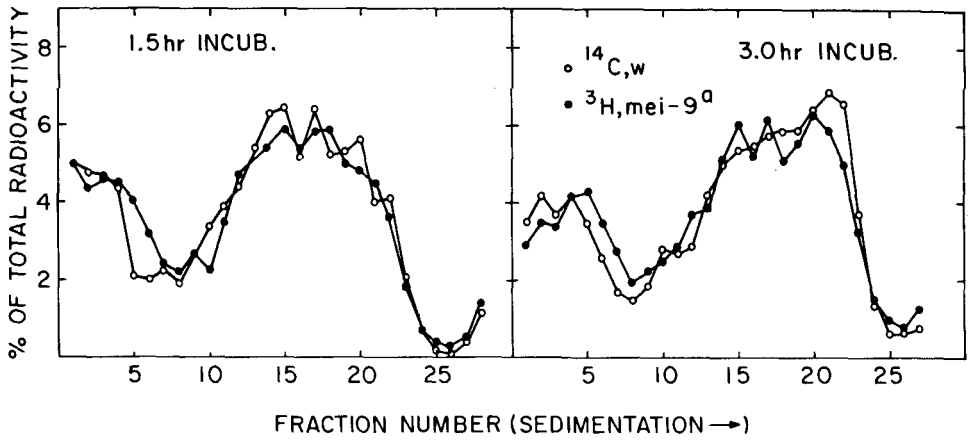

FIGURE 2.—Repair of single-strand DNA breaks in *mei-9^{a}*. Primary tissue cultures were labeled, irradiated, and incubated, as described (BOYD and SETLOW 1976) with the following exceptions: The mutant culture was labeled in 1 ml of medium with ^{3}H-thymidine at a concentration of 0.5 μCi/ml. Each culture was established from 17 mg of embryos which produced cells having a specific activity of 0.018 cpm/cell. Prior to centrifugation the mutant cells were mixed with control cells (*w*) which had been labeled with 0.5 μCi/ml ^{14}C-thymidine to a specific activity of 0.007 cpm/cell. Alkaline sucrose gradients were layered with a total of 3.5×10^{5} cells containing 1,000–2,000 cpm of each isotope. All cultures were irradiated with 10 kR of X-rays prior to incubation. Centrifugation was continued for 160 min at 25,000 rpm in the Beckman 50.1 rotor.

In this assay, newly synthesized DNA is identified as a lower molecular weight species which sediments near the top of an alkaline sucrose gradient immediately following a pulse labeling period of 30 min (BOYD and SETLOW 1976). All cultures employed here were incubated for an additional 3 hr following the 30 min pulse labeling period in order to determine the molecular weight increase of the labeled DNA. The results obtained with primary mutant cultures are compared with parallel experiments performed with control cells (*w*). Where appropriate, UV treatment preceded the pulse labeling period by 30 min, and caffeine was present at all times after the irradiation period. The data in Figure 3 reflect our failure to detect any significant difference between postreplication repair or DNA synthesis in *mei-218* and control cells (see also Table 2, BOYD and SETLOW 1976). Cells carrying the *X* chromosome from which the meiotic mutants were isolated (*y*) also behave identically to *w* cells under the two conditions involving UV radiation (unpublished observations). Thus, the *w* strain provides a satisfactory control for the meiotic mutants in these experiments. In the absence of caffeine the *mei-9^{a}* strain also exhibits normal rates of DNA synthesis and postreplication repair in this test (Figure 4). Both functions, however, appear to be slightly reduced in the presence of caffeine. Although we have encountered some variability between experiments with the use of caffeine, this difference is considered to be significant, because the data obtained from the *mei-9* and *mei-218* strains were derived from the same experiment. The fact that cultures from all three

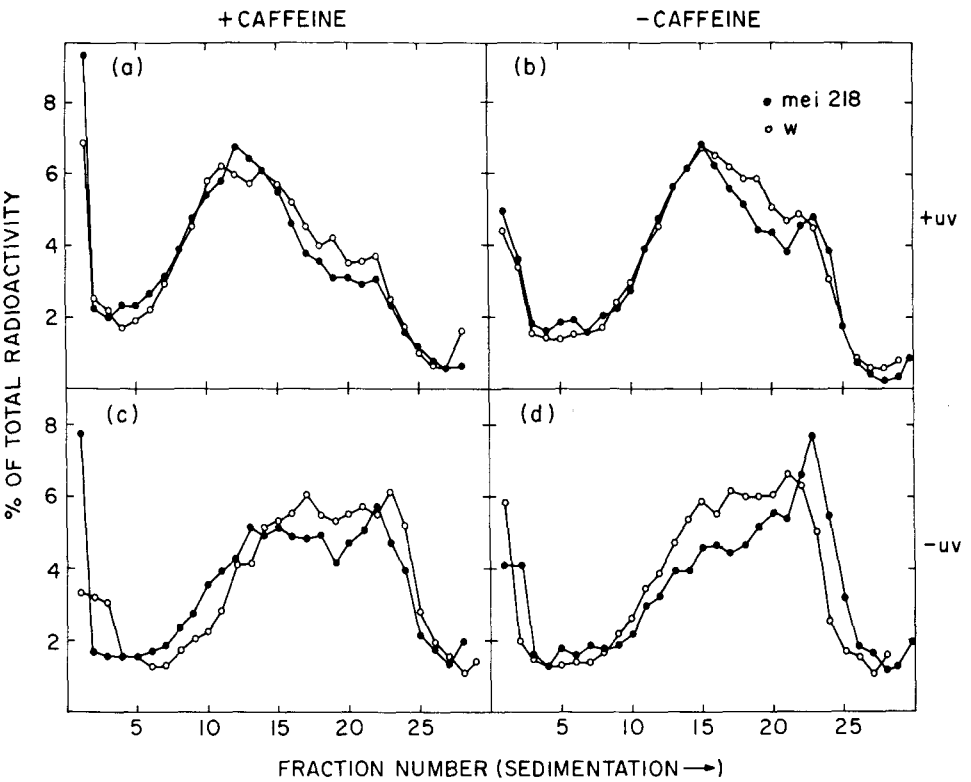

FIGURE 3.—Postreplication repair and DNA synthesis in *mei-218*. Primary cultures were irradiated, labeled, and incubated for 3 hr as described (BOYD and SETLOW 1976). Each curve was obtained from a separate gradient labeled with a single isotope. The control curves (*w*) also appear in Figure 5 of the previous report. Mutant cell cultures were prepared with 44–70 mg of embryos/dish. Gradients were layered with 4,500–8,500 cpm ^{3}H.

strains achieved equivalent specific radioactivities in these experiments reduces the possibility that the differences observed were due to variations in the metabolic state of the cultures.

Excision repair. The presence of pyrimidine dimers in UV-treated DNA can be detected by exposure of DNA to a repair endonuclease from *M. luteus* (CARRIER and SETLOW 1970). This enzyme can also be applied to monitor dimers in whole eukaryotic cells when the cells are first rendered permeable to the enzyme by osmotic shock (WILKINS 1973; BUHL, SETLOW and REGAN 1974). We have employed the latter approach to follow the level of endonuclease-sensitive sites in the DNA of irradiated cultures of established cell lines (Figure 5). In these experiments cultures were irradiated and incubated for various times prior to treatment with endonuclease. After endonuclease treatment the molecular weight of the cellular DNA was measured by centrifugation of irradiated (^{3}H labeled) and unirradiated (^{14}C labeled) samples in the same gradient. In the absence of any incubation, (Figure 5, top right panel) DNA of irradiated control

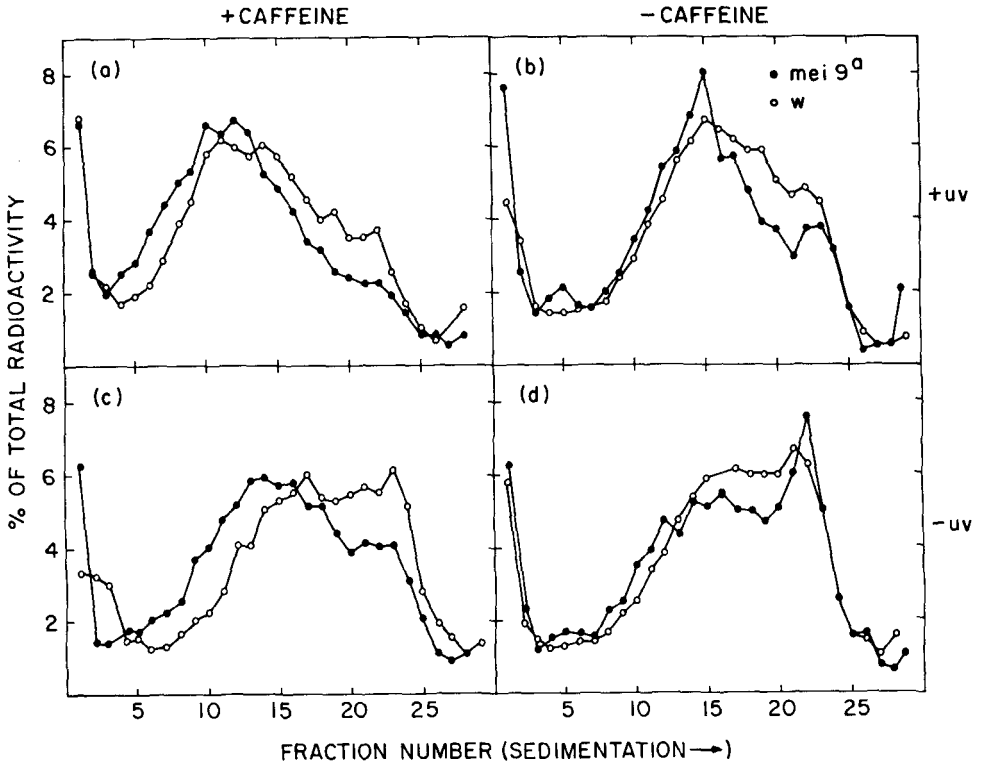

FIGURE 4.—Postreplication repair and DNA synthesis in *mei-9ª*. Experimental conditions are those described under Figure 3. The mutant cultures were prepared with 33–50 mg of embryos. Gradients received a total of 2,400–4,600 cpm ^{3}H.

cells sediments with a weight average molecular weight of 1.4×10^8 while the corresponding value for unirradiated cells is 3.12×10^8. The nuclease likewise recognizes radiation damage in cells derived from the *mei-9ª* strain (Figure 5, top left panel). In the latter case, however, a portion of the DNA is seen to have a molecular weight characteristic of unirradiated cells. This shoulder in the curve probably reflects a heterogeneous exposure of the cells to radiation as a result of the tendency of these cultures to form clumps. Alternatively, this difference between cultures may reflect the difference in their ages (6 and 10 months) or variable penetration of the nuclease.

As the control cultures are incubated for increasing periods of time after irradiation, the proportion of endonuclease-sensitive sites decreases (Figure 5) as indicated by the decreasing difference between irradiated and unirradiated cultures. Thus, between 3 and 6 hr of incubation the weight average molecular weight of the DNA from irradiated cells increases from 1.95×10^8 to 2.40×10^8 daltons, and by 23 hr it has achieved 96% recovery. The *mei-9ª* cultures, on the other hand, remove endonuclease-sensitive sites very slowly from their DNA. After 3 and 6 hr of incubation the weight average molecular weight of the irradiated DNA remains 47% that of the unirradiated DNA. At 23 hr this value

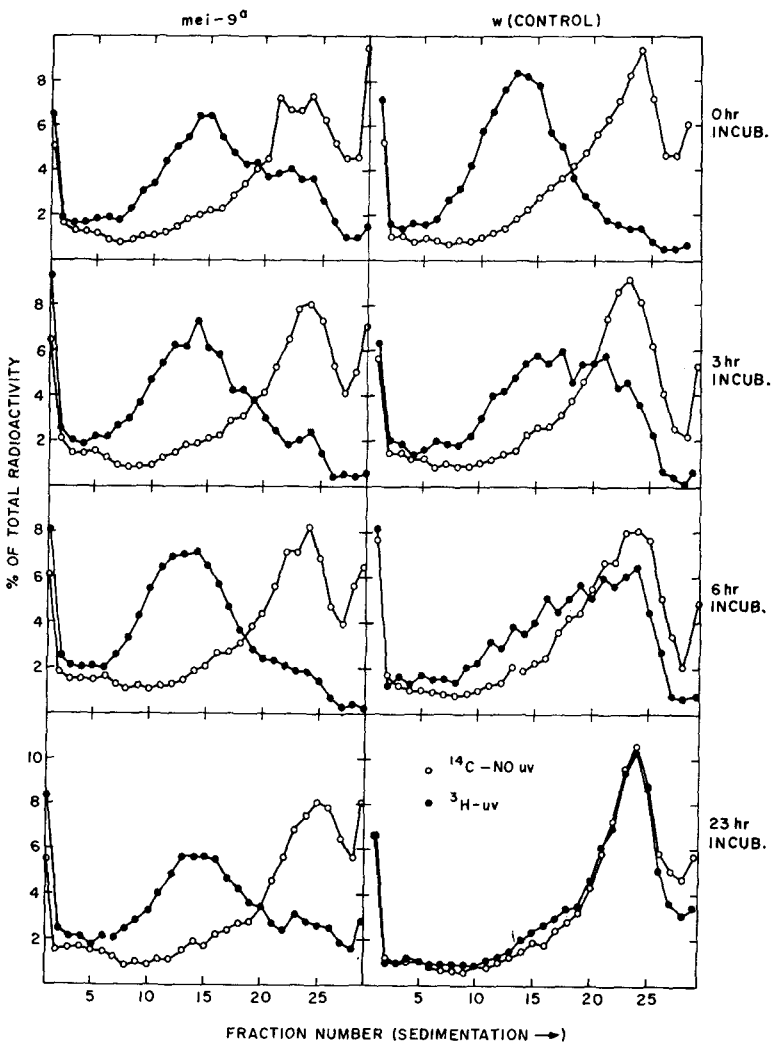

FIGURE 5.—Kinetic analysis of endonuclease-sensitive sites in the established cell lines *mei-9a-1* and *w-1*. Cultures of each cell line were labeled overnight with either ^{3}H or ^{14}C as described in MATERIALS AND METHODS. Mutant cultures contained 2×10^{6} cells/dish and the control cultures contained up to 5×10^{6} cells. The ^{3}H labeled cultures received 5 J/m^{2} of UV radiation, and all cultures were further incubated for the specified times. Cells from parallel cultures were then mixed and assayed for the presence of endonuclease-sensitive sites. Between 2,000 and 7,900 cpm of each isotope were layered on alkaline sucrose gradients in a maximum of 0.5×10^{6} cells. A single ^{14}C labeled culture from each strain was used to produce the control pattern for both the 3 and 6 hr incubation times. Each panel represents a single sucrose gradient. Different cell preparations were employed for the 0 and 23 hr incubations than were used in the 3 and 6 hr incubations.

is 56%, and only after 48 hr have the mutant cultures achieved a level of repair reached by the controls in 3 hr. Thus, *mei-9^a* is associated with roughly a 16-fold reduction in the repair rate of detectable endonuclease-sensitive sites. Irradiated *mei-9* cells do not spontaneously develop DNA nicks when assayed in the absence of exogenous nuclease (J. M. MASON, personal communication).

The data presented in Figure 6 extend the above conclusions to primary cultures of *mei-9^a* and *w*. Again, fresh cultures of the control strain lose most endonuclease-sensitive sites after one day's incubation while those of the *mei-9^a* cells retain such sites. The magnitude of the effect demonstrates that *mei-9^a* reduces repair in both sexes. If the embryos of only one sex were affected, the overall reduction in repair capacity of the cultures would be less than one half. These data are less precise than can be obtained with established cell lines, because the endonuclease assay is more difficult to control with primary cultures. The high molecular weight material found in the irradiated samples which were not incu-

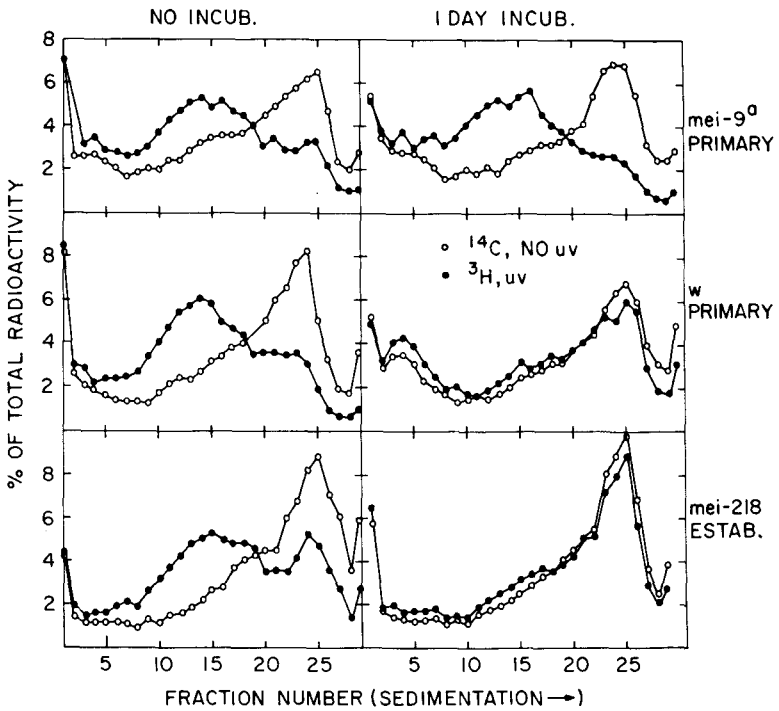

FIGURE 6.—Analysis of endonuclease-sensitive sites in primary cultures of *mei-9^a* and *w* and in an established cell line of *mei-218*. Analysis was performed as described in Figure 5 with the exception that freshly established primary cultures were employed from the *mei-9^a* and *w* strains. The *mei-9^a* cultures were each derived from 43 mg of embryos and were labeled to a specific activity of either 0.022 cpm/cell ^{3}H or 0.016 cpm/cell ^{14}C. The data for the *w* cultures are derived from two different experiments. Cultures of the established cell line *mei-218* were labeled to a specific activity of 0.013 cpm/cell ^{3}H. These cultures were highly clumped at a density of 4×10^6 cells/plate. The level of either isotope added to the gradients varied from 2.8–9.3 $\times$ 10^3 cpm.

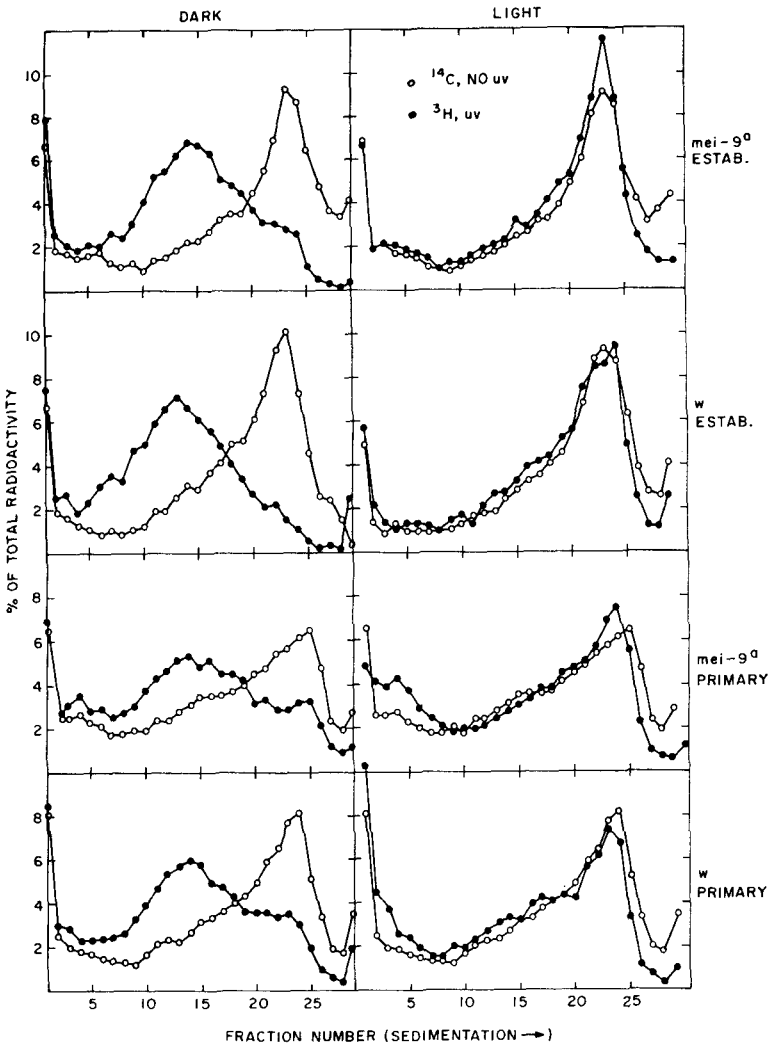

FIGURE 7.—Photorepair of endonuclease-sensitive sites in DNA of UV-irradiated cells. Cultures of established cell lines were labeled at densities of 4.9×10^6 cells/dish (*w-1*) and 2.3×10^6 cells/dish (*mei-9^a-1*). Cells of the *mei-9^a-1* culture were somewhat clumped. After UV treatment cultures of established lines were placed under saline and exposed to light or dark conditions for 30 min. Individual primary cultures were prepared from 43 mg (*mei-9^a*) or 37 mg (*w*) of embryos and labeled to equivalent specific activities. In the experiments employing primary cultures the only sample which was incubated for 30 min under saline was the one receiving both forms of irradiation. In these experiments a single unirradiated control was run in the same gradient with cells that had received no light. This control is reproduced with the data from light exposed cells for comparison. All other gradients contained two isotopes.

bated may be due to experimental problems or to the shielding of some of the cells from radiation in the complex cultures.

The lower two panels of Figure 6 reveal that the established cell line of *mei-218* is capable of removing endonuclease-sensitive sites. Again, the high molecular

weight DNA in the irradiated sample which was unincubated is undoubtedly due to the presence of large cellular clumps in this dense culture. DNA of irradiated cells recovers 92% of the molecular weight of unirradiated cells after a day's incubation. Therefore a deficiency in meiotic recombination is not a sufficient condition for the repair deficiency observed in *mei-9*^{a} cells.

Photorepair of endonuclease-sensitive sites. Exposure of cells to visible light for 30 min after exposure to UV radiation also abolishes the endonuclease-sensitive sites (Figure 7). This photorepair system is equally effective in primary cultures and established cell lines of both *w* and *mei-9* cultures. The more rigorous controls employed with the established cell lines prove that the observed repair is not due to a mechanism which is acting independently of light during the 30 min of light exposure. Such an explanation is also untenable for the primary cultures in view of the rate of dark repair seen in Figure 5. Thus, in each of these cell types photorepair is capable of eliminating the majority of the detectable endonuclease-sensitive sites.

DISCUSSION

Somatic effects of mei-9

Our principle conclusion is that the *mei-9* locus is essential for an excision repair process which acts on pyrimidine dimers. The extreme sensitivity of *mei-9* mutants to chemical mutagens further supports the conclusion that this locus is essential for a somatic repair function. The tests of chemical sensitivity were performed with three agents which generate different classes of lesions in cellular DNA. In mammalian systems AAF is a UV mimic (SETLOW and REGAN 1972), MMS is a monofunctional alkylating agent (SINGER 1975), and nitrogen mustard can cross-link DNA (BROOKES and LAWLEY 1961). Radiation studies performed in this (NGUYEN and BOYD, in preparation) and another laboratory (BAKER, CARPENTER and RIPOLL 1976) have demonstrated that this locus is also associated with an extreme sensitivity to UV and ionizing radiation. Somatic cells of *mei-9* larvae therefore have a reduced capacity to repair each of these forms of DNA damage. The broad spectrum of sensitivity exhibited by *mei-9* mutants may prove of value in screens for carcinogens (NGUYEN, under investigation). The sensitivity of *mei-9* males to AAF supports earlier suggestions (FAHMY and FAHMY 1972) that Drosophila can activate this procarcinogen to the proximate carcinogen.

The observation that larvae which carry either of the *mei-9* alleles are mutagen-sensitive indicates that the repair deficiency observed in these strains is also due to the *mei-9* mutation. Both alleles of the *mei-9* gene were recovered independently in experiments designed to recover recombination-deficient mutants (BAKER and CARPENTER 1972). The increased nondisjunction and reduced recombination observed in both strains has been mapped to a single chromosomal locus (CARPENTER and SANDLER 1974). Thus, mutagen sensitivity and a deficiency in recombination are both associated with two separately isolated mutants. This correlation makes it highly probable that the two phenotypes are

associated with an alteration in the same gene. Although the data on mutagen sensitivity reported here only demonstrate that males bearing *mei-9* mutants are sensitive to mutagens, controls for complementation analyses reported elsewhere (BOYD *et al.* 1976) have demonstrated that homozygous *mei-9* females are also sensitive to MMS. As described earlier in the text, the magnitude of the defect in excision repair observed in primary cultures demonstrates that the *mei-9* mutations disrupt excision repair in embryos of both sexes.

Relctionship of repair and meiosis

Our results clearly establish the involvement of a meiotic function in somatic cell repair. The two mutations *mei-9*^{a} and *mei-218* both reduce meiotic recombination to about 8% of control levels (BAKER and CARPENTER 1972). Extensive genetic analysis, however, has implicated these two loci in different aspects of the recombination process (BAKER and CARPENTER 1972; CARPENTER and SANDLER 1974). Those studies suggest that the *mei-9* mutations disrupt the exchange process itself whereas the *mei-218* mutant affects a precondition for exchange. Our observations of the repair capacity of these two mutant classes provide further support for this hypothesis. The excision-deficient property of *mei-9*^{a} cells is consistent with the existence of a nuclease which is active in both meiotic recombination and somatic cell repair. Since we have been unable to detect any defect in somatic cells of *mei-218*, it is probable that the function defined by this mutation is restricted to the meiotic process. Studies of somatic chromosome exchange (BAKER, CARPENTER and RIPOLL 1976) have also failed to detect effects of *mei-218* in somatic cells whereas *mei-9* exhibits dramatic somatic effects.

Excision analysis

There are two compelling arguments for equating the UV lesions recognized by the *mei-9* repair system with pyrimidine dimers. (1) The same lesions which are repaired by the normal dark repair process in control cells can also be photoreactivated. Thus, the dark repair system under study is probably acting on pyrimidine dimers, because enzymatic photorepair systems studied to date are specific for pyrimidine dimers (RUPERT 1975). (2) The nuclease preparation used in our assays has a strong specificity for pyrimidine dimers (CARRIER and SETLOW 1970). Since the endogenous repair mechanism removes lesions which are also recognized by the exogenous nuclease, the common lesion recognized by both systems is probably pyrimidine dimers.

The results obtained with the exogenous endonuclease suggest that excision repair of Drosophila DNA is normally complete one day after irradiation. The actual sealing of the single-strand nicks, which are presumably formed in the excision process, is apparently a rapid process, because we have been unable to detect such breaks 1.5 hrs after irradiation of wild-type cells with 15 J/m^2. If nicks are introduced in *mei-9* cells during *in vivo* incubation, they are rapidly resealed because analyses performed without exogenous nuclease produced no evidence of DNA nicks (J. M. MASON, personal communication).

Numerical analysis of data obtained from unincubated control cells (Figure 5, top right) indicate that excision may be less extensive than the gradient profiles suggest. The number average molecular weights of DNA obtained from irradiated and unirradiated cells have been used to estimate the number of breaks introduced by the nuclease (BUHL, SETLOW and REGAN 1974). These calculations indicate that the nuclease has made one nick/10^8 daltons in the DNA of unincubated control (w) cells. Extrapolation from chemical assays performed after high UV doses has shown that the UV fluence (5 J/m^2) administered to the cells in the endonuclease experiments converts 0.009% of the thymine in DNA of Drosophila cells to thymine-containing dimers (assay of CARRIER and SETLOW 1971). This value leads to the estimate that a dose of 5 J/m^2 produces 5.4 pyrimidine dimers/10^8 daltons (SETLOW *et al.* 1969). According to this calculation the endonuclease is acting on 1 out of 5 pyrimidine dimers. Similar results have been reported in applications of this assay to human (WILKINS 1973), marsupial (BUHL, SETLOW and REGAN 1974) and bacterial (GANESAN 1973) cells. It is probable that the principal reasons for these results is the non-uniform accessibility of the DNA in chromatin to exogenous enzymes, because the number of breaks made by the endonuclease in DNA extracted from irradiated cells equals approximately the number of dimers (PATERSON and LOHMAN 1975). The apparent inefficiency of the reactions in our experiments may also have been due to the fact that the assays were not performed with saturating concentrations of enzyme, because no detectable nicks are observed when the enzyme concentration is reduced only 5-fold.

Difficulties with the above calculation, on the other hand, suggest that excision is in fact more complete than those pessimistic estimates suggest. Since the larger DNA fragments accumulate near the bottom of the gradients, the molecular weight of that DNA may be greatly underestimated in our calculations. This error leads to an underestimate of the number of endonuclease-sensitive sites. Evidence for more complete excision in Drosophila cells comes from chemical analysis of dimers remaining in Schneider's cell line #2 one day after UV treatment (TROSKO and WILDER 1973). These data demonstrate that 40–60% of the dimers remain in the DNA after an exposure of 25–75 J/m^2. Excision may be even more complete in our studies than in those reported by TROSKO and WILDER (1973), because they employed UV doses which are ten times those reported here. We therefore presume that excision repair in our control cells (w) is at least as extensive as the value of 50% reported by TROSKO and WILDER (1973).

Related phenotypes in other organisms

The finding of mutagen sensitivity in strains which were originally isolated for deficiencies in meiotic recombination implicates a recombination function in the repair of somatic cell damage. This observation parallels those of prokaryotic mutants in which recombination deficiencies are frequently associated with mutagen sensitivity (HOWARD-FLANDERS 1975). However, in prokaryotes excision deficiency and recombination deficiency have not been associated by a single

mutational event (HOWARD-FLANDERS 1968) as they have in the *mei-9* mutants. An additional feature of the *mei-9* mutants which distinguishes them from the excision-deficient *uvr*⁻ mutants of *E. coli* (HOWARD-FLANDERS, BOYCE and THERIOT 1966) is that they are highly sensitive to MMS and ionizing radiation. The joint utilization of a single gene product for excision repair and meiotic processes does apparently have a parallel in a DNase-deficient strain of *Ustilago maydis* (BADMAN 1972) which is deficient in gene conversion (HOLLIDAY *et al.* 1974). The corresponding enzyme in wild type cells has been shown to act on UV-irradiated DNA (HOLLIDAY *et al.* 1974) and mismatched bases in native DNA (AHMED, HOLLOMAN and HOLLIDAY 1975). The excision-deficient forms of xeroderma pigmentosum (xp) in man also affects a function related to that defined by the *mei-9* mutants. Like the *mei-9* mutants, xp cells are sensitive to UV-radiation and AAF (CLEAVER and BOOTSMA 1975). Mutants in both classes exhibit nearly normal postreplication repair (this report and LEHMANN *et al.* 1975). The analogy is not complete, however, because at least some xp strains are insensitive to MMS whereas *mei-9* cells are highly sensitive to this mutagen.

Speculation on mechanism

Although the *mei-9* mutants also share the property of excision deficiency with the *uvr*⁻ mutants of *E. coli* they are associated with a genetic recombination deficiency which has not been demonstrated in the *uvr*⁻ mutants. Another feature of the *mei-9* mutants which distinguishes them from excision-deficient mutants of *E. coli* and man (Figure 1; CLEAVER and BOOTSMA 1975; HOWARD-FLANDERS, BOYCE and THERIOT 1966) is their extreme sensitivity to MMS and ionizing radiation. Since repair of single-strand breaks appears to be unaffected by *mei-9* (Figure 1), its sensitivity to X-rays probably reflects a failure to repair alternate forms of X-ray-induced damage, which are normally recognized by the excision mechanism. The difference in mutagen sensitivity between these mutant classes suggests that the specificity of the excision repair process in Drosophila may be much broader than that of other organisms. Whereas the mismatch and dimer-excision functions appear to be separate in *E. coli* (WILDENBERG and MESELSON 1975), these two related functions may be performed by the same mechanism in Drosophila. In fact, the *mei-9* locus may perform more than one function in meiotic recombination. Its excision-related function suggests that it could play a part in correcting heteroduplexes or breaking cross strands in recombination intermediates (HOTCHKISS 1974).

It is also possible that the *mei-9* locus itself controls a variety of functions as does the *rec A* gene in *E. coli* (RADMAN 1975). This possibility is supported by the observation that the *mei-9* mutants are associated with an unusually broad spectrum of phenotypes for excision-deficient mutants. A further alternative is that the *mei-9* product may be necessary to maintain the integrity of a molecular complex which incorporates more than one repair function. Finally, our data demonstrate that different recombination functions can participate in entirely different repair functions. Whereas *mei-9* disrupts excision repair without

influencing postreplication repair, other meiotic mutants exhibit defective postreplication repair (BOYD *et al.* 1976).

SUSIE F. BOYD provided invaluable technical assistance throughout the course of this work. ELEANOR GRIST, RANDY BOYD, KAREN E. SMITH, and ARTHUR FONG periodically participated in the experiments. K. H. THOMPSON assisted with the analysis of the data. DRS. R. L. SEECOF, I. SCHNEIDER and S. J. O'BRIEN generously provided the information that was essential for the tissue culture work. We are grateful to DRS. A. T. C .CARPENTER and B. S. BAKER for sharing their mutants with us. DR. L. SANDLER first called our attention to the potential importance of the *mei-9* locus in DNA repair. DRS. B. S. BAKER, A. T. C. CARPENTER, M. M. GREEN, S. G. SEDGWICK, D. V. MARTENSEN, and J. M. MASON contributed valuable comments on the manuscript. The work was supported in part by a fellowship from the Guggenheim Foundation and Grant No. E(04-3)34, #210 from the U. S. Energy Research and Development Administration (both to J. B. B.). The physical and chemical studies were carried out at Brookhaven National Laboratory under the auspices of the U. S. Energy Research and Development Administration. Mutagen sensitivities were measured in Davis.

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Corresponding editor: S. WOLFF