CHARACTERIZATION OF POSTREPLICATION REPAIR IN MUTAGEN-SENSITIVE STRAINS OF *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

Mutants of *Drosophila melanogaster*, with suspected repair deficiencies, were analyzed for their capacity to repair damage induced by X-rays and UV radiation. Analysis was performed on cell cultures derived from embryos of homozygous mutant stocks. Postreplication repair following UV radiation has been analyzed in mutant stocks derived from a total of ten complementation groups. Cultures were irradiated, pulse-labeled, and incubated in the dark prior to analysis by alkaline sucrose gradient centrifugation. Kinetics of the molecular weight increase in newly synthesized DNA were assayed after cells had been incubated in the presence or absence of caffeine. Two separate pathways of postreplication repair have been tentatively identified by mutants derived from four complementation groups. The proposed caffeine sensitive pathway (CAS) is defined by mutants which also disrupt meiosis. The second pathway (CIS) is caffeine insensitive and is not yet associated with meiotic functions. All mutants deficient in postreplication repair are also sensitive to nitrogen mustard. The mutants investigated display a normal capacity to repair single-strand breaks induced in DNA by X-rays, although two may possess a reduced capacity to repair damage caused by localized incorporation of high specific activity thymidine-^{3}H. The data have been employed to construct a model for repair of UV-induced damage in Drosophila DNA. Implications of the model for DNA repair in mammals are discussed.

AMONG the known dark repair mechanisms, excision repair is believed to be the most nearly error-free process for eliminating many types of damage from single DNA strands. If an advancing replication fork reaches a lesion before excision can act, however, the cell must resort to a temporary means of neutralizing DNA damage. The term postreplication repair refers to those cellular mechanisms which permit synthesis of high molecular weight DNA in the presence of a damaged parental strand (review by LEHMANN 1975). This process has been well characterized in UV-irradiated *E. coli* in which the synthetic apparatus presumably leaves a gap in the daughter strand opposite a pyrimidine dimer in the parental strand (RUPP and HOWARD-FLANDERS 1968). These gaps are subsequently repaired by recombination between the newly replicated sister

[1] ABBREVIATIONS: MMS—methyl methanesulfonate; Tris—tris(hydroxymethyl)aminomethane; EDTA—ethylenediaminetetraacetic acid; UV—ultraviolet irradiation; HN2—nitrogen mustard (mechlorethamine); AAF—2-acetylaminofluorene (2-acetamidofluorene).

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chromosomes (RUPP *et al.* 1971). This process essentially provides a mechanism for recovering information that has been disrupted in one strand from the complementary strand of the original parental duplex. It does not effect a complete repair, however, because the lesion is retained in one of the two daughter chromosomes.

Higher organisms also possess mechanisms for coping with the problem of synthesizing DNA in the presence of single-strand lesions (CLEAVER and THOMAS 1969). The importance of this type of repair is indicated by its implication in inherited disease (LEHMANN *et al.* 1975) and carcinogenesis (MCCANN *et al.* 1975). These mechanisms in eukaryotes are less thoroughly understood than those of bacteria, in part because their genetic dissection has been limited. In an attempt to alleviate this restriction the isolation of Drosophila mutants which are potentially deficient in DNA repair has been undertaken (SMITH 1973; SMITH 1976; BOYD *et al.* 1976). Initial screening of the *X* chromosome for mutants sensitive to methyl methanesulfonate (MMS) has produced over 50 mutants which fall into about 9 complementation groups. Nine MMS-sensitive mutants linked to the third chromosome have been assigned to 5 complementation groups (BOYD and GOLINO, unpublished). Mutants of the *mei-9* and *mei-41* loci (BAKER and CARPENTER 1972), which were recovered in a screen for mutants defective in meiotic processes, also exhibit MMS sensitivity (BOYD, GOLINO and SETLOW 1976; SMITH 1976). Our initial approach to the characterization of DNA repair in these mutants has been to study those strains which readily form homozygous stocks. Postreplication repair has been investigated in primary tissue cultures derived from embryonic cells. This technique has permitted us to assign mutants from 10 complementation groups to 4 classes which differ with respect to repair capacity.

MATERIALS AND METHODS

A. *Stocks and culture*

The mutant stocks employed are listed in Table 1. Nomenclature and unreferenced stocks are described in LINDSLEY and GRELL (1968).

The mutagen-sensitive strains were selected from *w* and *st* stocks. In most cases outcrossing has randomized all but the *X* chromosome. The *mu-2* mutation is a third chromosomal mutator which does not exhibit hypersensitivity to MMS, HN2, or AAF (GREEN and GOLINO, unpublished observations). The *mei-41*^{D5} stock carries a *B*^{S}*Y* chromosome because the fertility of this homozygous stock is vastly improved over the original isolate of this mutant.

Flies were cultured at room temperature on media containing corn meal, molasses, dried yeast, and agar, with propionic acid as a mold inhibitor.

B. *Collection of Embryos*

The following procedure was employed with the strains *mei-9*^{a}, *mei-218*, *w*, and *mei-41*^{D5}. Flies from 20–40 bottles were maintained in population cages and fed a paste of live yeast smeared on a medium which contains grape juice (A. B. BLUMENTHAL, personal communication). The flies received fresh food daily and were subjected to a 12 hr light-dark cycle at 25°. Collection was initiated at the beginning of the dark cycle on fresh grape medium smeared with autoclaved yeast paste. After 16 hr the embryos were removed and held at 25° for an additional 2 hr prior to sterilization. The remaining mutant stocks were exposed to the same medium in bottles for two days prior to embryo collection. Embryos were collected in individual bottles

TABLE 1

Mutant designation	Abbreviation used in text	Origin or description
X chromosome *Y* chromosome		
w/w	*w*	
w mus(1)101^{D1}/w mus(1)101^{D1}	*mus101*	BOYD *et al.* 1976
w mus(1)102^{D1}/w mus(1)102^{D1}	*mus102^{D1}*	BOYD *et al.* 1976
w mus(1)102^{D2}/w mus(1)102^{D2}	*mus102^{D2}*	BOYD *et al.* 1976
w mus(1)103^{D1}/w mus(1)103^{D1}	*mus103^{D1}*	BOYD *et al.* 1976
w mus(1)103^{D2}/w mus(1)103^{D2}	*mus103^{D2}*	BOYD *et al.* 1976
w mus(1)104^{D1}/w mus(1)104^{D1}	*mus104*	BOYD *et al.* 1976
w mus(1)105^{D1}/w mus(1)105^{D1}	*mus105*	BOYD *et al.* 1976
w mei-41^{D5}/w mei-41^{D5}	*B^{s}Y*	*mei-41^{D5}* BOYD *et al.* 1976
y mei-9^{a}/y mei-9^{a}; spa^{pol}/spa^{pol}	*y+Y*	*mei-9^{a}* BAKER and CARPENTER 1972
y mei-218/y mei-218; spa^{pol}/spa^{pol}	*y+Y*	*mei-218* BAKER and CARPENTER 1972
y/y; spa^{pol}/spa^{pol}	*y+Y*	*y+Y/y; spa^{pol}* BAKER and CARPENTER 1972
w^{a} mw mit/w^{a} mw mit	*Y*	*mit* GELBART 1974

Mutant designation	Abbreviation used in text	Origin or description
Third chromosome		
st mu-2/st mu-2	*st*	M. M. GREEN (unpublished)
st mu-2 mus(3)301^{D1}/st mu-2 mus(3)301^{D1}	*mus301*	BOYD and GOLINO (unpublished)
st mu-2 mus(3)302^{D1}/st mu-2 mus(3)302^{D1}	*mus302*	BOYD and GOLINO (unpublished)
st mu-2 mus(3)303^{D1}/st mu-2 mus(3)303^{D1}	*mus303*	BOYD and GOLINO (unpublished)
y^{71a9} w sn^{3}/y^{71a9} w sn^{3}; mu-1/mu-1	*mu-1*	GREEN 1970
Miscellaneous		
UV sensitive	UV-sen	GHLELOVITCH 1966
Df(3R)sbd^{105} p^{p} sbd^{105} bx sr e^{s}/T(2,3)ap^{Xa}	*sbd^{105}*	

under the conditions described for the population cages. Sufficient embryos (0.1–0.5 g wet weight) were generally obtained from young flies derived from 8 half-pint bottles.

The resulting embryos were suspended in water with a paint brush and strained through 400 μ nylon screens (Nitex HC-3-400, Tetko, Inc., Elmsford, N. Y.). They were then collected on 140 μ screens (Nitex HC-3-140) and washed first with one l water, then with 100 ml 0.8% NaCl-0.1% Triton X-100, and finally with one l of sterile distilled water. Any remaining larvae and debris were removed by hand. The embryos were weighed and held under humid conditions prior to sterilization.

C. *Preparation of primary tissue cultures*

Ingredients for tissue-culture media were obtained from Grand Island Biological Co. (Grand Island, N. Y.) unless otherwise noted. Cultures were prepared and labeled in Medium A which consisted of Schneider's Revised Medium, 16% (v/v) heated (56°, 30 min) fetal bovine calf serum (Flow Labs., Rockville, Maryland), 2 mM glutamine, 0.005% phenol red, 4 mUnits insulin/ml (Calbiochem, San Diego, California), 100 Units/ml penicillin, and 0.1 mg/ml streptomycin. Insulin was employed as a result of the observations of SEECOF and DEWHURST (1974). Bactopeptone was omitted from this medium, because it seriously inhibits the incorporation of labeled thymidine. Tissue-culture dishes were obtained from Corning (Corning, N. Y.). Cultures were maintained in a water-saturated air atmosphere at 25°.

Embryos (2–18 hr post-fertilization) were sterilized with a procedure derived from that of S. J. O'BRIEN (personal communication). A stream of 70% ethanol was employed to transfer

embryos from the Nitex screen to a 15 ml conical polystyrene centrifuge tube (Corning). The embryos were shaken from 2 min in 2.6% NaClO (diluted Clorox), pelleted by short centrifugation, and suspended in 70% ethanol. They were next soaked in 70% ethanol containing 0.05% $HgCl_2$ for 8 min and finally in 70% ethanol for 8–15 min.

Primary cultures were prepared according to the procedure of SEECOF (personal communication). Up to 0.75 g of embryos were transferred in 70% ethanol to a 7 ml Dounce Homogenizer (Kontes, Vineland, N. J.). They were washed with 0.8% NaCl, suspended in 5 ml of Medium A, and homogenized 10 complete strokes with a loose fitting pestle (A). The cell suspension was filtered through sterile nylon screen (Nitex 100μ) into a sterile polystyrene tube. The 15 ml tube was filled with medium and spun at 2,000 rpm (clinical centrifuge) for 4 min. The pellet of cells was resuspended in fresh medium with 20 complete cycles of pipetting in a 5 ml plastic pipette. Aliquots of the suspension (1.5 ml) were distributed to plastic culture dishes (35 mm diameter) at a density of about $1–2 \times 10^6$ cells/dish (75 mg embryos). Larger dishes (60 mm diameter) were occasionally used with 3.0 ml of medium. Cell counts are imprecise because of the presence of cell clumps and debris. Somewhat more accurate counts were obtained after trypsinization of the cells. Attached cells reacted negatively to a dye exclusion test (PHILLIPS 1973).

D. *Assay for repair of single-strand breaks*

Fresh cell cultures were labeled either with 1 μCi/ml thymidine-methyl-^{3}H (14–16 Ci/mmol, Schwarz Mann, Orangeburg, N. Y.) or with 0.5 μCi/ml thymidine methyl-^{14}C (50 mCi/mmol, New England Nuclear, Boston, Mass.). After 20 hr the cells were incubated in fresh unlabeled medium A for over an hour. The medium was removed, and the cultures received 10,000 R from a 50 KV Picker X-ray source. The machine was fitted with a Machlett X-ray tube (OE6–60), a W target, a 1.0 mm Be window and an Al filter constructed from 10 layers of commercial light weight foil. The source was calibrated with an ionization chamber. Fresh medium A was again added to the cultures and they were incubated for 1.5 or 3.0 hr prior to analysis by alkaline sucrose gradient centrifugation. The cells of two different strains were labeled with different isotopes, treated identically, and mixed prior to centrifugation (REGAN and SETLOW 1974).

E. *Analysis of postreplication repair and DNA synthesis*

Primary cultures, that had been established 20 hr previously, were exposed to 10 J/m^2 (fluence, 1 J/m^2/sec) from a germicidal lamp after removal of the medium. Fresh medium was added and the cultures were incubated for ½ hr. Thymidine-methyl-^{3}H (14–16 Ci/mmol) was then added to the cultures to produce a concentration of 12 μCi/ml. After a period of ½ hr the labeled medium was removed, the cells were rinsed once with fresh medium and covered with unlabeled medium. When caffeine was employed, all media to which the cells were exposed after UV treatment contained 0.3 mg/ml caffeine (Eastman). Unless otherwise stated, the period of incubation which followed pulse labeling was 3.0 hr. At the completion of the chase period the molecular weight of the labeled DNA was monitored by centrifugation in alkaline sucrose gradients. Yellow room lights were employed throughout to reduce photorepair.

F. *Sedimentation in alkaline sucrose gradients*

Cells were harvested by removing the medium and gently washing the plates twice with 1.5 ml of cold saline-EDTA (Puck's Saline A with 0.2 mg/ml EDTA, adjusted to pH 7.4 with $NaHCO_3$). In some experiments the cultures were then exposed to 1,500 R of X-rays to accelerate subsequent denaturation of the DNA. The cells were suspended in additional saline-EDTA and harvested by centrifugation from 7 ml at 2,000 rpm and 4° for 4 min. The pellet was resuspended at a concentration of 1,000–10,000 cpm/50 μl. When both ^{14}C and ^{3}H labeled cells were to be added to the same gradient, the two cell populations were mixed prior to centrifugation in equal proportions of detectable isotope.

Linear sucrose gradients (5 ml; 5–20% sucrose, w/v) containing 2.0 M NaCl, 0.01 M EDTA, and 0.33 N NaOH were pumped into polyallomer tubes. Each gradient was underlayered with 0.1 ml of 60% sucrose containing 1 N NaOH and overlayered with 0.3 ml of lysing solution

containing 0.25 M NaCl, 0.5 N NaOH, 0.01 M EDTA and 1% Sarkosyl. Up to 10^6 cells and greater than 1,000 cpm of each isotope were gently layered onto the lysing solution in 50 μl of saline-EDTA. Centrifugation was initiated after 20 min when the cells had previously been exposed to 1,500 R of X-rays. In the absence of this X-ray treatment the layered gradients were held for one hr prior to centrifugation. These two procedures produced equivalent results.

Gradients were centrifuged in the 50.1 rotor of the Beckman ultra centrifuge at 20° and 25,000 rpm for 160 min. The brake was disconnected at speeds below 5,000 rpm. Gradients were fractionated by pumping from the bottom of the tube, although fraction numbers start from the top of the gradients to permit alignment of the top fractions in plots of the data. The gradients were calibrated using DNA of bacteriophages T4 and T7 as molecular weight markers. Counting was performed on Whatman 17 paper as described (CARRIER and SETLOW 1971). Sedimentation data were subjected to computer analysis which subtracted background, converted the data to "% of total radioactivity" (recovered from gradient), and calculated molecular weights. Data obtained from the top four and bottom four fractions of each gradient were excluded in calculations of molecular weights. The data derived from pulse chase experiments have been plotted with the top fraction of parallel gradients aligned. Because this first fraction contained between one and two times the volume of the remaining fractions, the curves may be displaced by one fraction. The total number of fractions differs between gradients both because of variations in total gradient volume and due to slight variations in the volume of the fractions collected.

RESULTS

A. *Repair of single-strand breaks*

MMS-sensitive mutants have been tested for their capacity to repair single-strand breaks in DNA that has been exposed to X-rays *in vivo*. In these experiments the DNA of primary cultures was labeled by overnight incubation with thymidine-^{3}H or thymidine-^{14}C. Breaks were introduced by exposure of the cells to X-rays and the molecular weight of the DNA was monitored as a function of time by sedimentation in alkaline sucrose gradients. The parameters of the assay are defined by the experiment presented in the Figure 1a. In this experiment, irradiated cells labeled with ^{3}H were mixed with untreated ^{14}C-labeled cells prior to centrifugation. The bulk of the DNA from untreated cells sedimented with a weight average molecular weight of 270×10^6 to a position near the bottom of the tube. Radiation reduces this molecular weight to 70×10^6. The pattern obtained with irradiated cells is not changed by exposure of the cultures to 0.001 M NaCN immediately after irradiation. Within a wide range these patterns are unchanged by varying the number of cells added to the gradients. Irradiated cells derived from the *w* stock increased the size of their DNA to 220×10^6 daltons during 1.5 hr of incubation after irradiation (Figure 1b). In this experiment two identically treated cultures were labeled with different isotopes. Since the patterns can be superimposed there is no specific isotope effect at a labeling concentration of 1.0 μCi/ml thymidine-^{3}H. A concentration of 5 μCi/ml sharply reduces the observed molecular weight of the ^{3}H-labeled DNA, and a clear effect is observed at 2.5 μCi/ml (unpublished observations).

Although the rate of recovery varies somewhat between experiments, the use of two isotopes has permitted a precise comparison of mutant and control cells. Figures 1c and 1d represent a comparison between the rates of DNA repair in mutant (*mus302*) and control (*w*) cells. At two stages of recovery there is no

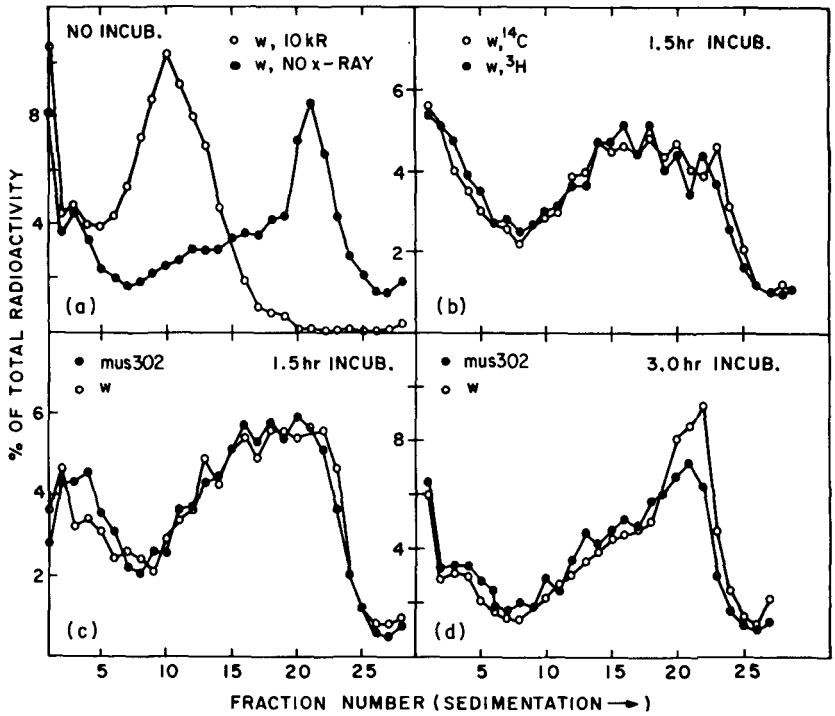

FIGURE 1.—Repair of single-strand breaks. Primary tissue cultures were labeled, irradiated, and incubated as described in MATERIALS AND METHODS. The data in each panel were derived from a single gradient. Experimental conditions differ for the unincubated samples (Panel a) in that both cultures were labeled with 0.5 μCi of isotope in 1 ml cultures and about 10^6 cells were layered on this gradient in 100 μl of saline-EDTA. An equivalent pattern was obtained when half this number of cells were layered on a parallel gradient. Cultures employed to produce the remaining three gradients (Panels b, c, d) all received 10 KR of X-rays prior to incubation. The total detectable radioactivity of individual isotopes applied to these gradients varied from 1,500 to 4,300 cpm.

detectable difference between these strains. Additional strains tested for the ability to repair single-strand breaks are marked with an asterisk in Table 3. Each of the 11 mutagen-sensitive strains tested exhibits a repair rate very similar to that of the controls. Since some of these strains are sensitive to X-rays (BOYD *et al.* 1976), they are probably deficient in repair of a form of X-ray-induced damage other than single-strand breaks. A difference of 10% or more in the weight average molecular weight of mutant and control populations was noted at both incubation times in the following strains: *UV-sen*, *sbd*^{105} and *Xa/Sb Ubx*. Since we have encountered difficulty in culturing cells from these strains, the significance of these results is presently in question.

B. *Postreplication repair and DNA synthesis in the absence of caffeine*

When primary cultures from the *w* stock (control) are pulsed with thymidine-^{3}H for 1/2 hr, the newly synthesized DNA has a weight average molecular weight of about 95 × 10^6 (Figure 2d, open circles). If these cells are then incu-

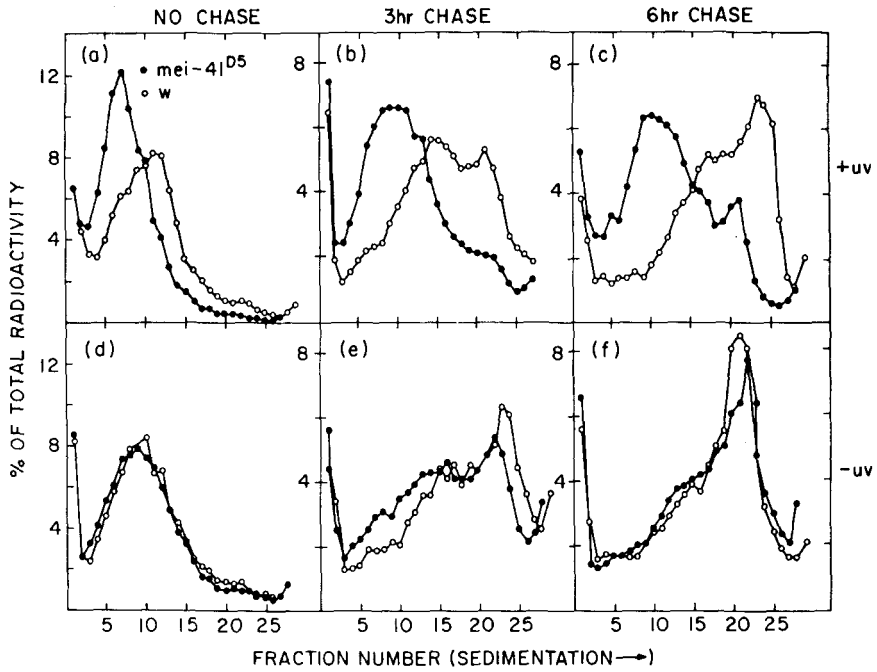

FIGURE 2.—Kinetic analysis of postreplication repair and DNA synthesis in *mei-41^{D5}*. All cultures were labeled with thymidine-^{3}H for 30 min. Data obtained from parallel experiments performed with *mei-41^{D5}* cells and control cells (*w*) have been plotted together. The total radioactivity applied to individual gradients varied from 6.5 to 11.7 $\times$ 10^3 cpm.

bated in the absence of labeled precursor, the size of the pulse labeled DNA increases as the labeled replicons are completed and joined (Figures 2e and f). A similar rate of increase in molecular weight (unpublished observation) has also been observed with SCHNEIDER's (1972) established cell line #2.

Control cells, which have received 10 J/m^2 of UV radiation 1/2 hr prior to the pulse-labeling period, initially synthesize DNA that is about 10% smaller than that produced by unirradiated cultures (Figure 2a, open circles). The increase in molecular weight observed during subsequent incubation also lags slightly behind that of unirradiated control cells (Figures 2b, c). We have further observed that UV treatment reduces the overall level of thymidine-^{3}H incorporation by about 30%. Cells of primary cultures that have received as much as 40 J/m^2 remain attached and retain a normal appearance for more than a week after irradiation. No variation in pattern has been observed by varying the number of cells added to a gradient (maximum—10^6 cells).

Repetition of the above experiments with unirradiated cells of the MMS-sensitive mutant *mei-41^{D5}* has failed to reveal any significant difference in the growth rate of pulse labeled DNA from these two strains (Figures 2d, e, f). The rates with which unirradiated cultures of the two strains incorporate thymidine-^{3}H are equivalent. After the 3 hr chase *w*-derived cells in this experiment had a specific activity of 13 $\times$ 10^{-3} cpm/cell and the corresponding value for

mei-41^{D5} cells was 14×10^{-3} cpm/cell. The patterns observed after UV irradiation are strikingly different (Figures 2a, b, c). At each time investigated, the observed weight average molecular weight of the mutant-derived DNA is less than 60% of the control value. A related but unexplained observation is that DNA synthesis (unpublished observations) and survival (SMITH, personal communication) of *mei-41*^{A1} larvae are both abnormally reduced by hydroxyurea.

Identical kinetic experiments have been performed with the MMS-sensitive mutants *mus101* and *mus104* (Figures 3, 4). After UV treatment the weight average molecular weight of the newly synthesized DNA is about 3/4 of the control value for both these mutants. They are, therefore, somewhat weaker than *mei-41*^{D5} in their effect on DNA synthesis following UV treatment. All three mutants exhibit a normal pattern of DNA synthesis immediately after the 1/2 hr pulse (Panel d of Figures 2, 3 and 4). However, DNA derived from unirradiated cultures of *mus101* and *mus104* has a slower rate of gain in molecular weight than the control (Panel e and f of Figures 3 and 4). These two mutants thus appear to affect normal DNA synthesis as well as DNA synthesis perturbed by radiation (see DISCUSSION). The *mei-41*^{D5} mutation, on the other hand, disrupts only the latter function.

Table 2 contains molecular weights derived from the data in Figures 2–4. These values are subject to a variation of about 13% (95% confidence limits

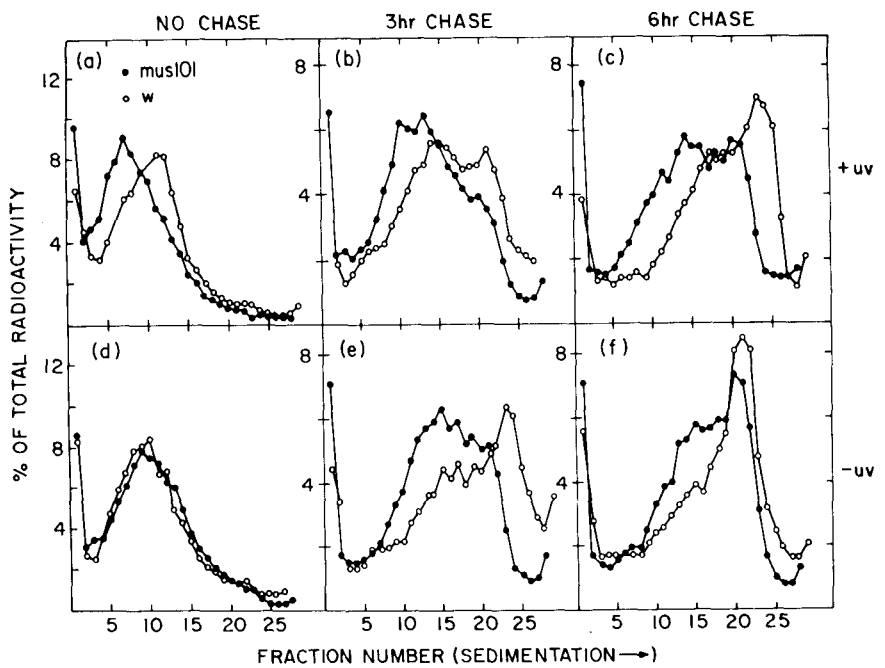

FIGURE 3.—Kinetic analysis of postreplication repair and DNA synthesis in *mus101*. Experimental details are found under Figure 2 and in MATERIALS AND METHODS. The control gradients represented in Figure 2 are reproduced here. Each gradient was layered with greater than 7.3×10^3 cpm ^{3}H.

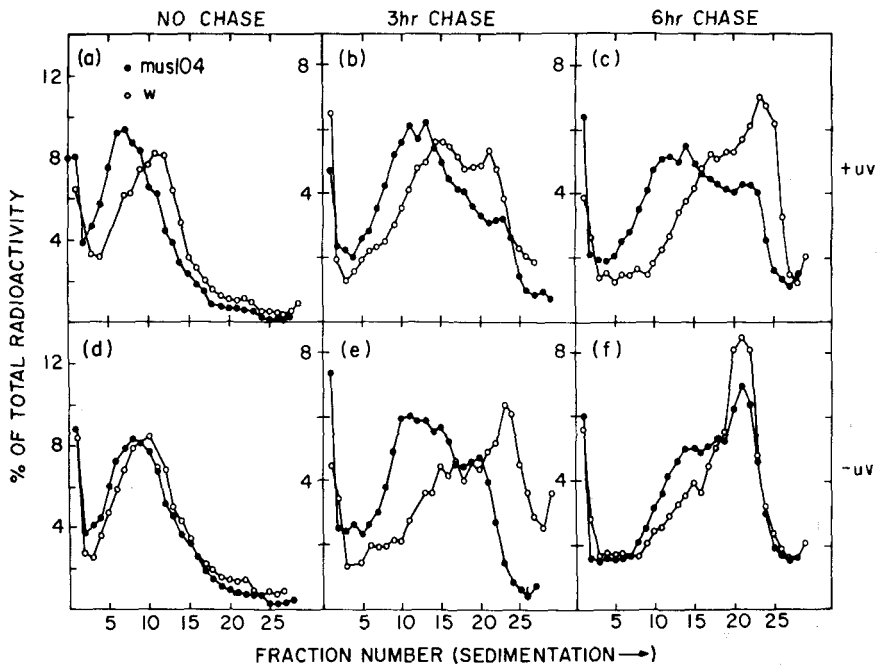

FIGURE 4.—Kinetic analysis of postreplication repair and DNA synthesis in *mus104*. Experimental details are found under Figure 2 and in MATERIALS AND METHODS. The control gradients represented in Figure 2 are reproduced here. Each gradient was layered with greater than 8.9×10^3 cpm ^{3}H.

TABLE 2

DNA synthesis and postreplication repair in primary cell cultures
Weight average molecular weight ($\times 10^{-6}$) of pulse-labeled DNA *vs.* time of chase following a 30 min pulse

Treatment Mutant	Time of chase 0 hr	3 hr	6 hr
With UV			
w	85	204	258
mei-41^{D5}	53 (0.62)	120 (0.59)	134 (0.52)
mus101	65 (0.76)	145 (0.71)	184 (0.71)
mus104	63 (0.74)	146 (0.72)	176 (0.68)
Without UV			
w	96	238	231
mei-41^{D5}	86 (0.90)	205 (0.86)	239 (1.03)
mus101	95 (0.99)	184 (0.77)	209 (0.90)
mus104	79 (0.82)	162 (0.68)	215 (0.93)

These data were taken from the experiments presented in Figures 2–4. Numbers in parentheses refer to the ratio of molecular weight obtained with mutant cells divided by the corresponding molecular weight of DNA from control (*w*) cells.

determined from six control gradients). Preliminary evidence suggests that where large differences are noted between the mutant and the control, the differences are accentuated by high concentrations of thymidine-^{3}H.

C. *Postreplication repair and DNA synthesis in the presence of caffeine*

The type of experiment described in the previous section has also been performed with caffeine present throughout the incubations following UV treatment. The results of a control experiment involving the *w* stock are presented in Figure 5. At a concentration of 0.3 mg/ml caffeine has no significant effect on the increase in molecular weight of newly synthesized DNA in unirradiated cells (3 hr chase period). It does, however, inhibit the increase observed after UV treatment by about 12%. In both cases caffeine decreases the overall incorporation of ^{3}H-thymidine in the culture by about 30%. Control cultures have been irradiated and exposed to caffeine for over a week without any change in appearance. The mean values for the weight average molecular weights ($\times 10^{-6}$) determined after 3 hr of incubation follow:—caffeine—UV, 225±6(6);—caffeine + UV, 199±5(6); + caffeine − UV, 227±8(3); + caffeine + UV, 176±10(3). Means and their standard errors were obtained from the number of separate determinations which appear in parenthesis. These data have been analyzed for significance at the 7% level in the students' t test. At this concentration caffeine has no significant effect in the absence of UV. All other pair-wise combinations of the data are significantly different. Irradiation alone reduces the molecular weight by 12% and caffeine plus radiation produce a total reduction of 22%. Higher concentrations of caffeine have been avoided, because they inhibit the molecular weight increase of DNA in unirradiated cultures. At 0.9 mg caffeine/ml, for example, the molecular weight of DNA from unirradiated cells is 15% below that of control cells. The corresponding value for irradiated cells is 26%.

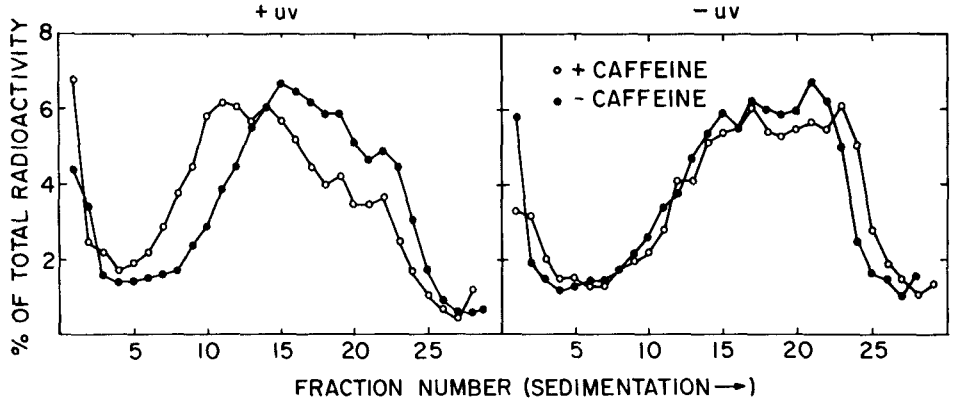

FIGURE 5.—Influence of caffeine on postreplication repair and DNA synthesis in control cells (*w*). The patterns were obtained after a 3 hr chase. Those without the use of caffeine are derived from a different experiment than that used to prepare Figures 2–4. Each gradient was layered with greater than 2.9 $\times$ 10^3 cpm ^{3}H.

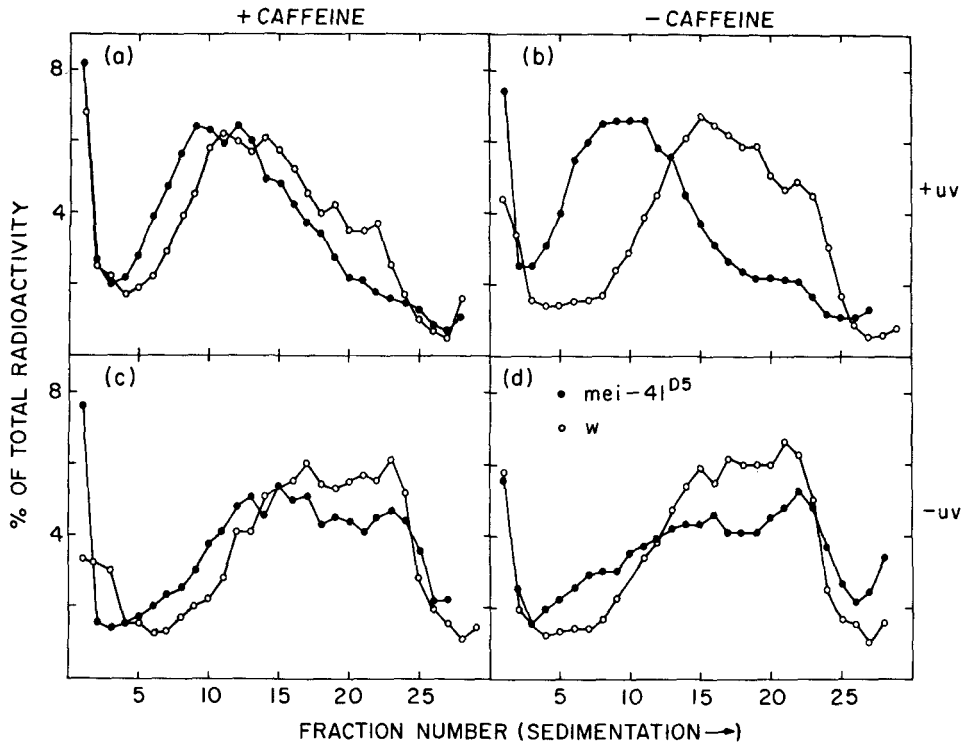

FIGURE 6.—Effect of caffeine on postreplication repair and DNA synthesis during a 3 hr chase in *mei-41*^{D5}. The control profiles are those presented in Figure 5. The right-hand panels, which were obtained without the use of caffeine, were taken in part from the two center panels in Figure 2. Each gradient derived from mutant cells was labeled with greater than 9.6×10^3 cpm ^{3}H.

The effect of caffeine on cells of the *mei-41*^{D1} strain was compared with its influence on control cells after a 3 hr chase period (Figure 6). The two right-hand panels correspond to the two center panels in Figure 2 and are provided to facilitate a comparison. The lower panels demonstrate that at 0.3 mg/ml caffeine has no significant effect on the molecular weight increase of DNA in unirradiated cells of the mutant. It does alter the relative positions of the control and mutant-derived peaks in irradiated cells (Figures 6a, b). The difference observed between the two after UV treatment is diminished by exposure to caffeine; implying that caffeine has little effect on the mutant and an inhibitory effect on the control. The important point is that caffeine has less of an effect on cells of the mutant than it has on control cells.

Analysis of the mutants *mus101* and *mus104* has revealed a possible deficiency in DNA synthesis occurring in unirradiated cells (Figures 3, 4). When caffeine is present under otherwise identical conditions this defect is further accentuated in both mutants (Figures 7c, d). Irradiation of the cells prior to caffeine exposure, however, reveals a clear difference between these strains (Figures 7a, b). Postreplication repair is more strongly inhibited in *mus104* by caffeine than it is in

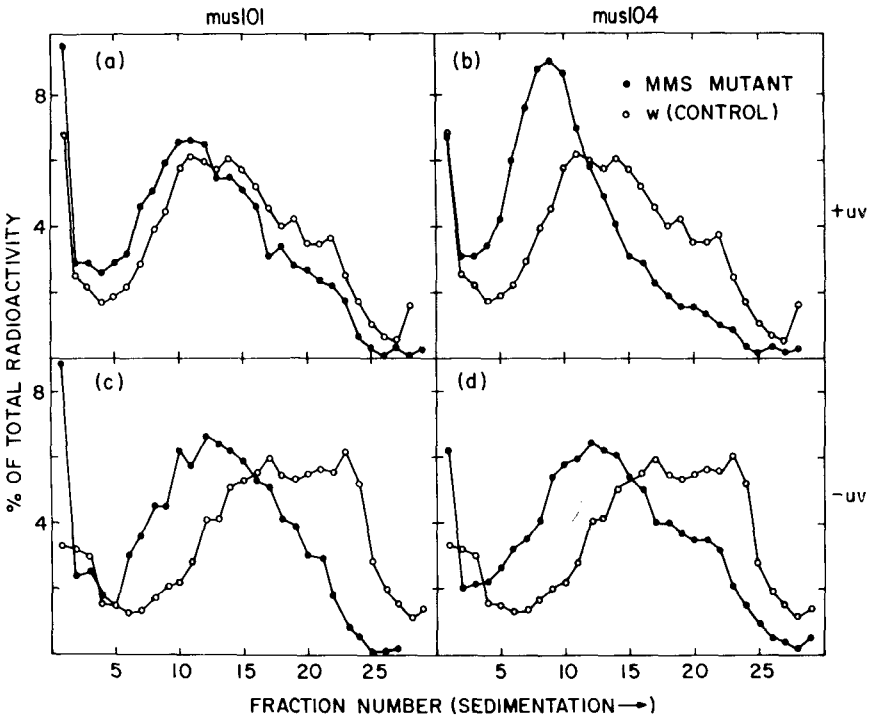

FIGURE 7.—Influence of caffeine on postreplication repair and DNA synthesis, during a 3 hr chase, in the mutants *mus101* and *mus104*. All samples were incubated with caffeine. The control profiles (*w*) are taken from Figure 5. The conditions used in this analysis correspond to those used to produce the center panels in Figures 3 and 4 except that cells were not incubated with caffeine in those experiments. Each gradient received greater than 2.2×10^3 cpm ^{3}H.

mus101. Under the above conditions the weight average molecular weight of DNA from *mus104* is 88×10^6, which is probably close to its preincubation value (Table 1).

D. *Comparison of the weight average molecular weights of DNA from mutant and control cells* (w)

The molecular weight ratios listed in Table 3 provide a qualitative comparison of the mutant effects. A ratio of 1.0 indicates a lack of effect and the minimum ratio representing the strongest possible effect is probably 0.3–0.5, depending on the molecular weight of the newly synthesized DNA immediately after the pulse (Table 2). On the basis of these results and the studies of nondisjunction (BOYD *et al.* 1976) the tested complementation groups have been tentatively assigned to 4 classes. It should be recalled at this point that the genetic assignment of the mutants to functional groups is itself tentative at this time.

Class 1 (postreplication repair-deficient, meiotic-defective, residual postreplication repair not strongly sensitive to caffeine). The three mutants *mei-41*^{D5}, *mus101*, and *mus302* are assigned to this pathway. *mus101* also shares an apparent sensitivity to ^{3}H with the Class 2 mutant.

TABLE 3

Ratios of DNA molecular weights

Weight average molecular weight of DNA from mutant cells divided by weight average molecular weight of DNA from control cells (chase time 3 hr)

Mutant Designation	Class	Without caffeine —UV	Without caffeine +UV	With caffeine —UV	With caffeine +UV
Homozygous *X* chromosomal mutants					
**mus101*	(1)	0.82†	0.69†	0.64	0.65
**mus102*^{D1}	4	1.11	0.99	0.99	1.03
**mus102*^{D2}	4	1.14	1.09	0.95	1.03
**mus103*^{D1}	(3)	0.97	0.89	0.89	0.79
**mus103*^{D2}	(3)	1.05	0.91	0.75	0.86
**mus104*	2	0.80†	0.71†	0.59	0.50
**mus105*	4	0.97	0.95	0.94	1.04
**mei-41*^{D5}	1	0.91	0.57†	0.90	0.69
**mei-9*^{a}	3	0.89	0.88	0.81	0.76
**mei-218*	4	0.99	0.89	0.95	0.85
spa^{pol}; *y*$^{+}$*Y/y*	4		0.96		0.91
mit	4		1.05		0.96
Homozygous third chromosomal mutants					
**mus301*	4	1.03	1.12	0.91	0.97
**mus302*	1	1.03†	0.80†	0.99	0.70
**mus303*	4	1.04	1.09	0.97	1.13
**st*	4	1.03	0.97	0.92	0.84
**mu-1*	4	0.91	0.93	1.03	1.07

Primary cultures were tetsed with one of four separate experimental conditions as described in MATERIALS AND METHODS. The weight average molecular weight of DNA derived from a given treatment of a mutant culture is divided by the corresponding value obtained with the white (*w*) stock. Uniformly labeled DNA from untreated cells has a weight average molecular weight of 290×10^6 under these experimental conditions.

The mean values used in these calculations for the 4 treatments of the *w* cells are those stated in RESULTS (all values in 10^6 daltons).

— caffeine — UV, 225 ± 14; — caffeine + UV, 199 ± 13;
+ caffeine — UV, 227 ± 13; + caffeine + UV, 176 ± 18.

Errors represent one standard deviation.

† Values derived from duplicate mutant assays. All other values were determined from a single analysis.

Underline: Values are significantly lower than the controls at the 5% level as determined with Student's *t* test.

* Assayed for repair of single-strand breaks.

( ) Class assignment uncertain—see text.

Class 2 (postreplication repair deficient, normal meiosis, residual postreplication repair strongly sensitive to caffeine). The only mutant assigned to this pathway is *mus104*.

Class 3 (postreplication repair proficient, DNA metabolism partially caffeine sensitive). Of the three mutants assigned to this class (*mei-9*^{a}, *mus103*^{D1}, *mus103*^{D2}), *mei-9*^{a} has been shown to be deficient in excision repair (BOYD, GOLINO and SETLOW 1976). An established culture of the *mus103*^{D1} mutant expresses normal excision repair (MASON, personal communication).

Class 4 (no detected defect in postreplication repair). In addition to the mutants of this class listed in Table 3, we have been unable to detect any deficiency in embryos derived from the following stocks: UV sensitive strain of GHLELOVITCH (1966), *mus101/C(1)DX, y f, c(3)G st ca/TM3*, or $ca^{nd}/TM3$. SMITH (1976) has produced evidence suggesting that an allele of the *mus102* mutants (Class 4) functions independently of $mei\text{-}41^{A1}$ (Class 1) in repair of MMS-induced damage.

DISCUSSION

DNA Synthesis in Drosophila

The molecular weight profiles obtained from unirradiated cultures are similar to patterns derived from mammalian systems (LEHMANN 1972). The first phase of DNA synthesis is the generation of Okazaki type fragments which have a molecular weight of about 5×10^4 (KRIEGSTEIN and HOGNESS 1974). These fragments are generated on replication forks, which advance at a rate of 0.85×10^6 daltons/min at 25°. This value is expected to apply to the cells employed here, because the above authors have observed the same rate of chain elongation in two diverse cell types (BLUMENTHAL, KRIEGSTEIN and HOGNESS 1974). As the replicons increase in size and are fused together the newly synthesized DNA advances to the center of the gradient from whence it is converted to a peak near the bottom of the gradient. The mean replicon size varies between cell types (BLUMENTHAL, KRIEGSTEIN and HOGNESS 1974), and has not been determined for primary cultures.

Uniformly labeled DNA obtained from primary cultures that have received 10 J/m^2 of UV radiation contains 0.018% of its thymine as pyrimidine dimers (determined by the method of CARRIER and SETLOW 1971). Extrapolation from mammalian studies indicates that the latter number corresponds to one pyrimidine dimer per 9.3×10^6 daltons (SETLOW *et al.* 1969) or 2.7 dimers in the 26×10^6 daltons (number average) that is synthesized during a 30 min pulse following UV treatment. Thus, normal replication in *w* appears to proceed through the dimers, because the molecular weight of the pulse-labeled DNA is only slightly reduced by this UV treatment. The majority of dimers remain in the DNA during the first hour following irradiation (BOYD, GOLINO and SETLOW 1976). In spite of the minimal inhibition (10%) of UV on the length of newly synthesized chains, this level of irradiation inhibits overall DNA synthesis by about 30%.

A model for DNA repair in Drosophila

A model for pathways of UV repair in *Drosophila melanogaster* is presented in Figure 8. Our data indicate that at least three dark repair mechanisms are capable of responding to UV-induced lesions. The complementation groups associated with each of the postulated pathways are indicated. All tested mutants that are sensitive to nitrogen mustard (BOYD *et al.* 1976) have proven to be deficient in postreplication repair or excision repair. The excision-deficient mutant *mei-9* is reviewed in more detail elsewhere (BOYD, GOLINO and SETLOW 1976). The

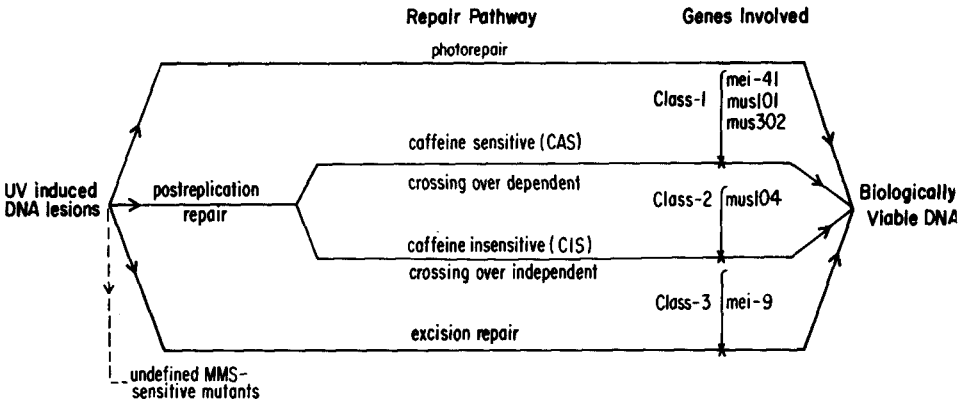

FIGURE 8.—Proposed DNA repair pathways in *Drosophila melanogaster*.

two postreplication repair pathways are designated CAS (caffeine sensitive) and CIS (caffeine insensitive). In this model it is assumed that one of two pathways of postreplication repair is disrupted by single mutations and that the residual capacity for postreplication repair is due to the unaffected pathway. The caffeine sensitivity of a given pathway was deduced from observations of the effect of this compound in mutants that block the alternate pathway. As an example, the Class 1 mutants are postulated to disrupt a pathway (CAS) that is sensitive to caffeine. This conclusion arises from the observation that when the alternate pathway (CIS) is disrupted by the Class 2 mutant, the residual repair is highly sensitive to caffeine. Evidence for multiple forms of postreplication repair is also available in bacteria (SEDGWICK 1975; YOUNGS and SMITH 1976) and yeast (COX and GAME 1974; BRENDEL and HAYNES 1973).

Postreplication repair in the absence of caffeine

Physical studies of postreplication repair in most mammalian systems have demonstrated that newly synthesized DNA has a reduced molecular weight when it is synthesized on a template containing UV lesions (LEHMANN 1975). In a marsupial (BUHL, SETLOW and REGAN 1974) and a chick cell line (LEHMANN and STEVENS 1975) these lesions have been identified with pyrimidine dimers. These observations are currently interpreted as being due to the presence of gaps opposite dimers in the newly synthesized strand (LEHMANN 1975). Mouse L cells, on the other hand, are like Drosophila in that they do not exhibit a marked reduction in the molecular weight of newly synthesized DNA following UV radiation (CHIU and RAUTH 1972).

In *E. coli* gaps opposite pyrimidine dimers are eliminated by recombination mechanisms which leave the dimers in the DNA (GANESAN 1974). Our observations of the CAS mutants taken together with their meiotic effects (BAKER and CARPENTER 1972; BOYD *et al.* 1976) implicate a recombination function in postreplication repair in Drosophila. We currently favor the hypothesis that in Drosophila replication also leaves gaps opposite UV damage and that in control cells

these gaps are rapidly sealed by recombination-mediated repair. In the CAS mutants the efficiency of this repair is decreased and the DNA synthesized during the 30 min labeling period has a reduced molecular weight. The normal rapid sealing of presumptive gaps is thought to be sensitive to caffeine, because the CAS mutants and caffeine have a similar influence on the molecular weight of the DNA. Finally, these mutants do not appear to affect functions critical to normal DNA synthesis, because their effect is observed only after UV treatment.

A second distinct form of repair is tentatively defined by the CIS mutant *mus104*. Unlike the CAS mutants this mutant is not defective in meiosis. It exhibits a normal level of nondisjunction (BOYD *et al.* 1976) and is therefore very likely capable of normal meiotic recombination (CARPENTER and BAKER 1974). This mutant appears to be normal in the initial stages of DNA synthesis and in repairing gaps left by single-strand breaks.

Two mutants, *mus101* and *mus104*, which have been assigned to different classes, display what appears to be a defect in normal DNA synthesis. Although they synthesize a normal product after pulse labeling, the subsequent increase in the molecular weight of the DNA is reduced in both strains in the absence of UV. Preliminary data support the suggestion that they synthesize DNA normally, but are sensitive to ^{3}H decay in addition to their UV sensitivity (J. FARMER, in preparation).

Influence of caffeine on postreplication repair

In Drosophila, caffeine enhances the frequency of X-ray-induced dominant lethals, but lowers the recovery of translocations (MENDELSON 1974). These effects may be due to abortive recombinational repair, since caffeine also reduces the frequency of meiotic crossing over (YEFREMOVA and FILIPPOVA 1974). In our experiments caffeine has been used at a concentration which does not have a significant effect on the molecular weight increase of newly synthesized DNA in control cultures. It does, however, have an inhibitory effect on the overall rate of DNA synthesis. In the CIS mutant *mus104* it potentiates what may be a form of ^{3}H-induced damage in unirradiated cells (Figure 7) and has a striking inhibitory influence on the molecular weight increase of DNA in UV-irradiated cells of this mutant.

Comparison of postreplication repair in Drosophila and mammals.

LEHMANN *et al.* (1975) offer two models to explain their observations that the variant cell lines of xeroderma pigmentosum are deficient in postreplication repair. The simplest model consistent with their data involves a single postreplication repair pathway. The second model, which we favor in the interpretation of our results, assumes that there are "two separate postreplication repair processes, one caffeine sensitive, the other caffeine resistant". If this model is applicable to both organisms, it predicts that in man the caffeine-sensitive pathway may be dependent on recombination functions. In Chinese hamster cells KATO (1974) has found that the induction of sister chromatid exchanges by UV, 4-nitroquinoline 1-oxide, and mitomycin C is mediated by a caffeine-sensitive mechanism.

The UV-induced lesions which are responsible for this effect are stable and only have their effect during the S phase of the cell cycle. In Drosophila UV has also been shown to stimulate somatic crossing over (MARTENSEN and GREEN 1976) and meiotic crossing over (PRUDHOMMEAU and PROUST 1974). The postulated caffeine-sensitive mechanism in man is thought to be independent of excision repair and appears to be different from known postreplication repair mechanisms in bacteria (WOLFF, BODYCOTE and PAINTER 1974).

A potential analogue of the CIS pathway has been studied in mammalian cells by LEHMANN (1972) and BUHL *et al.* (1972). Their data indicate that gaps in newly synthesized daughter DNA strands are filled by *de novo* DNA synthesis rather than by recombinational repair. Further support for this analogy is provided by studies of the effect of caffeine on the xeroderma pigmentosum variants (LEHMANN *et al.* 1975). At a caffeine concentration that has little influence on normal synthesis, the molecular weight increase of DNA in the variants is dramatically reduced just as it is in the CIS mutant. The caffeine-insensitive repair pathway in Drosophila (CIS) is presently thought to operate independently of recombination. Within the framework of this model the xeroderma variants are associated with the caffeine-insensitive repair pathway, which may also function independently of recombination. One further parallel between repair in Drosophila and mammals is that all tested mutants display a normal capacity to repair single-strand breaks.

Mouse L cells probably compensate for their relative lack of excision repair (TROSKO, CHU and CARRIER 1965) by the possession of a more efficient postreplication repair mechanism. Postreplication repair in tested rodent cells (ROBERTS, STURROCK and WARD 1974) is extremely sensitive to caffeine. In this property they are similar to our CIS mutant and to the xeroderma pigmentosum variants. Hence, if we assume that there are two modes of postreplication repair, we infer that the CAS pathway predominates in rodents. Normal human cell lines which exhibit little synergism between mutagens and caffeine (WILKINSON, KIEFER and NIAS 1970) are postulated to emphasize the CIS approach to postreplication repair. It is thus possible to rationalize much of the available data on eukaryotic repair in terms of the four repair pathways which have been postulated from the Drosophila data: photorepair, excision repair, CAS postreplication repair and CIS postreplication repair (Figure 8). The major differences in repair in higher organisms may therefore lie in the relative emphasis which each species places on the same basic group of repair mechanisms.

The molecular mechanism of postreplication repair in eukaryotes is currently in question (LEHMANN 1972; BUHL *et al.* 1972; PAINTER 1974; ROMMELAERE and MILLER-FAURÉS 1975). Elucidation of this process is apparently complicated by the existence of redundant mechanisms as is the case in microorganisms (CLARK 1973; YOUNGS and SMITH 1976). The use of caffeine has permitted a partial resolution of the suspected mechanisms. The clear distinction between CAS and CIS mutants in Drosophila further defines two parallel and partially redundant pathways of postreplication repair. These experiments also implicate genetic recombination in one form of postreplication repair in higher organisms.

Further analysis of these mutants may provide a clearer understanding of this form of repair.

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