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DNA Mismatch Repair Catalyzed by Extracts of Mitotic, Postmitotic, and Senescent *Drosophila* Tissues and Involvement of *mei-9* Gene Function for Full Activity

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Extracts of *Drosophila* embryos and adults have been found to catalyze highly efficient DNA mismatch repair, as well as repair of 1- and 5-bp loops. For mispairs T · G and G · G, repair is nick dependent and is specific for the nicked strand of heteroduplex DNA. In contrast, repair of A · A, C · A, G · A, C · T, T · T, and C · C is not nick dependent, suggesting the presence of glycosylase activities. For nick-dependent repair, the specific activity of embryo extracts was similar to that of extracts derived from the entirely postmitotic cells of young and senescent adults. Thus, DNA mismatch repair activity is expressed in *Drosophila* cells during both development and aging, suggesting that there may be a function or requirement for mismatch repair throughout the *Drosophila* life span. Nick-dependent repair was reduced in extracts of animals mutant for the *mei-9* gene. *mei-9* has been shown to be required in vivo for certain types of DNA mismatch repair, nucleotide excision repair (NER), and meiotic crossing over and is the *Drosophila* homolog of the yeast NER gene *rad1*.

DNA mismatch repair has long been known to be involved in two important cellular processes: the repair of mismatches generated by misincorporation of nucleotides during DNA replication, and the processing of recombination intermediates, resulting in novel configurations of genetic markers (24, 37). More recently DNA mismatch repair has been found to be involved in three additional processes: regulation of recombination between divergent DNA sequences associated with genetic instability (40, 47); nucleotide excision repair (NER) involved in repair of physical and chemical damage to DNA (14, 22, 34); and the recognition of DNA damage and the initiation of responses to this damage, such as cell cycle arrest (1, 19).

The best-characterized mismatch repair pathway is the *Escherichia coli* MutHLS system (36, 37). The MutHLS system recognizes and repairs all single-base mismatches as well as insertions and deletions ≤3 bp in size. The efficiency of mismatch repair depends on the specific mispair formed; e.g., Pu · Pu mispairs are repaired at higher efficiencies than Py · Py mispairs. Repair of a given mispair generally depends on surrounding sequence context (21), but C · C mismatches appear not to be repaired in the sequence contexts that have been studied (36, 51). Repair proceeds through mismatch-dependent nicking of the unmethylated DNA strand opposite a GATC site containing a methylated adenine, degradation from the nick through the mismatch site, and then resynthesis of the excision tract (36); these excision repair tracts can span as many as several thousand nucleotides (53). The MutS protein recognizes and binds to DNA at the mismatch. MutL interacts with MutS bound to the mismatch and is required for optimal activity of MutH endonuclease which nicks the unmethylated strand.

Eukaryotes possess a mismatch repair system related to the bacterial MutHLS system (24, 37). Multiple homologs of MutS and MutL (but not MutH) have been identified in yeast and in mammals, and certain of these genes have been demonstrated to be required for mismatch repair in vivo and in vitro. Mutations in the gene encoding a human MutS homolog (*hMSH2*) are common in hereditary nonpolyposis colorectal cancer families (15, 31). This observation is consistent with the idea that defects in the mismatch repair system may lead to genomic instability which predisposes the affected individuals to certain types of cancers. DNA methylation does not appear to play a role in eukaryotic mismatch repair, and the in vivo mechanism by which eukaryotes distinguish the newly replicated DNA strand from the parental strand is currently unknown. In vitro, strand-specific repair is initiated from a nick in the DNA of one of the strands (37).

NER is the repair of damaged DNA involving excision of oligomers with lengths of 27 to 29 nucleotides in eukaryotes and 12 to 13 nucleotides in prokaryotes (44, 56). In eukaryotes, there appears to be some functional overlap between NER and DNA mismatch repair, and certain gene products are required for both processes. NER systems appear to be highly conserved through evolution from yeast to mammals. Genes required for NER in yeast were first identified as UV-sensitive rad mutations (16). In humans, genes required for NER have been identified as the seven repair-deficient complementation groups (A to G) of the disease xeroderma pigmentosum (XP) (44). XP is characterized by extreme UV sensitivity and a predisposition to certain types of cancer. In *Drosophila*, several genes required for DNA repair have been identified in screens for mutations which confer increased sensitivity to mutagens (8). One of these genes, *mei-9*, had previously been identified as a gene required for meiotic crossing over and normal meiotic chromosome disjunction (4). Mutant *mei-9* females are able to generate heteroduplex DNA in recombination intermediates but are unable to repair mismatches within the heteroduplex regions and to resolve the recombination intermediates as reciprocal exchanges (11, 43). *Drosophila mei-9* mutants have also been shown to be slow in clearing UV-induced pyrimidine dimers from genomic DNA, indicating that mei-9 is required for *Drosophila* NER in vivo (9). The *mei-9* gene has been

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cloned from *Drosophila* and found to encode a homolog of the yeast NER protein Rad1 (46) and the human NER XP complementation group F protein (10, 48). We report here an in vitro system (51) using extracts of mitotic, postmitotic, or senescent *Drosophila* tissues, which efficiently catalyzes DNA mismatch repair. The specific activities of extracts derived from the entirely postmitotic cells of young and senescent adults were similar to those of extracts derived from rapidly dividing embryos. These results suggest that the mismatch repair system may function throughout the *Drosophila* life span. Specific activities of *Drosophila* extracts were considerably greater than those of HeLa cell extracts. Mismatch repair in *Drosophila* may be inducible, as specific activities are increased five- to sixfold by X-ray irradiation of flies. There are specific defects in repair in extracts generated from *mei-9* mutant animals, consistent with previous reports that *mei-9* is required in vivo for certain types of DNA mismatch repair (11, 43) and NER (9).

MATERIALS AND METHODS

***Drosophila* strains and culture.** All *Drosophila* strains are as previously described (32). *mei-9*^{AT2} and *mei-9*^{a} *Drosophila* stocks were obtained from R. Scott Hawley, University of California, Davis, Calif. Oregon-R strain flies were grown on cornmeal-molasses-agar medium (2). Embryos were collected on molasses-agar plates coated with yeast paste. Flies were cultured and aged as previously described (55). To obtain flies of defined ages, flies were cultured at 25°C until 0 to 2 days posteclosion, and then the male flies were maintained at 29°C, at 50 flies per vial. Aging flies were transferred to fresh vials every 4 days to prevent growth of bacteria or fungus in the cultures. "Young" refers to flies 4 to 5 days posteclosion, and "old" refers to flies 35 days posteclosion. At 35 days posteclosion at 29°C, only ~15% of the cohort is still surviving, and thus these flies are highly senescent.

X-ray treatment. Young male flies were separated from female flies and distributed at 50 flies per vial. These flies were X-ray irradiated for 4 h at 320 rads/min. The flies were allowed to recover for 48 h before preparation of extracts. The mock-irradiated control flies were handled and cultured identically to the irradiated flies.

***E. coli* strains.** *E. coli* NR9099, NR9162, and CSH50 and bacteriophage M13mp2 have been previously described (7, 26, 27, 29, 41). Mutant M13mp2 derivatives, previously described (26–28), were obtained from T. A. Kunkel, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, N.C.

Preparation of single-stranded DNA. To prepare phage stocks, mutant phage were grown for 5 to 6 h by adding 50 μl of plaque suspension to 5 ml of an early-log-phase culture of CSH50 cells in LB medium at 37°C. Cultures were then transferred to sterile microcentrifuge tubes and centrifuged at 10,000 rpm for 5 min at room temperature. Supernatants were aliquoted to sterile tubes; these stocks can be stored indefinitely at 4 or −20°C without loss of infectivity. One-half milliliter of the appropriate phage stock was added to an early-log-phase culture of CSH50 cells (2.5 ml) and allowed to stand for 5 min at room temperature. These infected cells were diluted into 500 ml of fresh LB medium and incubated overnight at 37°C with constant vigorous shaking. The phage were precipitated from 500 ml of the supernatant by adding 0.25 volume of NaCl and 20% PEG-8000 and stirring for 1 h in the cold room, followed by centrifugation at 11,800 rpm for 30 min at 4°C. The virus pellet was resuspended by adding the cationic detergent cetyltrimethylammonium bromide (24 ml/pellet obtained from 250 ml of the supernatant) and vortexing vigorously. The suspension was centrifuged at 14,000 rpm for 15 min, and the pellet was resuspended in 7 ml of 1.2 M NaCl/250 ml of supernatant. DNA was precipitated by adding 17.5 ml of ethanol and incubating the sample at −20°C overnight. The DNA was pelleted by centrifuging at 14,000 rpm for 15 min at 4°C. The pellet was washed with 70% ethanol, spun for 5 min, dried, and resuspended in 2 ml of Tris-EDTA (pH 8.0), and the final DNA concentration was determined.

Preparation and purification of RF DNA. Replicative-form (RF) DNA was prepared and purified by using the Wizard Maxipreps DNA purification system.

Construction of heteroduplexes containing mispairs. Heteroduplex DNA was generated as described previously (50). Briefly, the appropriate mutant RF DNA was digested to completion with restriction endonuclease *Ava*II, which incises once in M13mp2 at position −264 (where position +1 is the first transcribed base of *lacZ*α). This genome-length linear (minus-strand) DNA was annealed to mutant, single-stranded, circular, viral (plus-strand) DNA, to generate a completely double-stranded but nicked heteroduplex molecule. This nicked, circular DNA species was purified by 0.8% agarose gel electrophoresis. The nicked heteroduplex migrated as a distinct band, well separated from other DNA species on the gel (data not shown). The nicked heteroduplex was then isolated by electroelution, ethanol precipitated, and resuspended in 10 mM Tris-HCl (pH 8.0)–1 mM EDTA.

Preparation of competent cells, transfection, and plating. Preparation of competent cells, transfection, and plating were done essentially as described previously (7, 42). NR9162 cells which are deficient in mismatch repair system were derived from MC1061 and yield a very high efficiency for transfection, ~10^5 plaques/ng of DNA. Plating efficiency was not affected by pretreatment of the DNA with HeLa cell or *Drosophila* embryo extracts (data not shown). Transfection was accomplished with a Bio-Rad Gene Pulser set at 1.8 kV, 400 Ω, and 25 μF. Typical time constant is 9.3 ms. Immediately following electroporation, 1 ml of SOC medium (42) (at room temperature) was added to the cells, which were kept at room temperature. An appropriate amount of the electroporated cells was used to yield 200 to 500 plaques per plate for this assay. The total number of plaques was determined by counting all plaques for which the color phenotype was obvious. Tiny plaques, plaques at the edge of the plate, or those on regions of the plate smeared by a drop of water were not counted.

Preparation of extracts. Extracts from *Drosophila* embryos and adults were prepared essentially as previously described (35) except that both of the centrifugation steps were performed at 10,000 × *g* instead of 100,000 × *g*. Briefly, adults of *Drosophila melanogaster* Oregon-R were cultured in population cages, and the embryos were collected for 0 to 18 h on apple juice-agar plates coated with a thin layer of live yeast paste. The embryos were washed extensively in distilled H_2O and then dechorionated by immersion in 2.25% sodium hypochlorite (bleach) for 90 s. The embryos were then rinsed extensively with 0.7% NaCl–0.04% Triton X-100 solution and finally with distilled H_2O. The dechorionated embryos were filtered through Miracloth (Calbiochem), blotted dry from below, resuspended in 25 to 50 ml of homogenization buffer (20 mM HEPES-NaOH [pH 7.5], 5 mM magnesium acetate, 50 mM potassium acetate, 1.0 mM EGTA, 0.5 mM EDTA, 0.1% [vol/vol] Triton X-100, 10% [vol/vol] glycerol, 10 mM $Na_2S_2O_5$, 1.0 mM dithiothreitol, 5 μg of leupeptin per ml, 5 μg of pepstatin A per ml), and incubated on ice for 10 min. The embryo slurry was refiltered, blotted dry and weighed. The embryos were then homogenized (1 ml of buffer and 0.04 ml of 100 mM phenylmethylsulfonyl fluoride/g of embryos) in a Dounce homogenizer with a tight-fitting (A) pestle. The volume of the homogenate was measured, and one-ninth volume of 5 M NaCl was added to produce a final concentration of 0.5 M NaCl. This mixture was then homogenized with a B pestle and incubated for 30 min on ice with occasional stirring with a sterile plastic pipette. The homogenate was then centrifuged at 10,000 × *g* for 1 h at 4°C. The supernatant was collected, carefully avoiding the loose chromatin pellet. The supernatant was recentrifuged at 10,000 × *g* for 1 h at 4°C, and the supernatant was collected. The crude extract was frozen in liquid nitrogen as drops and stored at −80°C. Protein estimation was done by Bradford's method.

For generation of extracts from adult flies, *Drosophila* young or old adult flies were weighed and then homogenized in homogenization buffer at 1 ml of buffer and 0.04 ml of 100 mM phenylmethylsulfonyl fluoride/g of flies. The homogenate was filtered through four layers of cheesecloth, and the volume of the filtrate was measured. Subsequent steps were performed as described above. The same procedure was followed for X-ray-treated flies as well as for the mutant flies (both homozygous and heterozygous). When small numbers of flies were used, as in the case of cephalothoraces and X-ray-treated flies and controls, 150 μl of homogenization buffer was used per 100 cephalothoraces or whole flies. The filtration step was omitted, and subsequent steps were performed as described above. Extracts prepared in this way from small numbers of whole flies generally had about half of the specific activity of extracts prepared from large numbers of whole flies as described above.

Mismatch repair reaction. The mismatch repair reaction was as previously described (51). The standard mismatch repair reaction mixture (25 μl) contained 30 mM HEPES (pH 7.8), 7 mM $MgCl_2$, 4 mM ATP, 200 μM each CTP, GTP, and UTP, 100 μM each dATP, dGTP, dTTP, and dCTP, 40 mM creatine phosphate, 100 μg of creatine phosphokinase per ml, 15 mM sodium phosphate (pH 7.5), 5 ng of purified heteroduplex DNA, and the extract. After incubation at 37°C for the desired time, the reaction was terminated by the addition of 25 μl of stop mix (2% sodium dodecyl sulfate, 50 mM EDTA-Na_2 [pH 8.0], 2 mg of proteinase K per ml) and further incubated for 30 min. To this, 30 μl of tRNA (800 μg of tRNA per ml–4.6 M ammonium acetate) was added, and the sample was precipitated with 80 μl of isopropanol, extracted twice with 80 μl of phenol-chloroform-isoamyl alcohol, reprecipitated, and finally resuspended in 40 μl of distilled water. Transfection of *E. coli* NR9162 with this DNA was carried out by using a Bio-Rad Gene Pulser electroporation system. Repair efficiency is expressed in percent as 100 × (1 − ratio of percentages of mixed bursts obtained from extract-treated and untreated samples) (51).

RESULTS

The mismatch repair assay. The mismatch repair assay is based on the analysis of plaque color phenotypes resulting from the transfection of a *mutS E. coli* strain with purified M13mp2 heteroduplex DNAs that have been treated with various extracts (45, 51). The heteroduplexes contain base mispairs in the *E. coli lacZ*α gene; this gene can carry out α complementation, yielding blue phage plaques on host indicator plates (51). The composition of the mispair is such that

TABLE 1. Repair of G · G and T · G heteroduplexes by *Drosophila* embryo extract for 2 min at 37°C

Repair of:	Amt of protein (μg)	Total no. of plaques	Value for plaque phenotype			Total repair efficiency (%)
			Mixed	Minus strand	Plus strand	
G · G heteroduplex	0	5,690	31.6	45.8	22.6	
	0.2	5,691	27.8	44.6	28.3	12.0
	0.5	2,364	23.9	46.5	29.7	24.4
	2	2,045	15.7	41.3	43.0	50.3
	25	1,450	7.2	38.3	54.5	77.2
	50	1,675	5.7	39.1	55.2	82.0
	75	1,235	4.1	39.7	56.3	87.0
T · G heteroduplex	0	2,032	46.0	32.2	21.9	
	0.2	2,102	37.7	27.6	35.0	18.0
	0.5	1,964	33.8	26.6	39.6	26.5
	2	1,335	24.3	26.4	49.2	47.2
	25	1,291	19.8	23.6	56.6	57.0
	50	847	15.1	18.1	66.8	67.2

expression of one strand yields a light or dark blue plaque phenotype whereas the other strand contains a stop codon and leads to a white plaque. Upon transfection, any unrepaired molecules are potentially capable of forming mixed-color plaques at a certain frequency, as well as pure-color plaques. Pure-color bursts are obtained even without extract treatment, and this is characteristic of M13 phage replication in *E. coli* (51). When the template is first treated with extract and repair occurs, the ratios change such that mixed bursts decrease, and one or both pure bursts will increase depending on the extent of repair of either strand (51).

Mismatch repair requires a nick and is directed toward the nicked strand. The methylation state of adenine in d(GATC) sequences serves as the signal for strand discrimination of mismatch correction by the *E. coli* methyl-directed MutHLS pathway; a nick is made by MutH endonuclease, in the presence of MutL, at the d(GATC) site in the unmethylated strand (25, 33, 36). However, a persistent nick or strand break can bypass the requirements for both d(GATC) sequences and *mutH* for the *E. coli* mismatch repair system in vitro (30, 54). Transfection experiments suggest that a nick may suffice to determine strand specificity of mismatch repair in mammalian cells (17). Nuclear extracts derived from *Drosophila* K_c and HeLa cell lines have been found to correct single-base mispairs within open circular DNA heteroduplexes containing a strand-specific, site-specific nick located 808 bp from the mismatch (20). To determine whether a strand-specific and site-specific nick is required for efficient repair of G · G and T · G heteroduplexes in the *Drosophila* embryo extract, the relative frequencies of repair of the nicked and unnicked strands were assayed.

For both the G · G and T · G heteroduplexes, increasing *Drosophila* embryo extract amounts from 0 to 75 μg or 0 to 50 μg increased the repair efficiency to 87 and 67.2%, respectively (Table 1). Repair efficiency (defined in Materials and Methods) is a function of the degree to which extract treatment reduces the number of mixed-phenotype plaque bursts. The nick was always present in the minus strand, and as expected, repair was specific for the nicked strand. The frequency of mixed and minus-strand plaque phenotypes decreased dramatically with increasing extract, while the frequency of plus-strand plaque phenotypes increased (Table 1). Repair of G · G and T · G was also specific for the nicked strand in adult *Drosophila* extracts (data not shown).

When the nicked heteroduplex was ligated, repair of G · G and T · G was greatly reduced, as expected for nick-dependent repair (data not shown). In the presence of a nick, the nicked strand of the C · C heteroduplex also exhibited some preference for repair. However, ligation of the nicked C · C heteroduplex did not reduce the efficiency of repair, and now both strands were equally repaired (data not shown). This suggests the presence of an additional activity for C · C repair, as was previously reported for *E. coli* (39). The nick dependence of repair for each mispair is summarized in Table 4.

Repair of mispairs G · A, C · A, A · A, C · T, and T · T is not specific for the nicked strand. When the G · A and C · A mispairs were assayed for repair of the nicked and nonnicked DNA strands, repair was found to be specific for the nonnicked

TABLE 2. Repair of A · G, G · A, C · A, and A · A heteroduplexes by *Drosophila* young adult fly extract for 2 min at 37°C

Repair	Amt of protein (μg)	Total no. of plaques	Value for plaque phenotype			Total repair efficiency (%)
			Mixed	Minus strand	Plus strand	
G · A heteroduplex	0	1,529	33.4	39.8	26.8	
	0.5	1,712	26.3	47.1	26.9	21.3
	1.5	664	23.5	52.4	24.1	29.6
C · A heteroduplex	0	1,057	34.3	43.7	21.9	
	0.5	797	25.0	53.3	21.6	27.1
	1.5	744	20.4	61.0	18.6	40.5
A · A heteroduplex	0	1,400	30.0	46.0	24.4	
	1.5	818	11.6	51.7	36.7	61.3
A · G heteroduplex	0	1,202	32.8	28.0	41.1	
	0.5	789	24.8	25.3	49.8	24.4
	1.5	772	20.7	26.0	53.4	36.9

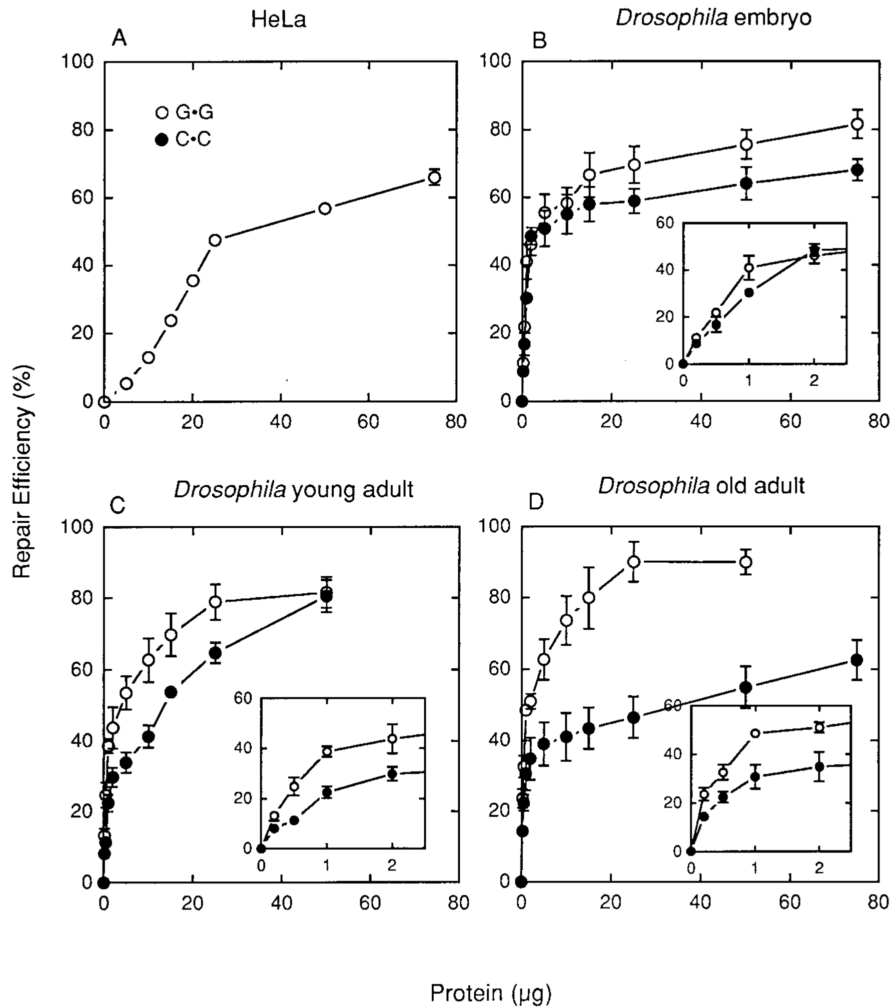

FIG. 1. Mismatch repair efficiencies of HeLa cell and *Drosophila* extracts. The percent repair efficiency was calculated as a function of amount of extract protein for the various types of extracts. Reaction mixtures were incubated for 15 min for HeLa cell extracts and for 2 min for *Drosophila* extracts. Results are expressed as total repair efficiencies and are based on counts of several hundred to several thousand plaques per data point. A small amount of C · C repair was observed in HeLa cell extracts, but it was not strand specific. The data are plotted as means ± standard errors. Open circles, repair of G · G; closed circles, repair of C · C.

(plus) strand (Table 2). A likely explanation for this result is that the G · A and C · A mispairs were being repaired by the activity of an A-glycosylase. Consistent with this idea, repair of the A · A mispair was found to occur on both strands (Table 2). Repair of the A · G mispair was found to be specific for the nicked strand; however, since the nicked strand also contains the A of the A · G mispair, this might or might not represent an A-glycosylase activity. Similarly, repair of mispairs C · T and T · T was not specific for the nicked strand. This repair may be the result of a T-glycosylase activity. A T-glycosylase has recently been purified from HeLa cell extracts (38).

Specific activity of mismatch repair in *Drosophila* extracts is maintained throughout development and aging. To compare mismatch repair activities during different stages of *Drosophila* development, it was necessary to measure the specific activities of extracts from embryos and from young and old adult flies. We also compared the specific activities of *Drosophila* extracts with those of HeLa cell extracts. The specific activities for correction of the mispairs G · G and C · C were determined by assaying repair in the linear range both for protein amount and time of incubation (Fig. 1). Conditions were identified for each type of extract (HeLa, *Drosophila* embryo, and *Drosophila* adult) for which repair activity was approximately linear with regard to protein concentration and time of reaction.

HeLa cell extract (0 to 25 μg of protein) shows an approximately linear response for percent repair efficiency when the reaction is carried out for 15 min (Fig. 1A). This range was used to determine the specific activity (percent repair efficiency/minute/microgram of protein) for the HeLa cell extract. For the more active *Drosophila* extracts (Fig. 1B to D), 0 to 1 μg of extract shows an approximately linear increase in the percent repair efficiency with 2-min incubation times. This range was thus used to determine the specific activities of mismatch repair in the various *Drosophila* extracts (Table 3). Mismatch repair activities were found to be approximately linear for repair efficiencies in the range of 0 to 20% (Fig. 1).

In *E. coli*, mismatch repair activity decreases as cells enter stationary phase because of a loss of MutS activity and, to a lesser extent, a decrease in MutH activity (13). It was of interest to determine if mismatch repair activity would be maintained in the postmitotic cells of the *Drosophila* adult and during aging. No significant difference in specific activity was found in extracts prepared from *Drosophila* embryos, young adults, and old adults (Table 3). Thus, expression of the mis-

TABLE 3. Measurement of specific activities of extracts for the repair of G · G heteroduplex

Extract	Sp act (% repair/min/μg of protein)	
	Extract 1	Extract 2
Drosophila embryos	24.2	16.3
Drosophila young adult	19.2	20.9
Drosophila old adult	22.5	21.7
Drosophila young cephalothorax	47.1	ND[a]
Drosophila old cephalothorax	44.7	ND
HeLa	0.14	0.12
HeLa (using *Drosophila* extract protocol)	0.08	ND
X-ray treatment		
Control[b]	7.5	12.4
Treated	44.0	69.2

[a] ND, not determined.

[b] Small-scale preparation of whole-fly extracts generally yields about half of the specific activity of large-scale preparations (see Materials and Methods).

TABLE 4. Specific activities of two different *Drosophila* embryo extracts with different mismatches

Mismatch[a]	Position[b]	Sp act (% repair/min/μg of protein)		Nick-dependent repair
		Extract 1	Extract 2	
A · A	89	55.0	53.0	No
T · G	89	23.8	26.5	Yes
G · G	88	24.2	16.3	Yes
C · C	88	15.5	13.7	No[c]
C · T	88	13.1	13.6	No
T · T	87	11.3	13.0	No
C · A	89	6.3	7.6	No
G · A	89	5.7	6.8	No
A · G	88	4.2	6.5	No[d]
Ω1 (+)	91	15.3	18.0	No
Ω5 (+)	127–131	16.0	10.0	No

[a] Heteroduplex DNA substrates (3′ nicked) with unpaired bases are depicted by the symbol Ω, followed by the number of unpaired bases. (+) indicates that the extra bases are present in the plus strand.

[b] Position of the mutation, where position +1 is the first transcribed base of the *lacZ* α-complementation gene in M13mp2 (51). Ω1(+) has A at position 91 of the *lacZ*α gene, and Ω5(+) has CACAT at positions 127 to 131.

[c] When a nick is present, the nicked strand is preferentially repaired; when the nick is absent, both strands are repaired (see text for details).

[d] Repair is strand specific but not necessarily nick dependent, as repair could have resulted from the activity of an A-glycosylase (see text for details).

match repair pathway factors is maintained throughout the adult *Drosophila* life span. All of the tissues of adult *Drosophila* are postmitotic with the exception of the gonads, which are located in the abdomen. To confirm that the mismatch repair activity in extracts of adults was indeed present in the postmitotic tissues and was not being contributed by the dividing cells of the gonads, extracts were prepared from young and old cephalothoraces, which consist entirely of postmitotic tissues. Specific activity of the cephalothorax extracts was even higher than that of whole-fly extracts, demonstrating that mismatch repair activities are indeed expressed in the postmitotic tissues of young and senescent adults.

For comparison, specific activities were calculated for HeLa cell extracts which had been prepared according to both published procedures (42) and by the same method as the *Drosophila* extracts were prepared (Table 3). The specific activity obtained for the HeLa cell extracts (maximum 0.14% repair/min/μg of protein) was comparable to published reports for activity of HeLa cell extracts (51). Strikingly, the *Drosophila* extracts averaged an approximately 150-fold higher specific activity than the HeLa cell extracts.

Mismatch repair activities in adults are induced by X-ray irradiation. It was of interest to determine whether the mismatch repair activity detected in the *Drosophila* extracts was an inducible system. To test for inducibility, extracts were prepared from adult flies which had been subjected to 76,800 rads of X-ray irradiation and from mock-irradiated control flies. The specific activity of the extracts for repair of G · G mismatch was found to be five- to sixfold induced by X-ray irradiation, indicating that the mismatch repair system detected in the extracts is inducible in adult flies (Table 3).

Relative efficiency of repair of different mismatches. To determine the relative efficiency of repair of different mismatches in *Drosophila* extracts, a variety of base mispairs were assayed for specific activity of repair in two independent embryo extracts (Table 4). A · A, T · G, and G · G were found to be the most efficiently repaired, C · C, T · T, and C · T were intermediate, and C · A, G · A, and A · G were the least efficiently repaired. In *E. coli*, the MutHLS system can also repair 1- to 3-bp loops but does not efficiently repair a 5-bp loop. The *Drosophila* embryo extract was found to efficiently repair both 1- and 5-bp loops; however, the repair was not nick dependent (Table 4).

Extracts of *mei-9* mutant animals exhibit defects in nick-dependent DNA mismatch repair. The *Drosophila mei-9* gene is homologous to yeast *rad1* and is required for meiotic recombination and certain types of mismatch repair and NER in vivo in *Drosophila*. To determine whether *mei-9* gene function is required for mismatch repair in the in vitro assay, extracts were prepared from animals homozygous for either of two strong *mei-9* alleles, as well as from heterozygous controls. Both the $mei\text{-}9^{AT2}$ and $mei\text{-}9^{a}$ mutant alleles were found to significantly reduce the repair of G · G and T · G mispairs. Relative to extracts of heterozygous flies, repair of G · G and T · G mispairs was reduced 5.7- and 9-fold, respectively, in $mei\text{-}9^{AT2}$ mutant extracts and reduced 12.5- and 10-fold, respectively, in $mei\text{-}9^{a}$ mutant extracts. The decrease in repair relative to wild-type flies may be even greater, as even the heterozygous fly extracts appear to be slightly reduced in activity relative to wild-type extracts. (Fig. 2). The *mei-9* mutations did not significantly affect the repair of the A · G or G · A mispairs (Fig. 2) or the other mispairs such as C · C, where repair was non-nick dependent (data not shown). Thus, *mei-9* mutations specifically reduce nick-dependent DNA mismatch repair in vitro.

DISCUSSION

We have characterized an in vitro system for DNA mismatch repair that uses extracts of *Drosophila* embryos and adults. Repair was nick dependent and specific for the nicked DNA strand of the heteroduplex for mispairs T · G and G · G. In contrast, repair of A · A, C · A, G · A, C · T, T · T, and C · C was not nick dependent and may be catalyzed by glycosylases. A-glycosylase activity has been observed in other systems, for example, the MutY activity of *E. coli* (3). A T-glycosylase has previously been purified from HeLa cell extracts (38). The range of relative activities for the repair of the different mispairs was similar to that previously reported for extracts of mammalian cells and *Drosophila* tissue culture cells. Strikingly, the *Drosophila* embryo and adult extracts have >100-fold-higher specific activity for nick-dependent repair than HeLa tissue culture cell extracts.

The developing *Drosophila* embryo exhibits the most rapid

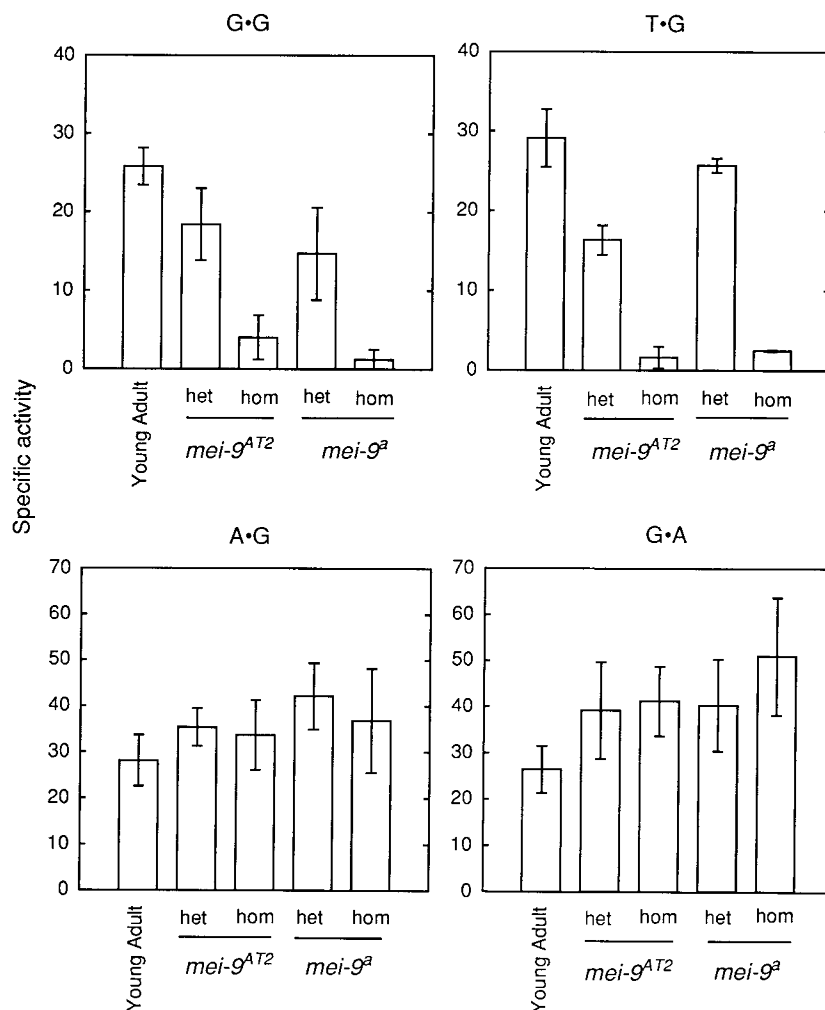

FIG. 2. Repair of G · G, T · G, A · G, and G · A mispairs by *Drosophila* wild-type and *mei-9* mutant extracts. Extracts were prepared from Oregon-R strain (wild-type) young adults and from flies heterozygous (het) and homozygous (hom) for *mei-9* alleles $mei\text{-}9^{AT2}$ and $mei\text{-}9^{a}$, as indicated. Results are expressed as specific activity (percent repair efficiency/minute/microgram of protein) and are based on counts of several hundred to several thousand plaques per assay. Reaction mixtures contained 0.5 μg of protein and were incubated for 2 min at 37°C, which is within the approximately linear range of the assay (Fig. 1C). The data are plotted as means ± standard errors of two to four independent measurements. All mispairs were efficiently repaired by a mixture of wild-type and *mei-9* mutant extracts (data not shown).

S phase known for a multicellular eukaryote, and it is therefore not surprising that these cells would express high levels of the factors required for postreplicative DNA mismatch repair. In *E. coli*, cessation of cell division by entry into stationary phase causes a down-regulation of DNA mismatch repair activities: a 10-fold decrease in *mutS* activity and a 3-fold decrease in *mutH* activity (13). In contrast, extracts of the entirely postmitotic cells of adult *Drosophila* had a specific activity for mismatch repair equal to that of the rapidly dividing *Drosophila* embryo. Moreover, equally high specific activity was obtained from extracts of highly senescent *Drosophila* adults. This finding indicates that the relevant activities are continuously expressed up to the end of the *Drosophila* life span. The continued high level expression of these DNA mismatch repair activities suggests that they may be required to maintain DNA sequence integrity throughout the adult *Drosophila* life span.

DNA mismatch repair activity in the postmitotic cells of adult *Drosophila* may be inducible, in that the specific activity of extracts was increased five- to sixfold by pretreatment of the adults with X rays. X rays produce hydroxyl radicals, which in turn cause double-strand breaks in DNA (5). In *Drosophila*, repair of double-strand breaks created by transposable element excision appears to involve exonucleolytic digestion of the free DNA ends and then resynthesis of the double-strand DNA gap, using homologous sequences as the template (12). X-ray-induced double-strand breaks may be repaired by a similar pathway, and we hypothesize that mismatch repair activities may be induced to correct any misincorporated nucleotides in the newly synthesized DNA. Consistent with this idea, double-strand break repair in yeast is associated with high rates of DNA synthesis errors (49). Alternatively, X-ray-generated oxygen radicals may directly damage certain DNA bases, and activities involved in both NER and mismatch repair may be induced to repair this damage.

Requirement for *mei-9* activity. Mutations in the *Drosophila mei-9* gene were first identified in a screen for mutations causing increased levels of meiotic nondisjunction (4). The meiotic nondisjunction results from a reduced level of meiotic crossing over (reciprocal exchange) to <10% of wild-type levels. While reciprocal exchange is greatly reduced by *mei-9* mutations, meiotic gene conversion is nearly normal. In addition, *mei-9* females exhibit high levels of postmeiotic segregation, where

progeny can inherit a single maternal chromosome yet be mosaic for both maternal alleles of a particular gene on that chromosome (11, 43). These data have been interpreted as indicating that *mei-9* females are able to generate heteroduplex DNA in recombination intermediates but are unable to repair mismatches within the heteroduplex regions and to resolve the recombination intermediates as reciprocal exchanges (46).

Drosophila DNA repair genes, including *mei-9*, have also been identified in screens for mutations which cause increased sensitivity to mutagens (8). Strong *mei-9* alleles were found to decrease NER, as evidenced by a decreased rate at which pyrimidine dimers are cleared from genomic DNA of *mei-9* animals after UV irradiation (9, 18). The *mei-9* gene was recently cloned from *Drosophila* and found to encode the homolog of the *Saccharomyces cerevisiae rad1* and *Schizosaccharomyces pombe rad16* genes, both of which are required for NER. *mei-9* is also homologous to human XP complementation group F, which is also required for NER (10, 48). In *S. cerevisiae*, *rad1* functions in combination with the product of another gene required for NER, *rad10*, as a single-stranded DNA endonuclease in vitro (52). It is hypothesized that the yeast Rad1/Rad10 endonuclease is responsible for generating the nick 5′ to DNA damage sites during NER (6), and it appears likely that *mei-9* has an analogous function in *Drosophila*. It has recently been reported that repair of a 26-base loop in yeast involves the action of both Msh2 and Rad1, demonstrating that mismatch repair and NER systems can act in concert to eliminate specific aberrant DNA structures (23).

One important aspect of the in vitro DNA mismatch repair system reported here is that highly active extracts can be prepared from whole adult *Drosophila* flies. This result allows extracts to be made from animals mutant for suspected or known DNA repair genes, such as *mei-9*. Our results demonstrate that in vitro DNA mismatch repair in extracts of *mei-9* adults exhibits specific defects. Nick-dependent repair of the mismatches G · G and T · G is reduced 5- to 12-fold. In contrast, the non-nick-dependent repair of the other mispairs such as A · G and G · A is not detectably affected. These results are consistent with a specific requirement for *mei-9* gene function during nick-dependent DNA mismatch repair. The availability of an in vitro system which is dependent on *mei-9* gene product for full activity should be useful in elucidating the exact role of *mei-9* function in DNA repair. Similarly, this system can potentially be used to characterize other DNA repair mutations for their affects on in vitro DNA mismatch repair.

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