*Proc. Natl. Acad. Sci. USA*
Vol. 83, pp. 3366–3370, May 1986
Genetics

# Photoreactivation rescue and hypermutability of ultraviolet-irradiated excisionless *Drosophila melanogaster* larvae

**(repair-dependent mutation/error-prone postreplication repair/excision repair/target size for mutation/somatic mutagenesis)**

HARUKO RYO AND SOHEI KONDO*

Department of Fundamental Radiology, Faculty of Medicine, Osaka University, Kita-ku, Osaka 530, Japan

*Communicated by Richard B. Setlow, December 27, 1985*

**ABSTRACT** **There is accumulating evidence suggesting that expression of genes for repair of UV damage to DNA in mammals and fish is regulated developmentally. Therefore, the activity of excision repair and photoreactivation *in vivo* in young larvae of *Drosophila melanogaster* was examined in a strain carrying the mutation *mus201* that was unable to carry out excision repair. The photoreactivation activity in first-instar larvae was so high that UV-induced lethality in excisionless larvae was almost completely rescued by posttreatment with fluorescent light. Excision repair activity in first-instar repair-proficient larvae was so high that UV irradiation was scarcely able to produce somatic eye-color mutations. In contrast, excisionless larvae showed a high incidence of somatic eye-color mutation after UV-irradiation, and this incidence was reduced to the spontaneous level by posttreatment with fluorescent light. Incorporation of a postreplication repair-defective mutation into the excisionless strain decreased the incidence of UV-induced somatic mutations by a factor of 3. The analogous repair dependence of UV mutagenesis in *Drosophila* and *Escherichia coli* is discussed. It is proposed that UV-induced somatic mutations in excisionless *Drosophila* larvae are caused primarily by pyrimidine dimers and that a constitutive, error-prone pathway for filling daughter-strand gaps opposite dimers is, at least partly, responsible for the fixation of mutations.**

The ability of living organisms to recover from lethal damage caused by external agents was first convincingly demonstrated in 1949 by Kelner (1), who observed rescue of UV-irradiated *Streptomyces griseus* by posttreatment with visible light. As early as 1952, Altenburg and Altenburg (2) reported that UV-induced lethal mutations in germ cells of *Drosophila melanogaster* were greatly reduced by posttreatment of UV-irradiated embryos with visible light. Setlow and Setlow (3) found that, in UV-induced inactivation of transforming DNA of *Haemophilus influenzae*, a major site of damage was thymine dimers. This finding led to studies on the molecular biology of UV effects and the finding that almost all living organisms studied have various mechanisms for repair of DNA damage caused by UV, x-rays, and various chemicals (4).

Studies on DNA repair at a molecular level have been confined mainly to microorganisms and in higher organisms to cells cultured *in vitro*. In a few cases, however, DNA repair has been studied *in vivo* in animals, and interesting findings have been obtained, often differing from those obtained *in vitro*. Pyrimidine dimers formed in human DNA are removed 1 order of magnitude faster in human skin *in vivo* than in cultured human fibroblasts (5, 6). Further, pyrimidine dimers formed *in vivo* in DNA of UV-irradiated human skin largely disappear after *in vivo* exposure to visible light (5, 7), but photoreactivation of pyrimidine dimers in cultured human cells is only rarely detected (4, 8). Photoreactivation of UV-induced pyrimidine dimers occurs in the skin (only in the dermis) of neonatal mice but not in that of adult mice of the same inbred strain (9). In mouse embryo fibroblasts, excision-repair activity decreases abruptly during progressive passage of the cells in culture (10). Cultured mouse fibroblasts have only low activity of excision repair (11). In adult mice, repair in the dark of DNA damage caused by UV and UV-mimetic chemicals, as measured by unscheduled DNA synthesis *in vivo* in skin, is considerably less active in fibroblastic cells than in epithelial cells (12, 13). Dark repair activity for UV-induced DNA damage is absent in cultured fish cells (14, 15) but present in fish embryos (15).

The above cited findings strongly suggest that the expression of repair genes in animals is regulated developmentally (4). Much information is available on both the developmental biology and genetics of *D. melanogaster*. In this species, about 30 DNA repair-defective mutations have been reported (16), and two mutant strains, referred to by the mutations *mei-9* and *mus201* that they respectively carry, are unable to excise UV-induced pyrimidine dimers (16, 17). Survival to eclosion of larvae of the *mus201* strain is drastically reduced after UV-irradiation as compared with parallel irradiation of repair-proficient larvae (17). In the present study, we used this excisionless strain in studies on photoreactivation and dark repair *in vivo* in *Drosophila* larvae. We report that the photoreactivation activity in *mus201 Drosophila* larvae is so high that UV-induced lethal damage can be almost completely repaired by photoreactivation and that the dark repair activity in repair-proficient larvae is so high that UV-irradiation scarcely induces somatic mutations, whereas the excisionless larvae are hypersensitive to somatic mutation induced by UV irradiation.

## MATERIALS AND METHODS

***Drosophila* Strains.** The strain with the homozygous genotype of $mus201^{D1}$ $cn^{35}$ (cinnabar eye color) used was obtained from P. D. Smith. It is extremely UV sensitive because of complete loss of the capacity of excision repair of UV-induced pyrimidine dimers (17). A stock with an X chromosome marked with *w* $mus104^{D1}$ (18) was obtained from J. B. Boyd. It is defective in the capacity of postreplication repair of UV-induced DNA damage (16). The loci $mus201^{D1}$ and $mus104^{D1}$ map to chromosome 2 and the X chromosome, respectively (19).

As previously reported (20), to assay somatic mutation, we used two strains: one bears the genetic markers $z^1$ (*zeste* mutant) and $w^{+(TE)}$ (a genetically unstable *white* allele, presumably caused by a transposable element) (21, 22), and the second bears the white-ivory mutation $w^i$ (23).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: kb, kilobase(s); bp, base pair(s).

*To whom correspondence should be addressed.

By incorporating the *mus104*$^{D1}$ and *mus201*$^{D1}$ mutations into strains bearing the genotype $z^l w^{+(TE)}$ or $w^i$, we constructed strains with the homozygous genotypes of ($z^l$ $w^{+(TE)}$; *mus201*), ($z^l$ $w^{+(TE)}$ *mus104*), ($z^l$ $w^{+(TE)}$ *mus104*; *mus201*), ($w^i$; *mus201*) and ($w^i$ *mus104*) and also DNA-repair-proficient strains with the genotypes of ($z^l$ $w^{+(TE)}$) and ($w^i$). In this paper, the symbols shown in parentheses above are used to denote the respective strains.

**Irradiation with UV and x-Rays.** Males and females were maintained separately for 2 to 4 days. Eighty pairs were placed overnight in a culture bottle (180 ml; 18 cm$^2$) containing sucrose/agar (3% sucrose/1.2% agar/1% propionic acid) for oviposition. Eggs were dechorionated by immersion in 2.5% NaOCl solution for 2.5 min at room temperature and then washed in water, placed on sucrose/agar, and incubated at 25°C for 24 hr. Larvae that hatched were collected. Larvae (200–300) were placed in a monolayer fashion in a glass Petri dish (9-cm diameter) containing a small amount of *Drosophila* Ringer solution and exposed to UV from two 15-W Toshiba germicidal lamps at a dose rate of 0.09 J/m$^2$ per sec for repair-deficient strains and of 1 J/m$^2$ per sec for repair-proficient strains. The larvae were transferred to bottles containing fresh standard medium and cultured in the dark at 25°C until eclosion. All procedures during and after UV irradiation were carried out under yellow light.

For x-irradiation, third instar $w^i$ larvae and second- to-third-instar $w^{+(TE)}$ larvae were used as described (20).

**Photoreactivation Procedure.** After UV-irradiation, larvae were exposed to fluorescent light from two 15-W Toshiba fluorescent lamps at a distance of 17 cm for 2 hr at 25°C. Larvae were then transferred to bottles containing fresh standard medium and, to ensure maximal photoreactivation, were cultured under fluorescent light at 25 cm from two 15-W fluorescent lamps until eclosion. Control larvae that received no posttreatment were kept identically to those subjected to the photoreactivation treatment, except that they were covered with aluminum foil.

**Assay of Lethality and Somatic Reversions.** Survival of larvae was scored as the fraction of larvae that survived to eclosion.

The frequency of somatic reversions was defined as the fraction of adult flies bearing mosaic, red spots on the yellow eyes of the genotype $z^l$, $w^{+(TE)}$ and on the ivory eyes of the mutant $w^i$ (see ref. 20 for details). In this paper, reversions mean those in male flies unless otherwise stated.

## RESULTS

**Photoreactivation Rescue of UV-Irradiated Excisionless Larvae.** The survival of UV-irradiated *mus201* larvae to adulthood was only slightly higher than the survival of colony-forming ability of UV-irradiated cultured *mus201* cells reported previously (24) (Fig. 1). The survival of UV-irradiated larvae of the repair-proficient strain was slightly higher than that reported for cultured repair-proficient cells (24) in the low-dose range but was lower than that reported for the latter in the high-dose range (Fig. 1). After posttreatment with fluorescent light, the survival of UV-irradiated excisionless larvae markedly increased, frequently above 80% (Fig. 1).

**Hypersensitivity of UV-Irradiated Excisionless Larvae to Somatic Mutation and to Photoreversal of UV-Induced Premutational Damage.** In the excisionless *mus201* strain, the frequency of reversion of $w^{+(TE)}$ increased to 19 and 28 times the spontaneous frequency at UV doses of 1 and 2 J/m$^2$, respectively (Table 1). In a repair-proficient strain, the frequencies of reversion of $w^{+(TE)}$ were only 2 and 3 times the spontaneous level, even at high doses of 20 and 25 J/m$^2$, respectively, and these increases were not statistically significant (Table 1). Like UV reversion of $w^{+(TE)}$, that of $w^i$ was significantly increased above the control level only in the excisionless strain but its frequency was 1 order of magnitude less than that of $w^{+(TE)}$ (Table 1).

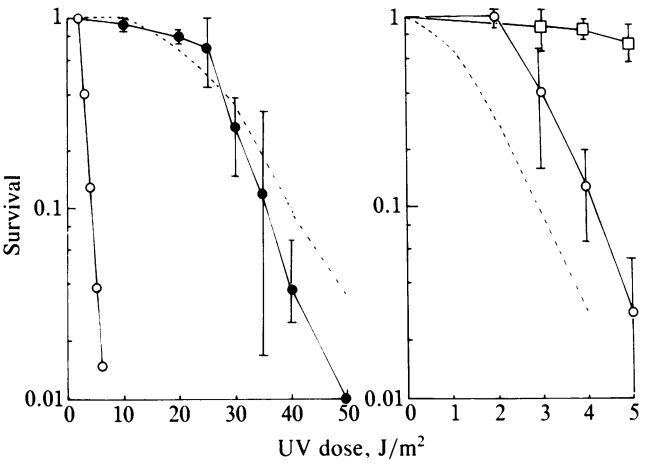


FIG. 1. UV effects on survival to adulthood after larval irradiation of *Drosophila* and photoreactivation rescue of UV-irradiated larvae. (*Left*) Comparison of UV sensitivities of excisionless (*mus201*) (○) and repair-proficient (Oregon R strain) (●) larvae after irradiation at the indicated doses in the first instar. The data are based on duplicate to triplicate experiments except for a point at 50 J/m$^2$ from a single experiment. The bars indicate standard deviations. The dotted line represents the dose–response curve of repair-proficient *Drosophila* cells for survival of colony-forming ability after UV irradiation (from ref. 24). (*Right*) Photoreactivation rescue of UV-irradiated larvae. Excisionless (*mus201*) larvae in the first instar were exposed to UV at the indicated doses from germicidal lamps. Half the UV-irradiated larvae were kept in the dark (○) and the rest were posttreated with fluorescent light (□). The bars indicate standard deviations. The dotted line represents the dose–response curve of cultured *mus201* cells for survival of colony-forming ability after UV irradiation in the dark (from ref. 24).

Posttreatment of UV-irradiated larvae with fluorescent light reduced the reversion frequency of $w^{+(TE)}$ in the *mus201* strain to the spontaneous frequency (Table 1).

It is puzzling that female larvae with two $w^i$ mutations did not show UV-reversion rates of twice those in males with one $w^i$ mutation, as was the case of x-ray-induced reversion of $w^i$ (Table 1).

**Reduction of UV- and x-Ray-Induced Mutation in Postreplication Defective Larvae.** The frequency of reversion of $w^{+(TE)}$ induced by UV-irradiation of first-instar larvae was reduced by about a factor of 3 by incorporation of the postreplication-defective mutation *mus104* into the *mus201* strain (Table 1). As an attempt to increase the reversion frequency, we shifted the time of x-irradiation to later stages, but reversion frequencies were increased only for the mutation $w^i$ (see ref. 20 for details). Therefore, the frequency of x-ray reversion of $w^{+(TE)}$ and that of $w^i$ in repair-proficient strains were reduced by about a factor of 2 by incorporation of the *mus104* mutation, but the reduction was significant only for reversion of $w^i$ (Table 1).

## DISCUSSION

We observed a marked reduction of UV-induced lethal damage in excisionless larvae by photoreactivation treatment (Fig. 1 *Right*): after a UV dose of 5 J/m$^2$, survival increased from about 1% in the dark to about 70% after posttreatment with visible light. However, it is difficult to assess quantitatively the observed fraction capable of photoreactivation because there is a big shoulder in the dose–survival curve after UV irradiation without photoreactivation (Fig. 1 *Right*). UV-induced somatic mutations were more suitable for use in quantitative estimation of the extent of the photoreactivation effect because they gave an approximately linear dose–

Table 1. Frequencies of somatic reversions of $w^{+(TE)}$ and $w^i$ in repair-deficient and repair-proficient strains after larval irradiation with UV during the first instar and with x-rays during later instars*

| Mutant locus and sex | DNA repair | Irradiation† | Frequency of reversion: No. of red spots/no. of flies | Frequency of reversion: % |
|---|---|---|---|---|
| $w^{+(TE)}$ in male | Wild type | Control | 13/18,214‡ | 0.07 |
| | | UV, 15 | 4/5,376 | 0.07 |
| | | UV, 20 | 10/6,523 | 0.15 |
| | | UV, 25 | 6/3,170 | 0.19 |
| | | X-ray, 300 | 7/2,100 | 0.33§ |
| | | X-ray, 600 | 12/2,311 | 0.52§ |
| | *mus201* | Control | 5/6,036 | 0.08 |
| | | UV, 1 | 68/4,441 | 1.5§ |
| | | UV, 2 | 54/2,486 | 2.2§ |
| | | PR¶ only | 0/1,218 | 0 |
| | | UV, 1 + PR¶ | 1/2,859 | 0.03 |
| | | UV, 2 + PR¶ | 1/724 | 0.14 |
| | *mus104* | Control | 6/6,104‡ | 0.10 |
| | | X-ray, 300 | 5/2,552 | 0.20§ |
| | | X-ray, 600 | 7/1,612 | 0.43§ |
| | *mus 104 mus 201* | Control | 0/632 | 0 |
| | | UV, 1 | 4/904 | 0.44§‖ |
| | | UV, 2 | 11/1,389 | 0.79§‖ |
| $w^i$ in male | Wild type | Control | 11/12,903‡ | 0.09 |
| | | UV, 10 | 8/6,098 | 0.13 |
| | | UV, 20 | 3/5,384 | 0.06 |
| | | X-ray, 300 | 29/1,337 | 2.2§ |
| | | X-ray, 600 | 55/1,096 | 5.0§ |
| | *mus201* | Control | 3/8,099 | 0.04 |
| | | UV, 0.75 | 4/3,637 | 0.11 |
| | | UV, 1.5 | 11/6,150 | 0.18§ |
| | *mus104* | Control | 3/1,633 | 0.18 |
| | | X-ray, 300 | 25/2,097 | 1.2§‖ |
| | | X-ray, 600 | 40/1,854 | 2.2§‖ |
| $w^i$ in female | Wild type | Control | 8/14,020‡ | 0.06 |
| | | UV, 10 | 7/5,846 | 0.12 |
| | | UV, 20 | 5/2,836 | 0.18 |
| | | X-ray, 300 | 71/1,273 | 5.6§ |
| | | X-ray, 600 | 111/1,082 | 10.3§ |
| | *mus201* | Control | 7/9,833 | 0.07 |
| | | UV, 0.75 | 5/4,529 | 0.11 |
| | | UV, 1.5 | 16/8,354 | 0.19§ |
| | *mus104* | Control | 5/1,866 | 0.27 |
| | | X-ray, 300 | 57/2,327 | 2.4§‖ |
| | | X-ray, 600 | 90/1,877 | 4.8§‖ |

*During the second to third and the third instar for reversions of $w^{+(TE)}$ and $w^i$, respectively.
†UV dose in J/m$^2$; x-ray dose in rad.
‡Pooled data from the present and previous experiments (20, 25).
§Significantly different from the corresponding control frequency at $P < 0.05$ by $\chi^2$ test. The $\chi^2$ test for the *mus104 mus201* strain used the control frequency in the *mus104* strain.
¶Photoreactivation (PR) treatment (see text for details).
‖Significantly different at $P < 0.05$ by $\chi^2$ test from the frequency of reversion in the corresponding *mus104*$^+$ strain at the same dose.

response relationship (Table 1). Taking the data from Table 1 at face value, we found that 97% of the UV-induced mutagenic damage at 2 J/m$^2$ of UV was repaired by photoreactivation after treatment.

Brown *et al.* (26) reported that cultured excisionless *mei-9 Drosophila* cells irradiated with 4 J/m$^2$ of UV and then for 60 min with a fluorescent light (from a 30-W fluorescent lamp at a distance of 25.5 cm) retained only about 9% of the pyrimidine dimers that were initially produced by UV. Furthermore, their data indicate that photoreactivation after treatment for longer than 60 min would decrease the percentage of dimers even further. Combining their results with ours on the photoreactivation effects *in vivo* on excisionless *mus201* larvae, we conclude that pyrimidine dimers are the major cause of UV-induced lethal and mutagenic damage in excisionless larvae and that the dimers produced at low UV doses are almost completely repaired by posttreatment with fluorescent light. In contrast, Brown *et al.* (26) concluded that much of the lethal effect of UV in cells cultured *in vitro* is nondimer damage because they found that photoreactivation increased the survival of *mei-9* cells irradiated at 4 J/m$^2$ of UV only to a level 1/40th of that expected from the photoreactivation-induced decrease in the dimers, assuming that dimers alone were the cause of lethality. The quantitative discrepancy between *in vivo* and *in vitro* data on decrease in UV-induced lethality after photoreactivation is not yet understood. However, it seems certain that both photorepair of dimers and photoreactivation of UV-induced biological damage occur in parallel *in vivo* in *Drosophila* larvae at very efficient rates.

In repair-proficient larvae, the frequency of reversion of $w^{+(TE)}$ at 20 J/m$^2$ after correction for the spontaneous rate was about 0.08%, whereas irradiation of excisionless larvae at 2 J/m$^2$ produced 2.1% (2.2–0.08%) of the induced frequency (Table 1). Thus, assuming a linear dose–response relationship, the rate of UV-induced mutation per unit dose in repair-proficient larvae was about 200 times less than that in the excisionless larvae. This indicates that repair-proficient larvae have a practically complete ability to repair mutagenic damage induced by UV in a low-dose range. The assumed high activity of DNA repair in larvae is compatible with the previous report by Baker *et al.* (27) that first-instar *mei-9* larvae produced by mothers possessing a homozygous *mei-9*$^+$ attached X chromosome, were phenotypically nearly completely wild type with respect to UV sensitivity, indicating that the amount of repair gene products supplied by mothers to their eggs is so great as to make the excisionless *mei-9* first-instar larvae repair-proficient.

When the chorion of the *Drosophila* egg is removed, germ cells at the polar cap stage are separated from the outside surface by only the thin transparent vitelline membrane. Therefore, the doses of UV used for induction of recessive lethal mutations in germ cells at this stage (28) can be taken as actually effective doses. The lethal mutation frequency increased almost linearly with increase in the UV dose from 3.8 to 18 J/m$^2$, but extrapolation of the linear regression line to lower doses intersected the line for the spontaneous frequency at about 2 J/m$^2$, indicating a threshold-like response in a low-dose range probably due to high activity of excision repair. Furthermore, the UV-induced mutation frequencies in embryos preirradiated with UV doses of 7.5, 13, and 18 J/m$^2$ decreased after posttreatment with visible light by factors of 2, 3, and 8 (27). In spite of large statistical errors, these data suffice to show that embryos possess high activity of dark repair and photorepair of UV damage.

Assuming that the major cause of UV-induced backmutation in excisionless *Drosophila* and *Escherichia coli* is pyrimidine dimers, we can compare the efficiencies of production of one mutational event per pyrimidine dimer in DNA. In Table 2, we tabulate reversion to prototrophy of an Arg$^-$ (arginine requiring) phenotype, which is caused by an amber-type nonsense mutation, in a *uvrA*$^-$ (excisionless) strain of *E. coli*. The reversion of the Arg$^-$ phenotype is caused by suppressor mutation of the amber type in *E. coli* (30). Under excisionless conditions, the rate of occurrence per J/m$^2$ of UV of a base substitution mutation, which

Table 2. Comparison of *E. coli* and *D. melanogaster* for mutation rates in excisionless strains after UV irradiation and for those in repair-proficient strains after x-irradiation

| Type of mutation | Reversion rate per locus | | Apparent target size for reversion, bp | |
|---|---|---|---|---|
| | UV (1 J/m$^2$) | X-ray (1 krad) | UV | X-ray |
| Suppressor mutation from Arg$^-$ to Arg$^+$ phenotype in *E. coli* | $1 \times 10^{-5}$* | $5 \times 10^{-8}$* | 1 | 0.5 |
| Reversion of $w^{+(TE)}$ in *D. melanogaster* | $2 \times 10^{-4}$† | $1 \times 10^{-4}$‡ | 20 | 1000 |
| Reversion of $w^i$ in *D. melanogaster* | $2 \times 10^{-5}$† | $3 \times 10^{-5}$‡ | 2 | 300 |

*From Kondo *et al.* (29); the UV mutations were in a *uvrA*$^-$ strain.
†From Table 1 assuming 80 eye imaginal discs per first-instar larva (20); the reversions were in *mus201* strains.
‡From Ryo *et al.* (20); the reversions were in repair-proficient strains after x-irradiation in the first instar.

suppresses the Arg$^-$ phenotype, is almost equal to that for reversion of $w^i$ in *Drosophila* and only 20-fold lower than that for reversion of $w^{+(TE)}$ in *Drosophila*. In contrast, the reversion rate per krad of x-rays for the Arg$^-$ phenotype is 3 orders of magnitude lower than those for $w^i$ and $w^{+(TE)}$ in *Drosophila*. In *Drosophila*, the error due to attenuation of UV beams before they reach target cells of the eye imaginal discs was small because, as seen from Fig. 1 *Right*, the survival–dose curve for UV inactivation of *mus201* larvae was close to that for UV inactivation of *mus201* cells in culture (24). As noted previously (24), the survival curve after UV irradiation of *mus201* cells is very close to that of another excisionless mutant *mei-9* cells (26). Therefore, we can use the value of 5.75 dimers per $10^8$ daltons of DNA at 4 J/m$^2$ of UV for *mei-9* cells (26) for estimating the rate of dimer formation in *mus201* cells. The efficiency of dimer formation $d$ per base pair (bp) per unit of UV dose can be calculated as $10^{-5}$ dimers per bp per J/m$^2$. This $d$ value is almost identical to the dimer formation efficiency in *E. coli* (31). Thus, the apparent target size of the gene, $x$(bp), can be calculated from the following equation, assuming that one dimer in the target gene leads to one mutational event:

$$f = dx, \qquad [1]$$

where $f$ is the mutation rate per locus per J/m$^2$. We assume that each of first-instar larvae has 80 cells in its imaginal eye discs (20). The male strains used have one locus of $w^i$ or $w^{+(TE)}$ per somatic cell. Thus, we obtain the $f$ values given in Table 2. Using the $f$ values and the $d$ values mentioned above, we obtained the $x$ values given in column 4 of Table 2. Similarly, assuming that one double-strand break, which is assumed to occur on the average once per 600 eV of x-ray energy absorbed by DNA (32), causes one mutational event, we can estimate the apparent target sizes for x-ray-induced mutations at the three loci (see the last column in Table 2). It is interesting to note that in *E. coli* the apparent target size for UV reversion and that for x-ray reversion are equal for inducing the base substitution mutation, but in *Drosophila* the target size for UV mutation is 150 and 50 times less, respectively, than the target size for x-ray mutation at $w^i$ and $w^{+(TE)}$. These results imply that the $w^i$ and $w^{+(TE)}$ alleles are highly sensitive to reversion by ionizing radiation. Based on the finding that the rate of reversion of $w^i$ per locus per krad for somatic reversion (20) is close to the previously reported rate (33) for germinal reversions, Ryo *et al.* (20) proposed that somatic reversion of $w^i$ results from precise excision of one of the tandemly duplicated 2.9-kilobase (kb) segments because the precise excision was previously demonstrated for germinal reversions of the $w^i$ mutant (34). As discussed previously (20), the high reversion rate of the $w^i$ mutant after x-irradiation is compatible with the hypothesis that the precise excision of one copy of the 2.9-kb duplicated fragments is caused by intrachromosomal recombination at any point along a double loop formed by pairing of the duplicated sequence (33, 34). Then, the much lower rate of UV reversion of $w^i$ than that of x-ray reversion of $w^i$ may be explained by the assumption that the precise excision of one 2.9-kb copy occurs efficiently only at DNA strand breaks, which are the major damage induced by x-irradiation, but which are not induced appreciably by UV-irradiation in an excisionless strain. Similarly, the higher reversion rate of $w^{+(TE)}$ after x-irradiation than after UV-irradiation can be explained by the assumption that a hypermutable "hot spot" structure of DNA similar to the double loop mentioned above is formed by the transposable element thought to be responsible for the unstable mutant phenotype of $w^{+(TE)}$ (21, 22).

In *E. coli*, the mutation *recA*$^-$ completely nullifies postreplication repair of UV damage (4, 35) and completely blocks induction of suppressor mutations from auxotrophy to prototrophy after irradiation with UV (35, 36) and x-rays (35). In *Drosophila*, the postreplication repair-deficient mutation *mus104* reduced the rate of UV-induced reversion of $w^{+(TE)}$ by a factor of about 3 (Table 1). The *mus104* mutation also reduced the x-ray-induced reversion rates of $w^{+(TE)}$ and $w^i$ by a factor of about 2, but the reduction was significant only for the high incidences of reversion of $w^i$ after x-irradiation in the third instar (see Table 1 and *Results* for details). The *mus104* mutation markedly reduced the incidences of reversion of $w^{+(TE)}$ by alkylating agents (25). In *E. coli*, UV mutagenesis in excisionless strains is believed to be caused by error-prone postreplication repair of daughter-strand gaps opposite pyrimidine dimers or from error-prone semiconservative replication past a pyrimidine dimer or pyrimidine-pyrimidine (6-4) photoproduct, and the error-prone functions are induced by participation of the *recA*$^+$ and *lexA*$^+$ genes activated by the presence of pyrimidine dimers (4, 37, 38). Boyd and Setlow (39) analyzed the modes of postreplication repair after UV irradiation of embryonic cells from various repair-defective strains of *Drosophila* and proposed that replication proceeds through UV-induced pyrimidine dimers by sealing of gaps in daughter strands opposite the dimers by two separate pathways: normal *trans*-dimer replication, which is rapid, recombination-dependent, and caffeine-sensitive; and a second pathway, which is slow, recombination-independent, and caffeine-insensitive. The *mus104* mutant is defective in normal *trans*-dimer DNA synthesis (39). From the above model of UV mutagenesis in *E. coli*, together with the model of Boyd and Setlow and our finding of reduction of UV mutagenesis by the *mus104* mutation, we propose as a working hypothesis that UV-induced somatic reversion of $w^{+(TE)}$ in excisionless *Drosophila* larvae is caused, at least in part, by an error-prone pathway for sealing daughter-strand gaps, which is defective in the *mus104* strain. Because of the

large contribution of maternal gene products to the repair capacity of first-instar larvae (27), the presumed error-prone *trans*-dimer DNA synthesis during the first instar must primarily be a constitutive process.

We thank J. B. Boyd, B. Rasmuson, P. D. Smith, and B. H. Judd for fly stocks, M. A. Yoo for competent help during the experiment, and M. Ninomiya for preparing the manuscript. This work was partly supported by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan and by a mutation-research fund from Takeda Yakuhin, Osaka.

1. Kelner, A. (1949) *Proc. Natl. Acad. Sci. USA* **35,** 73–79.
2. Altenburg, L. S. & Altenburg, E. (1952) *Genetics* **37,** 545–553.
3. Setlow, R. B. & Setlow, J. K. (1962) *Proc. Natl. Acad. Sci. USA* **48,** 1250–1257.
4. Friedberg, E. C. (1985) *DNA Repair* (Freeman, New York).
5. Sutherland, B. M., Harber, L. C. & Kochevar, I. E. (1980) *Cancer Res.* **40,** 3180–3185.
6. D'Ambrosio, S. M., Slazinski, L., Whetstone, J. W. & Lowney, E. (1981) *J. Invest. Dermatol.* **77,** 311–313.
7. D'Ambrosio, S. M., Whetstone, J. M., Slaziński, L. & Lowney, E. (1981) *Photochem. Photobiol.* **34,** 461–464.
8. Sutherland, B. M., Blackett, A. D., Feng, N. I., Freeman, S. E., Ogut, E. S., Gange, R. W. & Sutherland, J. C. (1985) *Ann. N.Y. Acad. Sci.* **453,** 73–79.
9. Ananthaswamy, H. N. & Fisher, M. S. (1981) *Cancer Res.* **41,** 1829–1833.
10. Peleg, L., Raz, E. & Ben-Ishai, R. (1976) *Exp. Cell. Res.* **104,** 304–307.
11. Setlow, R. B. & Regan, J. D. (1981) in *DNA Repair*, eds. Friedberg, E. C. & Hanawalt, P. C. (Dekker, New York), Vol. 1, pp. 307–318.
12. Ishikawa, T., Kodama, K., Ide, F. & Takayama, S. (1982) *Cancer Res.* **42,** 5216–5221.
13. Kodama, K., Ishikawa, T. & Takayama, S. (1984) *Cancer Res.* **44,** 2150–2154.
14. Mano, Y., Kator, K. & Egami, N. (1982) *Radiat. Res.* **90,** 501–508.
15. Kator, K. & Egami, N. (1985) *J. Radiat. Res.* **26,** 44.
16. Boyd, J. B., Harris, P. V., Presley, J. M. & Narachi, M. (1983) in *Cellular Responses to DNA Damage*, eds. Friedberg, E. C. & Bridges, B. A. (Liss, New York), pp. 107–123.
17. Boyd, J. B., Snyder, R. D., Harris, P. V., Presley, J. M., Boyd, S. F. & Smith, P. D. (1982) *Genetics* **100,** 239–257.
18. Boyd, J. B., Golino, M. D., Nguyen, T. D. & Green, M. M. (1976) *Genetics* **84,** 485–506.
19. Smith, P. D., Snyder, R. D. & Dusenberg, R. L. (1980) in *DNA Repair and Mutagenesis in Eukaryotes*, eds. Generoso, W. M., Shelby, M. D. & de Serres, F. J. (Plenum, New York), pp. 175–188.
20. Ryo, H., Yoo, M. A., Fujikawa, K. & Kondo, S. (1985) *Genetics* **110,** 441–451.
21. Rasmuson, B., Westerberg, B. M., Rasmuson, Å., Gvozdev, B. A., Belyava, E. S. & Ilyin, Y. V. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **45,** 545–551.
22. Rasmuson, B., Rasmuson, Å. & Nygren, J. (1984) in *Handbook of Mutagenicity Test Procedures*, eds. Kibey, B., Legator, M., Nichols, W. & Ramel, C. (Elsevier, Amsterdam), pp. 603–613.
23. Green, M. M. (1962) *Genetics* **47,** 483–488.
24. Todo, T., Ueda, R., Miyake, T. & Kondo, S. (1985) *Mutat. Res.* **145,** 165–170.
25. Fujikawa, K. & Kondo, S. (1986) *Genetics*, in press.
26. Brown, R. C., Harris, P. V. & Boyd, J. B. (1981) *Somatic Cell Genet.* **7,** 631–644.
27. Baker, B. S., Carpenter, A. T. C. & Ripoll, P. (1978) *Genetics* **90,** 531–578.
28. Browning, L. S. & Altenburg, E. (1962) *Genetics* **47,** 361–366.
29. Kondo, S., Ichikawa, H., Iwo, K. & Kato, T. (1970) *Genetics* **66,** 187–217.
30. Bridges, B. A., Dennis, B. E. & Munson, R. J. (1967) *Genetics* **57,** 897–908.
31. Setlow, R. B. (1966) *Science* **153,** 379–386.
32. Sawada, S. & Okada, S. (1972) *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **21,** 599–602.
33. Bowman, J. T. (1969) *Mutat. Res.* **7,** 409–415.
34. Karess, R. E. & Rubin, G. M. (1982) *Cell* **30,** 63–69.
35. Howard-Flanders, P. (1968) *Annu. Rev. Biochem.* **37,** 175–200.
36. Witkin, E. M. (1969) *Mutat. Res.* **8,** 9–14.
37. Witkin, E. M. (1975) *Bacteriol. Rev.* **40,** 869–907.
38. Walker, G. C. (1984) *Microbiol. Rev.* **48,** 60–93.
39. Boyd, J. B. & Setlow, R. B. (1976) *Genetics* **84,** 507–526.