COMPARISON OF SOMATIC REVERSIONS BETWEEN THE IVORY ALLELE AND TRANSPOSON-CAUSED MUTANT ALLELES AT THE WHITE LOCUS OF *DROSOPHILA MELANOGASTER* AFTER LARVAL TREATMENT WITH X RAYS AND ETHYL METHANESULFONATE

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ABSTRACT

Somatic reversion of strains with the ivory (w^i) allele, a mutation associated with a tandem duplication of a DNA sequence at the white locus, increased with the age of larvae at the time of X-irradiation as expected from the increase in the number of target cells. In contrast, two independently isolated strains with unstable w^+ loci associated with insertion of transposable elements showed higher reversion frequencies after treatment with X rays or ethyl methanesulfonate (EMS) at early larval stages than at late stages. Nevertheless, both the w^i strain and the two unstable w^+ strains reverted at nearly equal rates after treatment with X rays or EMS at early larval stages. Possible similarity in "hot spot" structure for the high reversibility of the two types of mutations is discussed in relation to production of presumed "mutator-type" cofactors specific to the transposon-caused mutations at early larval stages.

THE problem of the X-ray induction of back mutations in germ cells of Drosophila has a long history since the pioneering work by TIMOFEEFF-RESSOVSKY (1931) as argued in a concise article by GREEN (1961). Low frequencies of back mutations in germ cells are an obstacle for the study of the reversibility of mutant alleles. LEWIS (1959) originally reported germinal and somatic reversion of the white ivory mutant (w^i) by X-irradiation. GREEN (1962) showed that somatic reversion by X-irradiation occurs more frequently at the w^i allele than at the buff (w^{bf}) allele, approximately paralleling the germinal back mutation rates. Thus, somatic reversion of mutant alleles provides a convenient way for quantitative measurement of reversibility of mutants (LEWIS 1959; GREEN 1962).

An unstable mutant allele at the white locus isolated from a laboratory stock with the duplicated white loci *Dp* (w^{sp}) (w^{17G}) (RASMUSON, GREEN and KARLSSON 1974) has recently been shown to revert somatically after larval treatment with X rays and EMS (RASMUSON *et al.* 1978; RYO, KONDO and RASMUSON 1983).

Very recently, from a w^{DZL} revertant, LEVIS and RUBIN (1982) isolated an unstable mutant allele that reverts germinally and somatically. The genetic instability of these two unstable mutant alleles may be associated with transposable elements inserted in the white locus (RASMUSON *et al.* 1981; RASMUSON, RASMUSON and NYGREN 1984; LEVIS and RUBIN 1982).

The present paper reports that larvae at later stages were more sensitive than first instar larvae to somatic reversion of the w^i allele after X-irradiation, as expected from an increase in the number of target cells with the larval age (BECKER 1976). However, third instar larvae were more resistant than first instar larvae for reversion of the transposon-caused mutant alleles after treatment with X rays or EMS. The differential age-dependent reversibility of the two types of mutant alleles is discussed in relation to the difference in the molecular mechanism of mutagenesis involved.

MATERIALS AND METHODS

Drosophila stocks: The two strains of unstable zeste eye color mutants used were obtained from BERTIL RASMUSON and ROBERT LEVIS. Rasmuson's unstable strain, with the genotype *sc* z^1 w^+ *sn* (zeste unstable) will be abbreviated the UZ strain. This strain is thought to be unstable due to a transposable element inserted near the locus w^+ (RASMUSON *et al.* 1981). Levis' unstable strain with genotype *sc* z^1 $w^{y070181}$ was isolated as a single yellow-eyed male from a stock of a w^{DZL} revertant, *sc* z^1 w^{rD5} (LEVIS and RUBIN 1982). This strain will be named w^y here. However, when it is not necessary to make a distinction, the two strains will be loosely called zeste unstable w^+ strains. The w^i strains used here were a strain homozygous for the genotype y^2 w^i ct^6 derived from the strain with the genotype y^2 w^i ct^6/B^s Y y^+ and another strain homozygous for the genotype *y* w^i *ec* (from B. H. JUDD). Homozygotes and hemizygotes for the locus w^i and the loci z^1 w^+ (unstable), respectively, have ivory and zeste eyes.

The strains with the w^{DZL} allele (BINGHAM 1980) and w^{*6} allele (bleached white due to insertion of a defective *P* element) (SIMMONS and LIM 1980; RUBIN, KIDWELL and BINGHAM 1982) were also tested for somatic reversion.

Culture conditions: Female and male flies were aged separately for 3–4 days, mated for 24 hr at a ratio of 40 females to 40 males in culture bottles containing 40 ml of standard medium with brewer's yeast (Kirin Beer Company, Ltd., Osaka, Japan) and live yeast, and then transferred to fresh culture bottles to oviposit for 20 hr at 25°. After the oviposition, parental flies were discarded and resultant eggs were allowed to develop at 25°. The larvae that were at 24–44, 48–68 and 72–92 hr after oviposition, will be called the first, the second and the third instar larvae, respectively.

Treatment with EMS and X rays: EMS (from Tokyo Kasei Kogyo Ltd., Tokyo, Japan) was dissolved in phosphate buffer (1/15 M, pH 6.8) at appropriate concentrations immediately before use, and a 1-ml sample of the solution was pipetted onto the surface (18 cm^2) of the culture medium [agar, 10 g; dried yeast powder Ebios (Tanabe Seiyaku Company, Ltd.), 60 g; glucose, 60 g; propionic acid, 10 ml; water, 1 liter] in each bottle containing larvae at a given stage. EMS remained in the culture medium throughout larval development. X rays were applied with a Toshiba X-ray generator (180 kVp and 20 mA) at 1100 rads/min unless otherwise stated. Dosimetry was carried out by a Fricke dosimeter and Victoreen cavity chambers. Larvae cultured in shallow glass dishes (38 × 12 mm) or plastic Petri dishes (5 × 1.5 cm) were irradiated, transferred to bottles containing fresh medium and cultured at 25° until they emerged.

Frequency of somatic reversions: The frequency of somatic mutations is defined as the number of red sectors divided by the number of flies examined. In the UZ strain, red sectors consisting of four or more ommatidia that appeared on lemon yellow eyes of males were scored as mutant clones. In the w^i strains, both females and males and, in the w^y strain, only males were scored for all red sectors including as few as one red ommatidium. The differential criterion for the mutant assay is based on the findings that, in the control, phenocopies consisting of one or two light

TABLE 1

Frequencies of somatic reversion of the w^i *strain by treatment with X rays and EMS at different larval stages*

Mutagen	Larval instar	Dose (rads or mM)	Red spots/ females[a]	% (95% confidence interval)	Red spots/ males[a]	% (95% confidence interval)
Control			5/9227	0.054 (0.021–0.121)	4/9905	0.040 (0.014–0.097)
X rays	1st	1100	38/6356	0.60 (0.41–0.81)	19/5532	0.34 (0.20–0.52)
	2nd	1100	191/6515	2.93 (2.51–3.36)	92/6692	1.37 (1.11–1.66)
	3rd	1100	67/1657	4.04 (3.10–5.11)	42/1745	2.41 (1.72–3.18)
EMS	1st	10	75/1286	5.83 (4.56–7.27)	69/1370	5.04 (3.92–6.28)
	2nd	10	108/1209	8.93 (7.38–10.68)	123/1341	9.17 (7.58–10.82)
	3rd	10	65/1917	3.39 (2.63–4.26)	51/1850	2.76 (2.04–3.61)

[a] Flies scored, *i.e.*, no. of spots/no. of flies (male or female) scored.

reddish orange ommatidia appear at high frequency in UZ males but do not appear in either w^i or w^y flies.

RESULTS

Reversion of the white-ivory mutant: The frequency of somatic reversion of the w^i mutant increased with the increased larval age at which larvae were irradiated (Table 1). The frequency of X-ray induced reversions was about twice as high in females as in males. Since there was no difference in somatic reversibility in the two w^i strains used, the data were pooled.

The frequency of EMS-induced somatic reversion of the w^i mutant was only slightly higher following treatment at the second instar than at the first and decreased at the third instar (Table 1). Furthermore, female larvae were no more sensitive to EMS-induced reversions than males (Table 1).

Reversion of transposon-caused mutants: For the UZ and w^y strains, the frequencies of red spots induced by X rays at different stages are summarized in Table 2. The reversion frequency for the UZ strain showed negligible variation with the larval age at which X rays were given. The frequencies of somatic reversion of the w^y strain at various X-ray doses were almost equal after irradiation of first instar larvae and second instar larvae but markedly decreased after irradiation of third instar larvae. The mutant phenotypes appearing as red sectors on the zeste eyes were almost indistinguishable between the UZ and w^y strains.

The frequency of somatic mutations induced by EMS at 10 mM was higher at the first instar than at the second for both zeste unstable w^+ strains (Table

TABLE 2

Frequencies of somatic reversion of the UZ and w^y *strains by treatment with X rays and EMS at different larval stages*

Mutagen	Larval instar	Dose (rads or mM)	UZ strain		w^y strain	
			Red sectors/ males	% frequency[a]	Red sectors/ males	% frequency[a]
Control			9/11623	0.08 (0.04–0.15)	0/3603	0 (0–0.09)
X rays	1st	500			8/1477	0.5 (0.2–1.0)
		800			15/1712	0.9 (0.5–1.4)
		1100	74/8108[b]	0.9 (0.7–1.1)		
	2nd	500			4/665	0.6 (0.2–1.4)
		800			5/610	0.8 (0.3–1.8)
		1100	22/2997	0.7 (0.5–1.1)		
		1300			2/147	1.4 (0.2–4.6)
	3rd	1080			2/2197	0.1 (0.01–0.16)
		1100	33/3973	0.8 (0.6–1.1)		
EMS	1st	2.5	6/1215	0.5 (0.2–1.1)		
		5	14/1244	1.1 (0.7–1.8)		
		10	39/670	5.8 (4.1–7.8)	67/1772	3.8 (2.9–4.8)
	2nd	2.5	2/1600	0.1 (0.02–0.4)		
		5	19/1854	1.0 (0.6–1.6)		
		10	12/734	1.6 (0.9–2.8)	11/986	1.1 (0.5–1.9)
	3rd	10			5/1225	0.4 (0.2–0.9)

[a] Figures in parentheses give 95% confidence intervals.
[b] From RYO, KONDO and RASMUSON (1983).

2). Similar results were also observed for somatic mutations induced at 2.5 and 5 mM in the UZ strain (Table 2).

Distribution of mutant clone sizes: The size of mutant clones provides important information on the dynamics and the number of cells in the imaginal disc under study (GARCIA-BELLIDO and MERRIAM 1971; BECKER 1976; HAYNIE and BRYANT 1977). Distributions of the sizes of red spots induced after treatment with X rays and EMS at different larval ages are summarized in Figure 1. The sizes of red sectors, *i.e.*, the numbers of ommatidia within red sector areas, have been grouped into integral size classes with limits corresponding to 2^n, where n is a measure for the number of mitotic divisions required for a mutant cell to produce the corresponding clone size (HAYNIE and BRYANT 1977). After larval irradiation at the first and the second or third instar, the red sectors in the w^i strain had modal numbers of 17–32 and two to eight ommatidia, respectively (Figure 1A). Thus, the clone size was several times larger for revertants induced at the first instar than at the second or third instar. In inverse proportion to the clone size, the frequency of induced somatic mutations increased with larval age at the time of irradiation from the first to the third instar (Table 1).

The red sectors induced in the UZ strain after irradiation at the first and the second or third instar had modal numbers of nine to 32 and four to eight

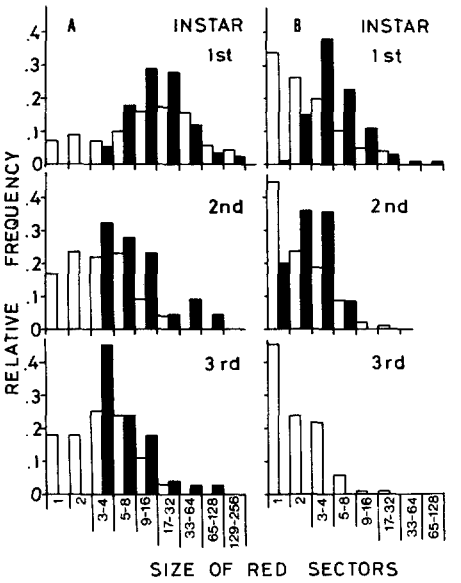

FIGURE 1.—Size distribution of red sectors induced by larval treatment with X rays and EMS. A, An X-ray dose of 1.1 krads was applied to larvae of the w^i (open bars) or the UZ (closed bars) strain at the indicated instars. B, EMS solution at 10 mM was administered to larvae of the w^i (open bars) or the w^y (closed bars) strain at the indicated instars.

ommatidia, respectively (Figure 1A), in agreement with the w^i strain excluding the unscored clones of one to three red ommatidia.

The size of mutant clones induced by EMS in the w^i strain had modal frequency at one ommatidium irrespective of larval age at the time of the onset of treatment (Figure 1B). In contrast, the w^y strain showed modal frequency at three to four and two to four ommatidia, respectively, for larvae at the first and the second instar at the time of the onset of EMS treatment (Figure 1B).

Dose-rate effects: To find a clue as to whether somatic reversion of the w^i allele occurs as the result of a single ionization, we studied the dose-rate effects on induction of red spots in the w^i strain. For chronic irradiation, one-ninth of a total dose was given nine times at hourly intervals to second instar larvae at 150-fold lower dose rate than acute irradiation. There was no dose-rate effect on the frequencies of induced reversions (Table 3), indicating that reversion of the w^i allele probably results primarily from the effects of a single ionization track.

Resistance of the w^{DZL} *mutant to reversion*: The w^{DZL} allele shows a dominant trait with phenotypic characteristics similar to those of the recessive gene z (BINGHAM 1980). Females with the genotype $z^+ w^{DZL}/z^+ w^{DZL}$, $z^+ w^{DZL}/z^+ w^+$ or $z w^{DZL}/z w^{DZL}$ all have zeste eyes, whereas $z^+ w^{DZL}$ males have dark red-brown eyes, $z w^{DZL}$ males zeste eyes, $z w^+$ males red eyes and $z^+ w^{\#6}$ males bleached white eyes (BINGHAM 1980; SIMMONS and LIM 1980). The w^{DZL} mutation was associated with insertion of a 13-kb transposable element upstream from the

TABLE 3

Dose-rate effects on somatic reversion of the w^i *strain by X-irradiation*

X-ray irradiation		Frequency of reversion				
Dose (rads)	Dose rate	Red spots/ males	%	Red spots/ females	%	Experiment
Control		2/4106	0.05	2/4228	0.05	
1100	Acute[a]	60/3531	1.70	113/3420	3.30	20
1100	Chronic[b]	35/2509	1.39	83/2503	3.32	20
1100	Acute[a]	18/2106	0.85	55/2122	2.59	22
1100	Chronic[b]	13/1275	1.02	37/1321	2.80	22

[a] With dose rate of 1.1 krads/min, 1-min exposure at the start or the end of the chronic exposure. Frequency average of two experiments is given.

[b] One-ninth of 1100 rads was given nine times at 1-hr intervals with dose rate of 30 and 7.3 rads/min for experiments 20 and 22, respectively.

TABLE 4

Somatic reversion of the w^{DZL} *allele in combination with various white alleles by X-irradiation of first instar larvae*

Genotype	Sex	Dose (rads)	Red spots/females	%
$z\ w^{DZL}$	Male	0	0/1886	<0.05
		800	2/1779	0.1[a]
$z\ w^{DZL}/z\ w^{DZL}$	Female	0	0/1349	<0.08
		800	0/2118	<0.05[a]
$z^+\ w^{DZL}/z^+\ w^{\#6}$	Female	0	0/447	<0.2
		800	5/986	0.5
$z^+\ w^{DZL}/z^+\ w^{DZL}$	Female	0	0/445	<0.2
		800	2/931	0.2
$z^+\ w^{DZL}/z^+\ w^+$	Female	0	1/1539	0.06
		1100	35/260	13.5
$z^+\ w^{\#6}$	Male	0	0/1329	<0.08
		1100	0/1803	<0.06

[a] After irradiation of second instar larvae.

white locus (LEVIS and RUBIN 1982). Since strain w^y is a derivative of revertant w^{rD5} from strain w^{DZL} and revertant w^{rD5} retains 2.9-kb portion of the 13-kb element (LEVIS and RUBIN 1982; R. LEVIS, personal communication), we tested the X-ray-induced reversion of the w^{DZL} mutant.

The rate of somatic reversion at the second instar with an X-ray dose of 800 rads was 0.1% for $z\ w^{DZL}$ males (Table 4), only about one-eighth of the reversion rate of w^y males (Table 2). The higher reversion rate observed for females with $w^{DZL}/w^{\#6}$ (Table 4) can be explained by entire loss of the locus $w^{\#6}$ as it is known that females with a w^{DZL} mutant and a deleted w locus have red eyes (BINGHAM 1980). The very high frequency (13.5%) of phenotypic reversion in heterozygotes with the genotype $z^+\ w^{DZL}/z^+\ w^+$ (Table 4) can be explained by X-ray-induced mitotic recombination as the observed reversion frequency is close to the recombination rate reported for the w^+/w heterozygotes (BECKER 1976).

DISCUSSION

The frequency of X-ray-induced reversion of the w^i mutant increased with the age of larvae (Table 1). The relative frequencies in first, second and third instar larvae at the time of irradiation were 1:4.5:7. This age-dependent increase in somatic reversibility is consistent with a report by BECKER (1976) that the relative frequencies of somatic recombination in the w^+/w eye color system induced by X-ray irradiation of first, second and third instar larvae were 1:4.5:15. The discrepancy between results on the relative sensitivities of third instar larvae (seven for somatic mutation but 15 for somatic recombination) is attributable to lower synchronization of larval age in the third instar in the present experiment, because the rate of reversion in third instar female larvae at 1 krad increased from 4.0% (Table 1) to 10% (80/783) on decreasing the age interval of third instar larvae for irradiation from 72–92 to 72–80 hr after oviposition. Therefore, as established for X-ray-induced mitotic recombination (BECKER 1976), we may conclude that the apparent increase in the reversion rate of the w^i allele per individual fly with age of larvae at the time of irradiation reflects an increase in the number of target cells, *i.e.*, imaginal eye disc cells, with larval age. This means that the reversion sensitivity of the w^i allele itself is virtually constant throughout larval development from the first to the early third instar.

In contrast, the frequency of X-ray-induced reversion of either the UZ strain or the w^y strain did not increase with the age at the time of irradiation (Table 2). This result leads to the conclusion that the two transposon-caused eye color mutant alleles are more mutable per target cell by X-irradiation at early larval stages than at late stages. This suggests that early larvae may have endogenous "mutator-type" cofactors specific to transposon-caused mutations. Since most transposon-mediated mutations in germ cells of *Drosophila melanogaster* occur premeiotically, that is, in early stages of germ cells (GREEN 1967; BINGHAM 1981; RASMUSON *et al.* 1981), cellular cofactors that are essential for transpositioning of transposable elements might be produced abundantly in somatic cells only in early developmental stages, as seems to be the case in germ cells.

In contrast to the age-dependent increase in X-ray-induced reversion of the w^i mutant, the frequency of EMS-induced reversion of the UZ or the w^y mutant decreased with larval age (Table 2). The relative frequencies of reversion of first, second and third instar w^y larvae exposed to 10 mM EMS were 1:0.3:0.1. As a first approximation, we assume that sensitivity of reversion of the w^y mutant is age independent (see X-ray-induced reversion of the w^y and the UZ mutant in Table 2). Then, the relative frequencies of EMS-induced reversion of the w^y mutant represent the relative ratios of the "effective" periods of exposure to EMS from the start of exposure at the three larval stages. On the other hand, relative reversion sensitivities of first, second and third instar w^i larvae measured by X-ray-induced frequencies were 1:4.5:7, as mentioned before. From these two sets of relative values, we can theoretically estimate the relative ratios of EMS-induced frequencies of first, second and third instar w^i larvae as 1:1.4 (= 0.3 × 4.5):0.7 (= 0.1 × 7). These theoretical values are fairly close to the observed ratios of 1:1.7:0.6 after treatment with 10 mM

EMS (Table 1) and explain why reversion of the w^i mutant after EMS treatment showed only slight age dependence.

The above conclusion that the effective period of EMS treatment was much longer at the first instar than at the second or third instar implies that after its administration EMS remains active in the culture medium throughout larval development. At the third instar, action of EMS on target cells of the w^i strain would produce one-ommatidium revertant clones at very high frequency, because at some time in this stage the most advanced imaginal eye disc cells of the w^i strain with ability to mutate, that is, stem cells that produce the smallest (one-ommatidium) revertant clones only, become the most numerous and, hence, must potentially have the highest capacity to produce revertants. In fact, EMS-induced revertant clones in the w^i strain had a modal frequency of one ommatidium irrespective of the larval age at the start of exposure (Figure 1B). This is in contrast to results on X-ray-induced reversion of the w^i mutant; in this case, revertant clones had a modal frequency of 17–32 ommatidia after irradiation at the first instar and of two to eight ommatidia after irradiation at second or third instar (Figure 1A).

On EMS-induced reversion of the w^y mutant, the modal frequency was at more than one ommatidium (Figure 1B). This was as expected since imaginal eye disc cells of third instar w^y larvae, which potentially have ability to produce one-ommatidium revertant clones, were resistant to reversion as measured by X-ray mutagenesis (Table 2).

There is another factor in EMS mutagenesis that tends to produce small mutant clones. This factor can be demonstrated in reversions of the UZ mutant exposed to EMS at the first instar because residual mutagenic action of EMS at later stages is negligible because of higher reversibility per target cell at this stage. The observed relative frequencies of reversion of first instar UZ larvae exposed to EMS were 0.1, 0.3, 0.3, 0.2, 0.1, 0.06, 0.02 and 0.01 for clone sizes of two, three to four, five to eight, nine to 16, 17–32, 33–64 and 65–124, respectively, when one-ommatidium spots were not scored (see also the distribution of revertants of the w^y mutant in Figure 1B). Thus, reversion of first instar UZ larvae had a modal frequency of three to eight ommatidia after EMS treatment, whereas it had a modal frequency of nine to 32 ommatidia after X-irradiation (Figure 1A). These results indicate that fixation of mutation occurs later by about two cell divisions on EMS mutagenesis than on X-ray mutagenesis. The delayed fixation of EMS-induced mutation explains the difference in the pattern of distribution of clone sizes between revertants of the w^y strain by EMS treatment and those of the UZ strain by X-irradiation (Figure 1). It also explains, when combined with the long-term activity of EMS, why larvae of the w^y and UZ strains showed much lower sensitivity to reversion by EMS treatment at the second instar than at the first instar despite their almost equal sensitivity to reversion by X-irradiation at these two stages (Table 2).

The observed X-ray-induced rate of reversion of the w^i mutant agrees with GREEN's 1962 results. Furthermore, the present findings that the frequency of X-ray-induced reversions was twice as high in females as in males (Table 1) confirm the previous conclusions (GREEN 1962; BOWMAN 1969) that the fre-

quency of somatic reversion of the w^i mutant increases in proportion to the number of w^i mutant loci per cell. However, EMS-induced somatic reversion of the w^i mutant showed no higher sensitivity in females than in males (Table 1). These contradictory results indicate that there is a substantial difference in the mechanism of reversion of the w^i allele induced by EMS and X-irradiation.

The resistance of the unstable w^+ allele in the UZ strain to reversion by X-irradiation of spermatozoa (RASMUSON *et al.* 1981) is worthy of reexamination by treatment of germ cells with appropriate mutagens at early stages, since somatic reversion of the same mutant allele occurred more frequently on EMS treatment of larvae at early stages than at late stages. This is an elaboration of the idea proposed by GREEN (1962). However, most unstable genes caused by transposable elements do not seem sensitive to exogenous mutagens (CAIRNS 1981). Their high mutability seems to be caused by endogenous factors. In fact, as summarized in Table 4, the w^{DZL} mutant with an insertion of a 13-kb element (LEVIS and RUBIN 1982) and the $w^{\#6}$ mutant with an insertion of a defective *P* element (RUBIN, KIDWELL and BINGHAM 1982; O'HARE and RUBIN 1983) were far more resistant to reversion by X rays than the UZ and w^y strains. Therefore, somatic reversibility of the w^y allele, which is a derivative of a revertant of w^{DZL}, should not be ascribed to a simple insertion of a fragment of the 13-kb element. Whatever the presumed complex element responsible for a somatically unstable mutation, if we could increase the reversibility of the mutant allele in germ cells by exogenous mutagens, we could readily identify the element using the recombinant DNA techniques.

The somatic reversion rate of the w^i allele is very close to that of the two zeste unstable w^+ alleles after treatment of first instar larvae with either X rays or EMS (*cf.* Tables 1 and 2). This suggests that reversion of the transposon-caused mutant alleles occurs by specific DNA rearrangement similar to that involved in reversion of the w^i allele. Germinal reversion of the w^i allele seems to result from precise excision of one of the tandemly duplicated 2.9-kb fragments in the regulatory domain of the white locus (KARESS and RUBIN 1982). The germinal reversion rate of $2 \times 10^{-5}/w^i$ locus/krad (BOWMAN 1969) is close to the somatic reversion rate of $3 \times 10^{-3}/2 \times 40/w^i$ locus/krad, where 3×10^{-3} is the reversion frequency per male fly per krad (Table 1), 40 stands for the number of imaginal eye disc cells at the first instar (MADHAVAN and SCHNEIDERMAN 1977) and 2 stands for two eye discs per fly. Therefore, we may assume that somatic reversion of the w^i allele occurs by the same mechanism as that proposed for germinal reversion, *i.e.*, precise excision of one copy of the duplicated fragments by intrachromosomal recombination at any point along a double loop formed by pairing of the duplicated sequence (BOWMAN 1965; KARESS and RUBIN 1982). Since the presumed precise excision at the w^i allele occurs at very high efficiency, that is, about 0.3% by a single ionization given at any point along the 2.9 kb $\times$ 2 length of the duplicated sequence, there must be a hypermutable "hot spot" structure of DNA such as the double loop mentioned above. For reversion of the zeste unstable w^+ strains, a stem loop structure made by the presumed transposable insert transposon may serve as a hot spot for precise (or imprecise) excision of itself or of another specific DNA sequence that is responsible for the unstable mutant phenotype.

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