X-RAY INDUCTION OF VISIBLE MUTATIONS (AT THE *YELLOW*, *WHITE*, *MINIATURE* AND *FORKED* LOCI) IN THE SUCCESSIVE STAGES OF OOCYTES OF *DROSPHILA*

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In *Drosophila*, it is well known that a considerable difference exists among the oocyte stages in the ability to repair damage induced by X-rays as well as in their radiosensitivity (see Sankaranarayanan and Sobels 1976 for a recent review). Among them, early oocyte stages at the synaptic stages are characterized by their highest radioresistant nature to the induction of dominant lethal mutations by ionizing radiation (Koch *et al.* 1970).

Recently, Miyamoto and Nakao (1978) have revealed that in the oocytes presumably corresponding to the synaptic stage the yield of the *dumpy* mutations induced by X-rays are markedly low as compared to that recorded in the subsequent cell stages of oocyte development. They have suggested that in the above oocytes a system is operating to cause reduction in the yield of *dumpy* mutations, namely, a system which has a tendency to interfere with the production or completion of the mutational events leading to these mutations.

In the present study, evidence supporting the above suggestion was obtained for the induction of visible mutations (at the *yellow*, *white*, *miniature* and *forked* loci) by X-rays in the successive stages of oocytes of *Drosophila*. It is this that is reported here.

MATERIALS AND METHODS

The experimental procedures are essentially the same as those used by Miyamoto and Nakao (1978). Briefly, females of *Drosophila melanogaster* having the constitution sc^{S1} *B InS* w^a sc^8 were irradiated with two different doses of X-rays (1500 and 3000 R), at the age of five days, using the dose rate of 130 R/min. (200 kV, 15mA, filter 1.0 mm Al plus 1.5 mm Cu). Immediately after treatment, the females were mated with *y w m f*; *dp* males en masse (30 females to 150 males per bottle). The parents were transferred to fresh vials daily for 12 days and allowed to lay eggs for 24 hours. According to this procedure, the first egg-laying period probably represents oocytes irradiated at stage 14, and the subsequent ones represent progressively earlier stages at the time of irradiation. From the above mating *y*, *w*, *m* and *f* mu-

tations were detected among the female progeny, although the stocks in the present study were used primarily for the detection of *dp* mutations (Miyamoto and Nakao 1978).

The F_1 flies were examined for complete and mosaic mutations at the four sex-linked loci, *yellow* (*y*), *white* (*w*), *miniature* (*m*) and *forked* (*f*). Since the yield of mosaic mutations detected at these loci was very low, the data pertinent to these mutations are excluded from the analysis and will not be further considered in this report. All the recovered mutants were tested for fertility in the heterozygous female and for viability in the male progeny.

The statistical tests of data obtained in the present study were made by using Kastenbaum and Bowman's tables (Kastenbaum and Bowman 1970).

RESULTS AND DISCUSSION

The data on the induction of complete mutations (at the *yellow*, *white*, *miniature* and *forked* loci) by X-rays with two different doses, 3000 and 1500 R, for 12 successive 1-day egg-laying periods are summarized for 6 successive two-day broods (broods A-F) in Table 1.

Table 1. Frequencies of complete mutations (at the *y*, *w*, *m* and *f* loci) induced by X-rays in 6 successive two-day broods

Dose (R)	Mutation frequency (%)					
	Brood A		Brood B		Brood C	
	y	w, m, f	y	w, m, f	y	w, m, f
3000	0.440** (8/1819)	0.275** (5/1819)	0.280** (12/4284)	0.140** (6/4284)	0.220** (15/6813)	0.176** (12/6813)
1500	0.107** (5/4661)	0.043 (2/4661)	0.086** (9/10415)	0.086** (9/10415)	0.038 (4/10429)	0.019 (2/10429)
Control	— (0/18349)	— (0/18349)				

(Table 1 continued)

Dose (R)	Mutation frequency (%)					
	Brood D		Brood E		Brood F	
	y	w, m, f	y	w, m, f	y	w, m, f
3000	0.027 (2/7469)	0.040 (3/7469)	0.014 (1/7342)	0.027 (2/7342)	0.019 (1/5252)	0.038 (2/5252)
1500	0.040 (4/9988)	0.020 (2/9988)	0.024 (2/8445)	0.024 (2/8445)	— (0/3642)	0.0110* (4/3642)

*, ** Significant at 5 and 1 % level from the control, respectively.

The mutation frequency at the *yellow* locus is given separately because of the relatively high rate at this locus. Since the numbers of *white*, *miniature* and *forked* mutants detected in each brood are not large, the mutation frequencies in the table are given as the sum of those obtained for these three mutants. The frequency patterns for the above mutations over the six sampling broods are graphically presented in Figs. 1 and 2.

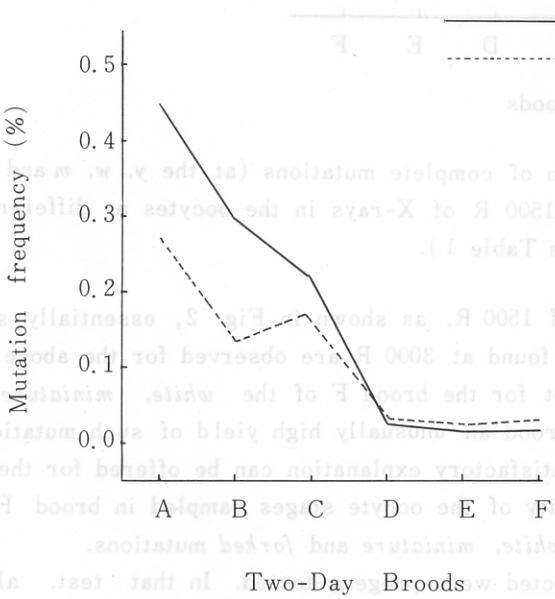

Fig. 1. Frequency pattern of complete mutations (at the *y*, *w*, *m* and *f* loci) induced by 3000 R of X-rays in the oocytes at different stages (Data from Table 1).

From Fig. 1, at the exposure level of 3000 R, the yield of the *yellow* mutations is at the highest level in the first brood, then decreases gradually in broods B and C, and reaches the lowest level in broods D, E and F. The frequency pattern for the *white*, *miniature* and *forked* mutations is almost similar to that for the *yellow* mutations. These findings are well in agreement with the recent observations on the *dumpy* mutations (Miyamoto and Nakao 1978).

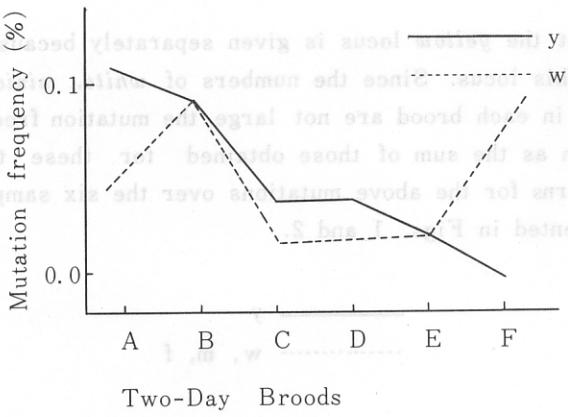

Fig. 2. Frequency pattern of complete mutations (at the *y*, *w*, *m* and *f* loci) induced by 1500 R of X-rays in the oocytes at different stages (Data from Table 1).

At the exposure level of 1500 R, as shown in Fig. 2, essentially similar frequency patterns to those found at 3000 R are observed for the above two classes of mutations, except for the brood F of the *white*, *miniature* and *forked* mutations. In that brood an unusually high yield of such mutations is recorded. At present no satisfactory explanation can be offered for the above unusually high radiosensitivity of the oocyte stages sampled in brood F only to the X-ray induction of *white*, *miniature* and *forked* mutations.

The mutant females detected were progeny-tested. In that test, all mutants that failed to produce enough offspring for the testing, for whatever reason, were lumped together and for convenience designated as "sterile". When the data were considered together (both exposure levels and oocyte stages sampled were ignored), on the *yellow* mutants, it was found that 12 (out of the 63) were sterile and 46 out of the rest bred as male-inviable. This probably suggests that the latter might be associated with small deletions containing the *yellow* locus (see Inagaki 1972).

Out of the 51 mutants recovered at the *white*, *miniature* and *forked* loci, 24 were sterile; out of the remainder 27, 19 were male-inviable. If the male-inviable mutants owe their origin to small deletions, as was presumed in the case of the *yellow* mutants, their ratio is somewhat small for these mutants.

A comparison was made on the relative distribution of the two types of mutants (male-viable type and male-inviable type mutants) recovered at the *yellow*, *white*, *miniature* and *forked* loci between the oocytes sampled in broods A-C and those sampled in broods D-F. The result is summarized in Tables 2 and 3. Clearly from the tables, in the oocytes sampled in broods D-F

Table 2. Progeny test of recovered mutants at the *yellow* locus

Brood	Sterile	Male-inviable	Male-viable	Total
A-C	11	38	4	53
D-F	1	8	1	10
Total	12	46	5	63

Table 3. Progeny test of recovered mutants at the *white*, *miniature* and *forked* loci

Brood	Sterile	Male-inviable	Male-viable	Total
A-C	18	11	7	36
D-F	6	8	1	15
Total	24	19	8	51

male-viable type mutant is detected little not only at the *yellow* locus but also at the *white*, *miniature* and *forked* loci, and male-inviable type mutant is recovered with relatively low yield at these loci, while in the oocytes sampled in broods A-C the above two types of mutant are recovered with relatively high frequency.

Recently, Miyamoto and Nakao (1978) have revealed that early oocyte stages presumably corresponding to the synaptic stage are characterized by their high radioresistance to the induction of *dumpy* mutations by X-rays. They have proposed that in these oocyte stages an efficient system which tends to repair the mutational events leading to such mutations. Present results have shown that the oocyte stages sampled in broods D-F are relatively radioresistant to the X-ray induction of *yellow*, *white*, *miniature* and *forked* mutations as compared to those sampled in broods A-C. Since the oocytes sampled in broods D-F presumably correspond to the ones at the synaptic stage under the brooding procedure employed in the present study (Miyamoto and Nakao 1978) and since oocytes at that stage are more efficient in repairing radiation induced damage than the subsequent oocytes with a system for the repair (stage 7 oocytes) (Koch *et al.* 1970), present findings may be interpreted to mean that mutational events leading to the *yellow*, *white*, *miniature* and *forked* mutations are also repaired efficiently by the presumed repair system. Consequently, present observations seem to lend some kind of support to the recent findings and suggestion of Miyamoto and Nakao (1978) mentioned above.

An additional interesting point in the present results is that in the oocytes sampled in broods D-F (presumably corresponding to the synaptic stage)

yellow, *white*, *miniature* and *forked* mutations which are often associated with small deletions (those of the male-inviable type in the progeny test) are recovered with a relatively low frequency and those which are seldom associated with such deletions (those of the male-viable type in the progeny test) are detected little (see Tables 2 and 3). This characteristic elucidated on the *yellow*, *white*, *miniature* and *forked* mutations shows a remarkable parallelism with that revealed recently by Miyamoto and Nakao (1978) on the *dumpy* mutations. According to their data, in the oocytes presumably corresponding to the synaptic stage the *dumpy* exceptions of the *ol*, *lv*, *ov* and *olv* types, which are often associated with structural changes (Inagaki *et al*. 1977; Miyamoto and Nakao 1978; Miyamoto 1978), are detected with a relatively low yield and those of the *o* and *v* ones, which are seldom associated with structural changes (Inagaki *et al*. 1977; Miyamoto and Nakao 1978; Miyamoto 1978) are rarely recovered. They have interpreted these findings as meaning that mutational events leading to gene mutations might be repaired efficiently by the presumed repair system, while the breakage events leading to structural changes might not be repaired so efficiently or some of them might involve changes which cannot be repaired by that system.

In view of this, one may speculate that presumed repair system which is operating in the synaptic stage oocytes has the following nature: it may repair to a considerable extent the mutational events induced by X-rays, whether they owe their origin to breakage events or not; and its repair efficiency may be relatively high to the mutational events leading to gene mutations.

However, since available information on the presumed repair system is still very limited, it seems judicious to consider that at present interpretation of this kind seems to be not so far from pure speculation.

SUMMARY

X-ray induction of visible mutations (at the *y*, *w*, *m* and *f* loci) was investigated in the oocytes of *D. melanogaster*. The frequency patterns for induced mutations in the successive stages of oocyte development were studied by transferring the inseminated females daily to fresh vials for 12 days. Under this transferring procedure, the first egg-laying period represents oocytes irradiated when they are at stage 14, and the subsequent ones represent progressively earlier stages of oocyte development at the time of irradiation.

The results obtained indicate that (1) the yield of *yellow* mutations recovered in the first six day egg-laying periods (lst-6 th day) is relatively higher than that in the subsequent six day periods (7 th-12th day); (2) the frequency pattern for the *white*, *miniature* and *forked* mutations through the sampling periods is practically similar to that observed in the *yellow* mutations.

These findings seem to indicate that the oocytes sampled in the subsequent six day egg-laying periods (7th-12th day) are relatively radioresistant to the induction of visible mutations (at the *y*, *w*, *m* and *f* loci) by X-rays as compared to those sampled in the first six day egg-laying periods(lst-6th day). This characteristic is very similar to that elucidated previously for the *dumpy* mutations.

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