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Characterization of fractional mutations in *Drosophila*: differential inhibition of complete and fractional mutations by inhibitors of repair synthesis

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SUMMARY

The 'maxy' technique was used for scoring the frequencies of whole body and fractional mutations in the progeny of males of *Drosophila melanogaster* that had been irradiated with 2000 R, irradiation being followed or preceded by treatment with caffeine or acriflavine. Three 2-day broods were used, and males irradiated without subsidiary chemical treatment were used as controls. Both chemicals whether given as post- or pre-treatment strongly reduced the frequencies of fractional mutants in all broods. The frequency of whole-body mutants was unaffected in the first two broods and suffered a moderate reduction in the third. These results are discussed in relation to the hypothesis that repair processes regulate the development of a primary lesion into either a whole-body or a fractional mutant and that the functioning of the repair enzymes depends on the state of maturity of the male germ cell.

1. INTRODUCTION

The occurrence of both complete and fractional mutations among all mutations has long been recognized (Muller, 1928; Auerbach, 1946; Altenburg & Browning, 1961). More recent work has shown that complete mutations differ from fractionals in several important respects. Firstly, unlike complete mutations, there is a lack of linear relationship between the dose of X-ray and the frequency of fractional mutations (Altenburg & Browning, 1961; Matsudaira *et al.* 1964; Inagaki & Nakao, 1966). Secondly, there is a higher proportion of complete mutations to fractionals after ionizing radiation than after treatment with chemical mutagens (Auerbach, 1946; Epler, 1966). Thirdly, in spermatozoa, O_2 promotes the induction of complete mutations while N_2 seems to favour fractionals (Inagaki & Nakao, 1966; Sobels, 1965).

No clear explanation has been offered for these differences in behaviour of the two mutational types. Muller, Carlson & Schalet (1961) suggested that complete mutations result from damage in both strands of DNA while fractionals arise from damage in only one of the two strands. On the other hand, on the basis of the differential behaviour of the two mutational types under different experimental conditions such as anoxia, treatment with N_2, dinitrophenol, storage, etc., several

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workers suggested that the realization of one or the other mutational type might be determined during the fixation of mutations by the action of different repair systems in the paternal germ cells (Sobels, 1965; Sobels *et al.* 1966; Brink, 1970; Mandal, Rahaman & Mukherjee, 1977). The repair mechanisms for point mutations and chromosome breakage may be different; the present discussion is restricted to point mutations.

In order to examine the validity of the proposed repair hypothesis and to distinguish it from the proposition of Muller *et al.* (1961), we have investigated the possibility of discriminating between the repair systems involved in the fixation of the two classes of point mutation by inhibition of repair synthesis by caffeine and acriflavine. These drugs are known to be inhibitors of repair systems in eukaryotes (Norman, 1971; Mendelson, 1973; Mendelson & Sobels, 1974; Chevaillier & Philippe, 1976; Malling & Wassom, 1975).

2. MATERIALS AND METHODS

The maxy stock of *Drosophila melanogaster* (Muller, 1954) was used for the present investigation. The X chromosome of the tested males was marked with l^{J1} sc^{J1+} Ind149 *ptg oc'* B^{M1} and the Y chromosome was marked with l^{J1+}. After or before the desired treatment (described below) the X-irradiated males were crossed to *y* sc^{S1} *car odsy f* g^2 *dy* ras^2 sn^3 ct^6 *cm rb ec w pn l* sc^8/l^{J1} sc^{J1+} Indl 49 *ptg oc'* B^{M1} females in the proportion of three females per male. The X-linked mutants appearing in the F_1 were scored in three consecutive 2-day broods. Mutants for only 11 of the 15 loci (excluding sc^{S1} and sc^8) were scored in order to avoid confusion with phenocopies. The 11 loci are: *y*, *car*, *f*, *g*, *ras*, *sn*, *ct*, *cm*, *rb*, *w* and *pn* (for description of these mutants see Lindsley & Grell, 1968). All mutants which appeared in the F_1, except clear-cut mosaics, were tested for transmissibility and allelism with known loci, with appropriate tester stocks. All mutants were examined for their somatic distribution.

X-irradiation was given with Picker's X-ray unit operated at 110 kV, 4 mA with 0·25 mm Al filter. The desired dose rate (750 QR/min) was obtained by varying the target distance from the source and was calibrated with a Victoreen dosimeter (Model No. 70). The total dose of X-rays was 2000 R (2 KR) for all experiments. For the pre- and post-treatment experiments, males were injected intra-abdominally before or after X-irradiation, with caffeine (1,3,7-trimethyl-2,6-dioxypurine; Orient Pharmaceuticals, Calcutta, India; batch no. 001) or acriflavine (a mixture of diamino-10,-methyl acridinium chloride and diamino-acridine; Arora Pharmaceuticals, India; batch no. 018), at a concentration of 100 μg/ml. The control males for only caffeine or acriflavine treatment either received 0·7 % NaCl or distilled water, those for other treatments received only 2 KR of X-rays. The statistical significance of the differences between the frequencies of induced mutations in control and various experimental sets has been tested by the Equality of Proportion Test (Snedecor, 1956) and the average frequencies of different replicates by analysis of variance and critical difference

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Table 1. *Data on the effect of caffeine and acriflavine, pre- and post-treatment, on the X-ray induced complete (W) and fractional (F) mutations in 3–4 replicate experiments and their statistical analysis*

Treatment other than 2 KR	Brood *a*			Brood *b*			Brood *c*		
	N	*W* (%)	*F* (%)	*N*	*W* (%)	*F* (%)	*N*	*W* (%)	*F* (%)
1. None	13109	0·096 (13)	0·128 (17)	10839	0·201 (22)	0·137 (15)	3901	0·434 (17)	0·147 (6)
2. Caffeine pre-treatment	8317	0·148 (12)	0·011 (1)	7322	0·190 (14)	0·009 (1)	3012	0·225 (5)	0 (0)
3. Caffeine post-treatment	8093	0·074 (7)	0 (0)	6808	0·170 (11)	0·008 (1)	1722	0·264 (5)	0·031 (1)
4. Acriflavine pre-treatment	6856	0·085 (7)	0 (0)	5788	0·111 (8)	0·015 (1)	1137	0·270 (3)	0 (0)
5. Acriflavine post-treatment	7091	0·061 (6)	0 (0)	5687	0·095 (6)	0·039 (2)	1341	0·056 (1)*	0 (0)
Analysis of variance: *F*-values and significance									
Effect of caffeine		2·57	49·4**		2·22	60·1**		2·44	7·26**
Effect of acriflavine		0·43	89·1**		2·88	16·3**		24·2	41·3**

All mean % values for fractionals are significantly different from the control (Row 1) at $P = 0{\cdot}01$ % by critical difference (CD) test.
* Only this mean % for complete mutations is significantly different from the control by CD test.
** *Significant at 1* % by the Fisher *F* test.

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tests for two-way classified data (Snedecor, 1956; Goon, Gupta & Dasgupta, 1968). For the Equality of Proportion Test, the z values were calculated from the pooled data.

3. RESULTS

Our data reveal that, at the concentration used in these tests, caffeine and acriflavine fail to induce mutations in *D. melanogaster*. This agrees with the results obtained by several earlier workers in higher systems (Cattanach, 1962; Janders & Seaten, 1962; Alderson & Khan, 1967; Clark & Clark, 1968). In Table 1 the results of the experiments in the three 2-day broods are presented as average frequency (per cent) in three (brood c) or four (broods a, b) replicates. The numbers shown in parentheses are the numbers of mutants obtained in the pooled data.

With the mating procedure used here and by others, germ cells of broods a, b and c represent sperms, late spermatids and early spermatids or late spermatocytes, respectively (Sobels, 1965). As is evident from the data and their analysis in Table 1, the frequency of fractionals is strongly reduced in all three broods by both caffeine and acriflavine, and by both pre- and post-treatments. The frequency of complete mutations is virtually unaffected by caffeine or acriflavine in broods a and b, but reduced significantly by acriflavine post-treatment in brood c. The F values for differences between replicates were in no case significant, suggesting homogeneity among different replicates. In none of the experiments was a difference between the effects of pre- and post-treatment observed.

In brood c, the Equality of Proportion Test on the pooled data (not shown in the table), however, revealed an absence or a marginal significance of the difference between the control and treated for both fractional (range of z values 1·52–1·99) and complete mutations (range of z values 0·96–1·96). This may be due to less sensitivity of the test caused by using the pooled data.

A locus-wise distribution of induced mutations shows that there is no preferential enhancement or reduction of induced mutations at any specific loci. Furthermore, the possibility of confusion of the fractional mutations with phenocopies can also be ruled out since in all fractional mutants the somatic distribution of the mosaic patches was symmetrical and occupied one-half or one-quarter (rarely one-eighth) of the target tissue. In no instance was there an irregular distribution or pleiotropism.

4. DISCUSSION

Our results clearly show that both caffeine and acriflavine strongly reduce the frequency of X-ray induced fractional mutations in all post-meiotic germ cells, but have no effect on the frequency of complete mutations, except possibly in brood c (spermatids and secondary spermatocytes). The two chemicals have the same effect whether used before or after X-irradiation. Such a difference in their effect on the two types of mutation cannot be explained as due simply to lack of restitution, as proposed by Mendelson & Sobels (1974), and requires further

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assumptions. Since both chemicals are known to act as inhibitors of repair synthesis, we suggest that the differences we have observed arise from a differential action of these chemicals on the repair system or systems present in the paternal germ cells: either because different repair systems recognize single and double strand breaks (Painter, 1974), or because a single repair enzyme acts differently on the two types of break.

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