

## SHORT COMMUNICATION

### THE INDUCTION OF SEX-LINKED RECESSIVE LETHALS BY FORMALDEHYDE IN TWO STRAINS OF *Drosophila melanogaster*

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#### ABSTRACT

Two strains of *Drosophila melanogaster* have been previously characterized as resistant (CO3) and sensitive (RC1) to the mutagenic action of chemical and physical agents (Andrade and Marques, *Brazil. J. Genet.* 3: 251-264, 1980). To evaluate the role played by excision repair in the differential sensitivity of these two strains, we compared their response to the induction of sex-linked recessive lethals (SLRL) by formaldehyde, a mutagen which induces DNA lesions mainly processed by excision repair. There was a significant increase in the frequency of SLRL in both strains. However, the resistant strain had a lower mutation frequency. We conclude that the RC1 strain is defective in the excision-resynthesis repair mechanism.

#### INTRODUCTION

Two strains of *Drosophila melanogaster*, CO3 (Columbia) and RC1 (Riverside), have shown differences in sensitivity to the induction of sex-linked recessive lethal (SLRL) and dominant lethal (DL) mutations, at different stages of oogenesis and spermatogenesis (Andrade and Marques, 1980; Benfato and Andrade, 1986; Reguly and Marques, 1987). The CO3 strain is more resistant than strain RC1 to SLRL and DL when exposed to different doses of ionizing radiation or to chemical mutagens.

However, when caffeine was administered before irradiation, the frequency of SLRS increased in the resistant strain (CO3) but remained unchanged in the sensitive one, indicating that the difference in sensitivity between the two strains is due to differences in the repair of premutational damage (Reguly and Marques, 1987).

On the other hand, Benfato and Andrade (1986), analysing oocytes, demonstrated a significant increase in the rate of DL induction in strain RC1, when exposed to fractionated doses of radiation. They suggest this is due to defective excision-repair in the RC1 strain.

Formaldehyde has been known as a mutagen since the work of Rapoport (1948) but its action at the molecular level is still not clear. A variety of mechanisms may be involved in the formaldehyde-induced lesions in different organisms. However, in bacteria and yeast, these lesions are repaired by an excision-repair system (Auerbach *et al.*, 1977; Benyajati *et al.*, 1983).

In view of these observations, we decided to study the response of these strains to the action of formaldehyde in order to verify the possible existence of defective excision DNA repair in the RC1 strain.

## MATERIAL AND METHODS

Two strains of *D. melanogaster*, maintained in the laboratory for more than twenty years, were used: CO3 (Columbia, New York) and RC1 (Riverside, California). Females of each strain, mass-mated in standard medium (Marques *et al.*, 1966) for 12 hours, were allowed to ovoposit for four hours in standard medium. Twenty four hours after the middle of the egg-laying period, the early first-instar larvae were collected, washed in saline to remove any trace of yeast, and placed in the treatment medium, which was prepared according to Auerbach and Moser (1953) and contained 0.25% formaldehyde. The larval density in the treatment medium was 100 larvae in 25 ml of medium. Seeding with live yeast was omitted. The same procedure, without formaldehyde, was used to determine the spontaneous mutation rates of each strain.

After eclosion of the adults, treated and control males were collected and individually crossed with 3-day females of the *Basc* marker strain (for description of the symbols, see Lindsley and Grell, 1968) during seven days to perform the SLRL test. Ten F1 cultures were established for each parental male. All cultures derived from a single treated larva were kept together in order to identify clusters of lethal mutations. When no round-eyed males were present in an F2 culture which contained more than 20 males of the non lethal type, the culture was scored as a lethal. When fewer than four round-eyed males were present a re-test was made. All experiments were run at 25°C. Two experiments on the induction of SLRL were performed. Since the results of each experiment did not differ statistically, the data were pooled. The statistical analyses were based on a 2x2 chi-square table in a one-sided test.

## RESULTS AND DISCUSSION

Treatment of male larvae with formaldehyde significantly increased the frequency of SLRL in both strains ( $P = 0.01$ , Table I). This confirms the mutagenicity of formaldehyde in *Drosophila* male larvae, as previously demonstrated by Rapoport (1948), Auerbach (1952), Herkowitz (1953, 1955), Slizynska (1957) and Szabad *et al.* (1983). The frequency of lethals was always lower in the CO3 strain ( $P < 0.01$ ). Therefore, the RC1 strain was sensitive and CO3 resistant to the induction of SLRL mutation after treatment with formaldehyde.

Table I - Frequency of sex-linked recessive lethals induced by formaldehyde in two strains of *Drosophila melanogaster*.

Strain	Treatment (%)	Chromosomes tested	Lethals	
			Number	%
CO3	-	562	1	0.18
RC1	-	533	2	0.37
CO3	0.25	460	10	2.17
RC1	0.25	522	24	4.60

Studies on the germ cells of males of these two strains have shown that CO3 is resistant to different types of damage (DL and SLRL) induced by mutagens such as gamma radiation, ethylmethanesulfonate (EMS) and intergerrimine (Andrade, 1976; Paula Ramos, 1977; Andrade and Marques, 1980; Reguly and Marques, 1987; A. Pacheco, personal communication).

Formaldehyde is a potent mutagen in *Drosophila*, inducing SLRL in mature sperm and mosaic spots in somatic cells only when applied during larval life. However, there has been no indication of mutagenicity of formaldehyde in female germ-lines (Rapoport, 1948; Szabad *et al.*, 1983). It induces point mutations, chromosome breaks and recombination. Its major mode of mutagenic activity appears to be the induction of single strand breaks in DNA through a metabolic process (Magaña-Schwencke and Ekert, 1978; Magaña-Schwencke *et al.*, 1978). In *Saccharomyces cerevisiae* and bacteria the formaldehyde-induced lesions are subjected to excision-repair (Auerbach *et al.*, 1977; Benyajati *et al.*, 1983).

In a previous study Benfato and Andrade (1986), analysing stage-seven oocytes found a significant decrease in the rate of DL induction in CO3 strain when fractionated doses of radiation were compared with unfractionated ones. In contrast, in RC1 there was a significant increase in DL induction rate after fractionated doses, suggesting that the premutational lesions induced in this strain are not repaired. Since stage-seven oocytes have a duplicated genome at the time of irradiation, the authors assumed that both excision and recombinational repair are acting at this stage. Thus, in the RC1 strain, defective excision-repair (after endonuclease action) leads to the maintenance of single strand breaks, which may generate double strand breaks, leading to a saturation of recombinational repair and a consequent increase in the frequency of lethal mutations.

We suggest that, as in bacteria and yeast, the damage induced by formaldehyde in *D. melanogaster* is repaired by excision-repair, which seems defective in the RC1 strain.

## RESUMO

Duas linhagens de *Drosophila melanogaster* foram previamente caracterizadas como resistente (CO3) e sensível (RC1) à ação de agentes mutagênicos químicos e físicos. Com o objetivo de avaliar o papel do reparo por excisão-ressíntese na sensibilidade diferencial destas duas linhagens foram comparadas as respostas de CO3 e RC1 na indução de mutação letal recessiva ligada ao sexo (LRLS), após tratamento com formaldeído - um mutagênico que induz lesões no DNA reparadas preferencialmente por este mecanismo. Foi observado um aumento significativo na frequência de LRLS induzidos pelo formaldeído em ambas as linhagens. Por outro lado, a linhagem CO3 apresentou, sistematicamente, uma menor frequência de mutação em relação à sensível RC1. Em vista destas observações, pode-se inferir que a linhagem RC1 é defectiva no mecanismo de reparação por excisão-ressíntese.

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(Received August 13, 1991)