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CYTOGENETIC ANALYSIS OF TWO STRAINS OF *Drosophila willistoni* TREATED WITH FORMALDEHYDE WITH SPECIAL REFERENCE TO THE ALCOHOL DEHYDROGENASE REGION

Cláudia ROHDE, Heloisa H. R. de ANDRADE and Vera Lúcia S. VALENTE

Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Caixa Postal 15053, CEP 91501-970, Porto Alegre, Rio Grande do Sul, Brasil

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ABSTRACT

Studies were carried out to establish the mutagenic effect of formaldehyde (FA) in inducing deletions preferentially in the *Adh* region as seen in the polytene chromosomes of *Drosophila willistoni*. The differential response to FA in two genetically different strains was also studied. One of the strains has been maintained in the laboratory for about 30 years and is quasi monomorphic for the already known paracentric inversions in this species. The other one which has high levels of chromosome polymorphism for inversions was recently obtained from the natural populations. The results are consistent with the idea that the *Adh* region is particularly sensitive to the effect of FA in *Drosophila willistoni*. Therefore, this mutagen may be useful in mapping this region in other species. Furthermore, the recently obtained strain showed a significantly higher proportion of FA-induced chromosome breaks (deletions) than the old laboratory strain which this clearly establishes that they differ in relation to their response to the clastogenic effect of formaldehyde.

RESUMEN

Se estudió el efecto mutagénico del formaldehído (FA) para inducir deleciones preferenciales sobre la región *Adh* en los cromosomas politénicos de *Drosophila willistoni* y la respuesta diferencial de este mutágeno en dos cepas que difieren desde el punto de vista genético. Una cepa ha sido mantenida en el laboratorio por alrededor de 30 años y es casi monomórfica para las inversiones paracéntricas conocidas en esta especie. La otra cepa fue recientemente establecida a partir de poblaciones naturales que poseen altos niveles de polimorfismo cromosómico para inversiones. Los resultados son consistentes con la idea de que la región *Adh* es particularmente sensible al efecto del formaldehido en *Drosophila willistoni*, por lo tanto es adecuado para mapear esta región en otras especies. Además, la cepa recién colectada manifiestó una proporción significativamente mayor de acumulación de rompimientos cromosómicos (deleciones) inducidos por FA que la cepa del laboratorio lo que demuestra que hay efecto diferencial del formaldehido sobre ellas.

INTRODUCTION

In the genus *Drosophila*, the *ADH* enzyme plays a paramount role in protecting larvae against the alcohols produced during the fermentation process of the substrates exploited by them. This assumption is based in a series of ecological studies (David *et al.* 1975, 1979, David and Bocquet 1975, Atkinson and Shorrocks 1977, Mckenzie and Mckechnie 1979, Parsons and Spence 1980) and experimental evidences from *Adh*-null mutants of *D. melanogaster* (Grell *et al.* 1968, O'Donnell *et al.* 1975, 1977).

The mutagen formaldehyde (FA), is present in the polluted air of the cities. It is widely used by the chemical and textile industries in the production of paper, resines, fertilizers, drugs and cosmetics. The first study concerning its mutagenic effect in *Drosophila* was performed by Rapoport (1946) and its effects were extensively studied by Auerbach *et al.* (review in 1977). According to these studies the effects of FA are observed in the male *Drosophila* only. It was demonstrated that, where fed to larvae, the primary spermatocytes were the only germ cells affected; on the other hand after adult feeding the results were negative. When adult injection of FA was used, then sperm, spermatids and primary spermatocytes showed a positive response.

FA is commonly found in the environment. It has high mutagenic and carcinogenic potential, the mechanism of which are, as yet, poorly understood. According to Auerbach *et al.* (1977) FA appears to produce bridges between DNA and proteins or between DNA-DNA, leading to point mutations and chromosomal aberrations.

O'Donnell *et al.* (1975, 1977) showed that several point mutations and chromosomal aberrations induced by FA were located at the *Adh* locus of *D.melanogaster*. In addition, it was observed that the majority of the *Adh* negative mutants induced by FA represented deletions inside the 35B3-5 region of the left arm of the chromosome 2 which corresponds to the position of the *Adh* locus. Later, Woodruff and Ashburner (1979a,b) and Ashburner *et al.* (1982 a,b) made an exhaustive study of this small region and genetically and cytogenetically characterized several other gene loci around the *Adh* locus.

By means of cloning and sequencing strategies, the *Adh* region from the mutants obtained by O'Donnell *et al.* (1977) were studied by Benyajati *et al.* (1983). This study showed that the mutants correspond to very small deletions (6 to 34 pb) inside the *Adh* gene sequence.

Drosophila willistoni is a Neotropical species, considered as a paradigm to evolutionary studies (review in Ehrman and Powell 1982). Whereas gene mapping by classical methods in this species is a hard task due to its extensive chromosomal inversion polymorphism, nonetheless Lakovaara and Saura (1972) were successful in mapping *Adh* to IIR using such approaches. Localization in IIR has more recently been confirmed by Rohde *et al.* (1995)

The aim of this study was to investigate the response of two strains of *D.willistoni* to the clastogenic effect of FA as well as to identify the chromosomal localization of the *Adh* region by means of the FA-induced deletions.

MATERIALS AND METHODS

Strains

Two strains of *Drosophila willistoni* (WIP4 and 17A2) were exposed to formaldehyde treatment. WIP4 is an old laboratory stock collected 29 years ago in Ipitanga, in northeastern Brazil and the 17A2 strain derived from a collection of South Brazil in 1990; both strains were maintained as mass matings.

Chemical and treatment procedures

Formaldehyde (CAS nº 9028-84-6), obtained from Vetec Química Fina Ltda, São Paulo, Brazil, was dissolved in distilled water.

Females and males of each strain, mass-mated in standard medium (Marques *et al.* 1966) for 24 hours, were allowed to oviposit for four hours in culture bottles (1/16 l) containing 12.5 g of soybean flour; 25.5 g of baker's yeast; 5.0 g of agar; 25.0 g of sugar and 250 ml of distilled water (according to Andrade and Reguly 1993). Twenty-four hours later, the early first-instar larvae were treated by dropping 200 µl of a solution of 0.25 % FA on the surface of the food. Forty-eight hours after the beginning of the oviposition period the larvae were fed with live yeast through the third instar stage.

Negative controls were submitted to the same experimental procedures, except by the fact that the surface treatment was made only with 200 µl of distilled water.

After the emergence of the imagoes derived from the treated and control groups, sexes were separated and the following matings made: untreated females with treated males (treated group) and untreated females with untreated males (control group). These crosses were made in 1/16 l standard culture medium (Marques *et al.* 1966) bottles and transferred every three days. All experiments were run at 25°C.

Cytogenetic analysis

The salivary gland of F1 larvae from both crosses were processed by the technique of Ashburner (1967) and the sealed slides were analyzed under contrast phase Zeiss III Photomicroscopy, according to the reference map of polytene chromosomes of Dobzhansky (1950).

Statistical analysis

The one-sided chi-square test for proportions was used to evaluate the clastogenic potential of the FA between treated and control groups of each strain, as well as between treated and control groups of 17A2 and WIP4.

RESULTS

Preliminarily all the five chromosome arms of the *D. willistoni* polytenic complement were screened to verify if there was any region with a high concentration of FA-induced deletions. Since IIR chromosome showed a larger concentration of these lesions, our analysis focused on this chromosome arm.

Figure 1 shows the results of the cytogenetic analysis of IIR of the WIP4 strain. Each wavy line above the polytene chromosome corresponds to a deletion detected in the corresponding bands in the control (A) and FA-treated (B) groups. No other type of chromosome aberration was observed.

Figure 2 represents the results obtained in the 17A2 strain between the control (A) and FA-treated (B) groups. In both figures the arrowheads show the sites of the highest deletion frequencies, suggesting the probable region of the *Adh* gene.

The results of the chi-square test applied to the comparisons between treated and control samples for each strain as well as between treated and control groups of 17A2 and WIP4 are shown in **table I**. The results obtained with this test suggest that FA causes a significant clastogenic effect in both strains (**Table Ia**) as reflected in the statistically significant increase in the frequency of FA-induced deletions over the entire length of IIR as compared with respective controls. Moreover, both treated and untreated 17A2 are significantly more sensitive

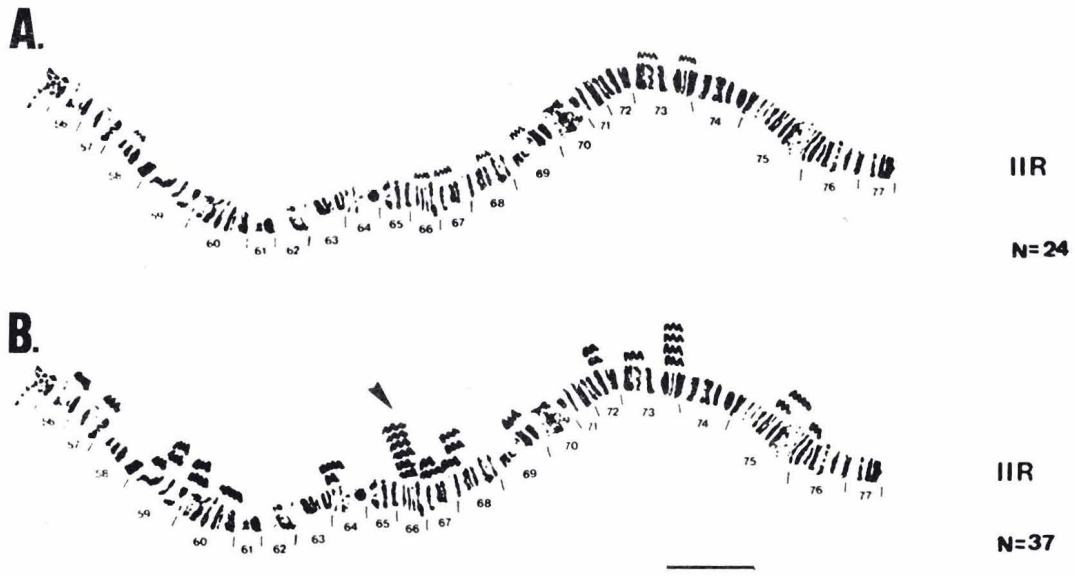

Figure 1. Right arm of the second chromosome (IIR) of *Drosophila willistoni* (WIP4 strain). In (A) each broken line over some of the bands indicate the spontaneous deletions observed in the control group. In (B) deletions detected after FA treatment are represented. The arrow shows the site of the major concentration of deletions and N corresponds to the number of IIR chromosomes analyzed. Bar = 10 μm

than the corresponding groups in WIP4. When the deletions within sections 66-67 are compared in the two strains (**Table Ib**) with their respective controls, a significant difference is found only with respect to 17A2.

The results suggest an interstrain difference in sensitivity to the deletion-inducing ability of FA.

DISCUSSION

It was found in this study with *Drosophila willistoni* male larvae that FA induces a significant increase in the frequencies of deletions along the entire length of IIR in both WIP4 and 17A2 strains. These results confirm the clastogenic effect of

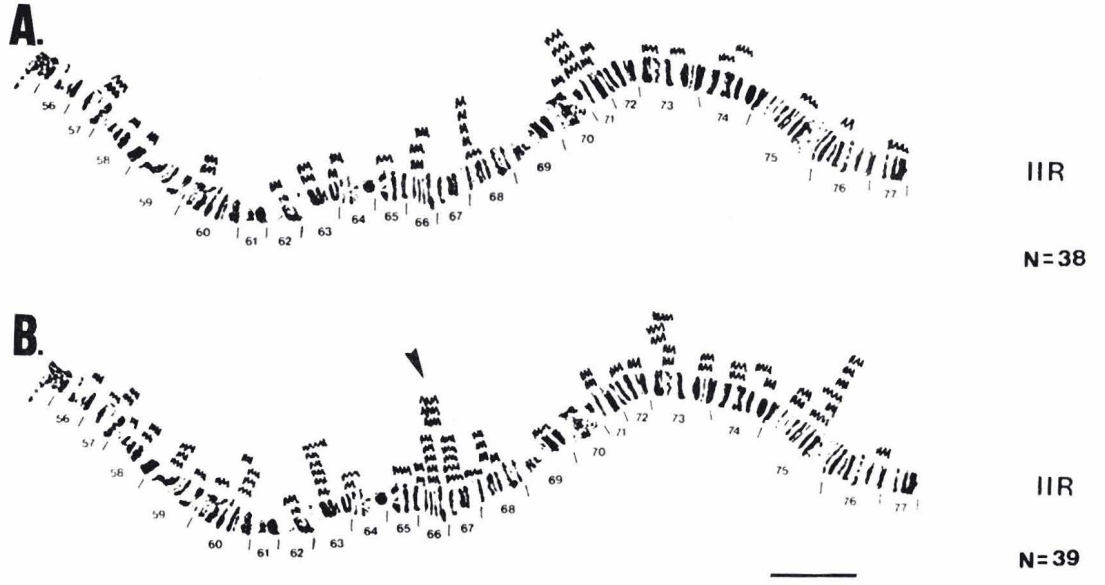

Figure 2. Right arm of the second chromosome (IIR) of *Drosophila willistoni* (17A2 strain). In (A) each broken line over some of the bands indicate the spontaneous deletions observed in the control group. In (B) deletions detected after FA treatment are represented. The arrow shows the site of the major concentration of deletions and N corresponds to the number of IIR chromosomes analyzed. Bar = 10 μm

TABLE I. FREQUENCY OF DELETIONS INDUCED BY FORMALDEHYDE IN TWO STRAINS OF *Drosophila willistoni*

A. In all sections of IIR chromosome arm

Strain	Treatment FA (%)	Chromosomes IIR analyzed	Number of deletions	Deletions per chromosome	Statatistical evaluation
WIP4	-	24	07	0.29	+
WIP4	0.25	37	32	0.86	
17A2	-	38	34	0.89	+
17A2	0.25	39	71	1.82	
WIP4	0.25	37	32	0.86	+
17A2	0.25	39	71	1.82	
WIP4	-	24	07	0.29	+
17A2	-	38	34	0.89	

B. In sections 66-67 of the IIR chromosome arm

Strain	Treatment (%)	Chromosomes IIR analyzed	Number of deletions	Deletions per 66-67 region	Statatistical evaluation
WIP4	-	24	02	0.08	-
WIP4	0.25	37	08	0.21	
17A2	-	38	03	0.07	+
17A2	0.25	39	13	0.33	

Chi-square test for proportion, one-sided: +, significant; -, not significant
Probability levels: $\alpha = \beta = 0.005$

FA in *Drosophila* male larvae, as previously demonstrated by Rapoport (1946) and might reflect the FA-induction of single-strand breaks in DNA that Magaña-Schwencke *et al.* (1978) identified in FA-treated yeast.

In addition, the response of both strains to the FA treatment showed that the more recently collected strain, 17A2, has a significantly higher frequency of spontaneous or FA-induced deletions in IIR compared with WIP4. In fact, similar interstrain differences in response to the treatment with FA were obtained in *Drosophila melanogaster* by Andrade *et al.* (1990) and Andrade and Reguly (1993). They used two strains that had been previously characterized as resistant (CO3) and sensitive (RC1) to the mutagenic action of chemical and physical agents (Andrade and Marques 1980). By means of a comparison of the frequencies of sex-linked recessive lethals and chromosome aberrations induced by FA in both strains the authors suggested that a defective excision repair mechanism is responsible for the high sensibility of the RC1 strain.

It is noteworthy to mention that the differential response of different strains to DNA-damaging agents depends on the genetic variability affecting not only the DNA repair mechanisms. Such is the case in which Margulies *et al.* (1989) demonstrated in *Drosophila melanogaster* the interaction of P elements with X-ray induced lesions leading to a significant increase in X and Y chromosome loss greater than the simple sum of the two effects. In other words, P elements may exert a sinergistic effect on the genotoxic activity of X-rays. Furthermore, Regner (1992) using a P element probe demonstrated that 17A2 has 24 insertion sites of such elements in the euchromatic arms of all the polytene chromosomes. In contrast, in the WIP4 strain only the chromocenter heterochromatin was able to hybridize with this probe element. Thus, we may speculate that transposable elements are responsible for the higher frequencies of spontaneous and FA-induced deletions on the 17A2 strain. In addition, these elements can also explain the high frequency of small size deletions on the 17A2 controls. This last interpretation is based on the study of chromosome aberrations induced by the mobilization of the hobo element in native Hawaiian populations of *Drosophila* (Lyttle and Haymer 1992) and in the high occurrence of microdeletions observed by our group in previous studies of chromosomal polymorphism of natural populations (unpublished).

The second aim of the experiment was to use FA and its potential to induce deletions as a tool to identify the *Adh* region in *D.willistoni*. For this purpose, we analyzed the deletion frequencies in each region of IIR chromosome arm

and identified a higher incidence of these lesions in sections 66 and 67 than in other sections of IIR in both strains, WIP4 and 17A2. However, this increase is statisticaly significant only in the 17A2 region. In spite of this fact the increment observed in these sections is in agreement with our previous study on the localization of the *Adh* locus. Using a sequence of the *Drosophila melanogaster Adh* gene as a probe for *in situ* hybridization (Moses 1986), we observed a consistent signal in both strains between sections 66 and 67 of IIR (Rohde *et al.* 1995). Thus, the results obtained by *in situ* hybridization strengthen the suggestion that sections 66-67 represent the locus of the *Adh* gene in *Drosophila willistoni*, since no other region of the chromosomic complement was more sensitive to the FA-induced deletions.

Although our results indicate that FA may be used to localize the *Adh* locus in *Drosophila willistoni* further experimental work is needed for a better evaluation of this phenomenon.

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