3H-Actinomycin-D Binding to Mitotic Chromosomes of Drosophila melanogaster

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Abstract. The binding of 3H-AMD to the metaphase chromosomes of Drosophila melanogaster has been analyzed after two different periods of exposure to photographic emulsion. The entirely heterochromatic Y chromosome was markedly less labelled than euchromatin and other heterochromatic regions. Moreover, the few grains present on the Y chromosome were clustered in two regions, one localized in the middle of Y5 and the other in the proximal third of Y4. This labelling pattern is not affected by removing histones with a 2-hour treatment with 2N HCl. It is suggested that the specific underlabelling of the Y chromosome reflects a peculiar AT richness.

Introduction

Actinomycin-D (AMD) binds specifically to double stranded DNA with a binding which requires guanine (Reich and Goldberg, 1964; Müller and Crothers, 1968). Because of its binding specificity tritiated actinomycin (3H-AMD) was used as a cytochemical label for DNA in autoradiographic experiments (Brachet and Ficq, 1965; Ebstein, 1967), proving to be a useful tool for the structural and functional analysis of chromatin (Berlowitz et al., 1969; Brachet and Hulin, 1969; Sieger et al., 1971).

Autoradiographic studies have shown that 3H-AMD is also able to bind to fixed chromosomes, and a nonrandom distribution of grains between and within chromosomes has been described (Miles, 1970; Cionini and Avanzi, 1972; Cionini, 1973; Rocchi et al., 1974; Prantera et al., 1976). It has been suggested that this nonrandomness reflects variations in DNA base composition and/or in protein distribution along chromosomes (Miles, 1970). In the present investigation the 3H-AMD pattern of fixation to the metaphase chromosomes of Drosophila melanogaster has been studied with the main purpose of determining the AMD binding capacity of the heterochromatic regions. In previous studies we characterized the heterochromatin of Drosophila melanogaster with various banding techniques and the induction of selective decondensation with Hoechst 33258 (Pimpinelli et al., 1975; Gatti et al., 1976; Pimpinelli et al., 1976).
Materials and Methods

Neural ganglia obtained from the dissection of male third instar larvae of Oregon-R stock were incubated in a 0.7% NaCl solution containing $10^{-5}$ M colchicine. After 2 h the ganglia were fixed and squashed under siliconized coverslips which were then removed by freezing on dry ice (Gatti et al., 1976). To reduce the radioactive background the ganglia were squashed on slides previously treated with cold AMD (10 μg/ml) (Ebstein, 1967). Three experiments were carried out as follows:

In the first two experiments the preparations were incubated in the dark for 30 min with a 5 μCi/ml $^3$H-AMD solution (Schwarz Bioresearch s.a., 8.4 Ci/mM). After washing in distilled H$_2$O they were covered with Kodak AR-10 stripping film, exposed in the dark at 4°C for 4 or 9 d, developed with Kodak D19 and then stained with Giemsa (Merck).

In the third experiment, before being treated with the above procedure, the preparations were treated for 2 h at room temperature with a 2 N HCl solution to remove histones (Comings and Avelino, 1974).

Results

Figure 1 shows neuroblast metaphases of *Drosophila melanogaster* after labelling with $^3$H-AMD and autoradiography. The pattern of grain distribution between chromosomes was analyzed directly under the microscope in all three experiments. In addition, in experiments 2 and 3 labelled metaphases were photographed and a further grain count performed on photographs (experiments 2PH and 3PH). The data in Table 1 clearly show that the Y chromosome is significantly less heavily labelled than the X chromosome and autosomes, and that this pattern is not affected by acid extraction of histones. The reduced

![Fig. 1a-d. Neuroblast metaphases of *Drosophila melanogaster* after labelling with $^3$H-AMD and autoradiography. a and b 4 d of exposure (expt. 2); c pretreatment with HCl and 4 d of exposure (expt. 3); d 9 d of exposure. The bar represents 5 μm](image-url)