Silver staining of Drosophila polytene chromosomes and the effect of hyaluronidase and lysozyme pretreatment

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Abstract

The Ag-AS technique was used for staining the polytene chromosomes of D. melanogaster and D. lummei. Bands were stained dark reddish-brown, interbands light yellow. A toromere was heavily stained on the sixth chromosome of D. lummei. The staining intensity of nucleoli was lower than that of chromosomes. During a prolonged staining ectopic threads and the nonhomogeneous structure of nucleoli were revealed. Pretreatment with RNase caused slight changes in the silver staining pattern of chromosomes; pretreatment with DNase did not result in any visible changes, while after preincubation with proteolytic enzymes chromosome morphology was destroyed. Hyaluronidase and lysozyme removed the silver-reducing components from chromosomes without destroying the general chromosome structure. Each of these two enzymes acts specifically: hyaluronidase affects the morphology of chromosomes, but not nucleoli and bands at heat shock puffs, whereas the action of lysozyme is probably evenly distributed between chromosomes and nucleoli.

Introduction

The ammoniacal silver staining (Ag-AS) technique has been extensively used for the localization of nucleolar organizer regions (NORs) on mitotic chromosomes in a wide variety of vertebrates (e.g. Goodpasture & Bloom, 1975; Tantravahi et al., 1976; Bloom et al., 1978; Nardi et al., 1978; Schmid, 1978), in the insect Achaeta domesticus (Howell, 1977; Czaker, 1978) and in plants (Schubert et al., 1979). It has also been used for the staining of the lampbrush chromosomes of Triturus cristatus carnifex (Varley & Morgan, 1978), as well as of synaptonemal complexes (e.g. Dresser & Moses, 1980) or of polytene chromosomes of two Dipterans – Sciara coprophila (Black & Ansley, 1964) and Rhynchosciara hollanderi (Stocker, 1978; Stocker et al., 1978). Although the mechanism of silver staining is not yet known in detail, it is supposed that silver is intensively absorbed by specific non-histone proteins of NORs which were active in the previous interphase (Howell, 1977, Schwarzacher et al., 1978; Olert et al., 1979).

In this study the Ag-AS technique was used for staining the polytene chromosomes of Drosophila. It was shown that the intensively stained component is removed after the chromosomes have been pretreated with hyaluronidase and lysozyme.

Material and methods

Drosophila melanogaster (Oregon R) and D. lummei Hackman (Moscow strain) salivary gland chromosome preparations of the late third instar larvae were made using the conventional squashing technique. For most of the experiments larvae of D. melanogaster had been preliminarily kept at 37°C for 30 min. i.e. we used larvae with developed ‘heat

shock' puffs. *D. lummei* larvae were cultured at 14 °C in order to obtain toromeres in the sixth chromosome.

Silver staining was carried out according to modified methods of the Ag-AS technique (Goodpasture & Bloom, 1975). Three drops of a 50% solution of silver nitrate were placed on the surface of each slide and covered with a coverslip. The slides were heated to 65 °C for 2–3 min until silver crystals were formed along the edges of the coverslip. Then the coverslip was removed and the slide rapidly washed and dried. After that 2 drops of an ammoniacal-silver solution (4 g AgNO₃ per 5 ml H₂O and 2.5 ml concentrated NH₄OH) and 2 drops of 3% formalin (neutralized with sodium acetate) were placed on the slide surface. The slide was covered with a coverslip and the staining process was monitored under a light microscope.

Giemsa staining: Two times diluted azure-eosine stock solution (according to Romanovsky-Giemsa) for 10 min, followed by 10 sec rinsing in distilled water and air-drying.

The conditions of pretreatment with enzymes and NaOH were as follows (Khachaturov et al., 1975; Howell, 1977; Byarugaba, 1978; Varley & Morgan, 1978):
(a) RNase purified by preparative polyacrylamide gel electrophoresis, 100 µg/ml in 2×SSC, 37 °C, 1 hour;
(b) Pancreatic DNase I (Serva), 100 µg/ml in 0.01 M MgCl₂ 37 °C, 1 hour;
(c) Trypsin, 100 µg/ml in 2×SSC, room temperature, 5 minutes;
(d) Pepsin (Schuchardt), 10 mg/ml in 0.9 M NaCl, pH 1.6, 37°C, 1 hour;
(e) Pronase E (Serva), 100 µg/ml, water solution, 37 °C, 1 hour;
(f) Testicular hyaluronidase (Calbiochem), 100 µg/ml in 0.1 M phosphate buffer, pH 6.0, 37 °C, 1 hour;
(g) Hen's egg-white lysozyme (Serva), 100 µg/ml in 0.1 M phosphate buffer, pH 7.2, 37 °C, 1 hour;
(h) 0.1 N NaOH, room temperature, 20 minutes.

After each treatment slides were washed in two changes of water, 70% and 95% ethanol, and air-dried.

The preparations were analyzed with a Zeiss NU-2 microscope (K. Zeiss, Jena) with a 100× oil immersion objective.

Results

Staining of non-treated preparations

The following changes were observed during the staining of polytene chromosomes. After the first stage ('the impregnation') chromosomes turned light yellow. During the very first moments of the second stage ('the development') the yellow colour grew more intense, the chromosome bands then turned reddish brown, while interbands remained yellow. Simultaneously nucleoli acquired a reddish tinge, but they were stained more lightly than the bands and their structural inhomogeneity was clearly visible. The protoplasm around chromosomes also turned yellow, but was paler than observed using usual methods of staining of polytene chromosomes (Fig. 1), although some

Fig. 1. Silver-stained chromosomes of *D. melanogaster*. Five nucleoli (N) and heat shock puffs (HS) can be seen.