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THE SILVER STAINING OF NUCLEOLUS FROM POLYTENE CHROMOSOME PREPARATIONS OF *DROSOPHILA MELANOGASTER*

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The nucleolus organizer regions (NORs) can specifically stained in routine chromosome preparations by a silver staining method[5,11]. The stained material is neither rDNA nor transcribed rRNA, but, proteins which rapidly associate with the freshly transcribed rRNA[7]. In mouse-human somatic hybrid cells, only those NORs which are functional active during the preceding interphase are stained by this method[8,9].

In the salivary gland polytene chromosomes of *D. melanogaster*, the rRNA genes can be found in the ramified nucleolus by *in situ* hybridization[16]. The nucleolus can be labelled, in the presence of high dose of α-amanitin, with ^{3}H-uridine (Fig. 1). Since it has been shown that the synthesis of many RNA species except rRNA are inhibited by α-amanitin[15], this result seems to suggest that the rRNA genes in the nucleolus are active. The *N*-banding technique which has been claimed to stain NORs specifically, does not selectively stain the nucleolus of *Chironomus* polytene chromosome preparation[6]. Here, we demonstrate that the active rRNA genes in the ramified nucleolus from *Drosophila* polytene chromosome prepartions can be stained specifically by the silver staining. Using the same method to stain the nucleolus of *Rhynchosciara* polytene chromosome preparations, Stocker *et al.* recently have also achieved the identical result[14].

Drosophila larvae are fed on sugar-agar medium[10]. The salivary gland polytene chromosomes from the third instar larvae are dissected in 45% acetic acid. Squashes are made by the conventional dry ice method. The chromosomal preparations are subsequently stained according to the method of Bloom and Goodpasture[2] with some modifications: the Ag solution (50% silver nitrate) is pipetted onto a slide, covered with a coverglass, and the slides are placed under a photo flood of 220 watts for 10 minutes at 50 to 60°C. The distance between the slide and photo flood is 19.5 cm. The coverglass is rinsed off with deionized water and the slide is allowed to dry in the air. 10 μl each of Ag solution (0.5 g of silver nitrate dissolved in 0.62 ml of water and 0.62 ml of 25% ammonia water) and 1% formalin (pH 5.92) are put onto the slide on two separated spots. The two solutions are mixed together quickly by using a coverglass which later will cover the chromosome preparations. The staining of the chromosomes is monitored under the microscope and the reaction is stopped by washing the slides with distilled water.

Fig. 2 shows the silver staining of the polyten chromosomes done by the modified Bloom and Goodpasture's method. The nucleolus becomes specifically decorated with black dots or granules. In contrast to the nucleolus, the chromosomal arms are only stained golden brown in color. Before performing the silver staining, if the chromosomal preparations are

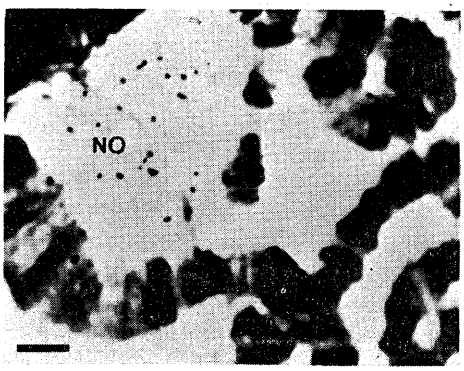

Fig. 1

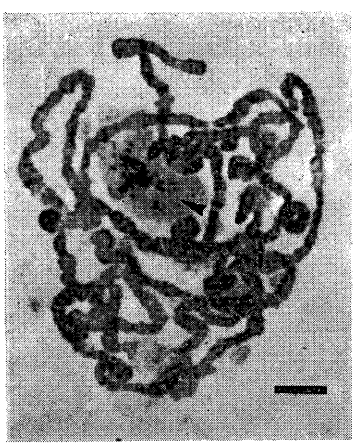

Fig. 2

Fig. 3

Fig. 1. The autoradiogram of salivary gland polytene chromosome. Selective labelling on the nucleolus (No) is demonstrated when the glands are preincubated in Robert's medium containing actinomycin D (5 μg/ml) for 15 minutes, then incubated in the same medium plus ^{3}H-uridine (41.6 Ci/mM, 0.2 mCi/ml) for 2 minutes at room temperature. The slides are coated with Kodak nuclear track emulsion (NTB-2) diluted 1 to 1 (V/V) with H_2O and developed after 24 hours' exposure. Bar=4 μ.

Fig. 2. The silver staining of salivary gland polytene chromosomes. The nucleolus (arrow) is decorated with black dots or granules, whereas, the chromosomal arms are stained golden brown in color. Bar=20 μ.

Fig. 3. The silver staining of salivary gland polytene chromosomes which are prepared from the third instar larva grown in sugar-agar medium containing actinomycin D. The nucleolus (arrow) is no longer stained black with dots or granules, but, becomes stained golden brown in color like that of the chromosomal arms. Bar= 20 μ.

treated either with pancreatic RN ase (100 μg/ml in 0.02 M acetate buffer pH 5.0, boiled for 7 min in water bath before use) for one hour at 37°C or with 0.2 N sulfuric acid for 30 min at 20°C, the silver-stainability of the nucleolus is unaltered. So the black stained material in the nucleolus would not be the RNA or basic proteins like histones in nature. Furthermore, if the chromosomal preparations are treated with Ellman's reagent[3,4] (10 mg/ml in 0.1 phosphate buffer, pH 8.0) for 1.5 hours at 20°C, then stained by the silver staining, the nucleolus is still stained the same as the untreated one. Since Ellman's reagent is known to block specifically the free sulfhydryl group of proteins, so the black dots or granules of staining in the nucleolus would not likely be resulted from the reaction of silver with free sulfhydryl group of the protein, though, silver salts like mercurials potentially can bind with the sulfhydryl group of proteins[1].

However, if the polytene chromosomes are prepared from the third instar larvae grown 3 hours at 20°C in the dark on one ml of sugar-agar medium[11] containing 220 μg of actinomycin D and stained with silver staining, the nucleolus no longer exhibits the black dots or granules as before, but, some golden brown fibrils instead (Fig. 3). In the presence of actinomycin D, the synthesis of rRNA in the cell is inhibited[12]. Since RNA itself could not likely be responsible for the staining observed in the nucleolus, it is tempting to speculate that a special nonhistone protein which could be involved in the synthesis of rRNA, plays the particular role in the silver staining demonstrated here.

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果蠅唾腺染色體細胞核仁之銀染色

溫五男　許美鳳　吳世祿

果蠅唾腺染色體細胞之核仁可用銀染色法染色。這種染色效果不受 RNase，酸以及 Ellman 試劑之影響，但與 rRNA 基因轉錄有關，因爲唾腺經 Actinomycin D 處理後，核仁銀染色物質消失不見。