Jpn. J. Human Genet. **25**, 229–233, 1980

AN IMPROVED SILVER STAINING TECHNIQUE FOR NUCLEOLUS ORGANIZER REGIONS BY USING NYLON CLOTH

Yoshiaki KODAMA, Michihiro C. YOSHIDA, and Motomichi SASAKI

Chromosome Research Unit, Faculty of Science, Hokkaido University, Sapporo 060, Japan

Summary A simple and reproducible silver-staining technique for nucleolus organizer regions (NORs) was developed, use being made of nylon cloth as a coverslip for even impregnation of the silver solution. Ag-NORs were clearly and selectively visualized in human and mouse chromosomes, without equivocal staining of centrometric heterochromatin and background silver grains.

INTRODUCTION

Nucleolus organizer regions (NORs) of chromosomes in various organisms can be selectively stained by the N-banding (Matsui and Sasaki, 1973; Funaki *et al.*, 1975) or silver-staining techniques (Howell *et al.*, 1975; Goodpasture and Bloom, 1975), the latter technique being simplified and much improved by Bloom and Goodpasture (1976). Even though this improved technique is widely used, there are still several problems in its practical use such as those hampered by occasional appearance of excessive background silver-grains and non-uniform staining in a given slide.

We have devised a simple and reproducible silver-staining technique to overcome the above problems by using nylon cloth as a coverslip during silver impregnation of the slides.

MATERIALS AND METHODS

Air-dried chromosome preparations were made from PHA-stimulated human lymphocyte cultures and from a hyperdiploid mouse Ehrlich asites tumor (EAT).

The staining procedure employed was essentially the same as the Ag-I method described by Bloom and Goodpasture (1976), with minor modifications. The silver nitrate (Ag-) solution was prepared by dissolving 1 g $AgNO_3$ in 2 ml distilled-

Received February 27, 1980

deionized (d.d.) water and filtered through a 0.2 μm Millipore filter immediately before use. The nylon cloth (50 × 22 mm) used were of seven different mesh sizes: 90, 150, 225, 300 and 500 μm of Nybolt nylon cloth (Swiss Silk Bolting Cloth Mfg. Co., Ltd., Zürich), and 94 and 148 μm of Nylon-mesh cloth (Kyoshinriko Co., Ltd., Tokyo).

Chromosome slides were mounted with 2 drops of the Ag-solution and covered with a nylon-slip which had been immersed in the same Ag-solution. The slides were then placed in a moist chamber added with d.d. water to the bottom, keeping away from the slides. The chamber was incubated for 1–2 hr at 50°C or 3–6 hr at 37°C. The progress of staining was empirically monitored by the color change of the nylon cloth, from white to brown, when NORs were sufficiently stained. The reaction was terminated by removing the cloth and rinsing off the excess Ag-solution under running d.d. water. The slides were then counterstained in Giemsa (3% in 1/15 M phosphate buffer, pH 6.8) for 30–60 sec, rinsed in running water and blotted dry.

RESULTS AND DISCUSSION

Figure 1A shows a representative feature of human metaphase chromosomes stained with the present method, using the 148 μm nylon cloth, with 1 hr incubation at 50°C. The Ag-NORs were intensely and unequivocally stained, without showing deleterious effect on chromosome morphology and precipitation of silver grains on background. It should be mentioned that the present technique yielded a uniform staining reaction across the slides and that the results were fairly consistent and highly reproducible. Similar results were obtained by using the nylon cloth of smaller or larger mesh size. In general, the larger the mesh size, the shorter reaction time was sufficient for appropriate Ag-staining, excepting the 500 μm mesh which was of no use due to excessive precipitation of silver grains all over the slide. The smaller meshes, 90 and 94 μm, were also useful, but they required prolonged staining time and were unpractical. In conclusion, satisfactory Ag-NORs could be obtained by using the 148, 150, 225 and 300 μm meshes so far tested.

Since the present method did not cause distortion of chromosome morphology, the NOR-bearing chromosomes were easily identifiable by a Q-banding method as follows. After Ag-stained metaphases were photographed, the slides were immersed in methanol : acetic acid (3 : 1) for 5–10 min to remove the Giemsa stain, rinsed in running water and blotted dry. The destained slides were restained by means of the combined quinacrine mustard (QM) and 33258 Hoechst (H) technique (Yoshida *et al.*, 1975). The previously photographed cells were located and rephotographed (Fig. 1B). It was demonstrated by this sequential staining that the Ag-NORs were located in the secondary constrictions or stalks of the short arms of acrocentric chromosomes and that the number, size and distribution of Ag-NORs

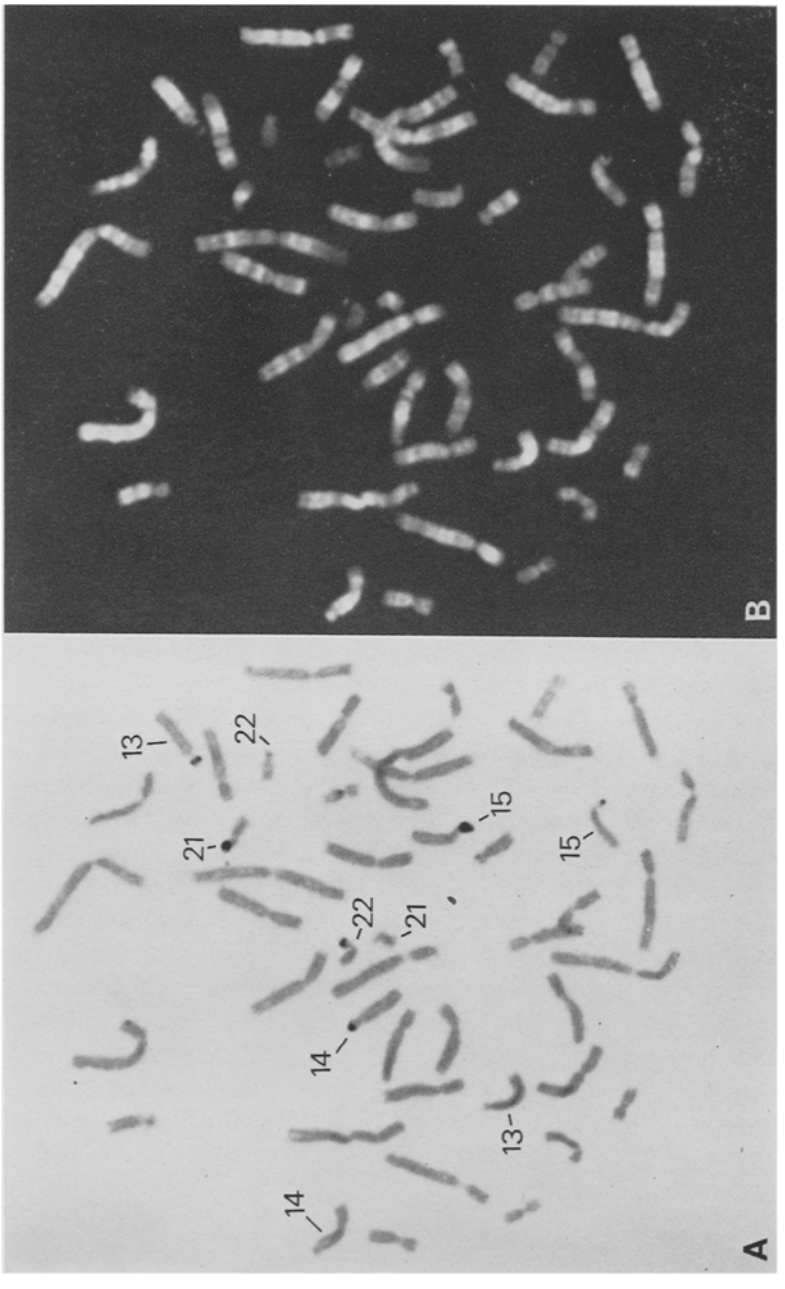

Fig. 1. Sequentially stained human chromosomes, for Ag-NORs (A) and Q-banding (B).

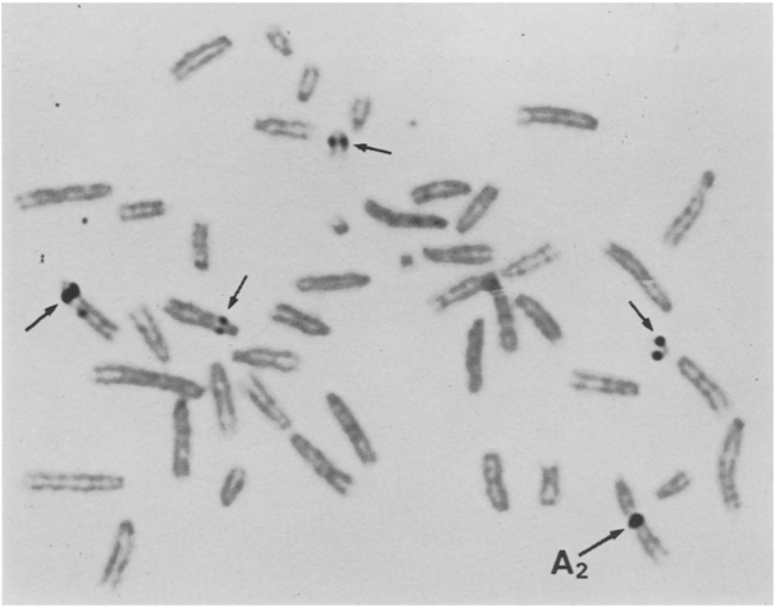

Fig. 2. Mouse EAT chromosomes stained by the present Ag-staining method.

were constant within an individual, confirming the conclusion of Bloom and Goodpasture (1976) and Schnedl (1978).

The present technique was applied to the mouse EAT chromosomes. Ag-NORs were found in 4 to 7 chromosomes with a modal number of 5 (Fig. 2). The acrocentric A_2 marker designated by Sasaki *et al.* (1974) was consistently present in the EAT cells here investigated. It showed an interstitial NOR located at the secondary constriction region of the long arm. A similar result has been obtained by the N-banding technique (Funaki *et al.*, 1975). Other Ag-NORs appeared to be fairly consistent from cell to cell, although there were noted a certain extent of variations in their number and distribution, probably reflecting the karyotypic instability of the present EAT line. A merit of the present technique was that the NORs were selectively demonstrated in the mouse chromosomes, without showing intensified staining reaction to the centromeric heterochromatin (C-band). Darkly stained centromeric heterochromatin regions were often observed in mouse chromosomes processed by the conventional Ag-staining which raised a considerable difficulty in the distinction of the NORs from the C-bands. Our nylon-slip method could overcome this problem (Fig. 2).

This work was supported by grants from the Ministry of Education, Science and Culture of Japan, and from the Ministry of Health and Welfare, Japan.

REFERENCES

Bloom, S.E., and Goodpasture, C. 1976. An improved technique for selective silver staining of nucleolar organizer regions in human chromosomes. *Hum. Genet.* **34**: 199–206.

Funaki, K., Matsui, S., and Sasaki, M. 1975. Location of nucleolar organizers in animal and plant chromosomes by means of an improved N-banding technique. *Chromosoma (Berl.)* **49**: 357–370.

Goodpasture, C., and Bloom, S.E. 1975. Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. *Chromosoma (Berl.)* **53**: 37–50.

Howell, W.M., Denton, T.E., and Diamond, J.R. 1975. Differentialstaining of the satellite regions of human acrocentric chromosomes. *Experientia (Basel)* **31**: 260–262.

Matsui, S., and Sasaki, M. 1973. Differential staining of nucleolus organizers in mammalian chromosomes. *Nature* **246**: 148–150.

Sasaki, M., Mori, M., and Oshimura, M. 1974. Chromosome banding patterns in Ehrlich and Yoshida ascites tumors. *J. Natl. Cancer Inst.* **52**: 1307–1315.

Schnedl, W. 1978. Structure and variability of human chromosomes analyzed by recent techniques. *Hum. Genet.* **41**: 1–9.

Yoshida, M.C., Ikeuchi, T., and Sasaki, M. 1975. Differential staining of parental chromosomes in interspecific cell hybrids with a combined quinacrine and 33258 Hoechst technique. *Proc. Jpn. Acad.* **51**: 184–187.