Preparation of Human Chromosomes for High Resolution Scanning Electron Microscopy

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Received January 21, 1992

Summary. The addition of ethidium bromide during the last 2.5-3 h of lymphocyte culturing restricted chromosome contraction and preserved the banding structure in scanning electron microscopy. Treatment of the chromosomes with trypsin and use of impregnation with osmium tetroxide and thiocarbohydrazide resulted in a structural preservation of high resolution quality.

A method for scanning electron microscopy (SEM) of metaphase chromosomes from human lymphocytes was designed by Harrison et al. (1985). Their standard technique for the preparation of chromosomes included incubation of the lymphocytes with colcemid for the collection of metaphases. Usually, however, the chromosomes contract when held in metaphase by colchicine, thus making many bands confluent. Since high resolution banding techniques, which give elongated chromosomes with many distinguishable bands, have been developed for light microscopy (Zabel et al., 1983; Ikeuchi, 1984), one aim of this work was to investigate whether a similar technique could be useful for SEM.

The fine surface structure of chromosomes can be examined by SEM if the metal coating of the preparations is replaced by an impregnation with osmium tetroxide and thiocarbohydrazide (IP and Fischman, 1979; Harrison et al., 1985). However, the attaining of high resolution imaging also requires a short trypsin digestion (Seabright, 1971), the duration of which has to be found empirically. Therefore, another of our aims was to study the effect of different durations of the trypsinization.

MATERIALS AND METHODS

Peripheral vein blood was sampled from humans in tubes containing heparin and was processed immediately. The erythrocytes were agglutinated for 30 min at room temperature with phytohemagglutinin and then separated by centrifugation. The leucocytes were cultured in RPMI 1640 medium, supplemented with phytohemagglutinin, penicillin, streptomycin, and 20% fetal calf serum at +37°C in humidified air with 5% CO₂.

A differential replication staining procedure, modified after Benn and Perle (1986), was applied after a culture period of 3 days. 5-bromodeoxyuridine (BrdU) was added at a concentration of 100 μM/ml medium, and the cells were grown for 16-17 h. Deoxycytidine was also added at the same time to the same concentration to reduce the toxicity of BrdU.

The cells were then rinsed and transferred into a prewarmed thymidine-enriched medium consisting of RPMI 1640, supplemented with penicillin, streptomycin, 20% fetal calf serum, and 2 μg/ml thymidine, and the cells were harvested by centrifugation 6 1/2 h later. Ethidium bromide (7 μg/ml medium) was added during the last 2.5-3 h of culturing to restrict the chromosome contraction.

Fifteen min before harvest, colcemid was added at a concentration of 5 ng/ml medium. The cells were then rinsed and placed into a solution of 75 mM KCl, where they were left for 15 min. After careful washings, the cells were fixed in a freshly made solution of methanol: glacial acetic acid (3:1). After centrifugation, the cells were suspended in a small volume of fixative, with 5 μl of this suspension placed on an ethanol-washed and polished circular cover glass (D=12 mm), which was air dried at about 40% humidity.

Before trypsinization, the cover glasses were rinsed with phosphate buffer to remove any residual fixative, treated with 0.1% Triton in buffer for 30 sec, and rinsed in 0.9% saline. The trypsinization was made with 0.025% trypsin in 0.9% saline for 5 or 10
Fig. 1. Light micrograph of elongated chromosomes derived by ethidium bromide retardation of the chromosomal condensation. Stained with Giemsa according to a differential replication staining method. ×1,200

Fig. 2. SEM of elongated chromosomes, trypsinized for 10 sec. ×3,000
Fig. 3. SEM of chromosomes after treatment with trypsin in A for 5 sec and in B for 10 sec. ×8,000

Fig. 4. High resolution SEM of chromosomes, trypsinized for 10 sec. Loops of chromatin fibres protrude from the surface of the chromosomes. ×25,000
sec at room temperature (SEABRIGHT, 1971). The action of trypsin was stopped with 3% glutaraldehyde in phosphate buffer and left there for 20 min.

The specimens were made electrically conductive by impregnation in osmic acid (1% OsO₄) for 5–10 min followed by treatment in tiocarbohydrazide (a saturated solution) for 5 min (MALICK and WILSON, 1975). The procedure was repeated until the cells became dark. A too lengthy treatment must be avoided as it tends to create precipitates. The specimens were dehydrated in a graded acetone series and critical point dried from liquid CO₂. The cover glasses were then mounted with silver paint onto stubs and examined in a Philips 525 scanning electron microscope.

RESULTS AND COMMENTS

Addition of ethidium bromide to the medium during the final culturing retarded the condensation of the chromosomes and resulted in elongated chromosomes as seen by light microscopy (Fig. 1). Preparation for electron microscopy of chromosomes, which were processed in the same way, also demonstrated elongated chromosomes, but to a less extent (Fig. 2). Thus, the results indicated that the addition of ethidium bromide to the culture medium was also an advantage when preparing chromosomes for electron microscopy.

Treatment of the chromosomes with trypsin removes a surface layer which otherwise hides structural details. HARRISON et al. (1985) used 20 to 40 sec for the trypsinization. With our protocol for preparation, a duration of the treatment for 5 sec was not sufficient (Fig. 3A), while a duration for 10 sec was optimal (Figs. 3B, 4).

In conclusion, the present protocol for preparation of human chromosomes results in a structural preservation which should have sufficient quality to serve as a basis for gene mapping with SEM (TRASK, 1991).

REFERENCES


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