DISCRIMINATION OF THE NUCLEOLUS BY A CRITICAL ELECTROLYTE CONCENTRATION METHOD

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A new technique for the visualization of the nucleolus based on a variant of the previously reported critical electrolyte concentration method for nucleoprotein complexes is proposed. The method consists of treating toluidine blue-stained preparations with Mg\textsuperscript{2+} ions in a concentration such that ribonucleoprotein complexes are metachromatically highlighted, while DNA-protein complexes attain their critical electrolyte concentration point, thus losing metachromasy. The technique, which is applicable to plant and animal cells, is proposed for studies involving identification, morphometry and eventually changes in substructure of the nucleolus at the light microscopy level.

A critical electrolyte concentration (CEC) method based on binding of toluidine blue molecules under Mg\textsuperscript{2+} competitive conditions has been proposed for discriminating differences in DNA-protein complexes in vitro (8) and associated with different states of chromatin supra-organization (1, 4). In the method proposed by Vidal and Mello (8), Mg\textsuperscript{2+} ions are added to the experimental staining solutions and the Mg\textsuperscript{2+} concentration at which metachromasy is prevented has been considered to be the CEC value.

When studying variants of this technique, it was found that ribonucleoprotein complexes could be metachromatically highlighted after the CEC value of DNA-protein complexes was attained. This report describes a variant of the CEC technique in which the nucleolus is significantly discriminated.

MATERIALS AND METHODS

Root tip cells of Allium cepa, salivary gland cells of Drosophila aracataca, Balb/3T3 cells, and NIH/3T3 cells transformed by transfection with DNA from carcinogen-treated human cells (5) were used.

All the materials were fixed in an ethanol-acetic acid (3 : 1, v/v) mixture for 1 min, with the exception of the onion root tips, which lasted 15 min in the fixative. The glands of D. aracataca were squashed in a drop of 45\% acetic acid after fixation, and frozen for removal of the coverslips, after which the preparations were rinsed in 70\% ethanol and air dried.

In the other materials, fixation was immediately followed by a 5-min bath in 70\% ethanol. The onion root tips were subsequently processed for paraffin wax embedding and cut into 12-\mu m thick sections. The cultured cells were air dried immediately after the 70\% ethanol treatment.

Staining was performed with a 0.025\% toluidine blue (Merck) solution in McIlvaine buffer at pH 4.1, for 15 min. Immediately after staining, the preparations were treated with 0.02 M to 0.10 M aqueous MgCl\textsubscript{2} solutions for 15 min, rapidly rinsed in distilled water (5s), air dried, cleared in xylene and mounted in Canada balsam.

Some preparations were treated with a 0.1\% RNase III (Sigma) aqueous solution at 37°C for 1 hr prior to staining.

Spectral absorption curves were obtained for the nucleolus and the chromatin of the onion cell nuclei in order to detect their absorption peak wavelengths, and consequently, metachromasy loss at the chromatin level. Microspectrophotometry was carried out with Zeiss equipment (EMI 6256 photomultiplier, photometer Ol, Planapo 100/1.25 objective, optovar 2, measuring diaphragm diameter of 0.25 mm, field diaphragm diameter of 0.1 mm, LD-Epiplan 16/0.30 condenser and Schott continuous monochromator filter ruler). The area of the specimen measured per plug point was 1.23 \mu m\textsuperscript{2}. Areas showing relatively homogeneous staining with maximal absorbance values not exceeding 1.200 were chosen for measure-
FIGS. 1–3. Metachromasy of nucleoli (arrows) in cells subjected to toluidine blue staining.

Figs. 1a–f. Allium cepa cells. a. Control. Only exceptionally is the nucleolus well distinguishable (black arrow). b–e. Staining followed by treatment with 0.05 M MgCl₂. f. Staining and Mg²⁺ treatment preceded by RNase digestion.

Figs. 2a–b. D. aracataca cells. a. Control. b. Staining followed by treatment with 0.05 M MgCl₂. Some polytene chromosome regions (puff, p) also display metachromasy.

Figs. 3a–c. Balb/3T3 cells (a–b) and transformed NIH/3T3 cells (c). a. Control. b–c. Staining followed by treatment with 0.05 M MgCl₂. Bars=10 μm.
RESULTS AND DISCUSSION

The visualization of the nucleoli in toluidine blue-stained preparations is often hindered by the strong affinity of the dye molecules for DNA (Figs. 1a, 2a, 3a). This makes that for ordinary preparations of some materials not subjected to enzymic digestions, nucleoli could not be distinguished from heterochromatic areas.

However, in those preparations in which toluidine blue staining is followed by treatment with Mg\(^{2+}\) ions, the nucleoli became easily detectable (Figs. 1b-e, 2b, 3b-c), provided that the Mg\(^{2+}\) concentration is sufficient to abolish the metachromasy due to DNA. It was observed that the metachromasy due to RNA is maintained at Mg\(^{2+}\) concentrations much higher than those required for the attainment of the CEC value for DNA or DNA-protein complexes. This observation is in agreement with Scott’s (6) report on CEC for 18S and 32S RNA compared to DNA in vitro, though different dye and inorganic cations having been used.

The Mg\(^{2+}\) concentrations most adequate for the visualization of the nucleoli in the materials studied were found to be 0.03–0.05 M. Under these circumstances, the spectral absorption peak was found to occur at \(\lambda = 570\) nm and 610 nm for Allium cepa nucleoli and chromatin, respectively. The other materials showed their nucleolar peaks positioned at \(\lambda = 580\) nm, and their chromatin peaks at 610 nm (D. aracataca and transformed NIH/3T3 cells) or 600 nm (Balb/3T3 cells). The part played by RNA in the metachromasy of the nucleoli was demonstrated in preparations treated with RNase prior to staining, in which nucleolar metachromasy was abolished (Fig. 1f).

This reasonably simple technique not only permits the identification of cell nucleoli but is also assumed to be useful for morphometric studies due to the good quality of the nucleolar contrast. In Allium cepa cells in particular the method highlights some details of the nucleolar substructure and its changes, which are not distinguishable in the few nucleoli discriminated with ordinary toluidine blue staining. These substructure details have only been described thus far with silver staining procedures and have been assumed to accompany redistribution of nucleolar components as a function of cell physiology (2, 3, 7).

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REFERENCES