# CYTOCHEMICAL CHARACTERISTICS OF RIBONUCLEOPROTEINS AND DESOXYRIBONUCLEOPROTEIN COMPLEX IN NUCLEOLI OF CULTIVATED PRIMATE RETICULAR CELLS

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Five fractions of ribonucleoprotein structures were detected histochemically in the nucleolus of reticular cells cultivated in vitro. Chrysomalin (an inhibitor of RNA synthesis) causes the disappearance of the first three fractions, and ethionine (an inhibitory of protein synthesis) causes four fractions to disappear. The fifth fraction – a complex of RNA with DNA of the heterochromatin type – is relatively stable.

In 1964, the writers reported the discovery of five fractions of ribonucleoproteins, differing in their isoelectric properties, by histochemical methods in the nucleoli of nerve cells [7]. Four of them were ribonucleoproteins (RNPs), while the fifth was considered to be a complex of heterochromatin type between RNP and DNA. More recently, the existence of such a complex has been verified by biochemical methods: RNP isolated from nuclei of calf thymus cells was found to contain about 4% of DNA which could not be removed from the complex either by treatment with desoxycholate or by gentle treatment with ribonuclease or desoxyribonuclease [8]. This paper describes data concerning RNP fractions in nucleoli of cultures of primate reticular cells treated with inhibitors of RNA or protein synthesis.

## EXPERIMENTAL

The following cells were used in the experiments: transplantable lines of J-96 cells obtained from the blood of a patient with subacute monocytic leukemia [9]; monkey tonsil cells (MTC) [2], and sublines L-41 and MTC-45, obtained from the above-mentioned lines by treating the cells with Coxsackie B3 and poliomyelitis type I viruses, respectively, as a result of which these sublines acquired specific resistance to the cytopathogenic action of the homologous virus [4]. The cells were cultivated on cover slips of Leighton's tubes in medium No. 199 with 10% bovine serum (100,000 cells/ml); on the second or third day of cultivation the cells were fixed and the isoelectric points of ribonucleoprotein structures (IEP RNP) determined by staining with methylene blue by Shabadash's method [6] in pH zones from 2.6 to 10.4 at intervals of 0.2 unit. Details of the method were described previously [7]. In some experiments the cells were treated either with chrysomalin (the Soviet analog of actinomycin D [1]), an inhibitor of DNA-dependent RNA synthesis, or with ethionine, an inhibitor of protein synthesis [3]. Chrysomalin was injected in a final concentration of 1 $\mu$g/ml and the cells were fixed 1, 6, 12, and 24 h later; ethionine was given in concentrations of 1, 2.5, 5, and 10 $\mu$g/ml and the cells were fixed 24 h later.

## EXPERIMENTAL RESULTS

As was observed previously [5], on the whole the IEP RNP of ribosomes and organelles in the cytoplasm of L-41 and MTC-45 cells resistant to a definite enterovirus were displaced toward alkaline pH value

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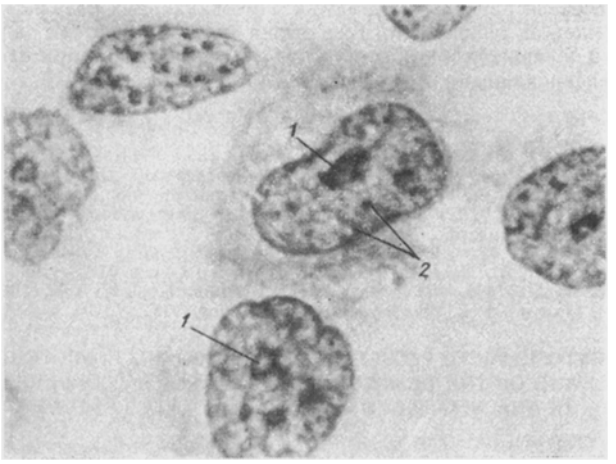


Fig. 1. Culture of monkey tonsil cells (2 days after seeding; treatment with chrysomalin for 1 h): perinucleolar chromatin visible as granules and as a "cap" against the background of coarsely granular chromatin of the nucleus. 1) "Cap;" 2) chromatin of nucleus. Stained with methylene blue at pH 3.5 by Shabadash's method, 200 ×.

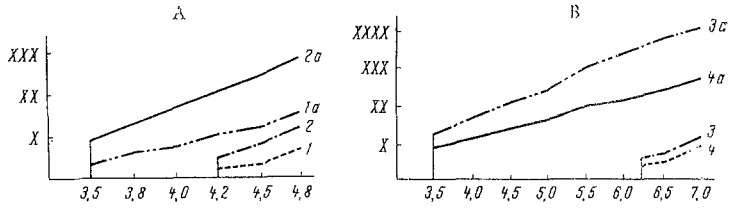


Fig. 2. Isoelectric points of ribonucleoprotein structures of J-96 cells retaining their structure after treatment for 6 h with chrysomalin. A: 1) cytoplasmic granules; 2) nucleus; B: 3) perinucleolar chromatin; 4) nucleolus; 1a, 2a, 3a, 4a) corresponding structures of control cells. Abscissa, pH values; ordinate, intensity of staining of structures (in excess).

compared with those of the original cell. However, the basic principles of detection of IEP for different categories of RNA were similar in all studied cell strains. In their nuclei, just as in the nuclei of nerve cells [7], five fractions of RNP-structures differing in their IEP were detected. The first RNA fraction had the appearance of a greenish-blue structureless mass, diffusely distributed throughout the nucleolus; this category of RNP stained at the highest pH values; its IEP lay within the range 2.5-2.7. The second fraction was found in pH zone 2.7-3.2 and consisted of dark blue granular structures in the central part of the nucleolus. The third was marked by a continuous, diffuse, and intensive staining of the whole nucleolus; it looked like a massive "eye" against the pale background of the relatively unstained nucleus. The fourth fraction consisted of the peripheral RNP of the nucleolus, forming an irregular mass or a dark circular rim; it was very variable. The high reactivity of the nucleolus makes it extremely probable that this RNP is not detected to an equal degree even in neighboring cells. The fifth fraction appeared at more alkaline pH values of the order of 4.0-4.5, as distinctive processes or "caps" of RNP-complex. These structures can break away from the nucleolus and migrate across the karyoplasm to the nuclear membrane, where they became rounded in shape and, in some cases, increased in size. In some cells this breaking away is accompanied by a marked decrease in the RNP content in the central part of the nucleolus. Judging from previous observations [7], this category of RNP is a special complex (or symplex) with DNA.

After treatment of the cells with chrysomalin, the first changes were found in the nucleolus. After 1 h they assumed an "empty" appearance: the RNA of the first, second, and third fractions no longer stained.

The perinucleolar chromatin (4th and 5th fractions) became granular in appearance; sometimes it resembled a broken ring or characteristic "cap" near the nucleolus (Fig. 1). However, no substantial change in the IEP RNP of the cell structures was observed under these circumstances. The action of inhibitor for 6 h caused degeneration of some cells; in the remaining morphologically intact cells, the IEP of the RNP structures of the cytoplasm and nucleus was displaced toward alkaline pH values; a particularly marked displacement was observed in the IEP of the nucleolus and perinucleolar chromatin (Fig. 2), which was stained a dull blue color. The more prolonged action of chrysomalin led to degeneration of most cells of the layer; neither the nucleolus or the perinucleolar chromatin stained in the morphologically intact cells. Only in a few cells could remains of the RNP complex (5th fraction) still be seen.

After the action of ethionine in a concentration of 1 $\mu$g/ml, a shift of IEP of the perinucleolar chromatin toward alkaline pH values by 1.0-1.2 unit of the pH scale was observed. Higher concentrations of the inhibitor led to corresponding changes in the IEP of the nucleolus also. If ethionine was given in a concentration of 10 $\mu$g/ml, marked degeneration of most cells was observed: the nucleoli appeared "empty" and deformed; the peripheral RNP of the nucleolus (4th fraction) disappeared and could not be detected at any of the pH values used. By contrast, a well-defined RNP-DNP complex (5th fraction) was clearly visible, and sometimes it could be seen to break away from the nucleolus and to migrate toward the nuclear membrane.

The five fractions of nucleolar ribonucleoproteins described above were thus found in cells which differed sharply in their functional and morphological properties. Since they were detected both in the nerve cells of animals and in cells of monolayer cultures, there is reason to suppose that the existence of these RNP fractions reflects the existence of general cytological principles. To judge from changes in these RNA fractions after treatment with inhibitors of cell synthesis, the RNA of the first three fractions is a morphological expression of ribonucleoprotein newly formed in the nucleolus: it disappears if synthesis of RNA or of protein is inhibited. The fourth fraction, the one which is changed to the greatest degree by inhibition of protein synthesis, evidently consists of an RNP complex in which the proportion of newly formed protein is high. The fifth fraction possesses relatively high stability of the action of the abovementioned inhibitors; it can be assumed that its components metabolize relatively slowly.

The pH values at which the different RNP categories in cultures of primate reticular cells appear do not correspond to the values at which they were detected in nerve cells [7]. This disagreement is evidently due to the high reactivity of the nucleolus, which makes it extremely likely, as was mentioned previously [7], that different results will be obtained even for neighboring cells at the same level of the nervous system, and still more for repeatedly dividing cells of an explanted tissue when compared with stabilized nerve cells.

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