Cytological characteristics of protein and nucleoprotein fractions of cell nuclei

In previous work from our laboratory it was shown that cell nuclei isolated in neutral citrate media from different sources can be separated into a nucleoprotein fraction extractable with 1 *M* NaCl and containing DNA, histone and non-histone protein, an acidic-protein fraction soluble in dilute alkali and containing RNA, and residual protein free from nucleic acids and insoluble in usual protein solvents[1-4]. In cell nuclei of different normal tissues the nitrogen distribution was in general similar, about 50–60 % of the total N being contained in the nucleoprotein, 30–40 % in the acidic protein and 5–10 % in the residual protein fractions. Tumour-cell nuclei differ from the above by their high content of residual protein (up to more than 50 % of the nuclear N) and lower content of the acidic-protein and especially nucleoprotein fractions[2,5,6].

Other authors[7], using similar methods of fractionation, described a globulin fraction obtained by preliminary extraction of nuclei with 0.14 *M* NaCl or other solutions of about the same ionic strength.

The cytological characteristics and intranuclear localization of these nuclear fractions remained obscure. The preexistence in intact nuclei of a nucleoprotein complex soluble in 1 *M* NaCl was also a matter of discussion[8].

These questions have now been studied by a combination of chemical and histochemical methods. Non-fixed sections (3–4μ) of different animal tissues were prepared with a deep-freezing knife and then extracted in the cold with the salt and alkaline solutions mentioned above. The method of preparation of these frozen sections made autolytic alterations unlikely, while their thinness was favourable for easy removal of soluble nuclear material. After such extractions the sections were fixed in absolute alcohol and stained for nucleic acids and protein with pyronin-methyl green[9], Feulgen reaction, bromphenol blue[10] and a combined Feulgen and bromphenol blue test.

The extraction with 0.14–0.4 *M* NaCl solubilized all the extra-chromatin protein and nucleic acid material, as evidenced by the combined staining method. 1–1.5 *M* NaCl readily extracted all the DNA-protein from cell nuclei in thin sections. From non-dissected nuclei the extraction was negligible.

The RNA-proteins of nucleoli and structures resembling the "residual chromosomes" of MIRSKY AND RIS[11] remained intact following the two extractions with NaCl solutions but disappeared after extraction with dilute alkali.

Our results clearly indicate a correlation of the globulin fraction with extra-chromatin proteins, of the DNA-protein fraction with chromatin, and of the acidic protein with nucleoli and residual chromatin and show that the extractability of DNA-protein is not an autolytic artifact.

In further studies, rat-liver nuclei free from cytoplasmic contamination have been isolated in a sucrose-0.05 *M* K glycerophosphate medium (d = 1.273), using as a homogenizer an all-glass bacterial mill rotated at 1000 rev./min. The native state of the nuclei was verified by their ability to form a gel after slight alkalinization[12].

Abbreviations: DNA, deoxyribonucleic acid; DNA-protein, deoxyribonucleoprotein; RNA, ribonucleic acid; RNA-protein, ribonucleoprotein.

The fractionation procedure included successive extractions of the isolated nuclei with 0.14 *M* NaCl or 0.05 *M* citrate followed by 1.5 *M* NaCl and 0.05 *M* NaOH. From the viscous DNA-protein solution a complex of DNA and non-histone protein was precipitated with dilute H_2SO_4.

Quantitatively the globulin fraction included about 20 % of the nuclear N and contained variable amounts (2.6–8 %) of RNA. The DNA-protein fraction included nearly 70 % of the nuclear N, approx. 18 % being represented by DNA, 34 % by histone and 17 % by non-histone protein. More than 99 % of the total nuclear DNA was found in 1.5 *M* NaCl extracts (DNA-protein fraction). Smears of material left after 1.5 *M* NaCl extraction showed many nucleoli and residual chromosomes stainable with pyronin. The small amount of DNA-protein not associated with these structures caused a faint background staining in some places. This was presumably due to residual DNA-protein which because of its high viscosity was incompletely extracted.

It is interesting to note that the acidic protein corresponding to nucleoli and residual chromosomes, and representing only about 6 % of the nuclear material, contained 14–20 % RNA, a value that is very characteristic for nucleolar material. The similarity in behaviour of the RNA-protein of nucleoli and residual chromosomes indicates a close relation between these two structures.

The residual protein fraction which remains after alkaline extraction does not contain any nucleic acid or other phosphorus compounds. It is most probable that it represents the nuclear-membrane protein[13].

The reported results show the correlation between certain chemical components of nuclei and definite nuclear structures. This correlation was revealed by successive extractions of protein and nucleoprotein fractions from thin frozen sections and isolated nuclei followed by histochemical staining.

The Severtzov Institute of Animal Morphology, Academy of Sciences of the USSR, Moscow (USSR)

I. B. ZBARSKY
G. P. GEORGIEV

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