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On the history of nuclear matrix manifestation

ZBARSKY IB
N.K. Koltzov Institute of Developmental Biology, Russian Academy of Sciences. 26, Vavilov Street, 117334 Moscow, Russia. e-mail: ibzba@ibrran.msk.su

ABSTRACT

The nonchromatin proteinous residue of the cell nucleus was revealed in our laboratory as early as in 1948 and then identified by light and electron microscopy as residual nucleoli, intranuclear network and nuclear envelope before 1960. This structure termed afterwards as "nuclear residue", "nuclear skeleton", "nuclear cage", "nuclear carcass" etc., was much later (in 1974) isolated, studied and entitled as "nuclear matrix" by Berezney and Coffey, to whom the discovery of this residual structure is often wronly ascribed. The real history of nuclear matrix manifestation is reported in this paper.

Key words: *Nuclear matrix, nuclear residue, nuclear fractions, history of nuclear fractionation.*

INTRODUCTION

The term "nuclear matrix" (NM)[1, 2] implies a structure consisting mainly of nonhistone proteins which remain after extraction of chromatin, casually lipids and residual DNA from isolated cell nuclei. Morphologically the NM consists of extracted nucleoli, nuclear envelope (lamina) and intranuclear fibrogranular network.

As early as in 1947 it was accepted that cell nuclei contained only nucleoprotein composed of thymonucleic acid (DNA) and basic proteins - protamins or histones.

Only after 1940 a few data on the presence of non-basic proteins in cell nuclei or nucleoproteins appeared. Primarily non-histone proteins in bacterial and plant nucleoproteins were described by Belozersky[3, 4]. Then a protein with high sulfur content was isolated from thymus nuclei[5]. However the desiccation of the tissue and its rough treatment with organic solvents suggest that it was a highly denatured

artifact. Later Stedman and Stedman[6] extracted isolated nuclei with mineral acid and termed the nonhistone protein remaining associated with nucleic acid as "chromosomin" supposing that it was a main constituent of the chromosomes.

Somewhat more reliable results belong to Mirsky and Pollister[7] and Mirsky and Ris[8] who isolated a non-histone tryptophane-containing protein from deoxyribonucleoprotein extracted from chromosomes and designated the "Residual from Chromosomes" remaining after this extraction.

Beginning from our studies on fractionation of cell nuclei we observed that nuclei isolated by non-aqueous methods as well as in acid medium usually used by previous workers contained denatured proteins and could not be resolved to fractions[9, 10]. Only the nuclei obtained by the method of Dounce[11] using very dilute citric acid could be fractionated.

However, as these nuclei were contaminated with cytoplasm, we elaborated, using citrate buffer, a method which produced much more purified nuclei[9, 12, 13]. Employing this method we fractionated isolated nuclei from normal and malignant rat and human tissues.

With 1-2 *M* NaCl we extracted a "nucleoprotein" fraction consisting of DNA, histone and a tryptophane-containing protein. The proteinous residue could be then resolved to "acidic protein" extracted with dilute alkali and insoluble "residual protein" (Fig 1).

Properties, quantitative ratio and amino acid compositition of these fractions were studied. The acidic protein (AP) sedimented at pH 5.0 - 5.3 and contained about 2.5% tryptophane; the residual protein (RP) was free of tryptophane and constituted 5-10% of nuclear nitrogen. However, in nuclei isolated from malignant tumors it contained tryptophane and amounted to 50% of nuclear dry weight[9, 13-15]. Later we showed that RP of tumors could be resolved by extraction with hot alcohol to tryptophane-containing and tryptophane-free moieties[16].

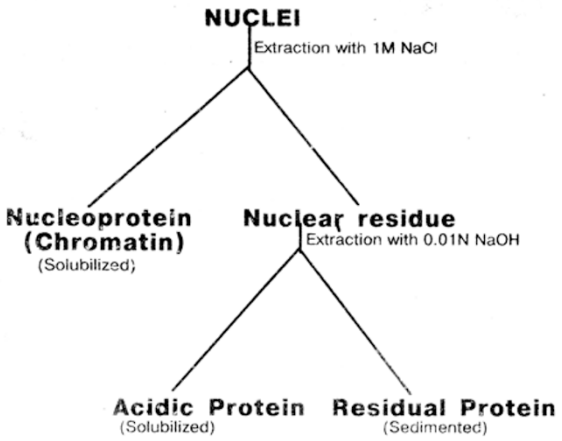

Fig 1. Scheme of nuclear fractionation [9]

Then, we developped the fractionation of cell nuclei by preliminary extraction of nuclear sap with 0.14 *M* NaCl and tentative treatment with DNase I. The fractions were, further, identified cytologically. Deep-freezed sections of various tissues were

extracted in the cold with solutions mentioned above, then fixed in absolute alcohol and stained for nucleic acids and proteins with pyronine-methyl green, Feulgen reaction, bromphenol blue and combined Feulgen-bromphenol blue test.

0.14-0.40 *M* NaCl extracted nuclear sap contained non-chromatin protein and RNA. 1.5 *M* NaCl exhaustively solubilized chromatin. Extracted nucleoli and intranuclear structure resembling "residual chromosomes" of Mirsky and Ris[10] contained ribonucleoprotein and remnants of the nuclear envelope which were further identified as 'lamina' remained[17-19]. Extraction with 0.01 N NaOH left only the latter[20-22].

Later, electron microscopy of nuclear fractions was studied in our laboratory and it was established that material remaining after extraction of nuclear sap and chromatin and DNase treatment consisted of extracted nucleoli and fibrogranular intranuclear network ("nucleonema") forming a so-called "nucleolo-chromosomal apparatus" attached to the remnants of the nuclear envelope[23-29].

Thus, in our studies (1948-1962) the initial data of non-chromatin nuclear proteins were obtained; their quantitative ratio, composition and accordance with cell organelles were established (Tab 1). Further, the cytological structures, representing nuclear skeleton (nuclear matrix) were verified by electron microscopy and confirmed in various laboratories[20, 30-33]. Our studies were cited and their priority acknow ledged by authoritative scientists[34, 35].

Tab 1. Composition of rat liver cell nuclei[26]

Nuclear structure	Corresponding fraction	Solvent	% dry nucleus	Composition of the fraction
Nuclear sap	"Globulin"	0.14*M* NaCl	20	RNA 2-8%
DNP of chromatin	"Deoxynucleo protein"	1-2*M* NaCl	70	DNA 33%,Histone 50% Nonhistone protein 17%
Nucleoli and residual chromosomes	"Acidic protein"	0.01*M* NaOH	5-6	RNA 16-20%

On 21 April 1988, these findings were registered by State Committee of the USSR on inventions and discoveries as a discovery No. 348: "Property of nonhistone proteins of the cell nucleus to form non-chromatin structural carcass (nuclear matrix)" with the priority of August 27, 1948 and November 13, 1959. Reviews of these studies appeared in our articles[36, 37] and monographs[1, 38].

However, in the majority of later published papers, the history of the identification of the NM is often misrepresented[39]. Really, the exploration of the nuclear skeleton (or nuclear matrix) is a result of many studies to which Berezney and Coffey joined after 14 or even 26 years. Their studies developped and enriched this important branch of cell biology, but have nothing to do with its revelation.

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