# PROTEINS AND FUNCTIONAL PROPERTIES OF CHROMATIN FRACTIONS FROM NUCLEI OF NORMAL LIVER AND EXPERIMENTAL HEPATOMAS

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In the modern view nuclear skeletal structures (the nuclear matrix) play an extremely important role in the structural organization of chromatin [12]. There is evidence that differences in the degree of compactness of active and inactive chromatin in the nucleus may be due not only to the specific nature of the protein composition, but also to local differences in interconnection between fibrils of deoxyribonucleoprotein (DNP) with protein fibrils of the matrix: The topography of diffuse and condensed regions of the nucleus is preserved after removal of both DNA and histone H1 from it [10]. The possibility of fractionating chromatin, based not on choice of conditions of selective degradation of the DNA of active chromatin, but on differences in solubility of DNP fragments having points of attachment to the matrix and not bound with it, is accordingly interesting. This possibility arises because of the fact that after nuclease treatment of a certain intensity (when the mean size of DNA fragments between neighboring double-stranded breaks is definitely less than the size of DNA of a single loop) under conditions of maximal solubility of DNP in the nucleus, chromatin enriched with points of attachment to the nuclear matrix remains in the nucleus [8].

It was shown previously that a buffer containing 10 mM Tris-HCl, pH 7.5, and 0.2 mM MgCl (TM medium) extracts from freshly isolated rat liver nuclei 60-75% of the chromatin, which is fragmented as far as mono, di-, and oligonucleosomes and contains hardly any nonhistone proteins [4, 5], whereas practically all the RNA-polymerase activity remains bound with DNP of the nuclear residue [5].

The investigation described below showed that chromatin fragments formed in nuclei of normal liver and of solid hepatomas by endonucleases, and in nuclei of ascites hepatomas by micrococcal nuclease, which differ in their ability to be detached from the matrix during extraction in TM medium, have a remarkably different composition of nonhistone proteins and RNA-polymerase activity.

## EXPERIMENTAL METHOD

Male Wistar rats weighing 150-200 g and male C3HA mice weighing 30-35 g were used. A solid hepatoma 27 was transplanted into noninbred rats. To isolate the nuclei, animals with Zajdela's ascites hepatoma (ZAH) were killed 5-6 days, animals with hepatoma 22 and 22A 7-8 days, and animals with hepatoma 27 were killed 24-30 days after inoculation of the tumors. Nuclei of the liver and solid hepatomas were isolated in buffer containing 30 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 70 μM 2-mercaptoethanol, 0.25 M sucrose (TMS medium), and purified through 2.2 M sucrose, as described previously [3]. To isolate nuclei from ascites hepatomas the cells were washed in 0.14 M NaCl, then subjected to osmotic shock in 10 mM Tris-HCl, pH 7.5, with 5 mM $MgCl_2$ for 30 min, subsequent procedures were carried out as in the experiments with liver nuclei. To study the protein composition of DNP fractions consisting of fragments with and without fixation points in the nucleus, the scheme of chromatin fractionation shown below was used. The conditions of centrifugation during fractionation were: 10 min, at 3000

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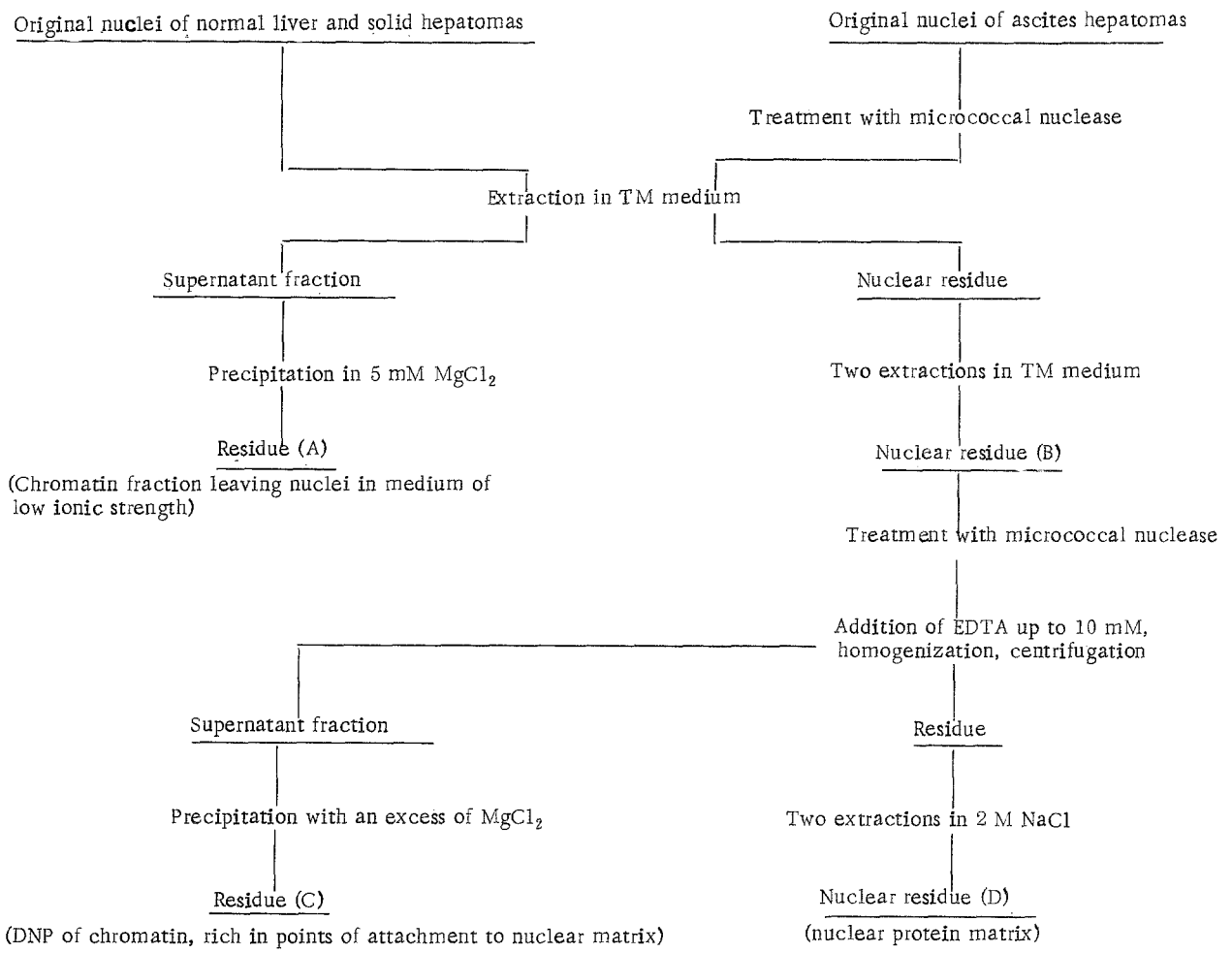
Scheme of chromatin fractionation




rpm, on a K-23 centrifuge (East Germany); the conditions for treatment with micrococcal nuclease (P-L Biochemicals, USA) were: 2-4 min at 37°C in TMS medium with 1 mM $CaCl_2$ and 30 units/ml of enzyme, and 1-2 mg/ml nuclear DNA. The template activity of fractions with RNA-polymerase from *E. coli* and their intrinsic RNA-synthesizing ability were determined as described in [3]. Electrophoresis of proteins was carried out in the presence of sodium dodecylsulfate in a gradient (10-20% as acrylamide) gel [9]. DNA was determined as in [7] and protein as in [11].

## EXPERIMENTAL RESULTS

The first stage of fractionation of chromatin by the scheme illustrated above is extraction of the nuclei in TM medium. Depending on the type of original tissue significant differences were found in the release of DNP from the nuclei. For instance, with the first extraction of isolated nuclei of the resting or regenerating liver in TMM medium about 50% (judging from the DNA content) of the chromatin was extracted, compared with about 50% from nuclei of solid hepatoma 27, about 20% from nuclei of solid hepatoma 22, whereas virtually no DNP was extracted under these conditions from nuclei of ZAH and ascites hepatoma 22A. The extractable chromatin was segmented as far as mono-, di-, and oligonucleosomes.

The absence of any significant release of DNP in TM medium from nuclei of ascites hepatomas was evidently due to a reduction (or absence) of their endonuclease activity, which is present in liver nuclei. According to the literature endonuclease activity was sharply depressed in nuclei of rapidly proliferating hepatomas [1], but usually activity of the enzymes was compared with respect to their ability to hydrolyze DNA as far as acid-soluble fragments. The results of the present experiments indicate a similar fall in activity of the isolated nuclei from ascites hepatomas. Definite correlation between endonuclease activity

TABLE 1. Template Activity (in relative units) of Chromatin Fractions with RNA-Polymerase of *E. coli* and Endogenous RNA-Polymerase (in % of corresponding level of RNA synthesis in original nuclei) (M ± m)

| Source of nuclei | Template for RNA synthesis | | | |
|---|---|---|---|---|
| | chromatin fraction extractable in TM medium (fraction A in scheme) | | chromatin fraction remaining in nuclei after three extractions in TM (fraction B on scheme) | |
| | endogenous RNA synthesis | RNA synthesis enzyme from E. coli | endogenous RNA synthesis | RNA synthesis with enzyme from E. Coli |
| Normal rat liver (n = 13) | Background | 98±16 | 160±30 | 110±25 |
| Solid hepatoma 27 of rats (n = 3) | " | 115±22 | 144±28 | 95±18 |
| ZAH of rats (n = 9) | " | 95±14 | 190±40 | 130±32 |
| Solid hepatoma 22 of mice (n = 3) | " | 86±17 | 120±15 | 99±16 |
| Ascites hepatoma 22 | " | 88±17 | 140±28 | 173±46 |

Legend. n) Number of experiments.

(similar in the character of degradation of DNP by Ca, Mg-dependent endonuclease) with the degree of malignancy must be noted: Whereas the nuclei of more malignant (ascites) hepatomas completely lost one of their characteristic features of normal (resting and regenerating) liver nuclei isolated in the presence of $Mg^{++}$, nuclei of the more malignant hepatomas (solid) preserved it to some degree.

Because of the absence of endogenous degradation of DNA in chromatin from ascites hepatoma nuclei, a stage of their preliminary treatment with micrococcal nuclease was introduced into the fractionation scheme before extraction of the nuclei in TM medium (see scheme). The conditions chosen for treatment (up to 2-3% yield of DNA into "nuclease" buffer) ensured maximal yield of DNP in TM medium (about 30% in the case of ZAH nuclei and about 50% in the case of hepatoma 22A), in which the DNA of the extractable DNP and DNA of the nuclear residue, as the results of electrophoresis in 1% agarose showed, were fragmented to about the same degree; the distribution of DNA by size of its fragments, moreover, was similar to that in chromatin fractions from normal liver. This last state of affairs indicates that the ability of chromatin fractions to be extracted under the given conditions from the nucleus is determined, not by the different sizes of the fragments of the DNP fractions, but by differences in their binding with skeletal structures. Such a possibility has been indicated before [6].

As Table 1 shows, although DNP extractable in TM medium (fraction A on the scheme) was capable of acting as template for *E. coli* RNA polymerase to almost the same degree as the original nuclei, its intrinsic RNA-synthesizing capacity was equal to background incorporation of [$^3$H]ITP. Since solutions capable of dissociating the complex of endogenous RNA-polymerase and chromatin molecules were not used in fractionation, the results are evidence that endogenous RNA-polymerase was not associated with the extractable DNP.

Under conditions of low ionic strength endogenous RNA polymerase thus remains with the unextractable DNP fraction (fraction B on the scheme) inside the nucleus and, as Table 1 shows, it preserves its activity.

To compare the protein composition of DNP fractions exhibiting such different RNA-synthesizing ability, the chromatin itself had to be extracted from the nuclear residue, i.e., it had to be separated from the nuclear skeletal structures. Additional treatment with micrococcal nuclease and EDTA enabled the greater part of the DNP from chromatin fragments not otherwise extractable in TM medium (fraction C on the scheme) to be solubilized. Meanwhile a considerable part of the endogenous RNA-polymerase activity also was solubilized from the nuclear residue.

The protein composition of the fractions was found to correspond to their functional properties. DNP fragments not exhibiting RNA-synthesizing ability were characterized by a protein/DNA ratio close to 1.1, and their content of nonhistone proteins was greatly reduced (see Fig. 1a-c) compared with fragments possessing RNA-synthesizing ability, which contained in their composition the greater part of the chromosomal nonhistone proteins (see: Fig. 1d-f), which also is reflected in an increased (over 2) protein/DNA ratio.

The composition of proteins of the DNP fraction successively removed, according to our scheme, from the nuclear matrix thus has common basic features in the case of nuclei both

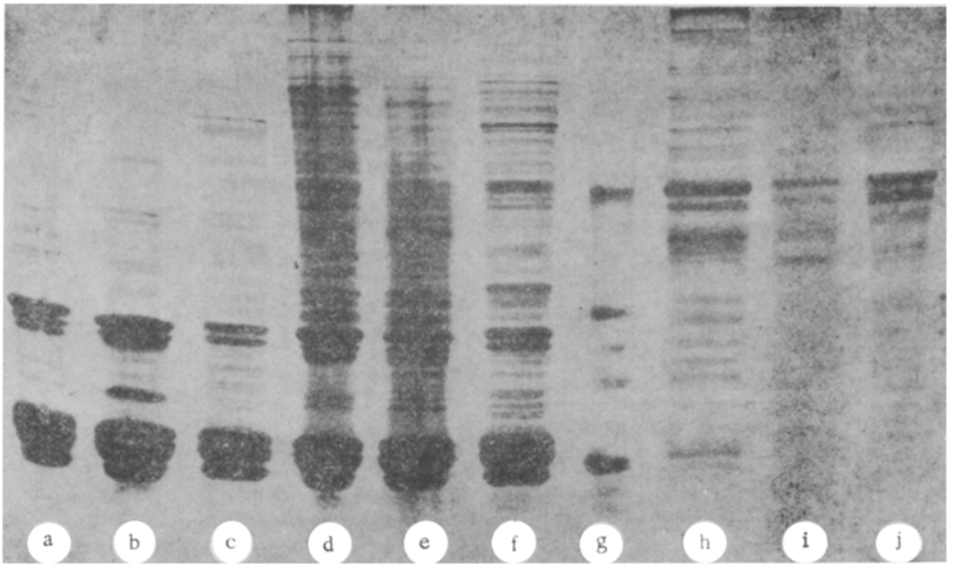



Fig. 1. Electrophoretic spectra of proteins: a, b, c) proteins of chromatin fraction extractable in TM medium (fraction A on scheme) from nuclei of normal liver (a), solid hepatoma 27 (b), and ZAH (c); d, e, f) proteins of chromatin solubilized from nuclear residue by micrococcal nuclease and EDTA (fraction C on scheme) of normal liver (d), solid hepatoma 27 (e), and ZAH (f); g) markers, from top to bottom: albumin (67 kilodaltons, DNase I (31 kilodaltons), RNase A (13.7 kilodaltons); h, i, j) proteins of nuclear matrix (fraction D of normal liver (h), solid hepatoma 27 (i), and ZAH (j).

from normal liver and from hepatomas. In addition, some quantitative and qualitative differences were found between the minor protein components of the DNP fractions of the hepatomas and the corresponding fractions of normal liver, which could reflect differences in the total proteins in nuclei from normal and tumor tissue [2]. Some differences also were discovered in matrix proteins, although the main polypeptides (in the region of 67 kilodaltons) were identical (Fig. 1h-j).

Chromatin fragments with endogenous RNA-synthesizing activity, obtained from nuclei both of normal liver and of hepatomas, thus remained mainly associated with nuclear skeletal structures in a salt-free medium and were enriched with nonhistone proteins.

As regards the possible site of the most important intranuclear processes, ever-increasing attention is being directed to the nuclear protein matrix [12]. We need mention only attachment of a replicating fragment of chromatin to it, taking place through a connection which is resistant even to a high ionic strength [13]. The suggestion that transcribable DNP fragments associates specifically with the matrix through a bond of DNA-(RNA-polymerase -- RNA or RNP)-matrix is in good agreement with the results of the present investigation.

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