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Phospholipid interactions in rat liver nuclear matrix

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Summary

Rat liver nuclear matrix has been isolated by salt extraction and nuclease digestion of nuclei. Under the electron microscope, the matrix appears as a spongelike network joined by thinner fibrils. Biochemical analysis shows a high protein content and low amounts of nucleic acid and phospholipid. Treatment of the matrix with phospholipase C results in a release of most of the nucleic acid, and a disappearance of the fibrils, however the appearance of the matrix is largely unaffected. It seems likely that phospholipids are responsible for the hydrophobic interactions between nucleic acids and matrix fibrils. From *in vitro* labelling studies the released DNA is more recently synthesised than the bulk material, however the matrix bound RNA appears to label less rapidly than total nuclear RNA.

INTRODUCTION

The nuclear matrix is a well defined framework structure which has been isolated from a variety of nuclei after low and high salt extraction steps and nuclease digestion (1,2). This matrix consists largely of protein with small amounts of RNA, DNA and phospholipids and reveals under the electron microscope, a structure composed of residual nucleoli with a fibrous internal framework derived from the interchromatinic structures of the nucleus (3).

Earlier investigations indicated that:

i. small molecular weight nuclear RNAs are actual matrix components (4) as well as newly synthesised DNA (5).

ii. heterogeneous nuclear RNA (HnRNA) is intimately associated with the nuclear network (6).

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In addition to its function as a skeletal framework, the nuclear matrix may participate in other nuclear activities ranging from maintaining a fixed site for DNA replication, providing a degree of organisation for the splicing of primary transcripts as well as establishing the orderly transport of mRNA into the cytoskeleton via nuclear pore complexes (7). In addition the phospholipids of the nuclear matrix have been implicated in the process of gene regulation by affecting the structural conformation of both DNA and nucleoprotein complexes (8,9,10). It has been observed that non-histone proteins, which are the major components of the matrix proteins, are bound to phospholipid molecules (11,12,13,14). Also the finding that sodium deoxycholate releases RNA from achromatinic nuclei (6,7) suggests a hydrophobic association between RNA and the matrix fibrils. In this paper we have carried out phospholipase C digestion on the nuclear matrix to investigate the role of phospholipid in the hydrophobic interactions between RNA, DNA and the matrix protein fibrils.

Materials and Methods

^{3}H-TTP and ^{3}H-UTP were purchased from New England Nuclear Corp., Boston, Mass. Micrococcal nuclease (EC 3.1.4.7) and Phospholipase C grade II from Clostridium Welchi (EC. 3.1.4.3), also containing sphingomyelinase activity, were obtained from Boehringer, Mannheim, West Germany.

Rat liver nuclei were purified according to the method of Marzluff (15). The nuclei were washed twice in 0.25 M Sucrose, 0.05 M Tris pH 7.4, 5 mM $MgCl_2$ and subjected to the following steps to obtain the nuclear matrix. The pellet, suspended in 0.01 M NaCl, 0.003 M $MgCl_2$, 0.01 M Tris pH 7.4, 0.0001 M $CaCl_2$, was digested with Micrococcal nuclease, 50 units/100 $O.D._{260}$, for 10 min. at 37°C. Digestion was terminated by centrifugation at 780 x g for 15 min. at 0°C.

Extractions of digested material were carried out at 0°C as follows. Each solution contained 10 mM Tris buffer pH 7.4:

i. two extractions with 0.2 mM $MgCl_2$ for 10 min., followed by centrifugation at 780 x g for 15 min.

ii. three extractions with 2 M NaCl containing 5 mM $MgCl_2$ for 10 min., followed by centrifugation as above.

Enzymatic digestion of nuclear matrix with phospholipase C was carried out at 37°C for 1 hr. in ethanolamine-HCl 0.05 M pH 6.5, $CaCl_2$ 0.02M, 4 units phospholipase C/100 $O.D._{260}$ of the starting material. The reaction was terminated by centrifugation.

Isolated nuclear matrix was also incubated in the same conditions in the absence of phospholipase C.

Incorporation of ^{3}H-TTP into isolated nuclei was carried out at 37°C for 10 min. in a final volume of 9.9 ml containing 25 mM Tris pH 8.0, 25 mM KCl, 5 mM 2-mercaptoethanol 10 mM Mg Acetate, 5 mM EDTA, 2mM ATP, 80 μM dATP, 80 μM CTP, 80 μM dGTP, 80 μM ^{3}H-TTP (125 cpm/pmol.), 4 x 10^8 nuclei.

The incorporation of both ^{3}H-TTP and ^{3}H-UTP was determined, at each step of matrix preparation and after phospholipase treatment, by spotting 100 μl samples onto glass fiber filters (Whatman GF/C) and determining acid insoluble radioactivity (16).

DNA and RNA were determined by the diphenylamine and orceine reactions, respectively (17), and proteins by the Lowry method (18).

Samples of matrix, before and after phospholipase C treatment were incubated with proteinase K (0.1%, w/v) and Sarcosine (1%, w/v).

The DNA and RNA were precipitated at -70°C with absolute ethanol. Electrophoresis of nucleic acids was carried out in agarose-acrylamide slab gel (19).

Phospholipids were extracted and separated by thin layer chromatography as previously described (14).

Samples of matrix before and after phospholipase treatment, were prepared for electron microscopy by fixation in glutaraldehyde 2.5% (0.05 M cacodylate pH 7.4, 5 mM $MgCl_2$) and postfixation in OsO_4 (1%) in the same cacodylate buffer.

Thin sections were double stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop 102 electron microscope operating at 80 kV.

Results and Discussion

Nuclei prepared in the presence of Triton X100 are devoid of an outer nuclear membrane. After micrococcal nuclease digestion followed by low and high salt extraction the final matrix preparation has the appearance of an internal ground matrix surrounded by a thin inner lamina which is closely associated with the matrix fibrils (Fig. 1).

The biochemical composition of rat liver matrix was found to be: protein 98%, DNA 0.15%, RNA 1.3%, phospholipid 0.5%. This analysis agrees with the values published by Berezney and Coffey (1). The phospholipids extracted from the nuclear matrix are mainly sphingomyelin and phosphatidylcholine as determined by thin layer chromatography (data not shown).

Fig. 2 shows the appearance of the nuclear matrix after incubation with phospholipase C as described in METHODS section. Despite the removal of the inner lamina by this treatment the structure maintains its nuclear shape. It can be seen however that the matrix fibrils have disappeared, the vacuoles have merged and that the nucleolus has expanded. Analysis shows that the phospholipase releases much of the matrix nucleic acid and affects a complete hydrolysis of matrix phospholipids. After digestion it is only possible to detect non-polar lipids in the residual matrix.

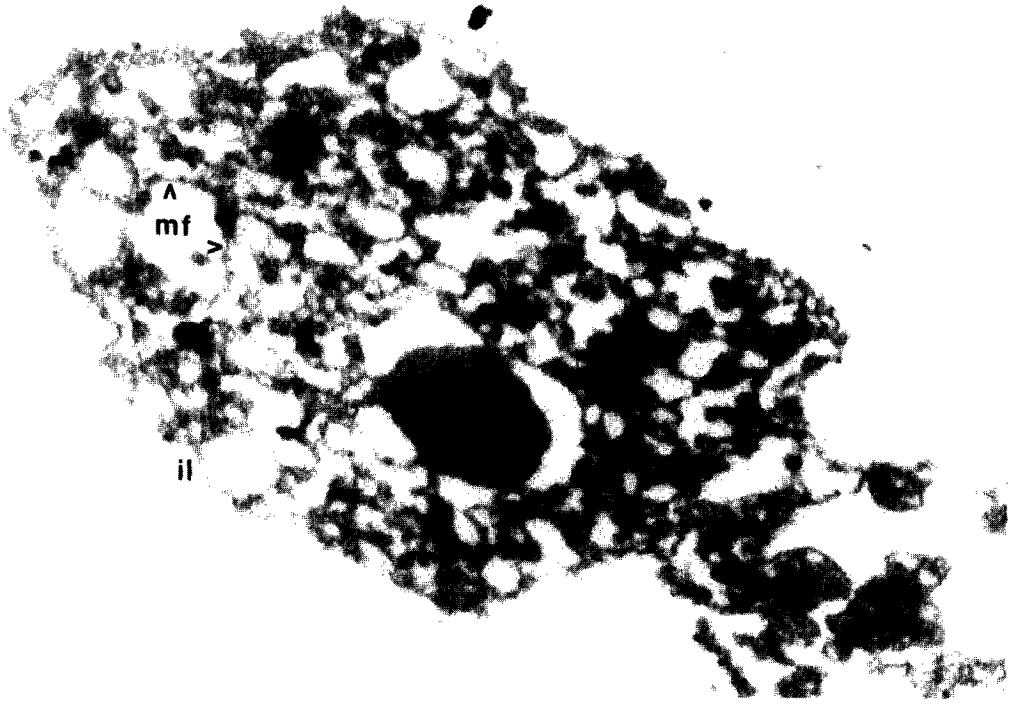

Fig. 1. Electron micrograph of rat liver matrix section (15,000 X). The original inner lamina (il) of the nucleus contains a spongelike ground matrix within which can be seen matrix fibrils (mf) and a nucleolus (N).

Fig. 3 shows a 3% agarose gel analysis of phospholipase released nucleic acid. This material was purified by SDS-proteinase K digestion followed by phenol extraction and Sephadex G50 chromatography. This material runs as a broad trailing band slightly behind the bromophenol blue marker. It is virtually all degraded by pre-incubation with ribonuclease A. On the other hand it is only slightly affected by pre-incubation with DNase I or micrococcal nuclease. This agrees with the finding that most of the matrix nucleic acid is RNA, however it would also suggest that this RNA is not protected against micrococcal nuclease during matrix isolation by its association with the matrix but rather that it is inherently resistant to this nuclease.

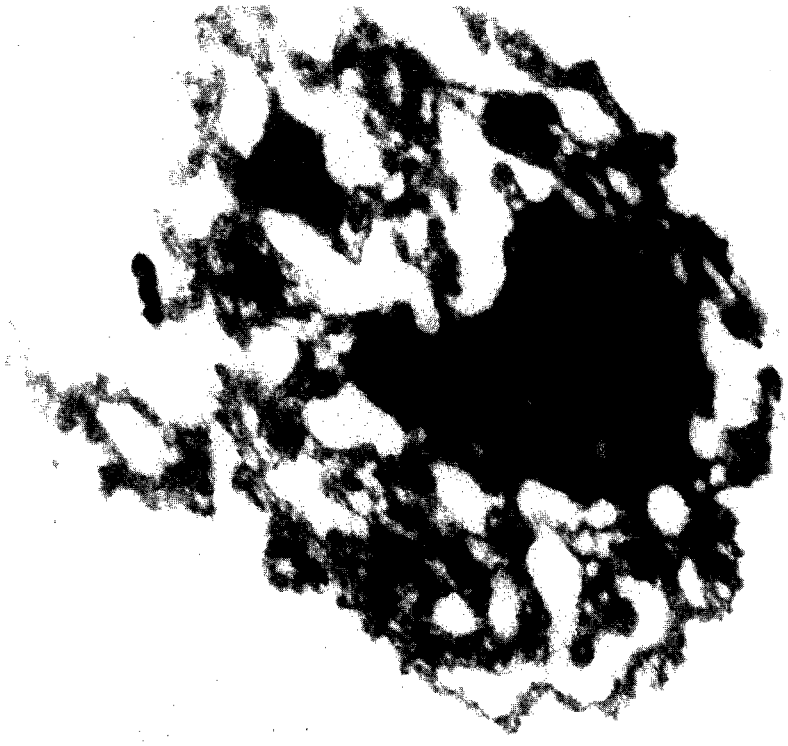

Fig. 2. Electron micrograph of phospholipase C treated rat liver matrix (15,000 X).
Both the inner lamina and the matrix fibrils have been released by phospholipase C digestion, however, the ground matrix maintains the general shape of the structure. The release of these elements is dependent on phopholipase C and does not occur in enzyme minus incubations. The phospholipase preparation is also free of proteinase activity as tested on bovine serum albumin and matrix protein substrates.

Table 1 shows the *in vitro* incorporation of radioactive precursor into the DNA and RNA fractions at various stages in the preparation of nuclear matrix. The relative specific activity of DNA in whole nuclei and the bulk extractable DNA is similar, however the small percentage of nuclear DNA associated with the matrix has a specific activity that is almost 200 times greater. A similar result has been reported for the matrix DNA in dividing 3T3 fibroblasts (5). One interpretation

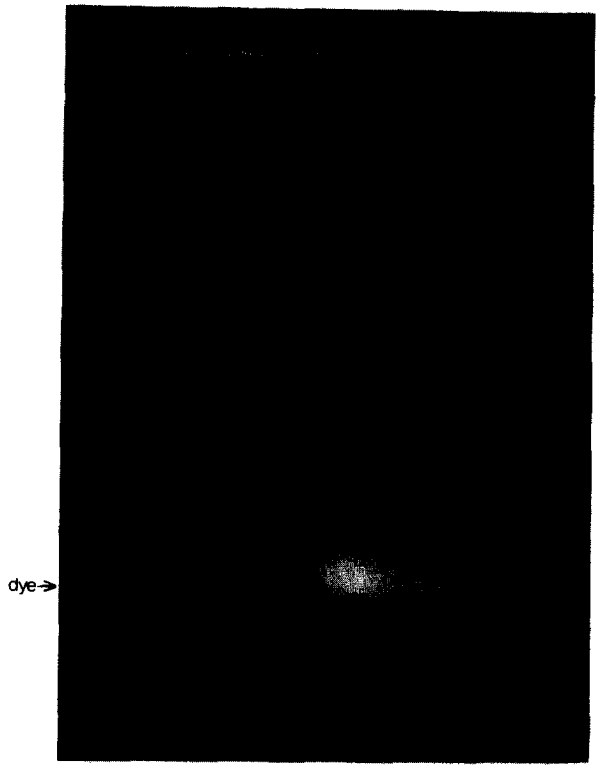

Fig. 3. Agarose gel analysis of phospholipase C released matrix nucleic acid.

lane 1 30 μg deproteinised matrix nucleic acid.

lane 2 effect of 400 μg/ml Ribonuclease A for 15 mins.

lane 3 effect of 400 μg/ml DNAse I.

lane 4 effect of 400 μg/ml micrococcal nuclease.

of these observations is that the site of DNA replication is located on the matrix and that the nascent DNA can be detached from the matrix by phospholipase C digestion.

In contrast, the relative specific activity for ^{3}H-UTP incorporation into RNA is approximately 100 times greater in the extractable fraction. This would suggest that the RNA released by phospholipase treatment is part of a more metabolically stable population. The occurrence of small molecular weight RNA associated with the nuclear skeleton has been demonstrated by others and a possible relationship with HnRNA has been suggested (2,4).

TABLE 1

DNA and RNA content of labelled nuclear fractions

	mg DNA/10^8 nuclei	^{3}H-TTP incorporated cpm/mg DNA	mg RNA/10^8 nuclei	^{3}H-UTP incorporated cpm/mg RNA
NUCLEI	10.0 ± 0.8	625	0.27 ± 0.03	1.66×10^5
TOTAL EXTRACTABLE MATERIAL	9.5 ± 0.2	500	0.20 ± 0.02	1.13×10^5
MATRIX	0.02 ± 0.001	9×10^4	0.04 ± 0.003	3.2×10^3
PHOSPHOLIPASE RELEASED MATERIAL	0.016 ± 0.001	8.2×10^4	0.03 ± 0.0001	2.0×10^3

We conclude from these preliminary studies that the phospholipid component of the nuclear matrix plays an important structural role in the tight binding of nucleic acid to the matrix either directly or indirectly through an association with the non-histone proteins of the matrix.

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