NUCLEAR MATRIX

Isolation and Characterization of a Framework Structure From Rat Liver Nuclei

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ABSTRACT

A nuclear framework structure termed the nuclear matrix has been isolated and characterized. This matrix forms the major residual structure of isolated nuclei and consists largely of protein with smaller amounts of RNA, DNA, carbohydrate, and phospholipid. The nuclear matrix can be further resolved by combined treatment with DNase and RNase. The remaining nuclear protein structure, after extraction of 90% of the nuclear protein, 99.9% of the DNA, and 98% of the RNA and phospholipid, is termed the nuclear protein matrix. Electron microscopy of this final nuclear protein matrix reveals an interior framework structure composed of residual nucleolar structures associated with a granular and fibrous internal matrix structure. The internal matrix framework is derived from the interchromatinic structures of the nucleus, and is connected to a surrounding residual nuclear envelope layer containing residual nuclear pore complex structures.

Sodium dodecyl sulfate-acrylamide gel electrophoresis of the nuclear matrix proteins demonstrates three major polypeptide fractions, P-1, P-2, and P-3, with average molecular weights of ~69,000, 66,000 and 62,000, as well as several minor polypeptides which migrate at ~50,000 and at higher molecular weights (>100,000). Polypeptides with molecular weights identical to those of P-1, P-2, and P-3 are also components of isolated nuclear envelopes and nucleoli, whereas isolated chromatin contains no detectable matrix polypeptides. This suggests that the major matrix polypeptides are localized in specific structural regions of the nucleus, i.e., nuclear envelope, nucleoli, and interchromatinic structures. The presence of cytochrome oxidase activity in the isolated nuclear matrix indicates that at least some integral proteins of the nuclear membrane are associated with the matrix.

Preliminary communications from our laboratory (12–14) have reported the isolation of a residual framework structure derived from rat liver nuclei, and termed the nuclear protein matrix, or more generally the nuclear matrix. Aside from serving as a skeletal framework, the nuclear matrix may

play an important role in nuclear functions. In this regard, newly replicated DNA was recently reported to be associated initially with the nuclear matrix (15–17). In this paper we present a more detailed study of the isolation, structure, and biochemistry of isolated rat liver nuclear matrix.

MATERIALS AND METHODS

Nuclei

Liver nuclei were prepared from male rats (Sprague-Dawley, 300–350 g, Charles River) based on procedures reported by Berezney et al. (9, 19). Livers were quickly excised, minced with a scalpel, and homogenized with 10 strokes at 1,200 rpm in a Potter-Elvehjem apparatus with a tissue ratio of 1 vol of liver to 2 vol of TM buffer (0.25 M sucrose, 0.05 M Tris, pH 7.4, 5 mM $MgCl_2$). After filtration through four layers of cheesecloth, the homogenate was centrifuged at 770 *g* for 10 min (Sorvall SS-34 rotor, DuPont Instruments, Sorvall Operations, Newtown, Conn.) to yield a crude nuclear pellet. The pellet was resuspended in 2.2 M sucrose TM buffer and centrifuged at 40,000 *g* for 90 min in a Beckman 21 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). This purified nuclear pellet was washed two times and stored in 0.25 M sucrose TM buffer at 0°C. Nuclei from 40–50 rat livers were isolated in this manner in ~6–8 h. The nuclei were used for isolating the nuclear protein matrix between 12 and 24 h after preparation.

Nuclear Protein Matrix

The procedure for the isolation of the nuclear protein matrix is presented in detail in the Results section.

Residual Nucleoli

The isolation of nucleoli from rat liver nuclei was based on the sonication procedures described by Busch (25). Isolated rat liver nuclei were resuspended to a final protein concentration of 1 mg/ml in 0.25 M sucrose, 10 mM Tris-HCl, pH 7.4, 1 mM $CaCl_2$. The nuclei were then sonicated in 30-s bursts at ~12 watts with a Branson sonicator (Heat Systems-Ultrasonics, Inc., Plainview, N. Y., model W-140) while the temperature was maintained below 4°C. The nucleoli were collected by centrifugation of 35 ml of nuclear sonicate through 20 ml of 0.9 M sucrose, 10 mM Tris, pH 7.4 in a Beckman SW 25.2 swinging bucket rotor at 3,000 *g* for 20 min. The nucleolar pellet was resuspended in the initial buffer and the centrifugation through 0.9 M sucrose, 10 mM Tris HCl, pH 7.4 repeated twice. The final nucleolar pellet was resuspended in TM buffer for subsequent analysis. Electron microscope studies of the isolated nucleoli confirmed the presence of relatively undamaged nucleoli and the virtual absence of visible contamination by other nuclear components.

Residual nucleoli were prepared by treating isolated nucleoli in a manner identical to that for the preparation of the nuclear protein matrix from control nuclei (see Table II). All nucleolar fractions were centrifuged at 3,000 *g* for 60 min at each step.

Residual Nuclear Membranes

Nuclear membranes were isolated as described by Berezney et al. (9, 19) with slight modifications. The isolated nuclei were suspended in 0.25 M sucrose TM buffer at a protein concentration of 2 mg/ml, and digested for 12–14 h at 2°C with 25 μg of DNase (Worthington Biochemical Corp., Freehold, N. J.) per ml. After centrifugation at 770 *g* for 30 min, the DNase-treated nuclear pellet was resuspended in 0.25 M sucrose, 0.5 M $MgCl_2$ in TM buffer to a final nuclear protein concentration of 4 mg/ml and layered over a discontinuous high-salt sucrose gradient of 1.6 M and 2.2 M sucrose containing 0.5 M $MgCl_2$ in TM buffer. After centrifugation for 1 h at 75,000 *g* in a Beckman SW 25.2 rotor, the nuclear membrane fraction was collected at the 0.25 M sucrose-1.6 M sucrose interface, suspended in 0.5 M $MgCl_2$ TM buffer, and centrifuged at 200,000 *g* for 30 min in a Beckman 65 rotor. The nuclear membranes were then resuspended in TM buffer for subsequent analysis.

Residual nuclear membranes were prepared by treating these isolated nuclear membranes exactly as in the preparation of the nuclear protein matrix from nuclei (see above). The nuclear membrane fractions were centrifuged at 200,000 *g* for 1 h at each step.

Chemical Fractionation of Nuclear Proteins

The nuclear proteins of isolated nuclei and the nuclear matrix were fractionated according to the sequential extraction procedures of Steele and Busch (71) as modified by Chung and Coffey (28). The general classes of nuclear proteins that are fractionated in this manner are termed soluble or nucleoplasmic proteins S_1 and S_2, histones, acidic proteins, and residual proteins. All extractions were performed at 0°C with 10 mg of nuclear protein in a total volume of 10 ml, followed by centrifugation at 15,000 *g* for 15 min.

Acrylamide Gel Electrophoresis

Sodium dodecyl sulfate (SDS) acrylamide gel electrophoresis was performed according to the procedures of Weber and Osborn (76), using 10% acrylamide gels in 0.1% SDS, 0.05 M Tris, pH 7.4 run at 4 mA per gel with a solution of 0.1% SDS, 0.05 M Tris, pH 7.4 in both the lower and upper chambers. Lyophilized samples were dissolved in 5% SDS, 0.05 M Tris, pH 7.4 containing 2% β-mercaptoethanol by heating at 100°C for 5 min, followed by incubation at room temperature for several hours. Addition of 6 M urea or guanidine HCl to the SDS samples, or alkylation of the polypeptides with sodium iodacetate (60) did not alter the polypeptide scans. Bromophenol blue was added to each sample to

serve as a tracking dye for R_f determinations and molecular weight estimations as described by Weber and Osborn (76). Reference proteins for molecular weight calibration included thyroglobulin, phosphorylase *a*, collagenase, bovine serum albumin, catalase, ovalbumin, α-chymotrypsinogen and cytochrome *c*. All gels were stained with Coomassie Blue (R-250) and scanned at 600 nm with a Gilford Linear Transport (model 2410, Gilford Instrument Laboratories Inc., Oberlin, Ohio) equipped with a fixed slit width of 0.05–0.10 mm. Densitometric comparisons of various nuclear protein fractions were always done with an equal amount of protein on each gel (50 μg).

Ureâ-acrylamide gels were used to detect histones in the nuclear matrix proteins, according to the procedure of Panyim and Chalkley (59).

Amino Acid Analysis

Protein samples were hydrolyzed in 5.7 N HCl for 24 h at 105°C *in vacuo*. Amino acid analysis of the protein hydrolysate was performed on a Beckman Model 120 Amino Acid Analyzer (Beckman Instruments, Inc., Fullerton, Calif.) by the procedures of Spackman et al. (68).

Chemical Determinations

RNA and DNA were separated as described by Munro and Fleck (57). DNA was determined by the Burton modification of the diphenylamine reaction (24), and RNA, protein, and phospholipid were analyzed as previously reported (19). Total neutral carbohydrate content was determined according to the phenolsulfuric acid procedure of Dubois et al. (32). Sialic acid was measured by the thiobarbituric acid method and corrected for deoxyribose as suggested by Warren (75). Protein was measured by the procedure of Lowry et al. (50).

Electron Transport Components

Assays for various electron transport components were performed as previously reported (19). NADH-cytochrome *c* reductase activity was assayed by following the reduction of cytochrome *c* at 550 nm. NADH-ferricyanide reductase activity was determined by measuring ferricyanide reduction at 420 nm. The enzymatic reduction rates were corrected for nonenzymatic reduction. Succinate dehydrogenase activity was assayed by measuring the reduction of phenazine methosulfate spectrophotometrically at 600 nm coupled to 2,6-dichlorophenolindophenol. All spectrophotometric determinations were performed with a Gilford Model 2000 recording spectrophotometer at 30°C. Cytochrome b_5 was determined by difference spectra in a Cary-15 double beam spectrophotometer (Cary Instruments, Monrovia, Calif.) after reduction by dithionite or NADH (19). Cytochrome *c* oxidase activity was measured polarographically by a modification of the assay of Chuang et al. (27) as previously described (19). Lipid was isolated from untreated nuclei by the procedure of Folch et al. (36). Lipid micelles for enzyme reconstitution studies were prepared in 10 mM Tris, pH 7.8 by sonication according to Rouser and Fleischer (63).

Estimation of Mitochondrial Contamination

Contribution of mitochondrial derived cytochrome *c* oxidase to the total activity of this enzyme in the nuclear matrix was routinely monitored by assaying for succinate dehydrogenase activity, or in some cases by the direct chemical determination of succinate dehydrogenase-associated flavin as previously described (19). Succinate dehydrogenase activity indicated <0.1% mitochondrial contamination on a protein basis. This correction indicated that <2% of the total matrix cytochrome *c* oxidase activity was due to mitochondrial contamination.

Electron Microscopy

Samples were prepared for electron microscopy by fixation in 4% glutaraldehyde (0.05 M cacodylate, pH 7.4, 5 mM $MgCl_2$) and postfixation in 1% OsO_4 in 0.05 M cacodylate, pH 7.4, 5 mM $MgCl_2$ at 0°C. The samples were embedded in Epon according to the method of Luft (51) or Spurr (70). Ultrathin sections were stained with both uranyl acetate and lead citrate according to Reynolds (61). To reveal nonchromatin structures in the nuclei, specimens were fixed in glutaraldehyde and treated by the regressive staining procedure described by Bernhard (20). All samples were observed and photographed on an AEI 801 electron microscope operating at 60 kV.

Light Microscopy

Sections of 1–2 μm were cut from the Epon-embedded blocks and stained with a 0.2% solution of toluidine blue 0 with 0.5% sodium borate. Samples were stained for 1 min at 60°C and rinsed in both distilled water and 70% ethanol.

Estimations of nuclear sphere radii were obtained by direct measurement on unfixed samples, utilizing Zeiss Nomarski interference optics. 100 random nuclear measurements were made for each reported value.

Materials

DNase I (EC 3.1.4.5) from bovine pancreas (~3,000 Worthington Kuntz U/mg) and RNase A (EC 2.7.7.16) from bovine pancreas (~3,000 Worthington Kalnitsky U/mg) were obtained from Worthington Biochemical Corp. Trypsin (3× crystallized) and collagenase (Type III) were also obtained from Worthington. Hyaluronidase from bovine testes was a product of Schwarz/Mann Div., Becton, Dickinson & Co. (Orangeburg, N. Y.). Triton X-100, calf thymus DNA, yeast RNA, sialic acid (*N*-acetyl neurominic acid), glucose, and trizma base were obtained from Sigma Chemical Co. (St. Louis,

Mo.). Sucrose, magnesium chloride, and sodium chloride were reagent grade products of J. T. Baker Chemical Co. (Phillipsburg, N. J.).

RESULTS

Chemical Treatment of Isolated Nuclei

In the course of some survey experiments in our laboratory, we discovered that isolated rat liver nuclei maintain their general spherical shape as observed under the light microscope after a wide variety of chemical treatments which remove many of the major components of the nucleus (see Table I). Nuclear spheres, of approximate dimensions of nuclei, were observed after combined treatment with 2 M NaCl which solubilizes most of the nuclear chromatin (8, 54, 71), and Triton X-100 which releases the nuclear envelope phospholipid as well as many of the membrane proteins (1, 2, 7, 12–14, 46, 74). In contrast, nuclear spheres were no longer observed after certain treatments known to solubilize or degrade proteins, such as 0.1 N NaOH, 5% SDS, or treatment with proteases such as pronase or trypsin (Table I). These observations suggested the presence of a supportive framework structure in the cell nucleus of an apparent protein nature. This supportive framework was not due to the presence of either the chromatin or nuclear membrane phospholipids. Furthermore, since extraction of histones from nuclei with either 0.25 N HCl or 2 M NaCl solutions (Table I) still left nuclear spheres visible, it appeared that residual nonhistone proteins might be responsible for the nuclear framework structure.

Our approach was to remove as much of the nuclear components without destroying nuclear spheres, thus isolating the minimal residual components necessary for maintaining the constitutive structural framework of the nucleus. This was accomplished by monitoring the progressive removal of various nuclear components in terms of chemical composition, while observing the integrity of nuclear structure with light and electron microscope procedures.

TABLE I

*Light Microscope Examination of Rat Liver Nuclei and Nuclear Matrix after Chemical Treatment**

Treatment	Presence of nuclear spheres	
	Nuclei	Nuclear matrix
Distilled H_2O	+	+
2 M NaCl	+	+
1% Triton X-100	+	+
2 M NaCl + 1% Triton X-100	+	+
0.25 N HCl	+	+
Acetone (95%)	+	+
0.01 N HCl in ethanol (80%, vol/vol)	+	+
1% β-Mercaptoethanol	+	+
DNase-RNase	+	+
Collagenase	+	+
Hyaluronidase	+	+
0.1 N NaOH	−	−
Trypsin or pronase	−	−
1% SDS + 1% (wt/vol) β-Mercaptoethanol	−	−

* Nuclear pellets containing 2 mg of protein from either isolated nuclei or nuclear matrix were resuspended in 1 ml of the appropriate solution and incubated at 22°C for 60 min. All the enzyme digestions were performed with 100 μg of enzyme per ml of suspension in TM buffer. The SDS-β-mercaptoethanol solution was in 0.05 M Tris, pH 7.4. Although many of the procedures removed large amounts of nuclear material, nuclear spheres were still observed where indicated (+). Absence of observable nuclear spheres is indicated as (−).

Isolation Procedures

The procedure for the isolation of the nuclear protein matrix is composed of four consecutive extractions as summarized in Table II. Aliquots of nuclear spheres obtained after each treatment are resuspended in TM buffer for subsequent biochemical and morphological analyses. During the treatments, the nuclear pellets are gently resuspended with a glass stirring rod. Vortexing of the nuclear suspensions causes excessive damage to the nuclear structures and should be avoided. An important requirement for obtaining high yields of spheres is to centrifuge at low *g*-force (770 *g*). Higher *g*-forces cause progressive compression and disruption of nuclear spheres.

Step one consists of resuspension of isolated rat liver nuclei (see Materials and Methods) in low magnesium buffer (0.2 mM $MgCl_2$; 10 mM Tris-HCl, pH 7.4) to a final concentration of 2 mg of nuclear protein per ml. After incubation on ice for 10 min, the nuclear suspension is centrifuged at 770 *g* for 30 min in a Sorvall SS-34 rotor. The extraction was repeated twice with approximately one-half the initial volume of buffer. The final pellet obtained is termed the low magnesium-treated nuclear sphere fraction (LM).

TABLE II

Steps in the Isolation of the Nuclear Matrix

Step	Consecutive extractions	Resultant nuclear sphere	Total nuclear material extracted (cumulative)*			
			Protein	DNA	RNA	Phospholipid
			%			
0	Isolated nuclei	Intact nucleus (N)	0	0	0	0
1	Low magnesium treatment (0.2 mM $MgCl_2$)	Low Mg^{++}-treated sphere (LM)	52.0 ± 2.23	75.8 ± 2.78	19.7 ± 1.83	2.5 ± 1.13
2	High-salt treatment (2 M NaCl)	High salt-treated sphere (HS)	83.7 ± 2.17	97.6 ± 1.21	66.0 ± 1.64	6.4 ± 1.65
3	Detergent treatment (1% Triton X-100)	Nuclear matrix (NM)	90.0 ± 1.57	97.7 ± 1.21	71.0 ± 1.68	97.8 ± 1.51
4	Nuclease treatment (DNase-RNase)	Nuclear protein matrix (NPM)	90.2 ± 1.55	>99.9	98.0 ± 1.12	98.0 ± 1.47

* Percent extracted determined relative to isolated nuclei centrifuged in 0.25 M sucrose TM buffer an equivalent number of times as each nuclear sphere fraction. SEM (±) for five separate preparations.

In step two, the low magnesium-treated nuclear spheres are suspended in high salt buffer (2 M NaCl, 0.2 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.4) to a volume corresponding to 4 mg of initial nuclear protein per ml of suspension. After ten min on ice, the suspension is centrifuged at 770 *g* for 60 min. The nuclear pellets are extracted two more times with one-half the initial volume of high salt buffer. The final pellet is termed the high salt-treated nuclear sphere fraction (HS).

In step three, the high salt-treated nuclear spheres are resuspended in TM buffer to a final volume corresponding to 4 mg of initial nuclear protein per ml. Triton X-100 (10%, wt/vol) in TM buffer is added to a final concentration of 1% while stirring on ice. After 10 min on ice, the nuclear suspension is centrifuged at 770 *g* for 30 min. The pellets are washed two times in an equivalent volume of TM buffer. The final pellet is termed the nuclear matrix fraction (NM).

Step four involves extensive digestion with high concentrations of DNase and RNase. The nuclear matrix is resuspended in TM buffer to a final protein concentration of 2 mg/ml. Electrophoretically purified DNase and RNase (200 μg each) are added to each milliliter of the nuclear matrix suspension. After a 60-min digestion at 22°C, the nuclear suspension is centrifuged at 770 *g* for 30 min and washed two times in equal volumes of TM buffer. The final pellet is termed the nuclear protein matrix fraction (NPM).

The final nuclear protein matrix represents the residual nuclear structure remaining after extracting 90% of the total nuclear proteins, 99.9% of DNA, and 98% of RNA and phospholipids (Table II).

The nuclear protein matrix isolation was developed for rat liver nuclei prepared in sucrose medium containing magnesium ions. The initial isolation procedures for nuclei are critical since endogenous nuclear endonuclease activity is an important factor for the subsequent removal of large amounts of DNA in the low magnesium extraction procedure of step one (6, 47, 48). In preliminary attempts to prepare nuclear matrix from other mammalian tissues, we have found that the bulk DNA is not released during low magnesium treatment (step 1). In the absence of endogenous nuclease activity, the high-salt buffer (step 2) generally leads to gel formation in the nuclear pellets. To overcome these difficulties we routinely preincubate isolated nuclei from various tissue sources (10^8 nuclei/ml) with low levels of DNase I (2–5 μg/ml) at 22°C for 15 min. This allows a more reproducible preparation of nuclear matrix from a variety of tissues including brain, prostrate, lung, spleen, thymus, and kidney. The details of these procedures and the properties of the isolated matrices will be reported elsewhere. (R. Berezney. Unpublished experiments.)

Gross Composition

A summary of the macromolecular composition of the various nuclear sphere fractions at each of the four extraction steps is presented in Table III. The final chemical composition of the nuclear protein matrix is 97.6% protein with traces of RNA (1.2%), phospholipid (1.1%) and <0.1% DNA. This is based on the assumption that these components represent 100% of the nuclear protein matrix. However, recently, small amounts of carbohydrate of nonnucleic acid origin have been detected in the nuclear protein matrix. No sialic acid (<0.1%) but significant amounts of neutral sugars

TABLE III

Gross Composition of Nuclear Sphere Fractions

Nuclear fraction	% Composition*			
	Protein	DNA	RNA	Phospholipid
Isolated nuclei	69.0 ± 1.78	23.9 ± 1.66	3.4 ± 0.14	3.7 ± 0.24
LM nuclear spheres	73.2 ± 1.18	12.6 ± 1.25	6.1 ± 0.51	8.0 ± 0.57
HS nuclear spheres	68.0 ± 2.02	3.4 ± 0.37	7.1 ± 0.55	21.4 ± 1.78
Nuclear matrix	80.8 ± 1.38	6.5 ± 0.72	11.7 ± 0.90	1.0 ± 0.26
Nuclear protein matrix	97.6 ± 0.38	<0.1	1.2 ± 0.07	1.1 ± 0.30

* Percent composition assumes total protein, RNA, DNA, and phospholipid to be equivalent to 100%. SEM (±) for five separate preparations.

(5.5%) are present. These neutral sugars consist largely of glucose, traces of mannose and galactose, and an unidentified carbohydrate.[1] We are currently investigating the nature of the association of the neutral carbohydrate with matrix proteins.

Nomarski Interference Microscope Measurements

Fig. 1 demonstrates the nuclear spheres observed with Nomarski optics at each step of the above extraction procedures. With this technique, the size of the various nuclear sphere fractions was measured as shown in Fig. 2. Isolated rat liver nuclei have a broad distribution of sizes corresponding to the mixed population of diploid and polyploid nuclei. The distribution of nuclear sphere sizes in the low magnesium and high salt-treated nuclear spheres and nuclear matrix fractions (steps 1-3) is remarkably similar to that of isolated nuclei. The mean radii are between 3.9 and 4.1 μm for all the fractions, and slight variations are not statistically significant ($P > 0.5$). In contrast, the final nuclear protein matrix spheres are decreased in size (mean radius of 2.7 μm) and the difference in size distribution between the protein matrix and isolated nuclei is highly significant ($P > 0.001$). This shift to smaller sizes in the protein matrix spheres may be related to the removal of the tightly bound residual DNA and/or RNA by the nuclease treatment, thereby permitting contraction of the spheres. The recovery of nuclear protein spheres after this nuclease digestion is >90%, indicating a minimal loss of spheres between these steps (See Table II).

[1] Shaper, J. H., T. Barry, R. Berezney, and D. S. Coffey. Manuscript in preparation.

Morphological Studies

Light micrographs of ~1-μm sections through untreated nuclei and the nuclear protein matrix are compared in Figs. 3 and 4. In agreement with the Nomarski interference microscope measurements, the sectioned nuclear protein matrix spheres are much smaller. It is also important to note that the matrix spheres are not empty but contain at least two distinguishable structures revealed by toluidine blue staining: intensely staining structures which appear to represent residual nucleoli, and a less intense staining material which forms a reticular-like meshwork throughout the residual nucleus. A continuous peripheral layer surrounding the internal matrix is also demonstrated with this toluidine blue stain. Although a number of the nucleolar-like bodies appear to have broken loose from the spheres, observation of a large number of sections revealed that over 75% are still associated with the interior of the nuclear protein matrix structures.

Comprehensive analysis by electron microscopy and measurement of various membrane marker components by techniques previously reported for bovine liver nuclei (9, 19) indicated a high degree of purity for the isolated nuclei. For example, succinate dehydrogenase assays indicated <0.1% mitochondrial contamination on a protein basis (see Materials and Methods). Electron microscope observations confirmed the virtual absence of contaminating membranes or other cytoplasmic structures in the nuclear fractions.

Low magnification survey electron micrographs of the isolated nuclei and various nuclear sphere fractions are shown in Figs. 5-9. These electron micrographs support the light microscope observations that nuclear sphere structures are maintained throughout the isolation procedure. A detailed

FIGURE 1 Nomarski interference light micrographs of nuclear sphere fractions. (*A*) Control nucleus, untreated; (*B*) low magnesium-treated nuclear sphere; (*C*) high salt-treated nuclear sphere; (*D*) nuclear matrix; and (*E*) nuclear protein matrix. × 870.

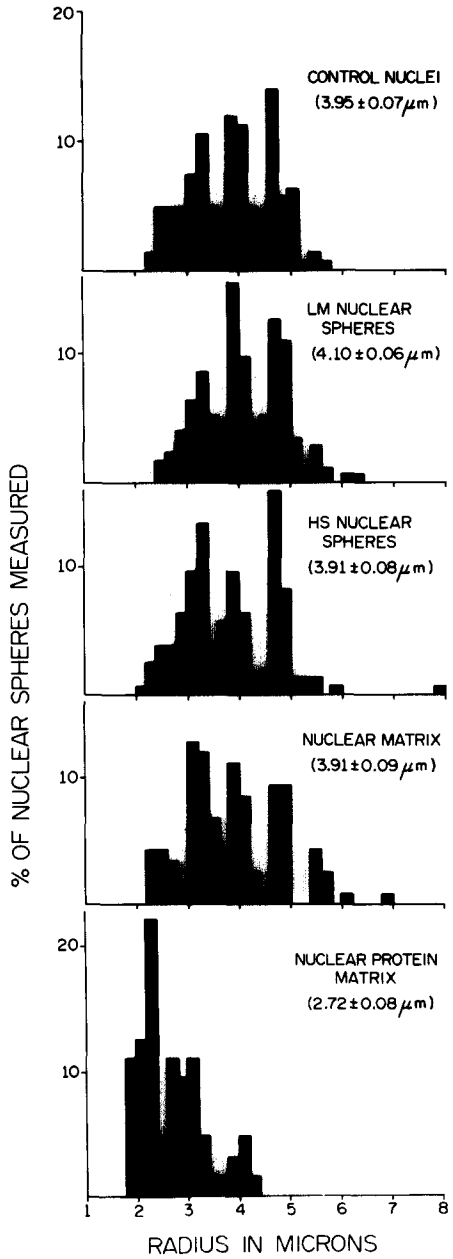


FIGURE 2 Size distribution of nuclear sphere fractions. The average radius of 100 nuclear spheres was measured for each fraction with Nomarski interference optics.

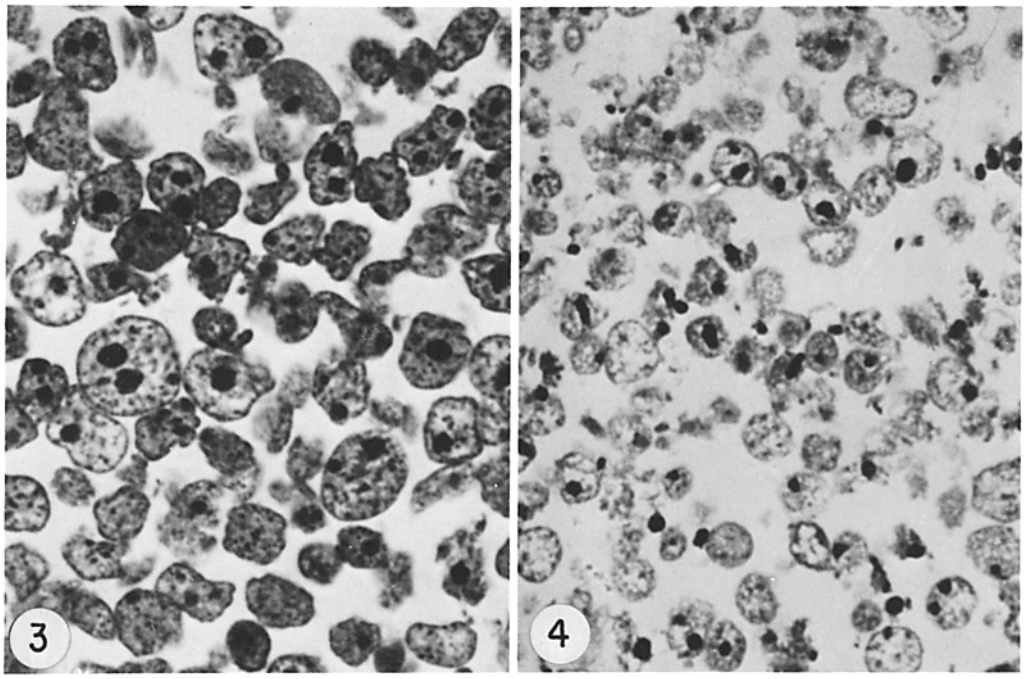
study of the structure of the various nuclear sphere fractions will be presented elsewhere.[2] In this analysis, we will concentrate on the structural organization of the final nuclear protein matrix, and the identification of the *in situ* nuclear components from which the nuclear matrix is derived.

The structural organization of isolated rat liver nuclei closely resembles the *in situ* appearance. Previous studies of nuclear morphology in liver and other mammalian cells have clearly defined several distinct regions in the nuclei (21, 22, 55, 73). Similarly, four main structural regions can be visualized in the nuclei of intact liver cells (Fig. 10*a*) and isolated nuclei (Fig. 10*b*): (1) a surrounding nuclear envelope (*NE*); (2) nucleoli (*N*); (3) dense chromatin (heterochromatin) patches beneath the nuclear envelope (*PC*) and surrounding the nucleoli (perinucleolar chromatin) (*PNC*), as well as in other interior regions of the nuclei; and (4) interchromatinic areas (*IC*), between condensed chromatin regions, which contain various granular and fibrous components, such as the interchromatinic granules and the perichromatinic fibers and granules (21, 22, 55, 73). The interchromatinic area is also presumed to contain the diffuse chromatin (euchromatin) of the nucleus (43).

The structure of the nuclear protein matrix consists of three main components (Fig. 11): (*a*) a residual nuclear envelope (*RE*) which forms a continuous structure surrounding the nuclear sphere, (*b*) highly condensed and electron-dense residual nucleoli (*RN*), and (*c*) an extensive granular and fibrous internal matrix structure (*IM*) which extends throughout the interior of the nuclear sphere from the residual nucleoli to the surrounding residual nuclear envelope (Fig. 12). This internal matrix framework appears to be derived primarily from the interchromatinic structures of the nucleus.

Higher magnification of the nuclear protein matrix interior (Fig. 13) demonstrates internal matrix

[2] Berezney, R., and D. S. Coffey. Manuscript in preparation.



FIGURES 3 and 4 Light microscope sections of untreated nuclei (Fig. 3) and the nuclear protein matrix (Fig. 4). 1-2-μm section stained with toluidine blue. × 1,450.

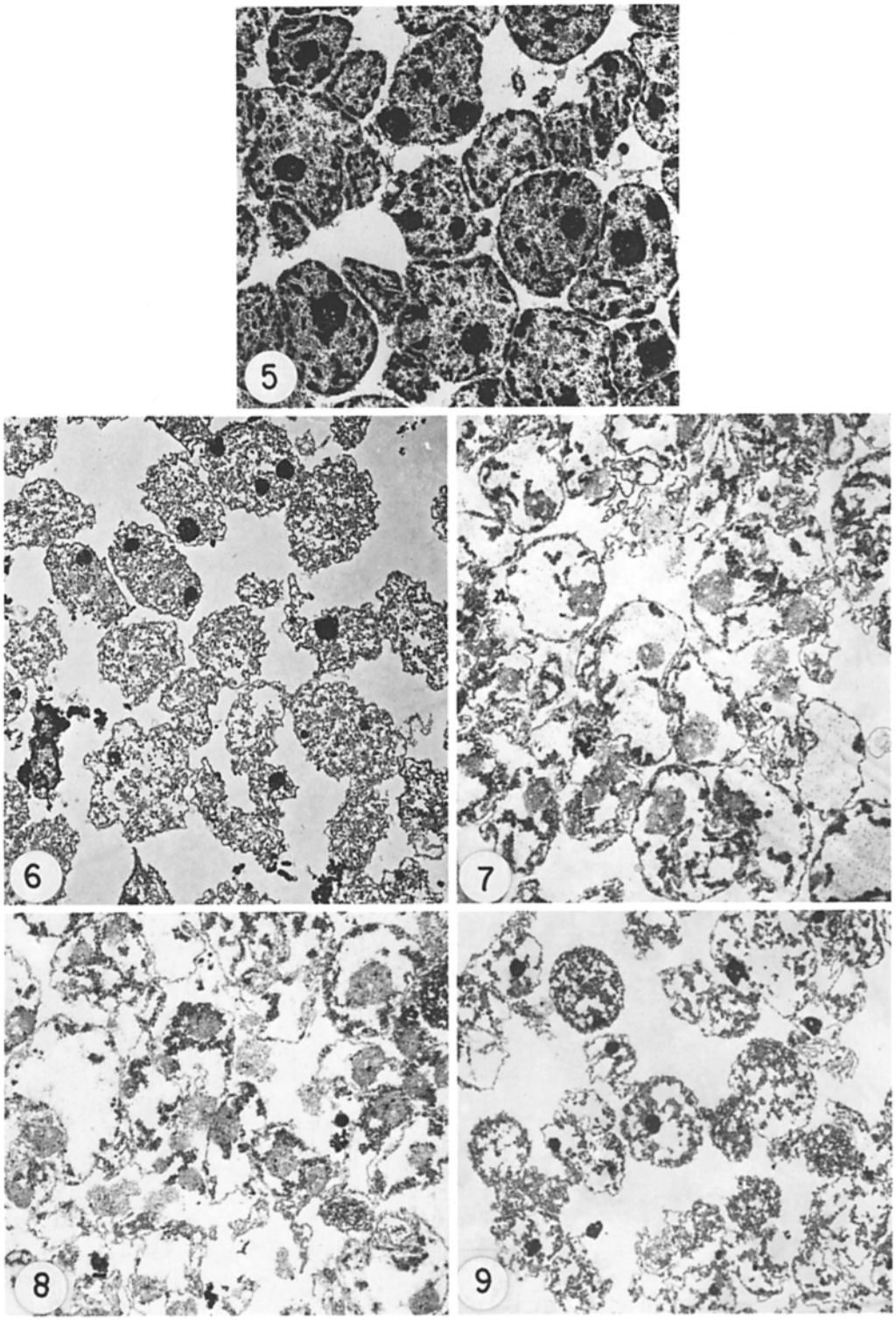
structures in close association with a residual nucleolus. The empty perinucleolar areas, which may correspond to sites of perinucleolar chromatin in isolated nuclei, are bordered by the residual nucleolus and the internal matrix structures. The internal matrix structures consist of densely packed fibers (matrix fibers) associated with electron-dense particulate structures termed matrix particles (Fig. 15). The fine structure of the internal matrix (Fig. 15) shows close resemblance to that of certain interchromatinic structures of isolated nuclei (Fig. 14) as well as nuclei *in situ* (Fig. 10*a* and references 21, 22, 55, 73). Although the internal matrix structures appear more condensed than the interchromatinic structures of isolated nuclei, both consist of distinct electron-dense particles of similar dimensions (150–250 Å in diameter) associated with a less electron-dense, tightly packed, fibrous substance (Figs. 14 and 15). Fibers as thin as 50 Å in width can be seen in the matrix of both the intact nuclei and the nuclear protein matrix.

The derivation of the internal matrix framework from the interchromatinic structure of the nucleus was also indicated by application of the Bernhard EDTA regressive staining technique (21, 55) to the various nuclear sphere fractions. With this procedure, the chromatin areas of the nucleus appear as unstained or slightly stained areas while the interchromatinic structures of the nucleus are highly electron dense. It was therefore possible to monitor the degree of preservation of the interchromatinic structure during isolation of the nuclear matrix. Our preliminary results indicate that components of the interchromatinic structures are still present in the internal matrix structure.[2]

The residual nuclear envelope of the nuclear matrix consists of a continuous electron-dense layer ~100–150 Å in thickness which completely surrounds the nuclear matrix. Associated with this continuous layer are distinct residual nuclear pore complex structures. In lateral view, characteristic annular structures ~800–900 Å in diameter and central granules of the nuclear pore complexes are visible (Figs. 16 and 17).

Chemical Treatment of Nuclear Matrix

In these experiments, isolated nuclear matrix (before final treatment with nucleases, see Table II) was exposed to a variety of chemical treatments as described earlier for isolated nuclei. Using Nomarski interference microscopy, we then determined whether the particular treatment re-



FIGURES 5, 6, 7, 8, and 9 Low magnification comparison of electron microscope sections of the nuclear sphere fractions. (Fig. 5), untreated nuclei; (Fig. 6), low magnesium-treated nuclear spheres; (Fig. 7), high salt-treated nuclear spheres; (Fig. 8), nuclear matrix; and (Fig. 9), nuclear protein matrix. × 3,100.

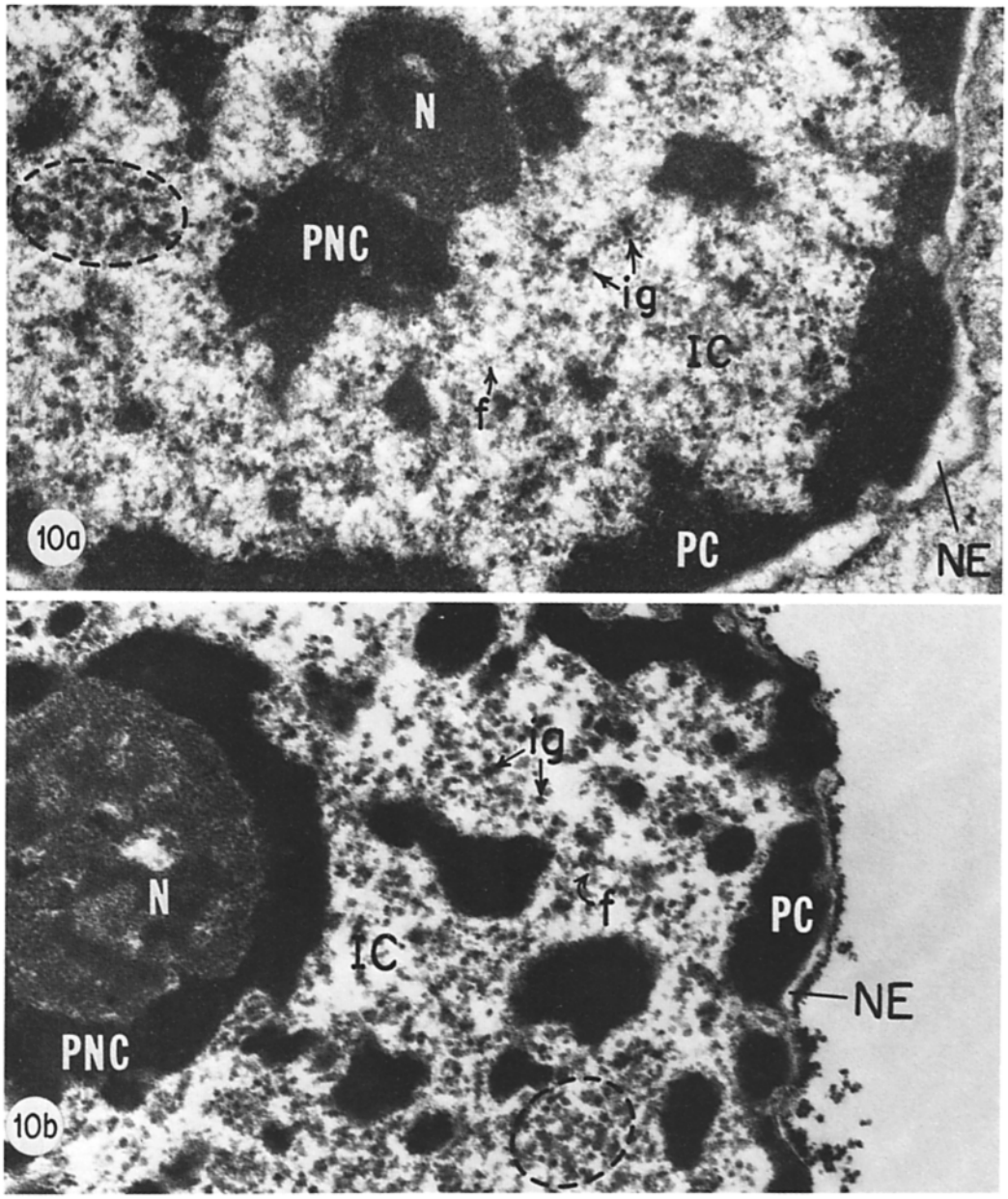
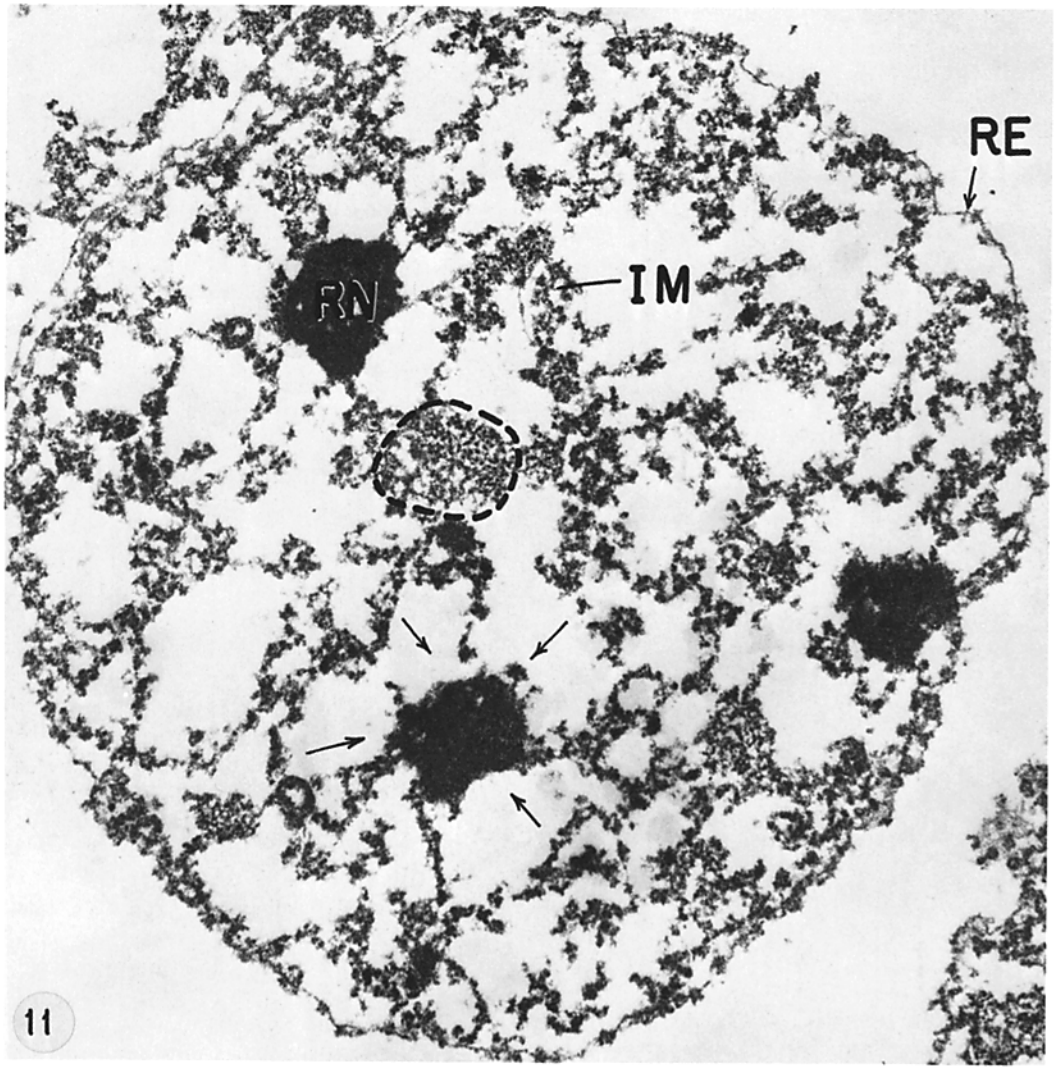


FIGURE 10 Electron microscope sections of rat liver nuclei *in situ* in liver tissue (Fig. 10*a*) and in the isolated nuclear fraction (Fig. 10*b*). The isolated rat liver nuclei maintain many of the structural features characteristic of nuclei *in situ*. These include: NE, nuclear envelope; N, nucleolus; PNC, perinucleolar condensed chromatin; PC, peripheral condensed chromatin; IC, interchromatinic areas. The interchromatinic areas contain electron-dense particles 150–250 Å in diameter termed interchromatinic granules (*ig*) as well as less electron-dense fibrous material (*f*). Clusters of interchromatinic granules are enclosed by a broken line. × 44,000.

sults in visible disruption of the matrix nuclear sphere structure. As indicated in Table I, the pattern is identical for isolated nuclei and nuclear matrix.

Protein Fractionation

Since the nuclear protein matrix is predominantly protein, it was important to establish what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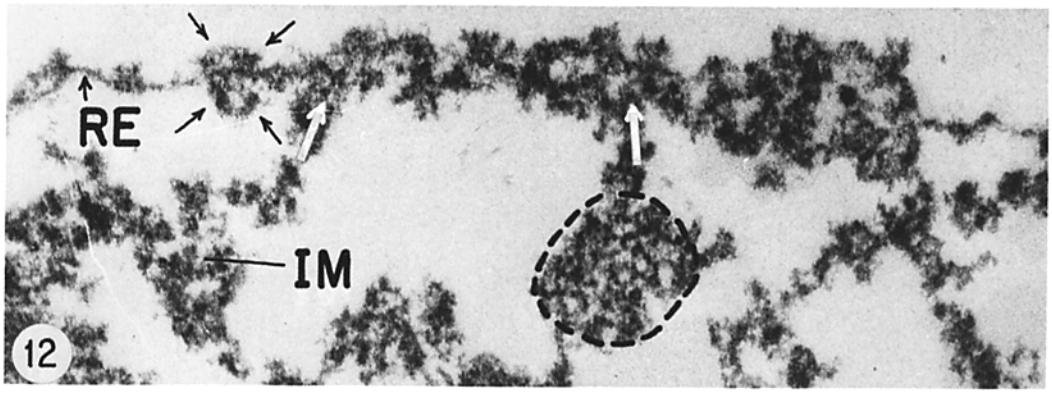

RE
IM
12

classes of nuclear proteins were present in this residual structure. 10 mg of the isolated nuclear protein matrix was subjected to a series of extractions which are commonly used to separate the major classes of nuclear proteins (28, 71). This extraction procedure was applied to the isolated nuclear protein matrix (*NPM*) and the results were compared to those obtained with isolated nuclei (*N*). The results are presented in Fig. 18 and are expressed as the percent of total proteins solubilized with each step of the fractionation. It is apparent that the nuclear matrix proteins fractionate predominantly as acidic proteins.

To determine whether acidic amino acids predominated in the nuclear matrix proteins, an amino acid analysis was performed and is presented in Table IV. The acidic to basic amino acid ratio is 1.46, and is consistent with an acidic nature for these proteins. Moreover, an earlier report by Steele and Busch (72) of the analysis of a fraction of residual acidic proteins extracted from rat liver nuclei is also presented for comparison.

The absence of histones as a significant component of the nuclear matrix proteins was established by resolution on urea acrylamide gels by the procedure of Panyim and Chalkley (59).

SDS-Acrylamide Gel Electrophoresis of Matrix Proteins

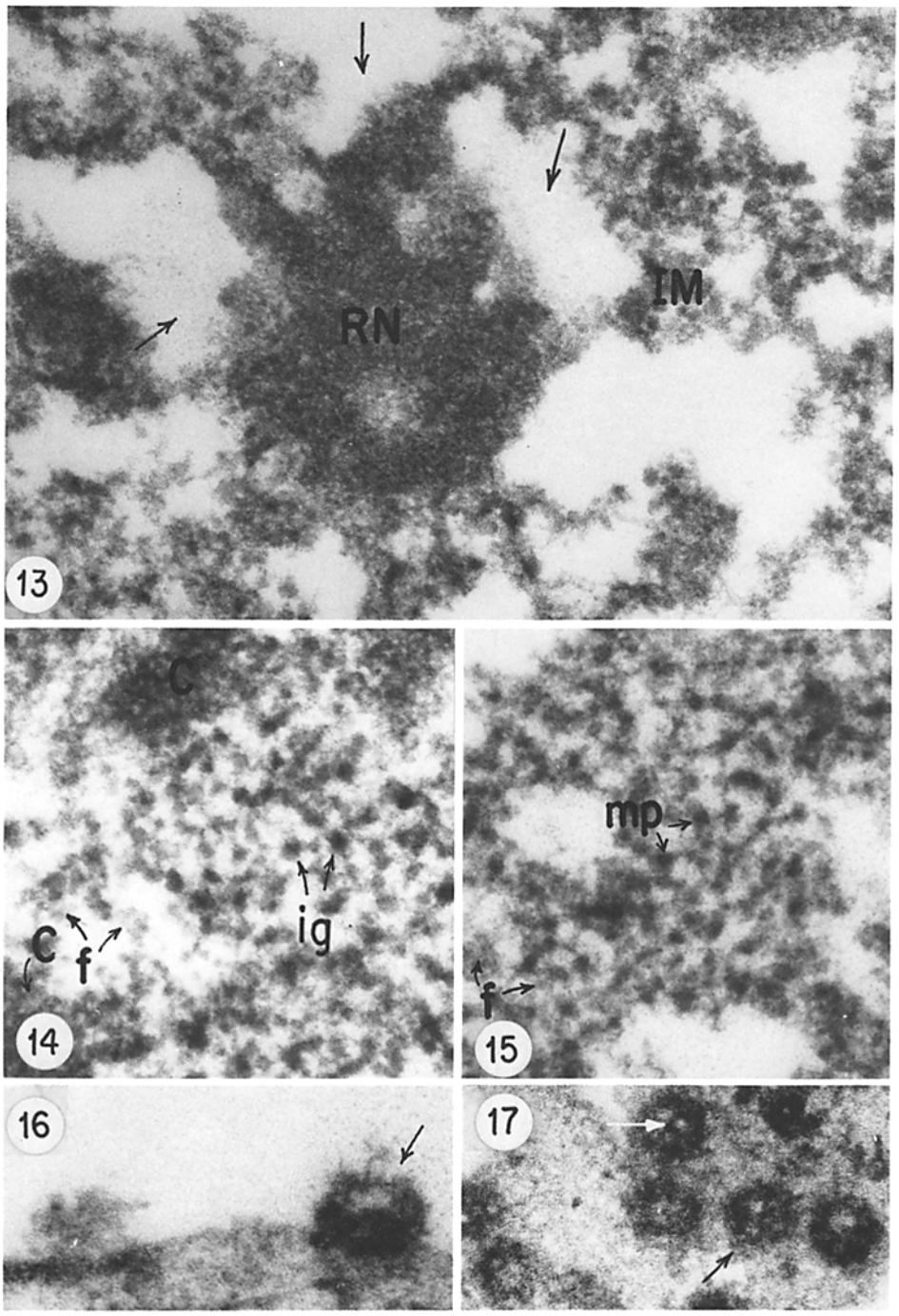
SDS gels of nuclear matrix and other nuclear fractions are compared in Fig. 19. Total nuclear proteins (gel 1) show a typical heterogeneous pattern with a multitude of bands. The low molecular weight histone-containing bands are particularly prominent. Nuclear matrix proteins (gels 3 & 4) are characterized by three major polypeptide bands in the molecular weight region of 60,000–70,000 and the absence of stained bands in the low molecular weight regions. Polypeptide gel patterns of the nuclear matrix (gel 3) or the nuclear protein matrix (gel 4) were compared in six separate preparations, and no significant differences were detected. This is consistent with the compositional data (see Table III) which demonstrate a high recovery of total protein in step 3 from nuclear matrix to step 4 in the nuclear protein matrix.

Comparison of total chromatin proteins (gel 5) prepared by the method of Shaw and Huang (65) as modified by Arnold and Young (5), and nuclear matrix proteins (gels 3 & 4) suggests the absence or chromatin polypeptides in the nuclear matrix and the corresponding absence of matrix polypeptides in chromatin protein. One exception, however, is a high molecular weight component whose molecular weight can only be roughly approximated as 200,000, which is found in all five of the gels.

Approximate molecular weights of the polypeptide bands were determined from gel scans by the method of Weber and Osborn (76). In each experiment, appropriate molecular weight protein standards (see Materials and Methods) were run in parallel gels or were comigrated in the same gel with the nuclear matrix proteins. The gel scans were also divided into five zones, e.g. Fig. 20 A–E, and the percent of total stained area in each region was determined. The three major polypeptide peaks in region B of the nuclear protein matrix are termed P-1, P-2, and P-3 and represent ~50% of the total stained area (Fig. 20). These polypeptide bands have approximate molecular weights of 69,000, 66,000 and 62,000, respectively. Other minor polypeptide bands are observed, primarily at ~50,000 daltons (zone C) and in the higher molecular weight regions >100,000 daltons (zone A).

FIGURE 11 Electron microscope section through the nuclear protein matrix revealing the internal structural components of the matrix. RN, residual nucleolus; IM, internal matrix framework; RE, residual nuclear envelope layer. Note the empty spaces surrounding the residual nucleoli and along the periphery (arrows). These may correspond to regions previously occupied by the perinucleolar and peripheral condensed chromatin in untreated nuclei (compare with Fig. 10*a* and *b*). × 21,000.

FIGURE 12 Higher magnification electron microscope section of the nuclear protein matrix in the region of the residual nuclear envelope. A close association of the internal matrix framework (*IM*) with the residual nuclear envelope (*RE*) is evident (white arrows). A residual nuclear pore complex structure is projecting through the residual nuclear envelope layer (black arrows). Regions of the internal matrix framework (enclosed in broken line) resemble clusters of interchromatinic granules seen in both isolated and *in situ* nuclei (compare with the regions enclosed by broken lines in Fig. 10*a* and *b*. × 50,000.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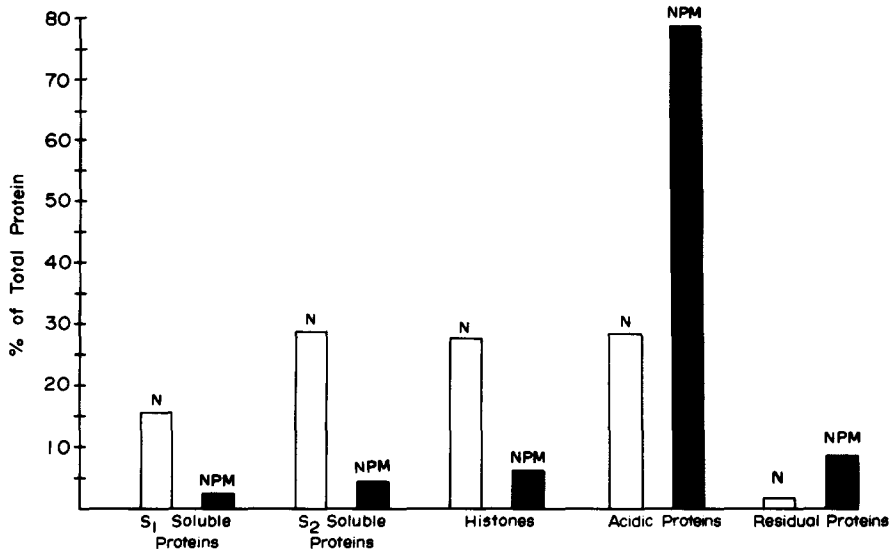

FIGURE 18 The chemical fractionation of the isolated nuclear protein matrix (black bars, *NPM*) compared to that of the total protein of isolated nuclei (white bars, *N*). The general classes of nuclear proteins were fractionated by the extraction procedures of Steele and Busch (71).

Comparisons of Nuclear Matrix, Nuclear Membrane, and Nucleolar Proteins

Our morphological studies indicated that the nuclear matrix contains components of the residual nuclear envelope which are continuous with an internal nuclear matrix connecting to residual nucleolar elements. This led us to determine whether these residual nuclear matrix protein components (P-1, P-2, and P-3) were present in isolated nucleoli or nuclear membrane preparations which had been subsequently extracted by the four steps used in the isolation of the nuclear protein matrix. Nucleoli were isolated by the method of Busch (25) and nuclear membranes by the procedure of Berezney et al. (9, 19). The final residual protein fractions of the nucleoli and nuclear membranes were subjected to solubilization and electrophoresis parallel with the nuclear protein matrix. The

FIGURE 13 High magnification of the nuclear protein matrix in the area of the residual nucleolus. The residual nucleolus, RN appears continuous with the internal matrix framework (*IM*). Empty spaces (arrows) surrounding the residual nucleolus may correspond to regions previously occupied by the perinucleolar condensed chromatin (compare with Fig. 10*a* and *b*). Note that these empty areas are bordered by the nucleolus and the internal matrix framework. × 102,000.

FIGURE 14 High magnification section of a region in the interior of an isolated nucleus. Distinct areas of condensed chromatin, C, are seen in the upper-center and lower-left regions of the micrograph. The regions between these condensed chromatin areas are the interchromatinic regions which contain electron-dense interchromatinic granules (*ig*) and less electron-dense fibrous structures (*f*). × 116,000.

FIGURE 15 High magnification of a section through the internal matrix framework of the nuclear protein matrix. This residual framework structure consists of electron-dense matrix particles (*mp*) and matrix fibers (*f*) which bear a close similarity to the interchromatinic structures of isolated as well as *in situ* liver nuclei (compare with Figs. 14 and 10*a* and *b*). × 116,000.

FIGURES 16 and 17 High magnification sections through the residual nuclear envelope layer of the nuclear protein matrix. Distinct residual nuclear pore complex structures are observed which still retain their characteristic annular structure (arrows). Central granules are often visible in tangential sections through the residual pore complex structures (white arrow in Fig. 17). × 158,000.

TABLE IV

*Comparison of Amino Acid Analyses of the Nuclear Protein Matrix with the Nonchromosomal Acidic Nuclear Protein Fraction of Steele and Busch**

Amino acid	(A) Nuclear matrix proteins	(B) Nonchromosomal alkali-soluble nuclear proteins*	Ratio A/B
	mol/100 mol of amino acids		
Lysine	6.55	6.27	1.04
Arginine	6.61	5.67	1.17
Histidine	2.26	2.29	0.99
Aspartic acid	9.23	9.25	1.00
Glutamic acid	13.34	12.04	1.11
Threonine	5.02	5.67	0.89
Serine	6.96	7.36	0.95
Proline	5.54	5.47	1.01
Glycine	9.34	7.96	1.17
Alanine	7.50	7.36	1.02
Valine	6.42	6.07	1.06
Isoleucine	3.94	4.58	0.86
Leucine	9.45	9.35	1.01
Tyrosine	2.56	2.79	0.92
Methionine	1.81	2.59	0.70
½ Cystine	0.24	1.29	0.19
Phenylalanine	3.21	3.98	0.81
Ratio $\frac{\text{acidic}}{\text{basic}}$	1.46	1.50	0.97

* Values under (B) from Steele and Busch (71).

residual nuclear membrane contains the three major polypeptide peaks (P-1, P-2, and P-3) of the nuclear matrix and, in addition, a prominent increase in the polypeptide peak at ~50,000 daltons (Fig. 21). In contrast, the residual nucleoli exhibit a wider variety of polypeptide peaks. Polypeptide bands were observed on the acrylamide gel of the residual nucleoli in the area of P-1 and P-2, but the P-3 band was deficient (Fig. 21).

Analysis of Electron Transport Components

Previous studies have shown that isolated nuclear membranes contain electron transport components including NADH cytochrome b_5 reductase, cytochrome b_5, and cytochrome *c* oxidase (9, 18, 19, 78). We therefore assayed the total nuclear matrix for these electron transport components. The specific activities of these factors in isolated nuclei increase from two- to fivefold in the high salt-treated nuclear sphere fraction, when expressed on a total milligram protein basis (Table V). These results are anticipated since the high-salt nuclear spheres are depleted in chromatin but still retain both the outer and inner nuclear membranes. When these membranes are disrupted with Triton X-100 and the final nuclear protein matrix is isolated, the specific activities of the NADH-ferricyanide and NADH-cytochrome *c* reductase are essentially abolished, as is the presence of cytochrome b_5. In contrast, considerable activity of cytochrome *c* oxidase is retained and the activity can be further increased by the addition of exogenous phospholipid. This indicates that at least some proteins of the nuclear membrane, such as cytochrome *c* oxidase, are still associated with residual components of the nuclear matrix.

DISCUSSION

Organization of the Nuclear Matrix

A number of investigators have previously reported the existence of residual nuclear structures after extraction of chromatin with high ionic strength solutions (26, 37, 58, 67, 80, 81). Re-

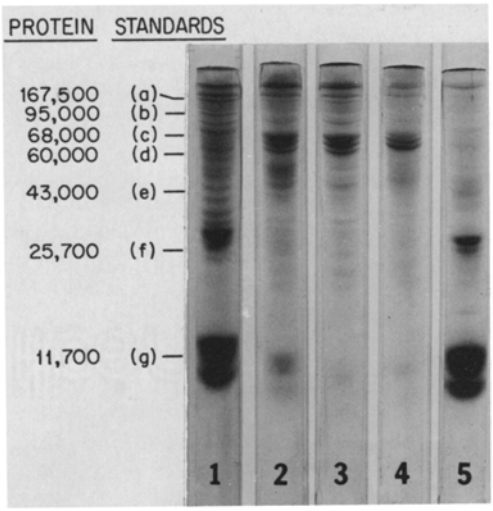

FIGURE 19 SDS-acrylamide gel electrophoresis of the nuclear matrix polypeptides. Approximately 80 μg of protein was placed on the top of each gel and resolved by electrophoresis. (1) Untreated control nuclei, (2) high salt-treated nuclear spheres, (3) nuclear matrix, (4) nuclear protein matrix, and (5) isolated chromatin. Standard proteins were (*a*) thyroglobulin (167,500); (*b*) phosphorylase a (95,000); (*c*) bovine serum albumin (68,000); (*d*) catalase (60,000); (*e*) ovalbumin (43,000); (*f*) chymotrypsinogen (25,700); and (*g*) cytochrome *c* (11,700). The high molecular weight component common to all the gels has an approximate molecular weight of 200,000.

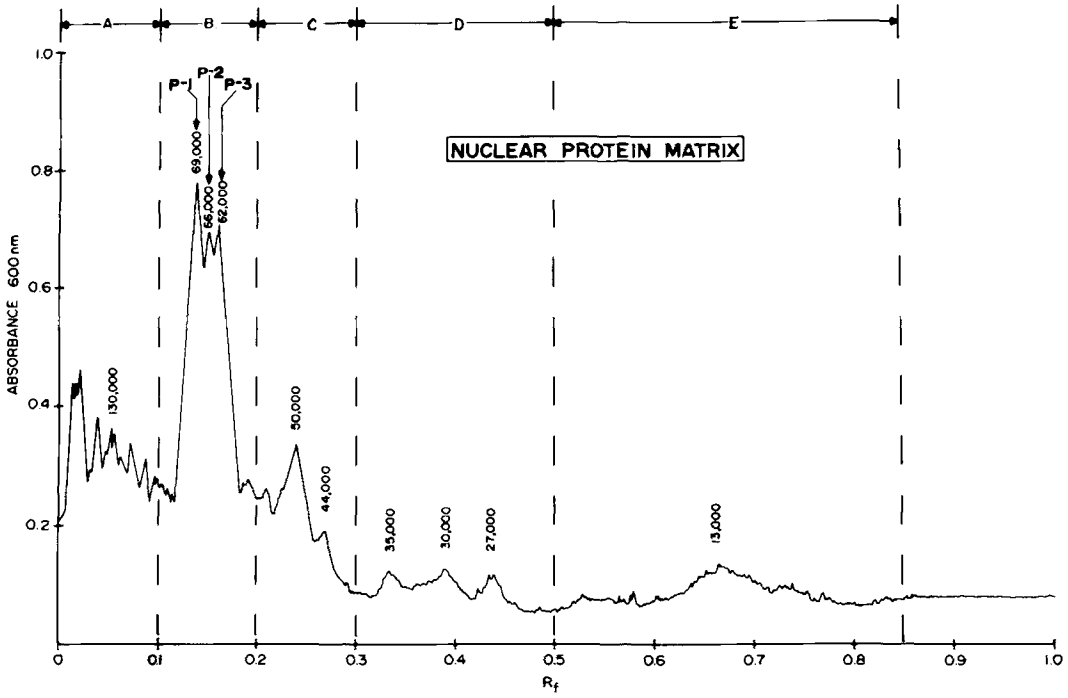

FIGURE 20 SDS-acrylamide gel scan of the nuclear protein matrix polypeptides. The gel was run with 50 μg of protein, stained with Coomassie Blue, and scanned with a Gilford Linear Transport at 600 nm. Molecular weight values were determined from a standard curve according to the procedures of Weber and Osborn (76) using the proteins listed in Fig. 19.

cently, we reported preliminary results on the isolation of a residual protein framework in the rat liver nucleus (12, 13) which we later termed the nuclear protein matrix (14). Particular emphasis is placed on understanding the structural derivation of the matrix from isolated nuclei. It might be argued, for example, that the nuclear protein matrix represents a fortuitous association of residual nuclear proteins, and bears no definitive relationship to the structure of the nucleus *in situ*. High magnification electron micrographs of the nuclear protein matrix (Figs. 11–17), however, clearly demonstrate that the structural components of the isolated matrix bear a remarkable resemblance to well-defined structures of the intact cell nucleus. These results are summarized in the following points: (*a*) *A residual nuclear envelope layer which still retains residual nuclear pore complexes.* High magnification of the residual pore complex (Figs. 16 and 17) demonstrates features typical of the intact nuclear pore complex such as the characteristic annular structure, central rodlets, and radial filaments connecting the annular and central rodlets (78). These findings agree with the results of Aaronson and Blobel (3) and Scheer et al. (64), who have demonstrated morphologically recognizable nuclear pore structures in isolated residual nuclear envelope layers, and support an earlier speculation by Wunderlich (77) that nuclear pore complexes contain permanent protein components. (*b*) *A residual internal matrix framework derived from the interchromatinic structure of the nucleus* (21, 22, 55, 73). Use of the Bernhard regressive staining procedure has revealed that the interchromatinic structure forms a reticulum or matrix throughout the nuclear interior (20, 55). In its structural organization the residual internal matrix observed in the nuclear protein matrix is strikingly similar to this *in situ* matrix as indicated by comparing Figs. 14 and 15. This structure consists of electron-dense matrix particles ~150–250 Å in diameter, associated with less electron-dense but tightly packed matrix fibers. The electron-dense matrix particles appear identical to the interchromatinic particles previously observed in nuclei *in situ* (21, 73). The matrix fibers which have a diameter of ~50 Å also have potential *in situ* structural derivatives in the interchromatinic re-

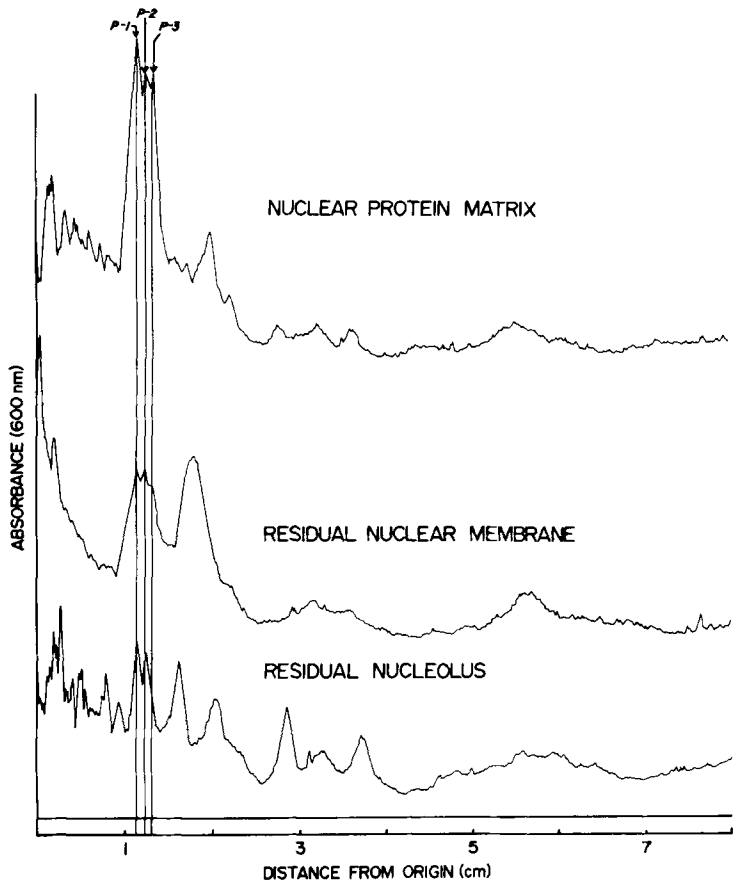

FIGURE 21 Comparative SDS-acrylamide gel scans of the nuclear protein matrix, residual nucleolar and residual nuclear membrane polypeptides. Treatment of isolated nucleoli and nuclear membranes was performed in the same way as treatment of isolated nuclei for the preparation of nuclear matrix. 50 μg of residual protein was placed on each gel.

TABLE V

Electron Transport Components

Assay	Nuclei	High salt-treated nuclear spheres	Nuclear protein matrix	NPM/N ratio
		sp act		
NADH-cytochrome *c* reductase (μmol cytochrome *c*/min per mg protein)	0.0604	0.278	0.001	0.017
+ Rotenone (10^{-4} M)	0.0589	0.273	0.001	0.017
NADH-ferricyanide reductase (μmol $K_3Fe(CN)_6$/min per mg protein)	0.379	1.97	0.04	0.106
Cytochrome b_5 (ΔA 425–410 nm/mg protein)	0.016	0.067	0.001	0.063
Cytochrome *c* oxidase (μmol O_2/min per mg protein)*	0.015	0.034	0.013	0.867
+ Phospholipid	0.017	0.037	0.025	1.47

* 0.5–1.0% of the total cytochrome *c* oxidase activity in the liver homogenate was recovered in the isolated nuclei.

gions of nuclei, but it has always been difficult to distinguish euchromatin fibers from "other interchromatinic substances".

Monneron and Bernhard (55), however, using the EDTA-regressive staining technique, identified 30–50-Å fibers as a basic component of various extrachromatinic structures. These include perichromatinic fibers, perichromatinic granules, coiled bodies, fibers associated with the nuclear pore complexes, and fibers which interconnect interchromatinic granules. Monneron and Bernhard further stressed similar staining properties of the fibers, and suggested a structural linkage between perichromatinic fibers and interchromatinic granules, as well as occasional connections between perichromatinic fibers and granules. The 50-Å matrix fibers may therefore represent residual components of these 30–50-Å fibers observed in intact nuclei. Recently, Comings and Okada (30) have suggested that these residual matrix fibers be termed matrixin. (*c*) *Residual nucleoli.* The identification of the highly condensed and electron-dense bodies as residual nucleoli is based on the monitoring of structural alternations which the nucleolus undergoes during the extraction procedures for the nuclear protein matrix. Recently, we have confirmed this interpretation by electron microscope examination of the isolated residual nucleoli prepared in this study. (R. Berezney. Unpublished experiments.)

The above considerations indicate that the isolated matrix is derived from well-defined structures of the intact nucleus. The question then arises as to what extent this *isolated residual matrix* corresponds to the matrix of intact nuclei. It is likely that the large decrease in the radius of the final nuclear protein matrix ($r = 2.72 \pm 0.08$ μm) in comparison to isolated nuclei ($r = 3.95 \pm 0.07$ μm) (see Fig. 2) is a reflection of massive configurational changes in specific components of the matrix. Further studies are therefore necessary to more precisely relate the structure of the isolated matrix to its *in situ* organization.

There is no doubt, however, that specific components of the matrix can be separated. This must occur, for example, in the isolation of nucleoli (25) or nuclear membrane (9, 19, 56). The effectiveness of 0.5 M $MgCl_2$ for the isolation of nuclear membranes (9, 19, 56, 78) suggests that high concentrations of the divalent cation magnesium may be critical in the separation of nuclear membranes from the matrix.

It is important to consider whether the nuclear matrix can be isolated from other types of eucaryotic cells. Recently, Herlan and Wunderlich (44) reported the isolation of a nuclear matrix from the macronuclei of the ciliate protozoan *Tetrahymena pyriformis,* using a modification of the procedure reported for rat liver (14). The isolated *Tetrahymena* matrix displayed a structural organization similar to that of the rat liver matrix. Comings and Okada (30) recently reported a similar structure for mouse liver nuclear matrix, and Hildebrand et al. (45) have reported the isolation of a nuclear matrix from cultured Chinese hamster ovary (CHO) cells.

The ability of the nuclear matrix to contract after nuclease treatment (Fig. 2) suggests that the matrix is not a rigid skeletal structure, but rather a flexible framework capable of vast changes in organization. In support of this view, Wunderlich and his co-workers have recently discovered that isolated *Tetrahymena* nuclear matrix can reversibly expand and contract when the concentrations of Mg^{++} and Ca^{++} are varied in the medium.[3] The presence of a flexible nuclear matrix framework in the cell nucleus is consistent with the observed swelling of the cell nucleus during the S period of the cell cycle (52) and the well-known phenomenon of nuclear swelling as a prerequisite for the initiation of RNA and/or DNA synthesis (23, 29, 39, 41, 42, 53).

It is therefore suggested that the nuclear matrix *in situ* is a dynamically changing structure closely coupled with nuclear functioning. Recently, we have found that newly replicated DNA is closely associated with the nuclear matrix in regenerating liver (15–17). Moreover the matrix proteins have been shown to phosphorylate to a maximal level at a time just preceding the onset of DNA replication in the regenerating liver (4, 10).

It has also been speculated that the nuclear matrix plays an important role in the transcription and intranuclear transport of RNA (26, 78). The association of residual nuclear pore complexes as an integral component of the nuclear matrix may provide a structural linkage for intranuclear transport to the cytoplasm. In accord with this view, Faiferman and Pogo (34) recently demonstrated that RNP particles containing rapidly labeled, heterogeneous nuclear RNA (hn-RNA) are associated with the nuclear matrix. In addition, we

[3] Wunderlich, F., and G. Herlan. 1977. A reversibly contractile nuclear matrix. Its isolation, structure, and composition. *J. Cell Biol.* **73**:271–278.

have recently observed that polyribonucleotides bind tightly to the isolated nuclear protein matrix.[2] These polyribonucleotides alter nuclear structure and function (29). Recently, Goidl et al. (38) have reported that certain of these polyribonucleotides release preformed polyribosomes from isolated nuclei.

Nuclear Matrix Proteins

The presence of the major matrix polypeptides P-1, P-2, and P-3 in isolated nucleoli and nuclear envelopes suggests that the 60,000–70,000-dalton polypeptides are distributed throughout the nuclear matrix as a major component of the framework structure. In support of these findings, Aaronson and Blobel (3), Dwyer and Blobel (33), Riley et al. (62), and Shelton et al. (66) have demonstrated similar 60,000–70,000-dalton polypeptides, and Comings and Okada (30) have recently reported that mouse liver nuclear matrix contains three major polypeptides with molecular weights similar to those of polypeptides of rat liver nuclear matrix.

The presence of nuclear membrane cytochrome *c* oxidase activity in the nuclear matrix suggests that at least some protein components of the nuclear membrane are still associated with the residual nuclear envelope layer of the matrix. This conclusion is in agreement with a view recently proposed by Scheer et al. (64). Our results, however, also demonstrate that some tightly bound nuclear membrane proteins are specifically removed during the isolation of the matrix. For example, cytochrome b_5, a tightly bound, integral membrane protein of both the endoplasmic reticulum (69) and the nuclear membrane (18), is largely extracted (Table V).

Since the nuclear membrane enzymes analyzed in this study make up only a small percentage of the total protein of the nuclear membrane, the total amount of membrane protein associated with the residual nuclear envelope layer cannot be determined from this analysis. A number of studies, however, have demonstrated that certain membrane proteins can maintain residual structures, albeit altered, after extraction of lipids with detergents (31, 49, 79), phospholipases (11), or organic solvents (35, 40). The large extraction of phospholipid (98%) from the nuclear envelope during matrix isolation, therefore, does not necessarily imply that a correspondingly large proportion of nuclear membrane proteins are also extracted.

Recent studies of the matrix polypeptides from other cells indicate possible differences in molecular weights. For example, Herlan and Wunderlich (44) reported three major polypeptides of 70,000, 57,000 and 54,000 in isolated matrix from the more primitive *Tetrahymena pyriformis* macronucleus, and Hildebrand et al. (45) found major polypeptide bands between 58,000 and 71,000 in nuclear matrix from CHO cells. It will be necessary to examine the matrix in cells from a large variety of organisms before interpreting any similarities or differences in matrix proteins, phylogenetically. It is important to emphasize, however, that other polypeptides aside from the 60,000–70,000-dalton fractions are components of the liver matrix such as the 50,000 and the ~200,000-dalton peptides.

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