COMPARATIVE STUDIES OF RAT LIVER AND SEA URCHIN EMBRYO NUCLEAR MATRICES: PARTIAL FRACTIONATION AND PROTEIN KINASE ACTIVITY DISTRIBUTION

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SUMMARY

Rat liver and sea urchin embryo nuclear matrices were found to differ in composition and in the strength of the association of their structural elements. Apart from the qualitative differences in composition, the embryonic matrices retained greater amounts of nuclear proteins and DNA, and were less susceptible to ultrasonic treatment than those of rat liver. They were essentially resistant to mild sonication, by which the rat liver matrix structure was resolved into two distinct fractions, referred to by Berezney (1980) as matricin and ribonucleoprotein (RNP). Both sub-fractions exhibited a protein kinase activity; the phosphorylating capacity of the RNP-associated protein kinases was found to be higher than that of the matricin-bound enzyme. The preferred substrate was among the secondary matrix proteins. In sea urchin embryos, sonication introduced no change in the type and location of the matrix proteins phosphorylated by the associated enzyme.

INTRODUCTION

The nuclear structural framework in eukaryotic cells, i.e. the nuclear matrix, resembles closely the nuclear structure observed in situ. While the general occurrence and ultrastructural similarities argue for a fundamental and a possible general function of the nuclear matrix, considerable tissue-related differences in the polypeptide composition (Berezney, 1979), as well as significant compositional differences between the nuclear matrices of lower (Herlan, Eckert, Kaffenberger & Wunderlich, 1979; Mitchelson, Beckers & Wanka, 1979; Poznanović & Ševaljević, 1980) and higher (Berezney & Coffey, 1977) eukaryotes, suggest that the composition of the nuclear matrix may depend on both the type and the functional state of the nucleus. Such an assumption is also supported by the discovery of alterations in the composition of the nuclear matrices following treatment with actinomycin (Herlan, Quevedo & Wunderlich, 1978), during embryogenesis (Poznanović & Ševaljević, 1980), differentiation (Long, Huang & Pogo, 1979), virus infection and different phases of the cell cycle (Hodge, Manicini, Davis & Heywood, 1977). The possibility of the existence of a composition-function relationship encouraged further, more profound studies of tissue and species-related nuclear matrix characteristics. The recently reported fractionation of the nuclear matrix (Berezney, 1980) and the discovery of the nuclear matrix-associated protein kinase(s) (Ševaljević, Poznanović,
Petrović & Krtolica, 1980), offered a novel tool for the structural and functional characterization of the nuclear matrix. They were used in this work for a comparative study of the susceptibility of the nuclear matrix structure to fractionation and the phosphorylating capacities of the subfractions in the tissue of lower (sea urchin embryo) and higher (rat) eukaryotes.

**MATERIALS AND METHODS**

**Preparation of nuclei and nuclear matrices**

Nuclei were prepared from rat liver and sea urchin embryos at the blastula and pluteus stages. Rats were decapitated and the livers perfused, excised, weighed and minced. One volume of tissue was suspended in 2 vol. of 0.25 M-sucrose, 50 mM-Tris-HCl (pH 7.4), 5 mM-MgCl₂. The tissue was homogenized in a motor-driven homogenizer (Ultra-Turrax, Janke Kunkel), twice at 120 V for 60 s and centrifuged at 770 g in an SS-34 (RC-5B Sorvall centrifuge), for 10 min at 4 °C. The pelleted nuclei were purified through a rough 2:2 M-sucrose gradient, washed and resuspended in 0.25 M-sucrose, 50 mM-Tris-HCl (pH 7.4), 5 mM-MgCl₂.

The method used for the preparation of nuclei from sea urchin embryos was essentially that of Lavtrup-Rein (1972). The embryos were suspended in a solution made up of equal parts of filtered sea water and 0.32 M-sucrose, 50 mM-Tris (pH 7.4), 3 mM-CaCl₂, and homogenized with a Dounce homogenizer. All operations were done at 0–4 °C and in the presence of 0.001 M-diisopropylfluorophosphonate. The homogenate was centrifuged at 1000 g for 5 min, the pellet was resuspended in five times the volume of 0.32 M-sucrose, 50 mM-Tris (pH 7.4), 3 mM-CaCl₂ and re-centrifuged at 1000 g. The pelleted nuclei were re-dispersed in 1:7 M-sucrose, 0.01 M-Tris (pH 7.4), 5 mM-CaCl₂ and centrifuged at 40000 £ for 90 min in a Beckman SW 25/1 rotor. The nuclei were washed twice with a solution containing 0.32 M-sucrose, 50 mM-Tris (pH 7.4), 3 mM-CaCl₂, at 1000 g.

To prepare the nuclear matrix, the nuclei were resuspended to 1 mg DNA/ml in 0.25 M-sucrose, 20 mM-Tris (pH 7.4), 5 mM-MgCl₂ and digested with 15 units of DNase I (Worthington Biochem. Corp. Freehold, N.Y.) for 10 min at 22 °C. After centrifugation, the nuclei were sequentially extracted for 10 min at 0 °C, three times with the low-magnesium (LM) buffer (0.2 mM-MgCl₂, 10 mM-Tris, pH 7.4) and three times with high-salt (HS) buffer (2 M-NaCl, 0.2 mM-MgCl₂, 10 mM-Tris, pH 7.4) buffer. The resulting nuclear pellet was extracted once with 1% Triton X-100 in LM buffer and washed twice with LM buffer.

**Matrix fractionation**

The nuclear matrices were resuspended in LM buffer and sonicated with one 30-s burst with an MSE sonicator at a peak-to-peak amplitude distance of 12 μm. The suspension was then centrifuged at 5000 g for 15 min in the SS-34 rotor. The resulting pellet and supernatant were referred to by Berezney (1980) as the matricin and the crude ribonucleoprotein (RNP) fractions, respectively. To obtain the RNP, the 5000 g supernatant was centrifuged at 100000 g in a Beckman 50 Ti rotor, at 4 °C.

**Electrophoresis and autoradiography**

The protein samples, prior to electrophoretic analysis, were phosphorylated in 2.5 ml of a reaction mixture containing 1 mg of protein and 1.1 nmol of [γ-³²P]ATP (1.1 μCi). Electrophoresis was carried out according to O'Farrell (1975). After a constant voltage at 100 V for 10 h, the gel was immersed in hot 10% trichloroacetic acid (TCA) for 30 min at 70 °C, stained in 0.1% Coomassie brilliant blue R, 50% methanol/10% TCA, destained in 50% methanol/10% acetic acid and dried. The pattern of ³²P radioactivity of the separated proteins was obtained after exposing the dried gel to a Kodak X-omat, X-ray film in the presence of an intensifying screen.
Protein kinase activity assays

The matrix, the matricin and the crude RNP fractions were assayed for enzyme activity in 1 ml of a reaction mixture containing 100 μg of the protein to be tested, 5 mM-MgCl₂, 30 mM-Tris-HCl (pH 7.4), 115 mM-NaCl and 2.4 nmol of [γ-³²P]ATP (NEN, lot 1300–294, 4.6 μCi/mmol). After incubation at 37 °C for 10 min the reaction mixture was chilled to 0 °C; ice-cold ATP was added to a final concentration of 1 mM and the proteins were precipitated with an equal volume of ice-cold TCA, 3 % Na₄P₂O₇. The precipitates were collected on Sartorius membrane filters (GMBH, 045 μm). The filters were subsequently washed three times with 5-ml portions of 5 % TCA, 1.5 % Na₄P₂O₇ and twice with ethanol. The dried filters were counted in 5 ml of a POPOP/PPO/toluene mixture.

Quantitative analysis

Protein, DNA and RNA were determined by the methods of Lowry, Roseborough, Farr & Randall (1951), Burton (1956) and Munro & Feck (1969), respectively.

RESULTS

Nuclear matrix

Sequential extractions of rat liver and sea urchin embryo nuclei with low magnesium and high sodium salts yielded residual structures with quantitatively different compositions. Numerical values presented in Table 1 show that rat liver and pluteus nuclear matrices retained approximately 17 and 25 % of the total nuclear proteins, respectively, whereas the blastula nuclear matrix retained 60 % of the nuclear proteins. The amount of the matrix-associated DNA was several-fold higher in embryonic than in rat liver tissue. However, the stage-related differences in the DNA were less pronounced than the differences in the protein and RNA contents.

Table 1. Relative proportions of nuclear macromolecules in the subfractions of rat liver and sea urchin embryo nuclei

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein</th>
<th>DNA</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blastula</td>
<td>Pluteus</td>
<td>Liver</td>
</tr>
<tr>
<td>Total nuclei</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Nuclear matrix</td>
<td>60-0</td>
<td>24-6</td>
<td>16-8</td>
</tr>
<tr>
<td>Matricin fraction (5000 g pellet)</td>
<td>43-0</td>
<td>19-9</td>
<td>4-8</td>
</tr>
<tr>
<td>RNP fraction (1000000 g pellet)</td>
<td>5-1</td>
<td>2-4</td>
<td>11-8</td>
</tr>
</tbody>
</table>

Fractionated nuclear matrices

Ultrasonic treatment and subsequent centrifugation of the isolated nuclear matrices at 5000 g resolved the nuclear matrix spheres into soluble and sedimenting fractions. The mass distribution of the nuclear matrix constituents among the subfractions is given in Tables 1 and 2. In accordance with the data reported by Berezney (1980), the rat liver matricin fraction, which comprised approximately 30 % of the total
nuclear matrix protein, was found to be composed largely of protein (90%), whereas the supernatant contained the bulk of the matrix protein and RNA (Table 2). Sea urchin embryo nuclear matrices showed a remarkably higher resistance to ultrasonic treatment than those from rat liver. Embryonic 5000 g pellets retained 70 to 80% of the total matrix protein and DNA and approximately 50% of the RNA (Tables 1 and 2). Centrifugation of the 5000 g supernatant at 100,000 g resulted in the formation of a gelatinous pellet referred to by Berezney (1980) as the RNP fraction. In the rat liver it comprised 90%, and in sea urchin embryo 50%, of the total 5000 g soluble proteins and RNA (Tables 1 and 2).

Table 2. Recovery of macromolecules in rat liver and sea urchin embryo nuclei and nuclear substructures

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein (mg/50 g wet wt of tissue)</th>
<th>DNA (mg/50 g wet wt of tissue)</th>
<th>RNA (mg/50 g wet wt of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nuclei</td>
<td>123±1 ±6.2</td>
<td>46.4±4.15</td>
<td>6.3±1.6</td>
</tr>
<tr>
<td>Nuclear matrix</td>
<td>20.6±2.5</td>
<td>0.98±0.17</td>
<td>2.4±0.11</td>
</tr>
<tr>
<td>Matricin fraction (5000 g pellet)</td>
<td>5.9±0.6</td>
<td>0.34±0.08</td>
<td>0.30±0.14</td>
</tr>
<tr>
<td>Crude RNP fraction (5000 g supernatant)</td>
<td>15.4±2.0</td>
<td>0.53±0.2</td>
<td>2.1±0.24</td>
</tr>
<tr>
<td>RNP fraction (100,000 g pellet)</td>
<td>14.6</td>
<td>0.21</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Rat liver (mg/50 g wet wt of tissue)  
Sea urchin embryo (μg/1×10⁶ spheres)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein (mg/50 g wet wt of tissue)</th>
<th>DNA (mg/50 g wet wt of tissue)</th>
<th>RNA (mg/50 g wet wt of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nuclei</td>
<td>34.8±0.43</td>
<td>4.46±0.12</td>
<td>5.55±0.54</td>
</tr>
<tr>
<td>Nuclear matrix</td>
<td>43.6±0.87</td>
<td>5.96±0.82</td>
<td>5.11±1.08</td>
</tr>
<tr>
<td>Matricin fraction</td>
<td>20.9±0.40</td>
<td>1.67±0.08</td>
<td>2.58±0.15</td>
</tr>
<tr>
<td>RNP fraction</td>
<td>10.7±0.77</td>
<td>1.95±0.09</td>
<td>1.34±0.45</td>
</tr>
</tbody>
</table>

* Average ± S.E. of three to six different preparations, except the values for the RNP fraction, which correspond to two experiments.

Phosphorylation of nuclear matrix subfractions in vitro

The kinetic aspects of the protein kinase activity of rat liver matricin (5000 g pellet) and the RNP (5000 g supernatant) fractions are presented in Fig. 1. While both nuclear matrix subfractions were capable of transferring the 32P from ATP to the substrate protein in an in vitro system, the phosphorylating capacity of the RNP-containing incubation mixture was remarkably higher than that containing an equal amount of the matricin proteins.

Unlike the rat liver, the 5000 g supernatant of the sonicated embryonic matrices exhibited a tenfold lower phosphorylating capacity than the pellet. It was found that the blastula and pluteus pellets incorporated 305 and 202 c.p.m. per μg of protein, respectively, whereas the specific radioactivities of the 5000 g soluble proteins were negligible at both stages. A more intensive labelling of the 5000 g pellet at the blastula than at the pluteus stage is in agreement with the higher protein kinase activity of blastula matrices reported previously (Sevaljević et al. 1980).
Rat liver and sea urchin embryo nuclear matrices

Fig. 1. The dependence of $^{32}$P incorporation into rat liver matrices ($5000 \text{ g}$ pellet) and RNP ($5000 \text{ g}$ supernatant) fractions on the time of incubation. $\times$ — $\times$, RNP; $\bullet$—$\bullet$, matricin.

Sodium dodecyl sulphate (SDS)/acrylamide gel electrophoresis and autoradiography of the polypeptides from the nuclear matrix subfractions

In Figs. 2–4 the electrophoretic patterns of the rat liver and sea urchin embryo nuclear matrix proteins are presented. The prominent feature is a qualitative difference in composition between the rat liver and sea urchin embryo matrix proteins. While most abundant sea urchin embryo bands had molecular weights of 13 and 16 ($\times 10^3$), those of the rat liver resided in the 60 to 80 ($\times 10^3$) molecular weight region of the gel. The triplet which have molecular weights of 62, 65, and 70 ($\times 10^3$) have been identified by Berezney (1980) as the primary matricin fibril constituents. In the electrophoretic system of Weber & Osborn (1969) the corresponding set of proteins exhibited molecular weights of 78, 71, and 68 ($\times 10^3$) (bands P-1, P-2, and P-3 in Fig. 2), whereas in the system of O’Farrell (1975), their molecular weights were 69, 63, and 57 ($\times 10^3$) (bands P-1, P-2, and P-3 in Fig. 3). It is likely that the lower values correlate with a better dissociation of proteins in O’Farrell’s system. Mild sonication of the rat liver matrices resulted in an enrichment of the $5000 \text{ g}$ pellet with the primary, and the $5000 \text{ g}$ supernatant with the secondary, nuclear matrix polypeptides (Fig. 2). The RNP was pelleted by centrifugation of the
Fig. 2. The Coomassie blue-staining patterns of rat liver proteins (from left to right): nuclear matrix, matricin (5000 g pellet), crude RNP fraction (5000 g supernatant RNP fraction (100000 g pellet) and 100000 g supernatant. Electrophoresis was carried out according to Weber & Osborn (1969). P-1, P-2, and P-3, primary matricin fibril polypeptides. K, 10^4 daltons.

5000 g supernatant at 100000 g. Electrophoretic analysis revealed that the 100000 g supernatant was enriched in the P-2 protein and in the low molecular weight polypeptides (Fig. 2, slot 5), whereas a preferential part of the high molecular weight proteins was associated with the pelleted RNP fraction (Fig. 2, slot 4).

The proteins that were released from the sonicated sea urchin embryo matrices represented an almost negligible fraction. Fig. 3 shows that the electrophoretic pattern of the proteins pelleted at 5000 g was indistinguishable from that of the nuclear matrix proteins; however, the 5000 g supernatant was slightly enriched with the minor matrix polypeptides.

Incubation of rat liver or sea urchin embryo matrices with labelled ATP resulted in the phosphorylation of the majority of the matrix proteins (Figs. 3, 4). Fig. 3 shows the Coomassie blue-staining and 32P-labeling patterns of proteins associated with the rat liver matrix (slots 1 and 2), matricin (slots 2 and 3) and crude RNP
fractions (slots 5 and 6). As expected from the kinetic data, the rat liver \(5000\,\text{g}\) soluble proteins (crude RNP fraction) were phosphorylated more intensely than those in the pelleted matricin fraction (Fig. 3, slots 4 and 6). As compared to the secondary polypeptides, the primary ones were less intensely phosphorylated even in the matricin fraction (Fig. 3, slot 4), where they represent the most abundant constituents (Fig. 3, slot 3). Autoradiographic analysis of the \(5000\,\text{g}\) soluble proteins revealed two bands in the region of the primary matrix proteins (Fig. 3, slot 6). Although the faster moving band appeared more intense, it remains unclear whether it corresponds to P-2 or P-3 proteins, or to both. The most heavily labelled protein class was found in the 130-140 \(\times 10^3\) molecular weight region of the gel and it corresponds to a minor constituent of the nuclear matrix structure.

Contrary to those of rat liver, the \(5000\,\text{g}\) soluble proteins of sea urchin embryos were phosphorylated to a virtually undetectable extent, whereas the \(^{32}\text{P}\)-labelling pattern of the matricin fraction was identical to that of the nuclear matrix proteins (Fig. 4).
DISCUSSION

Rat liver and sea urchin embryo nuclear matrices were found to differ in their quantitative and qualitative composition and in their susceptibility to fractionation by mild sonication. The embryonic matrices were resistant to ultrasonic treatment, which otherwise enabled the resolution of the rat liver nuclear matrix structure into a minimal spherical substructure, referred to by Berezney (1980) as the matricin and the RNP fractions. This suggests that the strength of the association among the nuclear matrix structural elements was stronger in embryonic than in rat liver nuclei. A decrease in the retention of nuclear protein in the sequence: blastula, pluteus and rat liver matrices, indicates also that the affinity of the nuclear matrix for the surrounding macromolecules decreased in the course of cell differentiation.

Phosphorylation of nuclear matrix proteins by endogenous protein kinases is probably a biochemical event related to some general nuclear function. In vivo phosphorylation of several classes of nuclear matrix proteins has been demonstrated in rat liver (Allen, Berezney & Coffey, 1977), as well as in the 3T6 cell line (Buckler-White, Humphrey & Pigiet, 1980). A nuclear envelope-associated protein kinase activity was described by Lam & Kasper (1979) and we reported for the first time the phosphorylation of nuclear matrix proteins by a matrix-associated kinase in an in vitro system (Sevaljević et al. 1980). Since the identical mobilities of three major nuclear envelope and three major nuclear matrix proteins on sodium dodecyl sulphate/acrylamide gels argue for their similarity, it is interesting to note that incubation of the nuclear envelope with $^{32}$P-ATP resulted in selective phosphorylation of the protein with the highest mobility, corresponding to the P-3 matrix polypeptide.
Rat liver and sea urchin embryo nuclear matrices

(Lam & Kasper, 1979). However, the stated similarity should be considered with caution, since the nuclear envelope and the nuclear matrix proteins were found to have different antigenic properties (Gerace, Blum & Blobel, 1978). The results presented in this work demonstrate that the primary matrix polypeptides are less intensely phosphorylated than the secondary ones, especially in the matricin fraction, where they constitute a polymeric network assembly. The soluble primary matrix polypeptides that remained in the 5000g supernatant of the sonicated matrices were more phosphorylated than those in the sedimenting fraction. This is consistent with the finding of a more intense phosphorylation of three lamina in the depolymerized (soluble) than those in the polymerized (insoluble) state (Gerace & Blobel, 1980). In vitro-phosphorylated nuclear envelope protein has also been reported to exist exclusively in a monomeric state (Lam & Kasper, 1979). It is highly probable that the matrix polypeptides that remained in the supernatants of the sonicated rat liver matrices were in a monomeric form. Phosphorylation and a possibly related reversible depolymerization of the fibrillar matrix proteins may be considered in the broader context of a dynamic flux that exists between the nuclear network structure and the surrounding environment. Such a mechanism could be involved in the nuclear functions related, not only to cell division, but also to the regulation of gene expression.

REFERENCES


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