Small Nuclear RNA-Protein Complex Anchors on the Actin Filaments in Bovine Lymphocyte Nuclear Matrix

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ABSTRACT. When the nuclear matrix from bovine lymphocytes was digested by RNase-depleted trypsin, the bulk of the matrix proteins, except actin, were hydrolyzed. The digestion left rapidly sedimented spherical structures (trypsin-treated nuclear matrix), which mainly were composed of actin (Nakayasu, H. and K. Ueda. Exp. Cell Res. 143, 55-62, 1983). Almost all the small nuclear RNAs of the original nuclear matrix remained associated with these actin spheres after trypsin digestion.

By sonication, the small nuclear RNPs (snRNPs) in both untreated and trypsin-treated nuclear matrices were solubilized in association with proteinous filaments of various size. The sedimentation pattern of these snRNP complexes was not changed by the digestion of the bulk of the proteins. The snRNP complex was adsorbed on rabbit muscle myosin-Sepharose then eluted by the addition of 5 mM ATP. We concluded that snRNPs are associated with actin filaments in the nuclear matrix of bovine lymphocytes.

Nuclei of eucaryotic cells contain a fibrogranular complex, generally called the nuclear matrix (2, 6, 14). Except for its surface lamina, which consists mainly of lamin proteins (12, 16, 35), little has been reported on the meshwork of fibrous materials that traverse the nuclear interior. It is likely that this interior meshwork participates in such nuclear functions as DNA replication (9, 15, 31), RNA processing (18, 21, 23).

Recently, we found that actin is a main component of the interior materials (27) in the bovine lymphocyte nuclear matrix. The existence of nuclear actin also has been reported in rat liver (9), Xenopus oocytes (8, 22), Physarum polycephalum (17), and dimethylsulfoxide-treated cells (11, 34). The function of this nuclear actin, however, has yet to be determined.

SnRNPs have been found in isolated nuclear matrices from rat liver (24), fibroblasts (37), chick oviduct cells (7) and bovine lymphocytes (26). In a previous paper (26), we demonstrated that bovine snRNPs could be dissociated both with a high salt buffer and a low salt buffer that contained ATP, calcium ion, EDTA and DTT and that their release was sensitive to magnesium ion. The conditions of release were closely related to the condition that causes the depolymerization of F-actin.

Abbreviations used: snRNA, small nuclear RNA; snRNP, small nuclear RNA-protein complex; hnRNA, heterogeneous nuclear RNA; hnRNP, heterogeneous nuclear RNA-protein complex; EDTA, ethylene-diaminetetraacetic acid; DTT, dithiothreitol; SDS, sodium dodecyl sulfate.
Therefore, we assumed that the snRNPs are anchored on actin filaments in the nuclear matrix. We here show evidence that small nuclear RNA-protein complexes (snRNPs) do anchor on the actin filaments of the bovine lymphocyte nuclear matrix.

MATERIALS AND METHODS

Materials. DNase I (DN-CL) and trypsin from bovine pancreas, and the trypsin inhibitor from soybean were obtained from Sigma Chemical Co. (St. Louis, MO). Possible contamination of RNase activity in these proteins was avoided by using affinity chromatography on Agarose-5'-{(4'-aminophenyl) uridine 2'(3') phosphate (Miles Laboratories Inc. Elkhart, IN) as described Brison and Chanbon (3). Proteinase K was obtained from E. Merck A.G. (Darmstadt, Germany). Rabbit muscle actin and myosin were purified as described in (30) and (19).

Rabbit muscle myosin-Sepharose was prepared by coupling myosin and cyanogenbromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) in 50 mM sodium carbonate buffer, pH 8.5, containing 0.5 M KCl and 1 mM EDTA (about 3-4 mg myosin/ml Sepharose). Following its preparation, the myosin-Sepharose was stored as a suspension in 0.1 M Tris-HCl buffer, pH 7.5, containing 50% (v/v) glycerol, 1 mM DTT and 1 mM EDTA at -20°C. Before use, the myosin-Sepharose was washed with 0.1 M Tris-HCl, pH 7.5, containing 1 mM DTT and 1 mM EDTA.

Preparation of bovine lymphocyte nuclear matrix. Unless otherwise stated, all procedures were performed at 0-4°C. Bovine lymphocyte nuclei (25) and nuclear matrix (26) were prepared as described elsewhere. Isolated nuclei (50 mg DNA) from bovine lymphocytes were incubated with RNase-depleted DNase I (1 mg) at 0°C for 60 min in GTM buffer (50 mM Tris-HCl buffer, pH 7.5, containing 2 mM MgCl₂, 25% (v/v) glycerol, 1 mM PMSF and 0.1% diethylpyrocarbonate). This mixture was centrifuged at 600 × g for 60 min, and the pellet obtained suspended in 8 ml of GTM buffer. Thirty-two milliliters of GTM buffer containing 0.5 M NaCl was added slowly to this suspension, with gentle shaking, to a final concentration of 0.4 M. The mixture was centrifuged at 600 × g for 90 min, and the resulting pellet (nuclear matrix) used immediately for the experiments without washing it with 2 M NaCl.

The small nuclear RNP (snRNP) fraction was obtained from the nuclear matrix by extraction with 50 mM Tris-HCl buffer containing 1 M NaCl and 1 mM MgCl₂ (26). The nuclear matrix (5 mg protein) was suspended in 10 ml of the buffer, and after 10 min the suspension was centrifuged at 20,000 rpm for 30 min. The supernatant was used as the snRNP fraction in which snRNPs were contained as 10 S snRNP core particles.

The trypsin-treated nuclear matrix was prepared as reported elsewhere (27), except that RNase-depleted trypsin and RNase-depleted trypsin inhibitor were used. Although considerable RNase activity contamination was detected in the commercial pancreatic trypsin, it was easily eliminated on an Agarose-5'-{(4'-aminophenyl) uridine 2'(3') phosphate (Miles Laboratories). The freshly prepared nuclear matrix was washed twice with 50 mM Tris-HCl buffer, pH 7.5, containing 0.4 mM MgCl₂ and 0.1% diethylpyrocarbonate to remove the PMSF contained in all the preparation media to inhibit protease activity, after which the matrix was suspended in the same buffer (1 ml/mg matrix protein).

The indicated amount of RNase-depleted trypsin (0-40 μg/mg matrix protein) was added and the mixture incubated at 30°C for 5 min. Digestion was stopped by the addition of an excess amount (more than 2-fold) of RNase-depleted trypsin inhibitor. The trypsin-treated nuclear matrix was collected by centrifugation (3,000 rpm for 10 min).
RNA extraction and electrophoresis. Samples from which RNA was to be extracted first were treated with DNase I and proteinase K as described by Tullis and Rubin (36). The same volume of incubated enzyme solution (0.2 mg/ml RNase-depleted DNase I, 0.5 mg/ml proteinase K in 50 mM Tris-HCl, pH 7.5, containing 10 mM CaCl₂, which protects DNase I from the attack of proteinase K) was added to the samples. This mixture was incubated at 30°C for 30 min to digest both DNA and proteins. Then a 1/5 vol of 0.1 M EDTA and 1/20 vol of 10 % SDS were added, and incubation continued for 30 min. An equal volume of phenol saturated with 50 mM Tris-HCl buffer, pH 7.5, containing 5 mM EDTA and 0.5 % SDS then was added. After centrifugation, phenol extraction was repeated when necessary.

RNAs were electrophoresed on a 10 % polyacrylamide gel in Tris-borate buffer, containing 8 M urea (4). After electrophoresis, the gel was stained with 0.001 % ethidium bromide for 30 min then photographed on a transilluminator (Model C60, Ultra-Violet Product Inc. San Gabriel, CA). Each snRNA was identified using bovine lymphocyte cytoplasmic 5S and 5.8S RNAs (25).

All the electrophoretic analyses of proteins were done as described (28) with a 13.5 % polyacrylamide separation gel and a 4.5 % stacking gel. The gels were stained with Coomassie Blue.

RESULTS

The nuclear matrix from bovine lymphocytes shows relatively simple protein bands on SDS-gel; 68K (lamin B), 53K, 43K (actin) and minor components. Therefore, it is a suitable material with which to study the locations and functions of proteins in association with the nuclear matrix.

![Fig. 1. Association of snRNAs with the trypsin-treated nuclear matrix. Freshly prepared nuclear matrices (0.5 mg protein each) were digested with the indicated amount of RNase-depleted trypsin as described in MATERIALS AND METHODS (lane 1, 0; lane 2, 0.02; lane 3, 0.05; lane 4, 0.1; lane 5, 0.2; lane 6, 0.5; lane 7, 1; lane 8, 2; lane 9, 5; lane 10, 10; and lane 11, 20 μg trypsin). These trypsin-treated nuclear matrices were suspended in 0.2 ml of the same buffer, after which 15 μl portions of the suspension were used for electrophoresis and 80 μl portions for RNA extraction. (A) Electrophoresis of proteins. Lane M, the molecular weight markers, phosphorylase-a (94,000), bovine serum albumin (67,000), Ig G heavy chain (50,000), ovalbumin (46,000), chymotrypsinogen (25,000), trypsin inhibitor (21,000) and myoglobin (17,000). The arrow indicates actin. (B) Electrophoresis of RNAs. Lane M, bovine lymphocyte cytoplasmic RNAs used as markers. Arrows, from top to bottom, indicate the U3, U2, U1, U4, 5S, U5 and U6 RNAs. Because we could not separate U1 RNA and 5.8S RNA with our system, the RNA band of the U1 RNA contains both U1 and 5.8S RNA.](image-url)
the nuclear matrix. We elsewhere have reported that the actin filaments in the nuclear matrix are located mainly in the matrix's interior, where they presumably interact with the fibrogranular structure and/or the residual nucleoli (27).

Trypsin digestion of the nuclear matrix. When the nuclear matrix was digested with trypsin, the bulk of the matrix proteins were hydrolyzed, but about 80% of the

![Image of gel electrophoresis](image)

**Fig. 2.** Trypsin digestion had no effect on the sedimentation pattern of snRNPs. Freshly prepared nuclear matrix (10 mg protein) was digested with 200 µg of trypsin as described in the text. The trypsin-treated nuclear matrix (C, D) and the same amount of untreated nuclear matrix (A, B) were each suspended in 3 ml of 50 mM Tris–HCl buffer, pH 7.5, containing 1 mM EDTA, 1 mM DTT and 0.1% (v/v) diethylpyrocarbonate, after which they were sonicated three times at 80 W for 30 sec in an ice bath. The sonicates were centrifuged at 3,000 rpm for 30 min, then their supernatant (1 ml each) were layered on a linear sucrose density gradient (10%–30% w/v, sucrose, 50 mM Tris–HCl, pH 7.5, 36 ml) and centrifuged at 27,000 rpm for 18 h in a SW 27 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Samples were fractionated in an ISCO fractionator (ISCO Instrumentation Specialities Co. Lincoln, Nebr.), then 1/5 of the volume of each fraction was used for protein electrophoresis and 4/5 for RNA extraction (left; top, right; bottom). (A, C) Respective electrophoreses of proteins from native and trypsin-treated nuclear matrix. Lane M, the molecular weight markers described in Fig. 1; lane W, whole protein of the native and trypsin-treated nuclear matrix; lane S, supernatants of the sonicates. The arrow indicates actin. (B, D) The respective electrophoreses of RNA from the native and trypsin-treated nuclear matrix. Arrows indicate the U1-U6 RNAs, as in Fig. 1B. Bovine lymphocyte cytoplasmic RNAs were centrifuged at the same time. Ribosomal 28S, 18S RNAs and tRNA were detected in fractions respectively numbered 12–15, 9–11 and 1–3.
actin remained associated with the rapidly-sedimented spherical structure, which had the same size as the original nuclear matrix (27). We also studied the chemical composition of the trypsin-treated nuclear matrix and found that considerable amounts of snRNAs and ribosomal RNAs remained associated with the spheres. During our studies, we became aware of RNase contamination in commercial trypsin. Therefore, digestion was carried out with RNase-depleted enzyme obtained by the affinity chromatography described in MATERIALS AND METHODS.

Fig. 1 shows the results. Except for actin, the bulk of the matrix proteins was hydrolyzed (Fig. 1A, lane 11), but almost all the snRNAs remained associated with the spheres (1B, lane 11). This suggests that snRNPs are anchored on the actin network of the matrix.

As described elsewhere (26), the snRNPs in the nuclear matrix sedimented as uniform, 10S core particles when they were extracted with high salt buffer. The snRNPs, however, sedimented as nonhomogeneous particles when prepared by sonication. This polymorphism of the snRNPs was not due to aggregation but to the association of snRNPs with a proteinous structure from the nuclear matrix (26). Sedimentation patterns of the snRNAs obtained by sonication of the native nuclear matrix.
matrix and the trypsin-treated nuclear matrix are shown in Fig. 2. They are almost identical in spite of the digestion of the proteins. Actin was detected in each fraction which contained snRNPs. The results indicate that the polymorphism of the snRNPs was due to association with actin filaments of various lengths. SnRNPs extracted with high salt buffer from the trypsin-treated matrix sedimented as uniform, 10S particles (data not shown).

**Binding experiments on myosin-Sepharose.** Results also were obtained by using myosin-Sepharose. If the snRNPs really do bind to the actin filaments, they would be adsorbed naturally on the myosin-Sepharose through its actin filaments. The actin filaments that came from the matrix (Fig. 3) were adsorbed on the myosin-Sepharose; almost all of the other matrix proteins were not. The snRNPs also bound to the myosin-Sepharose and were eluted together with actin when 5 mM ATP was added (Fig. 3B, lane 3). The same results were obtained with the trypsin treated nuclear matrix (3B, lane 7). About 1/3 of the actin and 1/3 of the snRNPs were present in the ATP-eluted fractions. Elution was not triggered by a change in ionic strength when ATP was added because little snRNPs were eluted with 0.2 M Tris-HCl buffer (data not shown). It is possible that the snRNPs bind directly to the myosin-Sepharose. It was however, very difficult to explain why the snRNPs were released by ATP. Thus, our results suggest that the adsorption and elution of the snRNPs took place through the actin filaments with which the snRNPs are associated.

The binding of the snRNPs to actin filaments might have been an artifact formed during preparations. If the snRNPs or snRNAs have an affinity for actin, the snRNPs could be transferred from their original positions to the actin filaments in the nuclei. To check this possibility, we tried to obtain actin from bovine lymphocytes. Our purified bovine actin, however, had lost much of its polymerizing activity. Therefore,

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**Fig. 4.** The association of snRNA(P)s with actin filaments is not an artifact. One hundred micrograms of snRNAs (lanes 1–4) and snRNPs (100 µg RNA, lanes 5–8) were prepared from bovine lymphocyte nuclear matrix, as described in the text then dissolved in 2 ml of 50 mM Tris-HCl buffer, pH 7.5, containing 0.1 M NaCl and 1 mM MgCl₂ with (lanes 1, 2, 5, 6) or without (lanes 3, 4, 7, 8) 3 mg of rabbit muscle F-actin. Samples were incubated at 0°C for 30 min then centrifuged at 120,000 × g for 3 h to precipitate the F-actin. RNAs were extracted from the supernatants (lanes 1, 3, 5, 7) and from the pellets (lanes 2, 4, 6, 8), electrophoresis being carried out with 1/4 the volume of each extract. Lane M, cytoplasmic RNAs as markers; lane N, RNAs from the snRNP fraction; lane M’, RNAs from the nuclear matrix. Arrows indicate the U1-U6 RNAs. Note that the quantity of snRNAs precipitated was not changed by an addition of actin.
Association of snRNP with Nuclear Actin

we experimented with rabbit muscle actin instead of lymphocyte actin. The purified actin filaments from rabbit muscle were not adsorbed on either the snRNAs or snRNPs from the bovine lymphocyte nuclear matrix. We used 0.1 M NaCl and 1 mM MgCl₂, but the ionic strength of the buffer used to prepare the nuclear matrix (0.5 M NaCl, 2 mM MgCl₂) is much higher than this buffer. Therefore, it is clear that the association of snRNP with actin filaments is not an artifact during preparation of the nuclear matrix.

**DISCUSSION**

Actin is present not only in cytoplasm but also in cell nucleus (8, 9, 10, 17, 22, 27), but little has been reported concerning the function of the nuclear actin. The results we have reported here indicate that the snRNPs in the bovine lymphocyte nuclear matrix anchor on the actin filaments of the nuclear matrix. It is still not clear whether these snRNP core particles (10S particles) are bound directly or indirectly to the actin filaments. We demonstrated, however, that bovine snRNPs are unable to form a complex with rabbit muscle actin. Because of the conservation of the amino acid sequence of actsins, it is difficult to believe that snRNPs bind directly to the lymphocyte actin filaments. We think that there is a yet unknown protein(s) with an affinity for both the snRNPs and actin. This snRNP binding protein(s) must be resistant to trypsin. Zeller et al. (38) found an snRNP-binding protein; but did not report whether it could bind to the actin filament.

When snRNPs were extracted from the nuclear matrix with high salt buffer, they acted as 10S core particles in which there was no actin. Therefore, binding between the snRNPs and the snRNP-binding protein might be salt labile. If this is true, it is important to determine whether the snRNP binding protein is common to each snRNPs. When actin filaments in the nuclear matrix were depolymerized with a low salt buffer containing ATP, DTT, EDTA, calcium ion and 10% formamide, the bound snRNPs released had different sedimentation constants in sucrose density gradient centrifugation (26). This may signify that the snRNP binding protein(s) differs for the corresponding snRNPs.

It is also possible that the snRNPs are bound to the actin filaments by high molecular weight RNAs, such as pre-mRNA or pre-rRNA. These RNAs might well remain with the nuclear matrix after trypsin digestion because they are visible at the top of the polyacrylamide gel (Fig. 1). The snRNPs might bind to these RNAs by a specific sequence, e.g. by a consensus sequence. The binding between the snRNPs and the nuclear matrix, however, is salt labile. In contrast, the binding of these high molecular weight RNAs is resistant to a high salt concentration (unpublished data). The hydrogen bond is resistant to large amounts of salt. Therefore, we believe that the snRNPs may bind to the actin filaments through some protein(s) that has an affinity for both the snRNPs and actin filaments, not through the high molecular weight RNAs.

Are all of the nuclear matrix snRNPs associate with actin? The quantity of snRNPs bound on the nuclear matrix was not changed by trypsin digestion (Fig. 1). Therefore, our results indicate that all the snRNPs are bound to the actin filaments. The possibility, however, remains that part of the snRNPs are anchored to a protein filament(s) that is resistant to trypsin. Even if this is true, such snRNPs can be neglected because actin is the only major protein left in the trypsin-treated nuclear matrix.
We have little information about the function of the actin-bound snRNPs; probably they act in the transport and processing of pre-mRNA. Many reports have described the association of hnRNA with the nuclear matrix (10, 11, 32, 33, 34), and Calvet and Pederson (5) have described the association of snRNA with hnRNA. Pagoulatos and Yaniv (29) found actin in the hnRNP from normal and Simian-Virus-40 infected monkey kidney cells. Gounon and Karsenti (13) have reported that there are hnRNP-like particles associated with the actin filament in the nuclei of Pleurodeles waltlìi. On the basis of these findings and our own results, we believe that the processing and transport of pre-mRNA takes place with the hnRNP-snRNP-actin complex.

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