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Nuclear ribonucleoprotein release and nucleoside triphosphatase activity are inhibited by antibodies directed against one nuclear matrix glycoprotein

(nuclear envelope/nucleocytoplasmic transport/lectins/ATP-dependent RNA release/fibrous lamina pore complex)

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ABSTRACT **Circumstantial evidence suggests that nucleocytoplasmic exchange or transport is an active process involving the nuclear pore complex of the nuclear envelope. To test this hypothesis, antibodies were generated against nuclear envelope components from a highly enriched pore complex fraction from *Spisula solidissima* oocytes. Some of these antibodies inhibited ATP-dependent ribonucleoprotein release from prelabeled, isolated rat nuclei and inhibited nucleoside triphosphatase activity essential in nucleocytoplasmic transport. Inhibition of both functions by lectins indicated that the antigen was a glycoprotein. It was identified as lamin B, a major component of the nuclear envelope and nuclear matrix. This glycoprotein may not only be a structural nuclear protein but also may have nucleoside triphosphatase activity. We speculate that lamin B represents the solid support for ribonucleoprotein transport. This protein is expected to be highly conserved if active transport in and out of the nucleus is essential in the eukaryotic system.**

Exchange between the two major compartments of the eukaryotic cell appears to be controlled by the double membrane of the nuclear envelope (NE). Aqueous channels of 80-nm diameter are the putative location of macromolecular exchange. These nuclear pore complexes are highly structured components of the NE (1). Briefly, eight fibrils traverse the pore complex, and central rings and radiating fibers span the waist, effectively reducing the apparent diameter of 80 nm to a patent hole of <9 nm (2) through which proteins of up to 43,000 daltons can diffuse (3). The small pore size available for diffusion compared with the large hydrated diameter of many macromolecules suggests an active transport mechanism between the nucleus and cytoplasm.

Our previous hypothetical models of nucleocytoplasmic exchange (4) assume a dynamic nuclear framework for RNA synthesis and processing (5). Ribonucleoprotein(s) (RNP) might be actively transported from the site of synthesis through the gate to the cytoplasm along a solid-phase framework. Such a transport mechanism would require (*i*) a support upon which transport could occur, (*ii*) the generation of movement and directionality, and (*iii*) a selection of the molecules to be transported from the nucleus. The transport system then must be present also in the nuclear pore complex. The structural orientation of the traverse fibrils of the nuclear pore complex suggests a rail or cable transport system, whereas the radiating structures at the waist of the pore suggest a sieving function (1). NE from oocyte nuclei are a rich source of these possible transport structures (6). We raised antibodies against the NE and pore complex constituents and tested these probes for their ability to inhibit the flow of RNP from the nucleus. We chose the ATP-dependent RNP release (7–11) as an assay to screen for antibodies with inhibitory activity. This release assay responds to ATPase inhibitors (8) and is therefore enzyme dependent. The cross reactivity of those antibodies that inhibit ATP-dependent RNP release but do not precipitate released RNP then can be used to determine the proteins involved in transport.

METHODS

Isolation of Clam NE Proteins. Clam germinal vesicles were isolated from mature eggs of *Spisula solidissima* by established procedures (12), and nuclear subfractions were obtained by using a discontinuous sucrose gradient (6). The NEs, recovered at the 45–50% sucrose interface fraction, were used fresh or after extraction with 1 M NaCl/1% Triton X-100. The fibrous lamina pore complex (FLPC) fraction obtained was comparable with that from rat liver nuclei. Rat liver nuclei were isolated by the method of Blobel and Potter (13). The procedure of Kaufmann *et al.* (14) to obtain rat FLPC was modified to obtain NEs by omitting the detergent step and centrifuging the NEs on a step gradient at 20,000 rpm in a SW-27 rotor for 20 min after the 1.6 M NaCl extraction. NEs were collected as a broad band between 35% and 40% sucrose.

Solubilized fractions were separated electrophoretically on 7–18% $NaDodSO_4$/polyacrylamide gels by using the discontinuous buffer system of Laemmli (15). After electrophoresis, the gels were briefly stained. Protein bands (Fig. 1) were cut out and extracted in a small amount of 0.1% $NaDodSO_4$/1 mM dithiothreitol. After lyophilization, distilled water was added to obtain a 1% $NaDodSO_4$ solution. Nine parts of ice-cold acetone were then added, and the precipitated protein was collected by centrifugation.

Production of Antibodies to Clam NE Proteins. The protein fractions eluted from acrylamide gels were prepared for immunization in White Leghorn chickens or New Zealand rabbits. Crosslinked antigens (16) were injected intramuscularly in the breast and legs of chickens. Rabbits were injected subdermally with $NaDodSO_4$-denatured protein or with fresh NE or FLPC in 40% sucrose. Injections (total, three) were given at 7-day intervals, and animals were bled 1 week after the third injection.

Ammonium sulfate-precipitated immunoglobulin was chromatographed on an ion-exchange column (DEAE-cellulose). The immunoglobulin was dialyzed against borate/saline, pH 8.2, and stored at −20°C.

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Abbreviations: RNP, ribonucleoprotein(s); NE, nuclear envelope; FLPC, fibrous lamina pore complex; WGA, wheat germ agglutinin; Con A, concanavalin A; NTPase, nucleoside triphosphatase.

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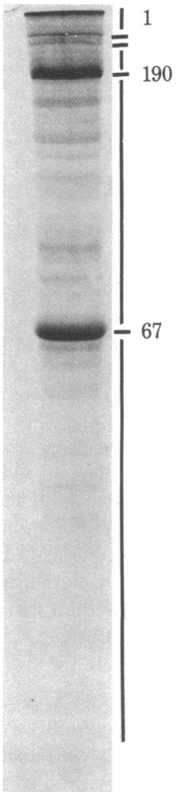

FIG. 1. The Coomassie blue-stained protein pattern shows the proteins of clam FLPC cut and eluted for injection into chicken and rabbits.

RNA Release Assay. For the RNA release experiments, 50 μCi (1 Ci = 3.7 × 10^{10} Bq) of [^{14}C]orotic acid (50 mCi/mmol; New England Nuclear) in sterile phosphate-buffered saline was injected intraperitoneally into rats weighing 150–200 g. Thirty minutes after injection, rats were sacrificed and liver nuclei were prepared (13) with 0.5 mM phenylmethylsulfonyl chloride and 5 mM iodoacetamide in the isolation buffers. Liver nuclei were washed in 50 mM Tris·HCl, pH 7.5/25 mM KCl/5 mM $MgCl_2$ containing 0.25 M sucrose and incubated with immunoglobulin or lectins [concanavalin A (Con A) or wheat germ agglutinin (WGA); Sigma] in the wash buffer for 15 min on ice. Test additives were then separated from nuclei by one washing with the same buffer. The nuclear pellets were resuspended in RNA release buffer containing 0.25 M sucrose, 10 mM KCl, 2 mM dithiothreitol, 2.5 mM $MgCl_2$, 1 mg of tRNA per ml in 50 mM Tris·HCl (pH 7.5) (10). The reaction was initiated by adding 2.5 mM ATP and allowed to proceed for 40 min at 32°C. Nuclei were removed by centrifugation, and the supernatant was precipitated with 10% trichloroacetic acid and assayed for radioactivity. Data from triplicate samples were normalized to cpm released per 1.5 × 10^6 nuclei. Approximately 4,000 cpm were released per sample with ATP and IgG obtained prior to immunization (preimmune IgG) added. Without ATP, about 2,000 cpm were released. The data in the tables are expressed as the percentage of ATP-dependent RNP (cpm released in the presence of ATP minus cpm released in its absence) from control nuclei. The pelleted nuclei were found to contain between 80% and 95% of the total counts depending on the test additives. The data from the release assay are the average of five experiments, except for the release experiments that used anti-rat lamin, which have been repeated twice. After the trichloroacetic acid-precipitable material was obtained from the supernatant, the quantity of P_i was determined in the resulting supernatant (17), and the data were expressed as μg of P_i released per mg of protein. Nucleoside triphosphatase (NTPase) activity was determined in a separate sample by continuously monitoring the oxidation of NADH in a Gilford spectrophotometer at a wavelength of 340 nm with a linked enzyme system (18). Rat NEs containing different antibodies were added to the tubes containing the enzyme system to begin the reaction. Reducing agents are present in the release buffer. This does not affect the antibody because, in the absence of denaturing agents, only 2 or 3 of the 23 disulfide bonds of the rabbit antibodies are reduced (19). As long as the number of reduced disulfide bonds is five or less, the antibodies maintain their specificity for the antigen (20).

Identification of Antigens on Nitrocellulose Blots. Polyacrylamide gels (7–18%) were transferred overnight at 250 mA to nitrocellulose sheets and stored in the blotting buffer transfer. Before reaction with the antibodies, all binding sites of the nitrocellulose sheets were covered with 20% bovine serum/3% bovine serum albumin/0.05% Tween in phosphate-buffered saline. The respective antisera were diluted 1:25 or 1:100 in this solution. Biotin-treated goat anti-rabbit antibodies were used as second antibody for 30 min at the manufacturer's (Vector Laboratories, Burlingame, CA) recommended concentration, followed by treatment with avidin-peroxidase in 1% calf serum in phosphate-buffered saline for 1 hr and three washes over 30 min before the substrate was added. For the demonstration of chicken antibodies, goat anti-chicken antibodies coupled to peroxidase were used (Cappel Laboratories, Cochranville, PA). This results in higher background and more nonspecific staining than in the case of the Vectastain system.

RESULTS

Initial testing of the ATP-dependent RNP release assay showed that slightly more than 50% of the released radioactivity ([^{14}C]orotic acid) was ATP-dependent in our hands. Rat nuclei exposed for 10 min to the preimmune IgG fraction showed the same ATP-dependent RNP release as did untreated nuclei. Rabbit anti-clam NE antibodies reduced the ATP-dependent release of RNP. Moreover, they also inhibited rat NE NTPase activity, which has been implicated in nucleocytoplasmic transport (8). Therefore, we raised antibodies against individual protein bands.

The inhibition of ATP-dependent RNP release obtained with the anti-clam antibodies is listed in Table 1. The chicken an-

Table 1. The effect of different antibodies on ATP-dependent RNP efflux and inorganic phosphate release

Antibodies	% ATP-dependent release	
	RNP	P_i
Chicken		
P IgG	100	100
P IgG*	0	0
Anti-clam 1	13	3
Anti-clam 67	100	100
Anti-rat lamin	12	20
Rat		
P IgG	100	100
P IgG*	0	0
Anti-clam NE	40	44
Anti-clam FLPC	104	100
Anti-clam 1	30	41
Anti-clam 190	100	100
Anti-clam 67	91	100
Anti-microsomes	100	95

P, preimmune (IgG prior to immunization). Isolated rat liver nuclei prelabeled (*in vivo*) with [^{14}C]orotic acid were incubated with the respective immunoglobulin fractions for 10 min on ice and washed once in 50 mM Tris·HCl, pH 7.5/25 mM KCl/5 mM $MgCl_2$/0.25 M sucrose before starting the reaction by resuspending the nuclei in the release buffer and adding ATP (reaction time, 40 min at 32°C). The percentage ATP-dependent release was calculated as described in *Methods*.
* Control without ATP.

tibody against high molecular weight components with the slowest mobility on the separating gel (anti-clam 1) gave the maximum inhibition, whereas chicken-derived anti-clam 67 showed no inhibition. This was surprising because Krohne *et al.* (21) claimed that the corresponding polypeptide in amphibian oocytes was the major constituent of the pore complex.

Though rabbit-derived anti-clam NE antibodies inhibited ATP-dependent RNP release, those against total FLPC did not. The FLPC is obtained from high-salt and detergent-treated NE; thus, the loss of antigenic activity could be due to the removal of antigens that are attached by salt-labile bonds or that reside in the intact membrane. However, antibodies with inhibitory activity were derived from those antigens of the separated FLPC proteins that barely entered the separating gel. The same component was also active when chicken was used to produce the antibodies. Thus, total FLPC does not induce inhibitory antibodies, but a high molecular weight fraction of FLPC does. None of these antibodies inhibited the ATP-independent RNP release. Antibodies against rat microsomes with the ribosomes removed were not inhibitory, excluding a crosslinked membrane as the site of inhibition. In this experiment, it was assumed that the outer nuclear membrane is equivalent to the rough endoplasmic reticulum and may generate movement at the pore waist. Antibodies against the 190,000-dalton protein doublet, the second most common clam NE protein, also did not inhibit ATP-dependent RNP release. However, antibodies against the salt and detergent extracts of clam NE inhibited both ATP-dependent RNP and inorganic phosphate release. When antibodies were raised against individual components of these extracts, again, only the high molecular weight component elicited inhibition.

Because the released P_i measured in our experiments could have been derived from sources other than ATP, we determined the quantity of ADP formation as a measure of NTPase activity (Table 2). After salt extraction (1.6 M NaCl) (14), rat liver NEs were assayed for NTPase (ADP formation) in the presence of antibody. When rabbit anti-clam NE and chicken anti-clam 1 antibodies were incubated with rat NE, the reduction in the quantity of ADP formation was similar to the reduction in the P_i released in our assays. This reduction was not seen with antibodies directed against clam protein 67.

We tested whether anti-clam 1 antibodies inhibited ATP-dependent RNA efflux by simply precipitating RNP in the nuclei. Even for incubation periods longer than those for intact nuclei, anti-clam 1 antibodies did not precipitate released RNP (data not shown). Crossreactivity also was tested by coating microtiter plates with the antibody and incubating the radiolabeled released RNP (≈10,000 cpm per well) for variable lengths of time at 5°C. Wells coated with anti-clam 1 antibody retained 168 cpm, whereas control wells coated with preimmune immunoglobulin contained 177 cpm. In parallel experiments, possible RNP degradation by the immunoglobulin fraction was examined by adding immunoglobulin to the radiolabeled released RNP and incubating under the same conditions. Only ≈15% of the acid-precipitable radioactivity was lost. Thus, anti-clam 1 antibodies do not crossreact immunologically with RNP, nor do they inhibit ATP-dependent RNP efflux by precipitating nuclear RNP.

Table 2. Comparison of phosphate release and ADP formation of rat NE NTPase

Antibodies	P_i, μg/mg of protein	NADH oxidized, mmol/hr per mg of protein
Control	8.4 ± 0.48	0.44 ± 0.02
Rat		
P IgG	9.0 ± 0.51	0.50 ± 0.01
Anti-clam NE	3.7 ± 0.25	0.30 ± 0.01
Chicken		
Anti-clam 1	4.0 ± 0.30	0.25 ± 0.07
Anti-rat lamins	5.1 ± 0.30	0.27 ± 0.03
Anti-clam 67	8.7 ± 0.40	0.47 ± 0.03

NTPase activity of isolated rat liver NE was continuously measured by monitoring the oxidation of NADH over a period of 60 min at 25°C. To start the reaction, NE incubated for 10 min with the respective antibody was added to the reaction buffer. The inorganic phosphate released was measured after precipitation of the proteins. These values reflect the amount of P_i after subtracting endogenous levels of P_i and hydrolyzed ATP as a result of nonenzymatic conditions.

Localization of Antigens Whose Antibodies Have Inhibitory Activity. Various cells were used for the localization of the anti-clam antibodies. The background staining with preimmune serum could not be overcome in whole clam eggs. However, isolated germinal vesicles showed strong NE and some intranuclear staining but no chromosomal staining for the chicken-derived anti-clam 1 antibody (Fig. 2*a*). Chromosomes are completely condensed in these nuclei and could be visualized by using antibodies directed against different sections of the FLPC protein spectrum (Fig. 2*b*). In bovine fetal aorta endothelial cells, a strong nuclear rim staining was seen with little or no cytoplasmic background for the anti-clam 1 antibody (Fig. 2*d*). This distribution corresponds to the one shown by others for NE-derived anti-lamin antibodies (21–22). Nuclei not completely flat showed fluorescence throughout.

Lectin Inhibition of ATP-Dependent RNP Release. The delipidated rat NEs reportedly contain 3–4% total carbohydrate (23). If proteins containing carbohydrate were involved in transport activity, they could be crosslinked with lectins and in this way inhibit RNP release. Both lectins tested, Con A and WGA, inhibited ATP-dependent RNP release and the oxidation of NADH, indicating inhibition of ADP formation (Table 3). This result indicates that a carbohydrate-containing macromolecule is involved in RNP release and NTPase inhibition. The lectins did not agglutinate the nuclei under our conditions. Because the major glycoprotein of the NE or matrix is a species

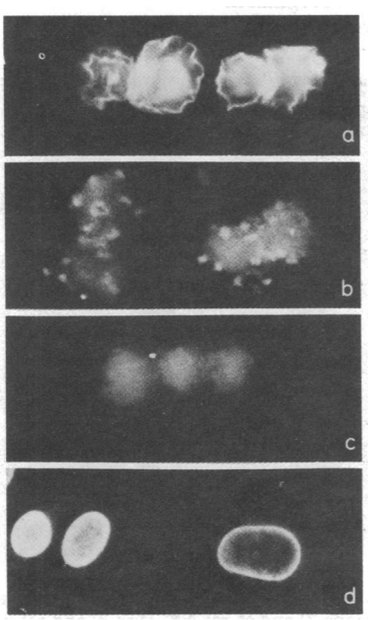

FIG. 2. (*a*) Micrograph of isolated clam germinal vesicles processed for indirect immunofluorescence localization with chicken-derived antibodies against clam 1 antigens, showing strong NE staining but no chromosomal staining. (*b*) When chicken-derived antibodies against those proteins are used that migrate faster than the 67,000-dalton clam NE protein, chromosomes but not NEs are labeled. (*c*) Preimmune chicken IgG served as a control and shows some background staining inside the nucleus. (*d*) When bovine fetal aorta endothelial cells are used with chicken-derived anti-clam 1 antibody, the nuclear periphery is fluorescent. (*a–d*, ×430.)

Table 3. The effect of lectins on RNP efflux and NTPase activity of rat liver nuclei

Conditions	% ATP-dependent RNP release	NADH, mmol/hr per mg of protein
Control nuclei	100	100
Control nuclei*	0	0
Nuclei + Con A		
0.5 mg/ml	19	46
1.0 mg/ml	5	37
Nuclei + WGA		
0.5 mg/ml	2	53
1.0 mg/ml	1	45

Prelabeled ([^{14}C]orotic acid) rat liver nuclei were preincubated on ice for 15 min with the test additives and washed once in 50 mM Tris·HCl, pH 7.5/25 mM KCl/5 mM $MgCl_2$/0.25 M sucrose before resuspension in the release buffer and initiation of the reaction by addition of ATP (40 min at 32°C).
*No addition of ATP.

with an apparent molecular mass of ≈180,000 daltons (24, 25) and because our antibodies against these species did not inhibit those functions, a protein with minor carbohydrate content seems to be involved.

Identification of Antigen Reacting with RNP Release-Inhibiting Antibodies. Because of their previous extractions, all of the NE proteins and those of the FLPC were insoluble in solutes used for immunoprecipitation. Therefore, we used the Southern technique (26) to transfer separated proteins of four fractions onto nitrocellulose sheets. The proteins of the total clam nuclei and NE and the corresponding fraction from rat were separated in sufficient quantity to allow their visualization on Coomassie blue-stained gels (Fig. 3*a*). Isolated nuclei were deemed essential as controls because they contain those antigens that are eliminated during the extraction procedure. Crossreactive antigens in these evolutionarily distant species could then be recognized.

The total clam NE antibody reacted with most clam NE proteins (even those not seen with the Coomassie blue staining), attesting to the sensitivity (Fig. 3*b*) of the assay. Surprisingly, only one major protein band of the rat NE, the largest of the three lamins, crossreacted. The second lower molecular weight protein appeared to be a breakdown product. This was shown by two-dimensional gel electrophoresis, where it had the same apparent isoelectric point as the largest of the three lamins and migrated as two charged isomers (not shown). This protein was also not present in total rat nuclei. The clam anti-67 antibody gave the reaction product at the expected place (Fig. 3*c*) and did crossreact with the two fastest migrating rat lamins and with their breakdown products. In our hands the two fastest migrating lamins corresponded to the two more basic lamins A and C of Gerace *et al.* (22), and the largest lamin corresponded to the more acidic lamin B as determined by two-dimensional gel electrophoresis (unpublished data). Antibodies to a fraction containing the second most prominent clam NE protein (≈190,000 daltons) reacted at their position but also crossreacted immunologically with rat lamins A and C (Fig. 3*d*). The major clam NE protein, then, is antigenically related to lamins A and C and not, as we had previously assumed, to lamin B (6). The chicken-derived antibody against clam high-dalton material (anti-clam 1) crossreacted with all three rat lamins (Fig. 3*e*) and with a few other proteins derived from the clam and rat NE and nucleus.

The results then show that antibodies against clam antigen that crossreact with all three rat lamins and the one identified as anti-rat lamin B alone inhibit ATP-dependent RNP release and NTPase activity; antibodies reacting with the 67,000-dalton clam NE protein and those reacting with lamins A and C have no inhibitory activity. Thus, it seems likely that the interaction of anti-clam antibodies with rat lamin B accounts for the observed inhibition of RNP release and NTPase activity.

One prediction then was that antibodies prepared against rat lamins would inhibit the same activities. As shown in Table 1, those antibodies reduced the ATP-dependent RNP release and NTPase activity, further implicating lamin B in RNP translocation. If, in fact, the antibodies and the lectins reacted with the

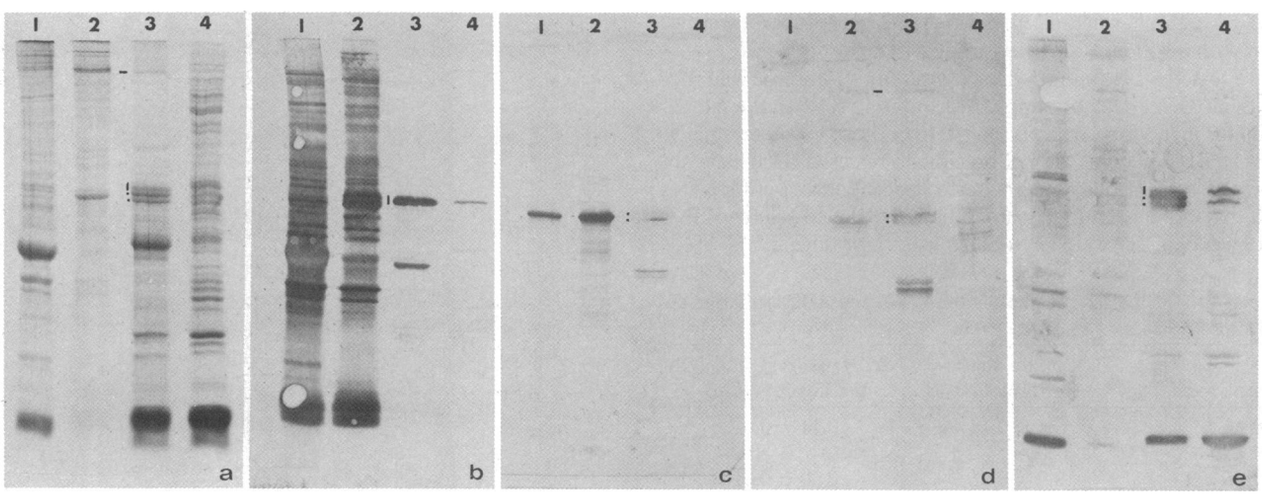

FIG. 3. Immunological characterization of proteins from nuclei and NE from rat and clam. (*a*) Coomassie blue staining of clam nuclei (lane 1) and clam NE (lane 2) and of rat NE (lane 3) and rat nuclei (lane 4). The same sequence was used on gels transferred to nitrocellulose sheets. (*b*) Test for crossreactivity of antibodies against total clam NE. In the clam fractions, most proteins react, whereas in the rat fractions, only one protein (lamin B) crossreacts. The lower band in lane 3 is a breakdown product of lamin B. (*c*) Test for crossreactivity of the anti-clam 67 antibody. The clam fractions show only one major band. The many minor bands in lane 2 are considered breakdown products. In rat NE, crossreactivity exists with two proteins, lamins A and C, as well as with their breakdown products. These two bands are also present in the nuclear fractions. (*d*) Test for crossreactivity with antibodies generated against the 190,000-dalton major protein doublet. Only a faint reaction product is seen at this site in clam NE, but the 67,000-dalton protein reacts, as do lamins A and C and their breakdown products. Two faint lines in lane 4 are present at that general location. The combined results shown in *c* and *d* indicate the antigenic relatedness between the lamins A and C and clam NE protein 67. (*e*) Test for crossreactivity with antibodies generated against slow-moving proteins at the top of the polyacrylamide gel (anti-clam 1 antibodies). Reaction products were seen over several clam protein bands and over all three rat lamins. The rat nuclei showed only two bands that crossreacted at the level of the lamins.

same protein in order to achieve release and enzyme inhibition, then lamin B must be a glycoprotein.

DISCUSSION

We have developed probes that prevent ATP-dependent RNP release from prelabeled isolated rat liver nuclei. Antibodies against only one protein from the total unextracted clam NE crossreacted with its counterpart in a rat nuclear fraction, and this antibody apparently prevented RNP release. The crossreacting rat antigen was identified as the lamin B (27). This result was confirmed by demonstrating that antibodies against rat lamins also have inhibitory activity. However, antibodies crossreacting with the rat NE proteins, lamins A and C, are not inhibitory.

Our results show that the clam NE contains antigens in common with the three rat lamins. Finding these antigens at the top of the separating gel (for all three lamins) and at the position where 190,000-dalton proteins would migrate (for lamins A and C) may be explained by the tendency of the rat lamins as well as the 67,000-dalton major clam NE protein to form polymers by disulfide interchain crosslinking.

The assay for lectin inhibition of ATP-dependent RNP release was done initially to determine whether inhibition would result if glycoproteins associated with the nuclear membranes (23) could be crosslinked. Only one of those lectins, Con A, binds to the nuclear membranes as judged by electron microscopy (28), whereas both WGA and Con A reduce the activity of NTPase and ATP-dependent RNP efflux. That the membrane was not involved was further suggested in the experiments with antibodies against microsomal membranes. Crosslinking of the outer nuclear membrane (the morphological equivalent of the rough endoplasmic reticulum) would have prevented transport if the outer nuclear membrane were directly involved.

By assuming that the two functions inhibited by the antibody and the lectin inhibit by attachment of these agents to the same protein, it followed that lamin B was a glycoprotein. This prediction was confirmed by direct incorporation studies (unpublished data).

At present we cannot say precisely what functions our antibodies inhibit. The assay used is that of ATP-dependent RNP release. We could clog the nuclear pore complex by crosslinking the orifice with anti-lamin B antibodies, which would suggest that lamin B is a constituent of the pore complex. This is contrary to the finding of Gerace *et al.* (22). However, unless lamin B is the NTPase, or if NTPase activity and RNP release are highly coupled, crosslinking with anti-lamin B antibodies does not explain why both functions are inhibited simultaneously. We could crosslink the solid support inside the nucleus upon which, according to our hypothesis, the transport takes place, implying that the nuclear matrix components contain lamin B. Gaps in the damaged nuclei must provide the access for antibodies because these molecules do not enter the nucleus through the pore complex (2, 3). Evidence for release through pore complexes despite severe gaps in the NEs comes from the work of Feldherr and Ogburn (29) with experimentally ruptured *Xenopus* germinal vesicles. Moreover, removal of the nuclear membranes by detergents does not increase the RNP release over that with intact NEs, implicating the pore complex as the site of release (30).

The question then, is whether the nuclear matrix might be the support structure for RNP transport to the pore complex. Because RNA is synthesized and processed there (5), movement also could be envisioned. The presence of all lamins throughout the nucleus would strengthen such a possibility. The available ultrastructural immunohistochemical evidence from isolated nuclei favors the concentration of the lamins at the nuclear periphery (22), but nuclei fixed *in situ* often show enough specific staining to suggest the presence of all lamins at lower density throughout the nucleus.

The NE contains kinase (31) and phosphatase activity which are thought to be part of the NTPase system (7, 32). Because the anti-lamin B antibodies also inhibit NTPase activity, lamin B might have enzyme activity. The extreme difficulties in solubilizing the NTPase activity from NE and matrix preparation experienced by Agutter *et al.* (8) may be due to the tendency of lamin B to form high molecular weight polymers (33). At present we have no evidence for the involvement of lamin C in the RNP transport as suggested by McDonald and Agutter (7). Experiments are needed to determine whether transport is blocked by inhibiting movement through crosslinking lamin B or by inhibiting the site of enzymatic activity of the same molecule. Several of these experiments and unequivocal proof that lamin B is involved in transport will come when monospecific anti-lamin B is available.

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