Ultrastructural Distribution of Nuclear Ribonucleoproteins as Visualized by Immunocytochemistry on Thin Sections

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ABSTRACT The ultrastructural distribution of nuclear ribonucleoproteins (RNP) has been investigated by incubation of thin sections of mouse or rat liver, embedded in Lowicryl K4M or prepared by cryoultramicrotomy, with antibodies specific for RNP. The antibodies were localized by means of a protein A-collodial gold complex. Anti-small nuclear (sn)RNP antibodies, specific for determinants of the nucleoplasmic snRNP species containing U1, U2, U4, U5, and U6 RNAs, were found associated preferentially with perichromatin fibrils, interchromatin granules, and coiled bodies. This indicates an early association of snRNP with structural constituents containing newly synthesized heterogeneous nuclear RNA. It also suggests a possible structural role of some snRNPs in nuclear architecture.

Antibodies against the core proteins of heterogeneous nuclear RNP particles associate preferentially with the border regions of condensed chromatin, and in particular with perichromatin fibrils and some perichromatin granules. These results are discussed in view of recent knowledge about the possible role of nucleoplasmic RNP-containing components in the functions of the cell nucleus.

MATERIALS AND METHODS

Material: Mouse or rat livers were fixed for 1 h with 0.5 or 1% glutaraldehyde in Sorensen phosphate buffer (0.1 M), pH 7.3, at 4°C. The pieces of tissue were then thoroughly washed in the same buffer. One part of the material was dehydrated through a series of ethanol concentrations at low temperature and embedded in the resin Lowicryl K4M (1, 14). Another part of the tissue was subjected to cryoultramicrotomy (32). The following antibodies were used for immunolabeling: (a) total human immunoglobulin from an anti-Sm (for definitions see 31, 12) obtained from a patients suffering from mixed connective tissue disease (courtesy of Dr. H.-J. Lakomek, Medizinische Klinik and Poliklinik, Universität Düsseldorf). This Ig precipitates U1, U2, U4, U5, and U6 snRNAs from cell extracts by virtue of protein determinants bound to these RNAs. The Ig binds to several polypeptides of molecular weight 25,000–35,000 on immunoblots of electrophoretically-separated cellular proteins; (h) mouse

Abbreviation used in this paper: RNP, ribonucleoproteins; sn, small nuclear RNA; hn, heterogeneous nuclear RNA.
monoclonal IgG of anti-Sm type from a hybridoma derived from an auto-
immune mouse (courtesy of Drs. Joan Steitz and Charles Janeway, Yale
University, New Haven, CT). This antibody has been characterized by Lerner
et al. (16) and precipitates snRNP containing Us, U2, U4, U5 and U6
snRNAs. The antibody used in these experiments was obtained from ascites fluid; (c)
Mouse monoclonal IgM from a hybridoma formed from spleen cells of a mouse
immunized with chicken 35S hnRNP core complexes and specific for the
hnRNP core group polypeptides of molecular weight 34,000-40,000 (17; and Leser,
Escaré-Wilke, and Martin, submitted for publication). This monoclonal IgM
binds protein A when associated with its antigen, and in terms of polypeptide
specificity and localization of antigens by immunofluorescence is essentially
identical to the previously reported polyclonal anti-hnRNP antibodies (13, 19).

Experimental Procedures: Ultrathin sections of Lowicryl-embedded liver
were placed on nickel grids and allowed to dry. They were then preincu-
bated in 1% ovalbumin in PBS for 15 min at room temperature, and the excess
of liquid was carefully removed and the grid was floated on a drop of the
incubation mixture consisting of diluted antibody (1:200-1:1,000, i.e., 50-100
μg protein/ml) in PBS with 1% ovalbumin. The incubation usually takes place
at 4°C, for 17 h, in a humid chamber. When human anti-Sm immunoglobulin
was used, herring sperm DNA (Sigma Chemical Co., St. Louis, MO, 500 μg/
ml) was added to adsorb traces of anti-DNA activity often present in these sera.
The preparations were then thoroughly washed with PBS and postincubated
for 1 h with the protein A-collodial gold complex (mean particle diameter 15
nm) at room temperature. After another wash with PBS, the grids were rinsed
with distilled H2O and dried. This procedure corresponds essentially to that
described by Roth (28). The sections were then stained with uranyl acetate
(2% aqueous) for 5 min and counterstained for 30 s with lead citrate.

When ultrathin cryosections were used, the nickel grids with sections were
first washed with phosphate buffer to remove sucrose. They were briefly soaked
with PBS-0.01 M glycine, incubated for 10 min with 5% BSA in PBS-glycine,
and then rinsed with PBS-glycine. The immunolabeling with the antibody
(1:200) was performed in PBS for 20 min at room temperature. The grids were
then extensively washed with PBS-glycine, PBS alone, and then floated on the
protein A-gold solution for 20 min. After this step, the preparations were again
thoroughly washed with PBS then with distilled H2O and stained directly
with uranyl acetate and lead citrate. Some grids were stained by the EDTA procedure
(4), as modified for cryosections (11).

Control experiments were carried out by incubating the sections with anti-
bodies preadsorbed for 30 min with the appropriate antigens: a 10-5 S nuclear
fraction for anti-Sm antibodies and a 30-5 S RNP fraction for the anti-hnRNP
core protein antibody isolated from sucrose density gradients essentially as in
(26). In addition, some sections were also incubated with the protein A-gold
complex alone. All the preparations were observed in a Philips EM 400 electron
microscope at 60 or 80 kV using a 40-10 μm objective aperture.

A quantitative evaluation of the distribution of labeling was performed on the
resin sections labeled with the human anti-Sm serum: nucleolus, 3.56 ± 0.94; condensed
chromatin, 1.54 ± 0.41; nucleoplasm, 8.84 ± 0.59; cytoplasm, 2.74 ± 0.37; (b) ultrathin frozen sections labeled with anti-
hnRNP monoclonal IgM, nucleolus 2.17 ± 0.31; condensed
chromatin, 4.77 ± 0.53; nucleoplasm, 23.79 ± 1.45; cyto-
plasm, 3.22 ± 0.47. Although these determinations show the
preferential labeling of the nucleoplasm by antibodies to both
hnRNP and snRNP (Wilcoxson signed rank, p < 0.01), they
do tend to underestimate the specificity of localization. In
effect, most of the label was concentrated on structures oc-
cupying only a limited minor portion of the nucleoplasm, the
surface of which is, e.g., for perichromatin fibrils, hardly
measurable. In addition, since it has been demonstrated that,
at least in the resin sections, the protein A-gold labeling is
restricted to the surface of the sections, only antigenic sites
exposed at the section surface were revealed. In ultrathin
cryosections, some penetration of antibodies might occur,
probably depending on the density of the cellular structural
components (for discussion of these questions, see reference
28).

RESULTS

The analysis of sections after Lowicryl embedding is favored because they display,
after a simple uranyl-lead staining, a contrast comparable with that of Epon sections stained by the
differential EDTA method for nuclear nucleoproteins (4). Consequently, chromatin appears grey-clear, whereas the
RNP-containing constituents exhibit high contrast and are easily
recognizable. The nuclear morphology is very well
preserved compared with routinely-used plastic embedding
media.

The results of control experiments using preadsorption of antibodies with the corresponding antigens on one hand, and
incubation with the protein A-collodial gold complex alone
on the other hand, are both negative, leaving only a very low
background.

Distribution of Anti-snRNP Antibodies

The patterns of topological distribution of the human anti-
Sm and of the anti-Sm monoclonal antibodies were identical.

The reactivity of the former was, however, much stronger,
presumably because more determinants are recognized.
The label did not occur in the nucleolar region and within
condensed chromatin areas. The antibodies were most
frequently associated with the clusters of interchromatin granules (Figs. 1 and 4) and with perichromatin fibrils (Figs. 1 and 2).
In the nuclei of mouse as well as rat liver, where a coiled body
was observed (~10-20% of nuclear sections examined), it
was usually strongly labeled as well (Fig. 3). Perichromatin
granules, which are found as individual structures or in small
groups, were in general only rarely labeled or remain unla-
beled (Figs. 1 and 2). The labeling of the cytoplasm was
generally low, except that some labeling could be detected in
erythrocytes.

Distribution of the Anti-hnRNP Core
Protein Antibody

This monoclonal antibody associated preferentially with
perichromatin fibrils (Fig. 6) and with perichromatin granules
(Fig. 7). It reacted strongly on ultrathin frozen sections, where
the gold particles decorated especially the perichromatin
region (Fig. 5). In these sections, the perichromatin fibrils were,
however, hardly distinguishable (11, 23). Coiled bodies often
appeared labeled in both types of sections, but with lower
intensity than after reaction with anti-snRNP antibodies. Clusters of interchromatin granules were occasionally labeled.
The nucleoli and areas of condensed chromatin remained
unlabeled. Cytoplasmic labeling was generally low with some
preference for mitochondria, and some labeling could be
observed in erythrocytes present in the liver tissue.

Quantitative Analysis of Antibody Distribution

The quantitative evaluation of the gross labeling distribution
on our cell sections reveals respectively the following
gold particle densities (±SEM): (a) resin sections labeled with
human anti-Sm serum: nucleolus, 3.56 ± 0.94; condensed
chromatin, 1.54 ± 0.41; nucleoplasm, 8.84 ± 0.59; cytoplasm,
2.74 ± 0.37; (b) ultrathin frozen sections labeled with anti-
hnRNP: monoclonal IgM, nucleolus 2.17 ± 0.31; condensed
chromatin, 4.77 ± 0.53; nucleoplasm, 23.79 ± 1.45; cyto-
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preferential labeling of the nucleoplasm by antibodies to both
snRNP and hnRNP (Wilcoxon signed rank, p < 0.01), they
do tend to underestimate the specificity of localization. In
effect, most of the label was concentrated on structures oc-
cupying only a limited minor portion of the nucleoplasm, the
surface of which is, e.g., for perichromatin fibrils, hardly
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at least in the resin sections, the protein A-gold labeling is
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exposed at the section surface were revealed. In ultrathin
cryosections, some penetration of antibodies might occur,
probably depending on the density of the cellular structural
components (for discussion of these questions, see reference
28).

DISCUSSION

This work makes the following novel observations concerning
the presence of RNP components within nucleoplasmic struc-
tures: (a) perichromatin fibrils contain snRNP as well as
hnRNP antigens; (b) perichromatin granules (or at least a
certain proportion of them) contain hnRNP, but only little

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Figures 1–3 Sections of mouse liver embedded in Lowicryl K4M and labeled with human anti-Sm serum. Bar, 0.5 μm. Fig. 1: An overview of a nucleus showing label associated preferentially with perichromatin fibrils (small arrows) and clusters of interchromatin granules (large arrows). The nucleolus (nu) and areas of condensed chromatin (c) remain unlabeled. × 30,000. Fig. 2: A detail of a nucleoplasmic region with labeled perichromatin fibrils (arrows) and occasional labeled perichromatin granules (arrowhead). × 53,800. Fig. 3: A detail of a labeled coiled body. × 49,500.
The perichromatin fibrils provides additional evidence concerning that period (11). In addition, the frequency of perichromatin fibrils and their RNA undergoes processing to avoid confusion.

The term "interchromatin fibrils" used by certain authors (25), all the fibrils are morphologically identical, we do not employ it any more. Towards the interchromatin space (11, 24), and because the migration of fibrils formed in the perichromatin areas (11, 22) towards the interchromatin space (11, 24), and because all the fibrils are morphologically identical, we do not employ the term "interchromatin fibrils" used by certain authors (25), to avoid confusion.

It has been previously demonstrated that perichromatin fibrils contain newly synthesized hnRNA (2, 11). After their formation the majority of the fibrils migrate towards the interchromatin space and their RNA undergoes processing during that period (11). In addition, the frequency of perichromatin fibrils in the nucleus reflects the rate of hnRNA synthesis in different cell systems (9; for review see reference 6). The fact that the anti-hnRNP core protein antibodies label the perichromatin fibrils provides additional evidence confirming the hnRNP nature of these constituents.

As far as the perichromatin granules are concerned, their role in nuclear functions still remains obscure. Hypotheses have been proposed as to their possible role in the transport of pre-mRNA within the nucleus (21), or in its intranuclear storage (9); in addition, it is not clear whether there are several populations of perichromatin granules with regard to the type of RNA that they contain (for review see references 10 and 25). Preliminary attempts to isolate biochemically the perichromatin granules have indicated that they may contain snRNP; (c) interchromatin granules and coiled bodies carry high amounts of snRNP. The latter constituents also contain a small quantity of hnRNP. The term "perichromatin fibrils" as used here includes all nucleoplasmic RNP fibrils regardless of whether they appear in the perichromatin areas or within the interchromatin space. Since it has been shown that the fibrils appearing within the interchromatin space result from the migration of fibrils formed in the perichromatin areas (11, 22) towards the interchromatin space (11, 24), and because all the fibrils are morphologically identical, we do not employ the term "interchromatin fibrils" used by certain authors (25), to avoid confusion.

The finding of snRNPs within clusters of interchromatin granules might suggest a storage role for this type of RNA in these constituents, a hypothesis in agreement with previous kinetic studies showing only a low level of [3H]uridine labeling in clusters of interchromatin granules (7, 8) and suggesting the presence of rather stable RNA species. The reaction of anti-snRNP antibodies with clusters of interchromatin granules may be the source of the commonly observed speckled pattern obtained in immunofluorescence studies with autoimmune disease sera (31, 16). Since interchromatin granules are the only clearly recognizable nucleoplasmic component within the network called "the inner nuclear matrix" (3, 20), a possible structural role of the snRNPs in nuclear architecture cannot be excluded.

Our present observations about the occurrence of snRNP and also of hnRNP antigens in the coiled body represent a new finding about this enigmatic structure, described many years ago as an RNP containing nucleoplasmic constituent (21). The possible function of this nuclear structure remains, for the moment, completely unclear.

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FIGURES 5-7  Sections labeled with anti-hnRNP core protein monoclonal antibody. Bar, 0.5 μm. Fig. 5: A general view of a rat liver cell nucleus from a section prepared by cryoultramicrotomy and stained by the EDTA technique. The contrast of condensed chromatin (c) is reduced. The nucleolus (nu) and the clusters of interchromatin granules (arrows) are unlabeled. Labeling is associated predominantly with the border of condensed chromatin areas. ×31,300. Figs. 6-7: Lowicryl sections of mouse liver showing association of label with the fine network of perichromatin fibrils (Fig. 6, arrows), and with some perichromatin granules (Fig. 7, arrows). × 64,500.
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