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Isolation of the heterogeneous nuclear RNA–ribonucleoprotein complex (hnRNP): A unique supramolecular assembly

(RNA processing/nuclear proteins/monoclonal antibodies/immunoprecipitation)

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ABSTRACT **The packaging of heterogeneous nuclear RNA (hnRNA), the fate of hnRNA in the nucleus, and the conversion of hnRNA to mRNA are believed to occur as the hnRNA transcript is associated with specific proteins to form a ribonucleoprotein complex termed the hnRNP complex. The identity and organization of the protein constituents of the hnRNP have been a matter of considerable controversy. We report here the isolation of the hnRNP complex from vertebrate cell nuclei, employing immunoprecipitation with monoclonal antibodies against the major proteins that are in contact with hnRNA in HeLa cells. Rapid immunoprecipitation from HeLa nucleoplasm with two different monoclonal antibodies to the hnRNP C proteins (41 and 43 kDa) isolates a similar complex that contains proteins and hnRNA of up to ≈10 kilobases. The major steady-state [^{35}S]methionine-labeled proteins of the isolated complex are of 34 kDa, 36 kDa (A_1 and A_2), 37 kDa, 38 kDa (B_1 and B_2), 41 kDa, 43 kDa (C_1 and C_2), and doublets at 68 kDa and at 120 kDa. Additional proteins from 45 kDa to very high molecular mass are also seen. The major proteins of the complex appear identical by NaDodSO$_4$/polyacrylamide gel electrophoresis to genuine hnRNP proteins—those which become crosslinked by UV light to the hnRNA *in vivo*. Immunoprecipitation with a different, noncrossreacting monoclonal antibody to the 120-kDa protein isolates an apparently identical complex of proteins that are present at a similar relative stoichiometry. Similar hnRNP complexes are found in rodent and avian cells. Nuclease digestions indicate that RNA plays a role in maintaining the integrity of the structure and that intact RNA of ≈125 nucleotides is sufficient to hold the complex of proteins together. The coimmunoprecipitation of the hnRNA and of all of the proteins through antibodies against different genuine hnRNP proteins and from divergent species strongly suggests that the hnRNP complex is a unitary structure of consistent, defined, and conserved components.**

Heterogeneous nuclear RNA (hnRNA) is found in the eukaryotic nucleus associated with proteins to form higher order structures that are referred to as heterogeneous nuclear ribonucleoprotein complexes (hnRNPs) (1–18). It is widely believed that the hnRNPs are the structural entities that serve as substrates for hnRNA processing into mRNA or hnRNA degradation. hnRNPs or their components can be released from nuclei by mechanical disruption (e.g., sonication) or by limited RNase digestion (endogenous or exogenous RNases). The material obtained by sonication is substantially larger and sediments in sucrose gradients as heterodisperse material between 40 S and 250 S (1–6, 8, 12–17). Most of it is converted by mild RNase treatment to a form that sediments under the same conditions at 40 S, similar to the material that is released from nuclei by RNase (4, 5). The appearances by electron microscopy of the sedimented 40S particles (4–6) and of particles seen on nascent transcripts in spread chromatin preparations (19–24) were taken together to suggest a "beads on a string" structure for the hnRNA with its associated proteinaceous particles (3, 4, 7, 15, 17). One of the central and most controversial issues in the study of hnRNPs concerns the complexity (number and molecular weight) and arrangement of their protein components. Progress along these lines has been hampered to a large extent by the limitations of the commonly used experimental tools such as sedimentation in sucrose gradients and isopycnic banding in Metrizamide (5), which have not resulted in the complete isolation of intact hnRNPs or in the definitive identification of their proteins (for reviews, see refs. 7 and 15).

A different and more stringent definition of RNP proteins that overcomes the shortcomings encountered by the *in vitro* approaches involves identification of proteins in direct contact with RNA *in vivo* by UV-induced RNA–protein crosslinking in intact cells (25–32). The RNA of interest can then be isolated with its crosslinked proteins under protein denaturing conditions so as to ascertain that the proteins that do copurify with it are genuine RNP proteins in that they must have been directly associated with the RNA *in vivo* in order to have become crosslinked to it by UV light. The RNA-contacting proteins have been identified after RNase digestion of the RNA (25–32). To obtain probes for these proteins, we have recently described a general approach to generate monoclonal antibodies to genuine RNP proteins (32). Purified UV-crosslinked complexes of poly(A)$^+$ RNA with proteins were obtained from HeLa cells and were injected into mice. The antibody-producing cells from immunized animals were fused with myeloma cells to generate a library of hybridoma colonies secreting monoclonal antibodies to the RNA-associated proteins (32). The properties and intracellular localization of some of the major hnRNP proteins were studied with these antibodies (32). In the work described here, we have used several of these monoclonal antibodies to hnRNA-associated proteins as immunoaffinity reagents to isolate the hnRNP complex. Preliminary characterization of the isolated hnRNP structure enables the identification of its protein components and reveals some of its structural features.

MATERIALS AND METHODS

Cell Culture and Labeling. HeLa S_3 (human) cells, Chinese hamster ovary cells (CHO), and chicken cells (MSB) were cultured to subconfluent densities as described (32). Cells were labeled with [^{35}S]methionine at 20 μCi/ml (1 Ci = 37 GBq) for 20 hr in Dulbecco's modified Eagle's medium containing 1/10th the normal methionine level and 5% fetal calf serum. Labeling with [5,6-^{3}H]uridine (50 μCi/ml) was for 15 min in the presence of 0.04 μg of actinomycin per ml added

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Abbreviations: hnRNA, heterogeneous nuclear RNA; hnRNP, heterogeneous nuclear ribonucleoprotein complex.

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15 min prior to the addition of the isotope.

Preparation and Analysis of UV-Crosslinked Poly(A)$^+$ hnRNPs. Irradiation of cells on culture dishes and isolation and analysis of RNPs was carried out as recently described (31, 32).

Cell Fractionation. Procedures for preparation of the nucleoplasmic fraction were essentially according to Pederson (2). All steps were carried out at 0–4°C as rapidly as possible. The culture medium was removed and the cell monolayers were washed twice with cold phosphate-buffered saline (P_i/NaCl). Cells were scraped with a rubber policeman in 1 ml per 10-cm plate of cold buffer A (10 mM Tris·HCl, pH 7.4/100 mM NaCl/2.5 mM $MgCl_2$) containing 0.5% aprotonin (Sigma), 2 μg of pepstatin A per ml, 2 μg of leupeptin per ml, and 0.5% Triton X-100 and homogenized by four passages through a 25-gauge needle. The nuclei were separated by a brief spin at 3000 × g, resuspended in 0.5 ml of cold buffer A containing 0.5% aprotonin, 2 μg of pepstatin A per ml, and 2 μg of leupeptin per ml, and sonicated twice for 5 sec each using a microtip sonicator (model w-220 F, Heat System/Ultrasonics, Plainview, NY) set at scale 2 on ice. The sonicate was layered on a 30% sucrose cushion in buffer A and centrifuged at 5000 rpm (4000 × g) for 15 min in the HB-4 rotor on an RC5 Sorvall centrifuge to remove chromatin and nucleoli. The supernatant fraction that is operationally defined as the nucleoplasm was taken and used for immunoprecipitation.

RNase Digestion. Digestion with RNase of the nucleoplasmic fraction was carried out with micrococcal nuclease (P-L Biochemicals) at 2 or 100 units/ml for 10 min at 30°C in the presence of 1 mM $CaCl_2$. The reaction was stopped with 5 mM EGTA.

Preparation of Monoclonal Antibodies to UV-Crosslinked RNPs. Procedures were as described recently (31, 32). Purified poly(A)$^+$ material from UV-irradiated HeLa cells (nuclei and cytoplasm) was used for immunizations of BALB/c mice (32). The UV-crosslinked RNPs were partially digested with RNase A and with micrococcal nuclease (32). Hybridomas were formed by fusions with Sp2/0 myeloma cells, and tissue culture, cloning, and screening procedures were essentially as described (33, 34). Ascites fluids were prepared by intraperitoneal inoculation of hybridomas into pristane-primed BALB/c mice.

Immunoprecipitation. The hnRNP complex was immunoprecipitated from the nucleoplasm for 10 min at 4°C with monoclonal antibody 4F4, 2B12, or 3G6 bound to protein A–agarose. These monoclonal antibodies bind to *Staphylococcus aureus* protein A (35) directly. Ascites fluids containing the antibodies were passed through an aminophenylphosphouridine 2′(3′)-phosphate–agarose column to remove possible RNase (36), and then 2.5 μl of the antibody was bound to 25 μl of protein A–agarose (Boehringer Mannheim) for 1 hr at 4°C in buffer A containing 0.5% Triton X-100. The antibody–protein A–agarose complex was washed three times with buffer A and incubated with the nucleoplasm for 10 min at 4°C with gentle rocking. The beads were washed five times by resuspension in 1-ml aliquots of the same buffer and the bound material was eluted from the agarose beads with 50 μl of NaDodSO$_4$/PAGE sample buffer (31) for protein analysis or with 300 μl of TEL buffer (200 mM Tris·HCl, pH 7.4/25 mM EDTA/100 mM LiCl) containing 1% NaDodSO$_4$ for RNA analysis. The RNA was extracted twice with phenol/chloroform/isoamyl alcohol after digestion with proteinase K (350 μg/ml) for 15 min at 37°C and precipitated with 3 vol of cold ethanol at −20°C overnight. Standard immunoprecipitations in the presence of ionic detergent were carried out in P_i/NaCl containing 1% Empigen BB (alkyl betaine, Albright & Wilson) as described elsewhere (40). Immunoprecipitation with ascites fluid of a BALB/c mouse that was inoculated intraperitoneally with the parent myeloma line Sp2/0 and treated as described above for the ascites fluids containing the monoclonal antibodies was included as a nonimmune control with each experiment. Amounts of Sp2/0 ascites fluid that contain similar quantities of mouse (nonimmune) IgG to that found in the monoclonal antibody preparations, as judged by Coomassie blue staining, were used.

Gel Electrophoresis. Protein samples were electrophoresed on a NaDodSO$_4$-containing discontinuous polyacrylamide gel electrophoresis system (NaDodSO$_4$/PAGE) (31). The separating gel had a final acrylamide concentration of 12.5%. After electrophoresis of ^{35}S-labeled material the gels were stained with Coomassie blue and impregnated with 2,5-diphenyloxazole for fluorography (37). RNA was analyzed by electrophoresis on 1.4% agarose gel in the presence of formaldehyde (38).

RESULTS

Monoclonal antibodies 4F4 and 2B12 recognize the two hnRNP C proteins of 41 and 43 kDa in human cells through different epitopes (32, 40), and 4F4 also recognizes the corresponding proteins in other vertebrate species (40). Immunoprecipitations from HeLa cells with ascitic fluid 4F4 or 2B12 in the presence of ionic detergents precipitate C_1 and C_2 (32, 40) (Fig. 1). However, after brief incubation with HeLa nucleoplasm without ionic detergents at 100 mM NaCl, 4F4 and 2B12 isolate, in addition to the C proteins, a complex that contains several other proteins (Fig. 1). The signals are specific in that they are not detectable in control immunoprecipitation with ascites fluid that was prepared, like the other ascitic fluids, by intraperitoneal inoculation of mice with the parent myeloma line Sp2/0. In addition to C_1 and C_2, the most prominent bands detected by steady-state [^{35}S]methionine labeling have apparent molecular masses of 34, 36, 37, 38, 68, and 120 kDa (Fig. 1). The proteins at 34 and 36 kDa and at 37 and 38 kDa correspond to the doublets of the A and B proteins, respectively, of the hnRNP 40S subparticle (4). The major proteins detected by [^{35}S]methionine labeling are also major, as seen by Coomassie blue staining (data not shown). The lack of effect of EDTA on the composition of the isolated structure indicates that its integrity is not dependent on divalent metal ions (e.g., Mg^{2+}). The absence of histones and, in general, of proteins smaller than 34 kDa indicates that the isolated complex is free of chromatin and ribosomal protein contamination (see Fig. 4). The only detectable difference between the material that is isolated with 4F4 and 2B12 is that in the latter there is a slight in-

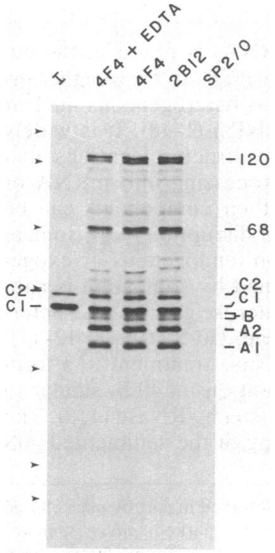

FIG. 1. Immunoprecipitation of the hnRNP complex with monoclonal antibodies against the C proteins (4F4 and 2B12). Lane I, immunoprecipitation with monoclonal antibody 4F4 in standard immunoprecipitation condition containing the ionic detergent Empigen BB at 1% (40); lanes 4F4 + EDTA and 4F4, immunoprecipitations with monoclonal antibody 4F4 from nucleoplasm with or without addition of EDTA (10 mM); lane 2B12, immunoprecipitation with monoclonal antibody 2B12; lane Sp2/0, control immunoprecipitation with ascites fluid of mouse inoculated intraperitoneally with the parent myeloma line Sp2/0. Arrowheads indicate the positions of size markers (increasing order): 14, 21, 30, 45, 69, 93, 116, and 200 kDa.

crease in a band of *ca.* 52 kDa, the migration of which is affected by that of the IgG heavy chain and is not likely to represent a major genuine hnRNP protein. Of the close doublet at 120 kDa, at least one of the bands corresponds to the recently identified 120-kDa hnRNP protein that becomes crosslinked *in vivo* to hnRNA and that is phosphorylated *in vivo* (32). This 120-kDa protein is recognized by another monoclonal antibody, 3G6 (32).

In addition to the proteins, the structure that is isolated by 4F4 also contains hnRNA. This identification is based on the findings that the co-isolated RNA is rapidly labeled nuclear RNA that is synthesized in the presence of 0.04 μg of actinomycin D per ml (and is therefore non-ribosomal) and is large and heterogeneous (Fig. 2). The size distribution of up to $\approx$10 kilobases suggests that it most likely includes also intact hnRNA, but the mean size of 1.5–2.5 kilobases suggests that most of it is likely to contain a small number of nicks. The [^{3}H]uridine-labeled material that is precipitated with 4F4 represents about 25% of the acid-precipitable nucleoplasmic RNA (data not shown). No discrete ribosomal RNA bands were detected in the immunoprecipitates, even after labeling for several hours without actinomycin D.

The authenticity of the major proteins that are isolated with the complex as genuine hnRNP proteins is supported by the fact that they appear by NaDodSO$_4$/PAGE to be the same as those that become crosslinked by UV to the hnRNA *in vivo* (Fig. 3). The UV-crosslinking procedure identifies genuine RNP proteins—those that are in direct contact with the RNA in the intact cell. The slight upshift in apparent molecular mass after such crosslinking (e.g., A_1, C_1, and C_2) is characteristic and diagnostic for proteins that are crosslinked by UV to RNA and is the result of the one or more protein-crosslinked RNase-resistant nucleotides (32). This conclusion is supported by immunoblotting data of the lanes shown in Fig. 3 with 4F4 and the anti-120-kDa antibody 3G6 (data not shown).

One of the major components of the hnRNP detected by both UV crosslinking and immunoprecipitation with 4F4 and 2B12 (Fig. 3) is a 120-kDa protein. In a previous study we identified a 120-kDa hnRNP protein and raised monoclonal antibodies to it (32). Immunoprecipitation from a nuclear sonicate with an anti-120-kDa antibody (3G6) (Fig. 4) demonstrates that the hnRNA–protein complex that is isolated by 3G6 is the same as that that is immunoprecipitated with anti-C proteins antibodies (4F4 and 2B12). The fact that different antibodies to different genuine hnRNP proteins immunoprecipitate the same set of proteins in what appears to be a similar relative stoichiometry strongly suggests that these proteins constitute a unique set, which defines the hnRNP complex. This qualitative and quantitative similarity of the material that is isolated with 4F4, 2B12, and 3G6 and of the UV-crosslinked complex (Fig. 3) argues that there is one basic hnRNP structure and that the detectable proteins are the major constituents of this structure. The finding of a relatively invariant composition of proteins demonstrates that hnRNP structure is a genuine and unique supramolecular assembly like other well-characterized cellular ribonucleoprotein structures such as the ribosome.

Antibody 4F4 recognizes the C proteins not only in human HeLa cells but also in other vertebrate species (40). This made it possible to use 4F4 to immunoprecipitate the hnRNP structures also from hamster and chicken cells. The protein patterns of the hnRNP proteins in the divergent species appear to be similar (Fig. 5). The interspecies differences in the mobilities of the proteins A, B, and C (indicated by arrowheads) are consistent with previous findings (39, 40); compared to mammals, the avian A and B proteins are somewhat larger and more closely spaced (39), whereas their C proteins are smaller (40). The 120-kDa protein is found in all vertebrate species tested and is larger in avian cells than in rodents and humans (unpublished data).

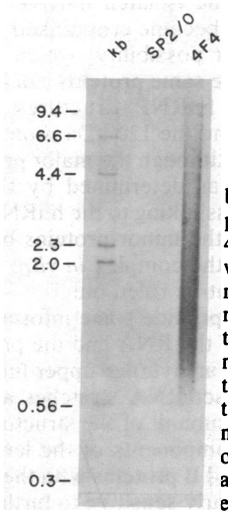

FIG. 2. Sizing of the rapidly labeled hnRNA that was immunoprecipitated with monoclonal antibody 4F4 or with Sp2/0 as control. Cells were labeled with [^{3}H]uridine for 15 min in the presence of 0.04 μg of actinomycin D per ml added 15 min prior to the addition of the isotope. The immunoprecipitated material was extracted with phenol/chloroform and the RNA was analyzed on a 1.4% formaldehyde/agarose gel. Bacteriophage λ DNA digested with *Hin*dIII and end-labeled with ^{32}P$_i$ was included as a size marker (kb, kilobases).

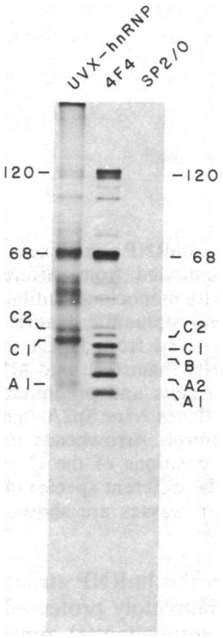

FIG. 3. Comparison of the proteins of the hnRNP complex immunoprecipitated with the anti-C proteins antibody 4F4 and the proteins that become crosslinked *in vivo* to poly(A)$^+$ hnRNA by UV irradiation (UVX-hnRNA). Molecular masses are shown in kDa.

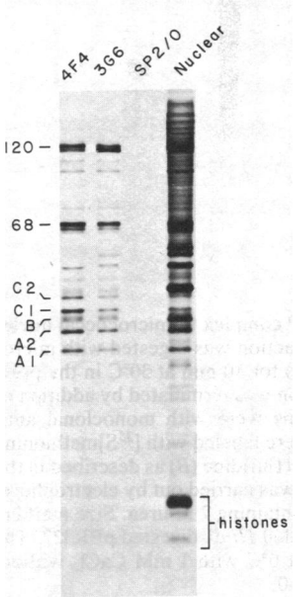

FIG. 4. Identity of the hnRNP structure isolated with monoclonal antibodies to the C proteins (4F4) and to the 120-kDa hnRNP protein (3G6). The nucleoplasm prepared from HeLa cells was divided in three and each third was incubated with the indicated monoclonal antibody or with Sp2/0 ascites fluid. Total nuclear proteins are included as reference. Molecular masses are shown in kDa.

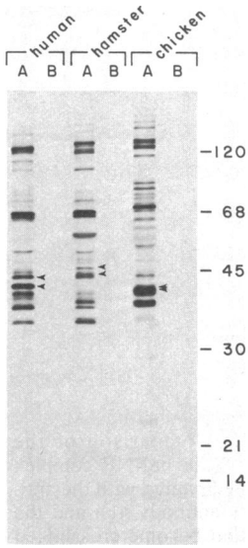

FIG. 5. hnRNP complexes immunoprecipitated from different species with monoclonal antibody 4F4. Nucleoplasmic fractions were prepared from HeLa (human), CHO (hamster), and MSB (chicken) cells and precipitated with 4F4 (lanes A) or Sp2/0 (lanes B) as control. Arrowheads indicate the positions of the C proteins in the different species (40). Molecular masses are shown in kDa.

The organization of the RNA in the hnRNP structure is such that no RNA stretches are completely protected from nuclease digestion and the RNA component(s) appears to play an important role in maintaining the integrity of the complex because extensive treatment with micrococcal nuclease leads to loss of some of the major proteins (Fig. 6). The RNase digestion experiment shows that the same proteins continue to coprecipitate by the anti-C proteins antibody, even when the average length of intact RNA is down to 100–150 nucleotides. This sets a limit on how far apart these proteins can be and argues that they must form a tight complex. Most obvious is the loss of the A and B proteins from the C proteins-containing domain after extensive nuclease digestion, whereas some of the others (e.g., 68 and 120 kDa) are less affected. The slight increase in the amount of C proteins that are immunoprecipitated after RNase digestion suggests that some additional C protein antigenic sites may become accessible to the antibody so that some C proteins either are buried inside the complex or are shielded by RNA. The RNA–protein and protein–protein interactions within the hnRNP can be further explored by salt dissociation. These studies indicate that at 500 mM NaCl some of the proteins, including the A and B groups, dissociate from the hnRNP, although the C proteins and several others are still associated with the hnRNA (unpublished data; see ref. 4).

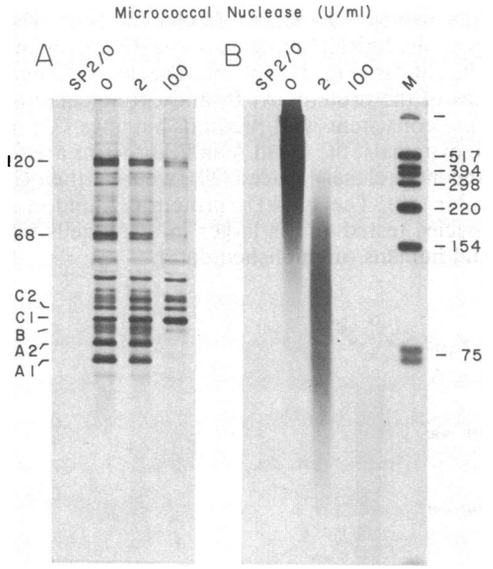

FIG. 6. Sensitivity of the hnRNP complex to micrococcal nuclease digestion. The nucleoplasmic fraction was digested with micrococcal nuclease (2 and 100 units/ml) for 10 min at 30°C in the presence of 1 mM $CaCl_2$ and the digestion was terminated by addition of 5 mM EGTA. Immunoprecipitations were with monoclonal antibody 4F4 or with Sp2/0. Proteins were labeled with [^{35}S]methionine (*A*) and the RNA was labeled with [^{3}H]uridine (*B*) as described in the legend to Fig. 2. RNA size analysis was carried out by electrophoresis on a 7.5% polyacrylamide gel containing 7 M urea. Size markers (lane M) (nucleotides) were ^{32}P-labeled *Hin*fI-digested pBR322. The nucleoplasmic fraction incubated at 0°C with 1 mM $CaCl_2$ without micrococcal nuclease is designated 0.

DISCUSSION

The findings presented here describe the isolation of a structure that contains hnRNA and a unique set of hnRNP proteins from the nucleus of vertebrate cells. This hnRNP structure was isolated from the nucleoplasm fraction of mechanically disrupted nuclei by using a rapid and specific immunoprecipitation procedure with different monoclonal antibodies to genuine hnRNP proteins (hnRNA-contacting proteins *in vivo*). Our procedure is drastically different from previous isolation procedures: it is specific and rapid and does not involve either sedimentations or column chromatography. It results in apparently complete purification of the hnRNP complex and permits the identification of its protein components. Analysis of the isolated hnRNP structure demonstrates that it is made up of hnRNA (and possibly also other RNAs) and ≈10 major and a number of minor proteins. These proteins appear to be present in the complex at fixed relative stoichiometry. In this sense they form a unique supramolecular assembly that is sufficiently stable to be isolated under the experimental conditions described. These unitary features are characteristic of genuine recognizable biological structures, including polynucleotide–protein organelles such as the ribosome. Since the hnRNP structure contains hnRNA, presumably including pre-mRNA sequences, it may serve as the structural unit on which processing of hnRNA occurs or as an intermediate in the pathway of mRNA formation. The absence of the hnRNP C proteins and the 120-kDa protein from nucleoli (32) and the lack of detectable tRNA and ribosomal RNA in the immunoprecipitate (Fig. 2) argue that the hnRNP structure is likely to be unique to large RNA polymerase II transcripts (hnRNA).

The possibility that other forms of hnRNPs exist which are composed of different proteins cannot presently be ruled out. However, these other forms, if extant, are likely to be minor because the proteins that make up the hnRNP structures isolated here are quantitatively the most abundant of the hnRNA-contacting proteins in the nucleus, as shown by UV crosslinking, and are found in the isolated hnRNP in similar relative amounts to those that become crosslinked to the hnRNA *in vivo* (Fig. 3). Another possibility, which at present cannot be excluded, is that the same proteins can be organized also into other forms of hnRNP structures in which, for example, the C proteins and the 120-kDa protein are not accessible to the antibodies. Although the major proteins are genuine hnRNP proteins, as determined by the stringent criterion of UV-induced crosslinking to the hnRNA *in vivo*, the possibility that some of the minor proteins became adventitiously associated with the complex *in vitro* in the course of cell fractionation cannot be ruled out.

The RNase digestion experiments provide some information about the overall organization of the RNA and the proteins in the complex. It suggests that an average upper limit of about 100–150 nucleotides of intact RNA stretches are sufficient to hold together the basic subunit of the structure that still contains all of the major components of the large structure. The association of the A and B proteins with the C protein-containing particle is particularly sensitive to further

nuclease digestion of the RNA. Other proteins within the complex probably interact with the C proteins also via protein–protein interaction or require only short stretches of RNA to "stitch" them together. Most of the proteins must, therefore, be physically close together to form tight assemblies rather than be dispersed along the hnRNA strand. This, together with the apparent similarity in the relative stoichiometry of the proteins, is consistent with a repeating subunit model (e.g., a multimer of 40S subunits) in which the subunit is made up of the identified proteins. Some of the C proteins must be exposed on the surface of the structure because they are accessible to the antibody and so is the 120-kDa protein, but RNase digestion may render additional C proteins accessible to the antibody.

The hnRNP structure has not been hitherto isolated and therefore several previous observations about its components and organization, some apparently conflicting, could not be easily reconciled. The features of the isolated structure described here tie together many of the previous observations and are consistent with various previous models. The "core" proteins (the A, B, and C groups) are clearly major constituents of the hnRNP, as suggested by several groups (1, 3–6, 27, 30, 32). But, as the data from several laboratories reflect, the structure also contains high molecular weight proteins (2, 5, 8, 11, 13–15, 30–32). The RNA, presumably the hnRNA itself, plays an important role in the structure, as suggested (1–6, 15, 17). The UV-crosslinking pattern suggests that the core (A, B, and C) proteins, particularly, the A_1, C_1, and C_2 proteins, are in close contact with the hnRNA (32) (Fig. 3), in agreement with the findings of LeStourgeon *et al.* (17); so are the proteins at 68 kDa and at 120 kDa.

The striking similarity of the protein patterns in divergent species suggests that the hnRNP is a highly conserved structural unit in vertebrates. Changes are seen in some of the individual proteins such as the A, B, and C proteins, which have been shown to have slightly different molecular weights in human, rodent, and avian cells (39, 40). These minor differences suggest that the overall features of the structure and many of the features of the individual proteins appear to be conserved but, that within these structural and functional constraints, some features of the individual proteins can vary from species to species.

The isolation of the hnRNP structure provides a definition of the components of this complex. Since it is the structure in which hnRNA exists in the nucleus, it is likely to play a major role in hnRNA processing. Our estimates from Na-DodSO_4/PAGE of [^{35}S]methionine-labeled proteins indicate that the hnRNP complex is also one of the major structures in the nucleus accounting for at least one-third of the protein in the nucleoplasm. It can be anticipated that some of the major proteins of the hnRNP complex may play a structural role and some of the minor proteins may also have enzymatic activities related to RNA processing and transport. The availability of isolated hnRNP and of antibodies to individual hnRNP proteins will make it possible to further characterize the hnRNP structure and its organization in the nucleus and to investigate the role of the hnRNP and its associated proteins in the pathway of mRNA formation.

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