Monoclonal Antibody Characterization of the C Proteins of Heterogeneous Nuclear Ribonucleoprotein Complexes in Vertebrate Cells

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ABSTRACT The C proteins (C\textsubscript{1} and C\textsubscript{2}) are major constituents of the 40S subparticle of heterogeneous nuclear ribonucleoprotein complexes (hnRNPs) (Beyer, A. L., M. E. Christensen, B. W. Walker, and W. M. LeStourgeon, 1977, Cell, 11:127-138) and are two of the most prominent proteins that become cross-linked by ultraviolet light to heterogeneous nuclear RNA (hnRNA) in vivo. Studies are described here on the characterization of the C proteins in vertebrate cells using monoclonal and polyclonal antibodies. Monoclonal antibodies to genuine RNP proteins, including the C proteins, were obtained by immunizing mice with purified complexes of poly(A\textsuperscript{+})hnRNA and poly(A\textsuperscript{+})mRNA with their contacting proteins in vivo obtained by ultraviolet cross-linking the complexes in intact cells (Dreyfuss, G., Y. D. Choi, and S. A. Adam, 1984, Mol. Cell. Biol., 4:1104-1114). One of the monoclonal antibodies identified the C proteins in widely divergent species ranging from human to lizard. In all species examined, there were two C proteins in the molecular weight range of from 39,000 to 42,000 for C\textsubscript{1}, and from 40,000 to 45,000 for C\textsubscript{2}. The two C proteins were found to be highly related to each other; they were recognized by the same monoclonal antibodies and antibodies raised against purified C\textsubscript{1} reacted also with C\textsubscript{2}. In avian, rodent, and human cells the C proteins were phosphorylated and were in contact with hnRNA in vivo. Immunofluorescence microscopy demonstrated that the C proteins are segregated to the nucleus. Within the nucleus the C proteins were not found in nucleoli and were not associated with chromatin as seen in cells in prophase. These findings demonstrate that C proteins with similar characteristics to those in humans are ubiquitous components of hnRNPs in vertebrates.

Heterogeneous nuclear RNAs (hnRNAs),\textsuperscript{1} the nuclear transcripts of which some are precursors to mRNAs, are associated in the cell with specific proteins to form heterogeneous nuclear ribonucleoprotein complexes (hnRNPs). The hnRNPs have been studied by both cytological (5, 7, 14, 37, 38) and biochemical (6, 10, 12, 13, 16, 20, 22, 25, 28–30, 32, 33, 35, 36, 39–42, 44–46, 48–50) approaches. These studies have revealed a major structural component of hnRNPs—the 40S particles. The major proteins of the 40S particles so far described are classified into three doublets: the A, B, and C groups (6). The C group proteins are the two proteins (by one-dimensional SDS PAGE) that appear to be the proteins most tightly associated with the hnRNA in vitro of the 40S particles, as determined by resistance to dissociation by salt (6). Because of their abundance and tight association with the hnRNA, the C proteins may play a central role in the formation of the ubiquitous 40S hnRNP subparticles and are potentially of cardinal importance in the structure and function of hnRNPs in vertebrates. The C proteins may also be involved in the attachment of the hnRNP complex to the putative nuclear matrix (12, 49).

The proteins in hnRNPs that are likely to be involved in the packaging of hnRNA and in its processing into mRNA can be identified by ultraviolet (UV)-induced RNA-protein cross-linking in intact cells (11–13, 36, 48, 49). We have recently obtained monoclonal antibodies to several of the proteins which are in direct contact with hnRNA in HeLa cells by immunizing mice with purified in vivo UV-cross-linked hnRNA-protein complexes (12). One of these antibodies, designated 2B12, recognized two of the major HeLa hnRNP proteins of molecular weight 41,000 and 43,000.

\textsuperscript{1} Abbreviations used in this paper: DME, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; hnRNA, heterogeneous nuclear RNA; hnRNP, heterogeneous nuclear ribonucleoprotein complex; UV, ultraviolet (light).
These two proteins were shown (12) to correspond to the C group proteins of the 40S hnRNP subparticle previously described by Beyer et al. (6).

The monoclonal antibody 2B12 recognized the C proteins (41,000 and 43,000 mol wt) only in higher mammals (12). In an attempt to define and study further the C proteins of other vertebrate species, we screened the anti-hnRNP monoclonal antibody-secreting hybridoma library and found monoclonal antibodies that recognize these proteins also in other species. This report makes use of such an antibody to identify the C proteins across vertebrate species and describes immunochemical studies which further characterize the C proteins of hnRNPs.

MATERIALS AND METHODS

Culture Cell and Labeling: HeLa (human), CV-1 (monkey), and Madin-Darby bovine kidney (bovine) cells were grown in monolayer culture in Dulbecco's modified Eagle's medium (DME) containing 10% fetal calf serum (FCS) at 37°C in 5% CO2 atmosphere. Cultures were supplemented with penicillin and streptomycin and used at subconfluent densities. Chinese hamster ovary cells were grown similarly in DME supplemented with nonessential amino acids. Chicken (MSB) cells (1) were grown in RPMI 1640 containing 10% FCS and 1% chicken serum. Lizard (Anolis carolinensis) myogenic cells (3) were cultured in F10 medium containing 10% horse serum and 4% chick embryo extract at 30°C in 5% CO2. Rat kangaroo Ptk2 cells were cultured in Eagle's minimum essential medium with nonessential amino acids and Earle's balanced saline solution with reduced bicarbonate (0.85 g/liter) supplemented with 10% FCS. Cells were labeled with [35S]methionine at 0.5 µCi/ml for 12 h in DME containing 1/10 the normal methionine level and 2% FCS. Labeling with [35S]orthophosphate (50 µCi/ml) was for 2 h in phosphate-free DME containing 2% FCS.

Preparation and Analysis of UV-cross-linked Poly(A)*-hnRNPs: Irradiation of cells on culture dishes was carried out in PBS for 3 min at room temperature as recently described (11, 12). After UV irradiation, the PBS was removed and the cells were allowed to swell for 5 min in ice-cold RSB (10 mM Tris-HCl pH 7.4, 10 mM NaCl, 1.5 mM MgCl2) containing the protease inhibitors aprotinin (0.5%; Sigma Chemical Co., St. Louis, MO), pepstatin A (1 µg/ml), and leupeptin (0.5 µg/ml) and the RNase inhibitor vanadyl-adenosine (10 mM) (4, 9). Samples were made 0.5% (vol/vol) Triton X-100, 0.5% deoxycholate, and 1% SDS and the cell suspensions were homogenized by four passages through a 25-gauge needle. Nuclei and cytoplasmic fractions were separated by low-speed centrifugation. The cytoplasmic fraction was discarded and the nuclei were resuspended in RSB and digested with RNase A (50 µg/ml) for 15 min at 37°C in the presence of 10 mM vanadyl-adenosine (10 mM) (11, 12). The DNase I (DPP7 grade, Worthington Biochemical Corp., Freehold, NJ) was treated with iodoacetamide (51) and digested with DNAse I (50 µg/ml for 15 min at 37°C) in the presence of 10 mM ethylenediaminetetraacetic acid (EDTA), 0.5% SDS. The sample was then sonicated twice for 5 min at 65°C, rapid chilling, and addition of LiCl to 0.5 M, the nucleic acid was removed from the nucleoproteins with a microtip and set to scale 2. The sonicate was centrifuged for 5 min at 10,000 g and the supernatant and incubated for 2 h at 4°C. Immunocomplexes were formed by adding the mono- and polyclonal antibodies, respectively, to the samples and allowed to incubate for 2 h at 4°C. Immunocomplexes were then precipitated by the addition of 5% polyethylene glycol (PEG) at 65°C for 6 h and dialyzed against PBS. 5 µg of the purified protein was mixed with 9 µl of complete Freund's adjuvant for intraperitoneal injections (47). Three immunizations were carried out at 2-wk intervals and ascites fluids were drained and used for immunoblotting at 1:200 dilution.

Immunoprecipitations: Immunoprecipitations were carried out in the presence of the non-denaturing switzen-solubilized detergent Empigen BB (alkyl betaine, Albright & Wilson, Whitehaven, Cumbria, England) which has been shown recently to have excellent solubilization properties (2, 23, 43). We found that immunoprecipitations in the presence of 1% Empigen BB are as efficient as, and much cleaner than, those with the standard "RIPA buffers" which as described below to be resolved on SDS PAGE. The gel was stained briefly with Coomassie Blue and destained, and the band at 81,000 mol wt was excised. The polyacrylamide was crushed and the protein was extracted in 50 mM Tris-HCl buffer, pH 8.0, containing 1 mM EDTA, 0.1% SDS, and 1% β-mercaptoethanol at 65°C for 6 h and dialyzed against PBS. 5 µg of the purified protein was mixed with 9 µl of complete Freund's adjuvant for intraperitoneal injections (47). Three immunizations were carried out at 2-wk intervals and ascites fluids were drained and used for immunoblotting at 1:200 dilution.

Immunoblotting: Blotting of proteins from polyacrylamide gels onto nitrocellulose paper (Schleicher & Schuell, Inc., Keene, NH) was carried out by electrotransfer at 0.15 A at room temperature for 6–12 h in 50 mM Tris-glycine, pH 9.1, containing 20% methanol (11, 12). The nitrocellulose blot was treated essentially according to Burnette (8) except that 0.5% gelatin or 5% nonfat dry milk was used instead of BSA as the blocking reagents, and the nitrocellulose blot was incubated first with monoclonal antibody-containing ascites fluid and then with 2H β2-microglobulin antibody (Fab')2 (Cappel Laboratories, Inc., Cochranville, PA) was added with 25 µl of Protein A-Agarose (Boehringer Mannheim Biochemicals, Indianapolis, IN) and incubation was continued for one more hour on a rocker at 4°C. Immunocomplexes with Protein A-Agarose were pelleted and washed five times with 1% Empigen BB in PBS. The proteins were removed from the beads by being boiled with 50 µl of SDS gel electrophoresis sample buffer.

Immunoeurofluorescence Microscopy: Immunofluorescence microscopy was carried out using a Zeiss Photomicroscope III equipped with the 63x Planapochromat objective. Cells were cultured on glass coverslips, rinsed with PBS, and fixed with 2% formaldehyde (methanol-free electron microscopy grade) and Poly-L-lysine (Worthington Biochemical Corp., Freehold, NJ) was fixed with 1% paraformaldehyde (in 0.1% Triton X-100 (v/v) for 10 min at room temperature). The tissue was then incubated for 5 min in acetic acid at ~20°C. Ascites fluid dilutions were 1:500 in PBS, and incubations with the first and second antibody were at room temperature for 30 min. Detection of the mouse antibodies was with fluorescent isothiocyanate-
RESULTS

A library of monoclonal antibody-secreting hybridomas was generated by fusion of SP2/0 myeloma cells with spleen lymphocytes of mice immunized with in vivo UV-cross-linked protein-RNA complexes of poly(A)*hnRNA and poly(A)*mRNA. Of these hybridomas, ~80 recognized the 41,000- and 43,000-mol-wt C proteins of HeLa cells as assayed by immunoblotting (12). One of these, designated 2B12, was further characterized and it was found to recognize the C proteins in human and monkey cells but not in lower vertebrate species (12). In an attempt to obtain an anti-C proteins antibody that would identify these proteins in other species, the culture supernatants of the colonies that recognized HeLa C1 and C2 by immunoblotting were tested by immunofluorescence on chick embryo fibroblasts. One colony, designated 4F4, which was positive and provided nuclear staining, was recloned and expanded. Immunoprecipitations of HeLa cell material with 4F4 and 2B12 are shown in Fig. 1 and demonstrate that the two monoclonal antibodies recognized two similar polypeptides.

Immunofluorescence microscopy on HeLa cells (Fig. 2, b and d) indicates that the cellular distribution of the proteins recognized by 2B12 and 4F4 was also similar. In addition 4F4, unlike 2B12, stained other vertebrate cells and extended the previous observation (12) that the C proteins (Fig. 2) in vertebrates are segregated to the nucleus but are not found in nucleoli. Nucleoli can be stained with monoclonal antibodies using the same fixation and staining procedures (data not shown) and, therefore, the absence of nucleolar stain indeed reflects the absence from nucleoli of the C proteins. Furthermore, since 4F4 is positive in human cells and in lizard cells whereas 2B12 is positive only in higher mammals (Fig. 2), the two monoclonal antibodies are apparently directed against two different epitopes. To determine whether the two antigenic sites reside on the same polypeptide chain of the 41,000- and the 43,000-mol-wt proteins (C1 and C2), or whether they are on different polypeptides of similar molecular weights, the antibodies were assayed by crossed immunoprecipitation-immunoblotting assay. HeLa cell material was immunoprecipitated with either 2B12 or 4F4, resolved by SDS PAGE, and electroblotted, and the blot was probed for each immunoprecipitation with the other antibody (Fig. 3). Since each antibody recognized the same two polypeptides that are immunoprecipitated by the other, the epitopes the two antibodies recognize must be on the same polypeptide chains.

That two different monoclonal antibodies recognize both C1 and C2 further suggests that the two polypeptides are highly related. To examine this relatedness, we purified the 41,000-mol-wt polypeptide (C1) from HeLa cells by immunoprecipitation with 2B12 and SDS PAGE. The 41,000-mol-wt band was excised from the gel and used to raise antibodies in mice. Immunoblotting analysis with the mouse polyclonal antibody 4F4 after SDS PAGE. The results, shown in Fig. 6, demonstrate that, as is the case in HeLa cells, the C proteins in other species are in contact with poly(A)*hnRNA in vivo. The low molecular weight forms are somewhat variable, they probably represent proteolytic fragments of the C proteins. These forms are usually much more pronounced after immunoprecipitation experiments (cf. Fig. 8). No signal could be detected by either immunoblotting or immunofluorescence with 4F4 in Xenopus laevis cultured cells (data not shown).

The C proteins of HeLa cells have been shown by UV cross-linking to be in contact with hnRNA in vivo (12). To determine if this is also the case in other species, we carried out UV cross-linking in hamster and chicken cells. The cross-linked poly(A)*hnRNP fraction from these cells was selected, digested with RNase, and probed by immunoblotting with 4F4 after SDS PAGE. The results, shown in Fig. 6, demonstrate that, as is the case in HeLa cells, the C proteins of other species are in contact with poly(A)*hnRNA in vivo. The bands at higher molecular weight are presumably homotypic or heterotypic oligomeric forms of the C proteins (12).

The immunofluorescence microscopy (Fig. 2) and the UV cross-linking data indicate that the C proteins are concentrated in the nucleus where they interact with hnRNA and are excluded from nucleoli. Immunofluorescence microscopy of rat kangaroo cells P1K2 (Fig. 7) shows that chromosomes...
in prophase cells did not stain. It can therefore be concluded that although hnRNP proteins, possibly including the C proteins, probably associate with chromatin-bound nascent RNA polymerase II transcripts (13), they nevertheless are not bona fide chromatin proteins.

The hnRNP C proteins in human HeLa cells have previously been shown to be phosphorylated in vitro (18, 28) and in vivo (12, 18). The autoradiogram shown in Fig. 8 demon-
Figure 3 Immunoprecipitation and cross-immunoblotting of 41,000- and 43,000-mol-wt proteins (41 and 43) with 2B12 and 4F4. Immunoprecipitations were from unlabeled HeLa cells. The immunoprecipitate was dissolved in sample buffer and subjected to SDS PAGE and blotted. Immunoblot analysis was carried out as described in Materials and Methods and antibodies iodinated directly were used. (A), 41,000- and 43,000-mol-wt proteins were immunoprecipitated with 2B12 (1) or with SP2/O (2) and probed with 4F4. (B) The two proteins were immunoprecipitated with 4F4 (1) or with SP2/O and probed with 2B12.

Figure 4 Immunoblot analysis of total cellular material of HeLa (human) and monkey CV-1 (monkey) with anti-41,000-mol-wt protein polyclonal antibodies. These antibodies were prepared as ascitic fluid as described in Materials and Methods.

Figure 5 Identification of group C proteins of hnRNP in different vertebrate cells with monoclonal antibody 4F4. Cells grown in culture were washed twice with PBS and dissolved in SDS PAGE sample buffer. An aliquot was resolved by SDS PAGE and immunoblotting was carried out as described in Materials and Methods. The cell lines used were: HeLa (human), CV-1 (monkey), Madin-Darby bovine kidney (bovine), Chinese hamster ovary (hamster), MSB (chicken), and Anolis carolinensis myogenic cells (lizard). Values at left, molecular weight \( \times 10^{-3} \).

Figure 6 Immunoblot analysis of group C proteins cross-linked in vivo to poly(A)\(^{+}\)hnRNA by UV irradiation of intact cells in different species. Cells grown in monolayer were irradiated for 3 min with UV and the nuclei were isolated as described in the text. The poly(A)\(^{+}\)hnRNA was selected by oligo(dT)-cellulose chromatography and digested with RNAses, and the released proteins were resolved by SDS PAGE, blotted onto nitrocellulose paper, and probed with monoclonal antibody 4F4.
strates that, as in HeLa cells, the C proteins of hnRNP in other species are also phosphorylated in vivo. Human, hamster, and chicken cells were incubated with $[^{32}P]$orthophosphate and the C proteins were immunoprecipitated from the disrupted cells in the presence of phosphatase inhibitors. The labeling experiment shown in Fig. 8 was carried out in the presence of 5 μg/ml actinomycin D to inhibit transcription of RNA polymerase II. This was found to be helpful because otherwise the background was too high as a result of the RNA that was precipitated with the C proteins. However, there was no effect on the intensity of the label in the C proteins due to actinomycin D (Y. D. Choi and G. Dreyfuss, unpublished results).

DISCUSSION

The most prominent structural feature of hnRNP complexes described so far is the proteinaceous 40S subparticles. Of the three recognized subgroups of proteins that are major components of the 40S particles (A, B, and C), the C proteins are unique. They remain associated with the hnRNA in vitro under salt conditions where the other subgroup proteins dissociate (6), they are phosphorylated (12, 18, 28), and they are acidic whereas the others are basic (6, 12). The classification of the A, B, and C proteins was based on observations made in human HeLa cells and likely candidates were pointed out also in rodents (6). The data obtained with the monoclonal antibody 4F4 demonstrate that proteins similar to the human C proteins are found across vertebrate species and the antibody defines them from humans to reptiles. Although the C proteins in the different species must have some common and highly conserved features, such as the antigenic site recognized by 4F4 and an hnRNA binding site, they are not completely conserved across vertebrates. Their apparent molecular weight in different species can differ by a few thousand per polypeptide chain (approximately a 40 amino acid size difference between the chicken and hamster C2 proteins) and the site against which antibody 2B12 is directed is conserved only in higher mammals. This is somewhat surprising because other proteins that are part of a complex higher-order structure (e.g., histones, actins, and tubulins) are usually of almost identical size in different species. Other proteins of the 40S subparticle of hnRNP, which most likely correspond to the A and B proteins, were recently identified in different species using polyclonal and monoclonal antibodies (19, 27, 31), and these also differ in size between human, rodent, and avian cells (27).

The two C proteins, C1 and C2, are very similar to each other and share antigenic sites. In all cases, with every anti-
body so far encountered, the same antibody recognizes both proteins. In addition, their partial peptide maps are somewhat related (12) and antibodies raised against purified C2 react with C2 in both human and monkey cells. Away it does not seem likely that the two C protein bands arise from the same polypeptide after posttranslational modification because their ratio is always the same under any biochemical and physiological conditions of the C proteins are indicated by arrowheads. 1, immunoprecipitation of group C proteins from HeLa (human), CHO (hamster), and M5B (chicken) cells labeled with [32P]orthophosphate. Cells were labeled with [32P]orthophosphate at 50 μCi/ml in phosphate-free Eagle's minimum essential medium containing 2% dialyzed FCS for 2 h in the presence of 5.0 μg/ml of actinomycin D. Immunoprecipitation was carried out as described in Materials and Methods. Phosphatase inhibitors (10 mM NaF and 20 μM ZnCl2) were included throughout the procedure. The positions of the C proteins are indicated by arrowheads. 1, immunoprecipitations with 4F4; 2, control immunoprecipitations with Sp 2/0.

As is the case in HeLa cells (12, 18, 28), the C proteins in other species are also phosphorylated and are associated with both poly(A)+ and poly(A)−hnRNA in vivo. The immunofluorescence microscopy data on the C proteins, the 120,000-mol-wt hnRNP protein (12), and the A and B proteins (19, 27, 31) indicate that the major hnRNP proteins so far examined are segregated to the nucleus. The RNA transcripts that emerge in the cytoplasm must, therefore, exchange the major proteins with which it is associated, as previously suggested (12, 25, 42). Within the nucleus these hnRNP proteins are excluded from nucleoli, which suggests that they are not involved in ribosomal RNA transcription or processing. The immunofluorescence micrographs of PK1 cells in prophase indicate that the C proteins and possibly also the other major hnRNP proteins are not chromatin proteins although they are likely to be associated with actively transcribing regions through association with nascent hnRNP transcripts.

The data presented here demonstrate that C1 and C2 have conserved properties across vertebrates. Taken together with the recent findings of Lesser et al. (27) on the relative conservation of the A and B proteins, they suggest that higher-order structures of hnRNP with nuclear proteins are highly conserved in these species.

We are grateful to Ermone J. Hussissian and Anjali Patel for excellent technical assistance. Empigen BB was a generous gift from Albright and Wilson, Ltd. (Marchon Division).

This work was supported by grants from the National Institutes of Health (GM3188), the National Science Foundation, the Leukemia Research Foundation, Inc., and the Searle Leadership Fund.

Received for publication 1 June 1984.

REFERENCES

1. Akiyama, Y. and S. Kato. 1974. Two cell lines from lymphomas of Marek's disease. Biken J. 17:105–116.
2. Allen, J. C. and C. Humphries. 1975. The use of tritium labelling in the agarose chromatography of biological membranes. FEBS (Fed. Eur. Biochem. Soc.) Lett. 75:158–162.
3. Bayne, E. R. and S. B. Simpson, Jr. 1980. Influence of environmental factors on the accumulation and differentiation of polyribosomes in vivo. Exp. Cell Res. 127:5–10.
4. Berger, S. L. and C. S. Birkenmeier. 1979. Inhibition of intactable nuclear bodies with ribonucleoside-adenosine complexes: isolation of messenger ribonucleic acid from resting lymphocytes. Biochemistry. 18:5435–1549.
5. Beyer, A. L., A. H. Bouton, and O. L. Miller, Jr. 1981. Correlation of hnRNP structure and nascent transcript cleavage. Cell 26:155–165.
6. Beyer, A. L., M. E. Christensen, B. W. Walker, and W. M. LeStourgeon. 1977. Identification and characterization of the packaging proteins of core 40S hnRNP particles. Cell 11:127–138.
7. Beyer, A. L., O. L. Miller, Jr., and S. L. McKnight. 1980. Ribonucleoprotein structure in nascent hnRNA is nonrandom and sequence-dependent. Cell 20:75–84.
8. Burnette, W. N. 1981. "Western Blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radiolabeled protein. Anal. Biochem. 112:195–205.
9. Cervera, M., G. Dreyfuss, and S. Penman. 1981. Messenger RNA is translated when associated with the cytoskeletal framework in normal and VSV-infected HeLa cells. J. Cell Biol. 90:18–24.
10. Christensen, M. E., W. M. LeStourgeon, M. Jamrich, G. L. Howard, L. A. Serunian, L. A. Swiny, and W. Deppert. 1983. Acylated simian virus 40 large T-antigen: a monoparticles from nuclear ribonucleoproteins containing premessenger RNA. J. Mol. Biol. 95:227–238.
11. Dreyfuss, G., S. A. Adam, and Y. D. Choi. 1984. Physical change in cytoplasmic messenger ribonucleoproteins in cells treated with inhibitors of mRNA transcription. Mol. Cell. Biol. 4:415–423.
12. Dreyfuss, G., Y. D. Choi, and S. A. Adam. 1984. Characterization of hnRNP-protein complexes in vivo with monovalent antibodies. Mol. Cell. Biol. 4:1104–1114.
13. Economides, I. V., and T. Pederson. 1983. Structure of nuclear ribonucleoprotein: heterogeneous nuclear RNA is complexed with a major sexet of proteins in vivo. Proc. Natl. Acad. Sci. USA. 80:1599–1602.
14. Fez, V. J., L. E. Wilkinson, and C. D. Laird. 1976. Comparative organization of active transduction units in Oncopelus fasciatus. Cell. 22:831–840.
15. Galfer, G. and C. Mijlsten. 1982. Preparation of monoclonal antibodies: strategies and procedures. Methods Enzymol. 73:1–46.
16. Gattrocini, R., J. Stevenin, G. Devilliers, and M. Jacob. 1978. Size heterogeneity of monoparticles from nuclear ribonucleoproteins containing premessenger RNA. FEBS (Fed. Eur. Biochem. Soc.) Lett. 80:318–323.
17. Greenwood, F. C., W. C. Hunter, and J. S. Glover. 1983. The preparation of [3H]-labeled human growth hormone of high specific radioactivity. J. Biochem. 89:1243–1247.
18. Holczewski, E. R. and D. L. Friedman. 1984. Phosphorylation of the C-protein of HeLa cell hnRNP particles. J. Biol. Chem. 259:31–40.
19. Jones, R. E., C. Okamuro, and T. E. Martin. 1980. Immunofluorescence localization of the proteins of nuclear ribonucleoprotein complexes. J. Cell Biol. 86:235–243.
20. Karn, J., V. I. Vidalis, L. C. Boffa, and V. G. Allfrey. 1977. Characterization of the non-ribosomal nuclear proteins associated with rapidly labeled heterogeneous nuclear RNA. J. Biol. Chem. 252:3707–3712.
21. Keeler, S. W. 1975. Rapid isolation of antigenic from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. 115:1617–1624.
22. Kish, V. M., and T. Pederson. 1975. Ribonucleoprotein organization of polyanlyde complexes in HeLa cell heterogenous nuclear RNA. J. Mol. Biol. 92:227–238.
23. Klockmann, U., and W. Deppe. 1983. Acetylated simian virus 40 large T-antigen: a...
35. Mayrand, S., and T. Pederson. 1981. Nuclear ribonucleoprotein particles probed in living HeLa cells. J. Cell Biol. 90:380–384.
36. Mayrand, S., B. Setyono, J. R. Greenberg, and T. Pederson. 1981. Structure of nuclear ribonucleoprotein: identification of proteins in contact with poly(A)5 heterogenous nuclear RNA in living HeLa cells. J. Cell Biol. 90:380–384.
37. McKnight, S. L., and O. L. Miller. 1976. Ultrastructural patterns of RNA synthesis during early embryogenesis of Drosophila melanogaster. Cell. 8:305–319.
38. Miller, O. L., and A. H. Bakken. 1972. Morphological studies of transcription. Kaunitzka Symp. Methods Reprod. Endocrinol. 3:155–167.
39. Pederson, T. 1974. Proteins associated with heterogeneous nuclear RNA in eukaryotic cells. J. Mol. Biol. 83:163–183.
40. Raoa, W., P. Symmons, H. Saumweber, and M. Frasch. 1983. Nopackaging and packaging proteins in hnRNA in Drosophila melanogaster. Cell. 33:529–541.
41. Sanmartin, O. P., E. M. Lukanidin, J. Molinar, and G. P. Georgiev. 1968. Structural organization of nuclear complexes containing DNA-like DNA. J. Mol. Biol. 35:313–326.
42. Setyono, B., and J. R. Greenberg. 1981. Proteins associated with poly(A) and other regions of mRNA and hnRNA molecules as investigated by crosslinking. Cell. 24:725–738.
43. Steffenhagen, M., and W. Depper. 1983. Different structural systems of the nucleus are targets for SV40 large T-antigen. Cell. 33:173–181.
44. Steitz, J. A., and R. Kamen. 1981. Arrangement of 35S heterogeneous nuclear ribonucleoprotein on Polyoma virus late nuclear transcripts. Mol. Cell. Biol. 1:21–34.
45. Stevens, J. H., R. Collin-Martinie, R. Gattioni, and M. Jacob. 1977. Complexity of the structure of particles containing heterogeneous nuclear RNA in demonstrated by ribonuclease treatment. Eur. J. Biochem. 74:589–602.
46. Tomczak, T., J. Molinar, and A. Tago. 1983. Structural characterization of nuclear poly(A)-protein particles in rat liver. Eur. J. Biochem. 131:283–288.
47. Tung, A. S. 1983. Production of large amounts of antibodies, nonspecific immunoglobulins, and other serum proteins in ascitic fluids of individual mice and guinea pigs. Methods Enzymol. 93:12–23.
48. Van Eekelen, C. A. G., E. C. M. Mailman, R. J. Reinders, and W. Van Venrooij. 1981. Adenoviral hnRNA is associated with host proteins. Eur. J. Biochem. 119:461–467.
49. Van Eekelen, C. A. G., and W. J. Van Venrooij. 1981. hnRNA and its attachment to nuclear protein matrix. J. Cell Biol. 88:554–563.
50. Walker, B. W., L. Lothstein, C. L. Baker, and W. M. LeStourgeon. 1980. The release of 40S hnRNP particles by brief digestion of HeLa nuclei with micrococcal nuclease. Nucl. Acids Res. 8:3039–3057.
51. Zimmerman, S. B., and G. Sandeen. 1966. The ribonuclease activity of crystallized pancreatic deoxyribonuclease. Anal. Biochem. 14:266–277.

new subclass associated with a detergent resistant lamina of the plasma membrane. EMBO (Eur. Mol. Biol. Organ.) J. 2:1151–1157.
24. Kohler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (Lond.). 256:495–497.
25. Kumar, A., and T. Pederson. 1975. Comparison of proteins bound to heterogeneous nuclear RNA and messenger RNA in HeLa cells. J. Mol. Biol. 96:353–365.
26. Laskey, R. A., and A. D. Mills. 1975. Quantitative film detection of 3H and 14C in polyacrylamide gels by fluorography. Eur. J. Biochem. 56:335–341.
27. Lesser, G. P., J. Escara-Wilke, and T. E. Martin. 1984. Monoclonal antibodies to heterogeneous nuclear RNA-protein complexes. J. Biol. Chem. 259:1827–1833.
28. LeStourgeon, W. M., L. Lothstein, B. W. Walker, and A. L. Beyer. 1981. The composition and general topology of RNA and protein in monomer 40S ribonucleoprotein particles. In The Cell Nucleus. H. Busch, editor. Academic Press, Inc., New York. 9:49–87.
29. Martin, T. E., P. Billings, A. Levey, S. Oszarsian, J. Quinlan, H. Swift, and L. Urbas. 1984. [Title of the paper not visible in the image.] The Journal of Cell Biology. 99:225–245.
30. Martin, T. E., P. B. Billings, J. M. Pullman, B. J. Stevens, and A. J. Kinniburgh. 1978. Substructures of nuclear ribonucleoprotein complexes. Cold Spring Harbor Symp. Quant. Biol. 42:899–909.
31. Martin, T. E., and C. S. Okamura. 1981. Immunochemistry of nuclear hnRNP complexes. In The Cell Nucleus. H. Busch, editor. Academic Press, Inc., New York. 9:119–144.
32. Martin, T. E., J. M. Pullman, and M. E. McMullen. 1980. Structure and function of nuclear and cytoplasmic ribonucleoprotein complexes. In Cell Biology: A Comprehensive Treatise. D. M. Prescott and L. Goldstein, editors. Academic Press, Inc., New York. 4:137–174.
33. Maundrell, K., and K. Scherrer. 1979. Characterization of pre-mRNA-containing nuclear ribonucleoprotein particles from avian erythroblasts. Eur. J. Biochem. 99:225–238.
34. Maxwell, I. H., F. Maxwell, and W. E. Hahn. 1977. Removal of RNase activity from DNAse by affinity chromatography on agarose-coupled aminophenylphosphoryl-uridine-2'(3')-phosphate. Nucleic Acids Res. 4:241–246.
35. Mayrand, S., and T. Pederson. 1981. Nuclear ribonucleoprotein particles probed in living cells. Proc. Natl. Acad. Sci. USA. 78:2208–2212.
36. Mayrand, S., B. Setyono, J. R. Greenberg, and T. Pederson. 1981. Structure of nuclear ribonucleoprotein: identification of proteins in contact with poly(A)5 heterogeneous nuclear RNA in living HeLa cells. J. Cell Biol. 90:380–384.