

Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus

(nuclear ribonucleoprotein/rheumatic disease/RNA processing)

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Communicated by Aaron B. Lerner, July 27, 1979

ABSTRACT Patients with systemic lupus erythematosus often possess antibodies against two nuclear antigens called Sm and RNP (ribonucleoprotein). We have established the molecular identity of these antigens by analyzing immune precipitates of nuclear extracts from mouse Ehrlich ascites cells labeled with ^{32}P and ^{35}S . Anti-Sm serum selectively precipitates six small nuclear RNA molecules (snRNAs); anti-RNP serum reacts with only two of these; and a third serum, characterized as mostly anti-RNP, precipitates a subset of three snRNA bands. Three of the six RNAs are identified by fingerprint analysis as the previously characterized and highly abundant nucleoplasmic snRNA species U1a (171 nucleotides), U1b, and U2 (196 nucleotides). The other three RNAs (U4, U5, and U6) likewise are uridine rich and contain modified nucleotides, but they are smaller, with lengths of about 145, 120, and 95 residues, respectively. Each of the six snRNAs is complexed with and apparently antigenic by virtue of association with specific proteins. All three sera precipitate an identical complement of seven different polypeptides ranging in molecular weight from 12,000 to 35,000; these proteins are abundant in nuclear extracts, but are neither histones nor the major polypeptides comprising the 30S heterogeneous nuclear RNP particles of mammalian nuclei. Our data argue that each of the six snRNAs exists in a separate small nuclear ribonucleoprotein (snRNP) complex with a total molecular weight of about 175,000. We find that human antisera also precipitate snRNAs from a wide range of vertebrate species and from arthropods. We discuss the antigenic snRNPs in relation to the published literature on snRNAs and nuclear RNPs and consider possible functions of snRNPs in nuclear processes.

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown etiology (see refs. 1 and 2 for reviews). Antibodies to nuclear components, many of which have been identified as known macromolecules or macromolecular assemblies, are a hallmark of the disease. Antibodies directed against DNA, RNA, and nucleohistones are common. However, the molecular nature of two other antigens commonly precipitated by sera from patients with SLE, called ribonucleoprotein (RNP) and Sm, has remained obscure.

Previous work on the RNP and Sm antigens has established that they are highly conserved nuclear components (3, 4). Immunodiffusion experiments using anti-RNP and anti-Sm sera have detected crossreacting material in a wide variety of mammalian tissues (3). Immunofluorescence studies (3, 4) have localized RNP entirely within the interphase nucleus, whereas Sm is reported to be largely, but not exclusively, nuclear. Most striking is the observation that the activity of RNP, as measured by immunodiffusion assays, can be destroyed by either RNase or trypsin (3-5). Sm is sensitive only to trypsin and is less heat labile, but appears antigenically related to RNP (3, 4, 6). Finally, velocity centrifugation and gel filtration studies (3, 4) have assigned a sedimentation constant of 7-10 S and a molecular

weight of about 200,000 to RNP; Sm appears smaller and more heterogeneous.

Here we show that anti-RNP and anti-Sm antibodies are directed against a distinct class of nuclear particles, each apparently consisting of a single small RNA and several small proteins. The RNA components of these complexes are unambiguously identified as the small nuclear RNAs (snRNAs) of eukaryotic cells.

MATERIALS AND METHODS

Sera were obtained from three patients with SLE as defined by the American Rheumatism Association (7). The IgG fraction was prepared by ammonium sulfate precipitation followed by DEAE-cellulose chromatography (8) and stored frozen at a concentration of 10-20 mg/ml.

Ehrlich ascites cells (2×10^5 /ml) in minimal essential medium (GIBCO) containing 2% the usual concentration of phosphate were labeled with [^{32}P]orthophosphate at 10 mCi/liter (1 Ci = 3.7×10^{10} becquerels) for 24 hr. Alternatively, 200 ml of cells in minimal essential medium were labeled with 5 mCi of [^{35}S]methionine (1000 Ci/mmol, Amersham).

Nuclear supernatants were typically prepared from 8×10^8 cells by the following procedure, with all steps carried out at 0°C. Cells were harvested at $160 \times g$ for 5 min and washed in 50 ml of phosphate-buffered saline (130 mM NaCl/20 mM Na phosphate, pH 7.3). Cells were then broken by 10 strokes of a tight Dounce pestle in 30 ml of 140 mM NaCl/10 mM Tris-HCl, pH 7.5/1.5 mM MgCl₂/0.5% Nonidet P-40, incubated for 10 min, and given 10 more strokes. The mixture was underlayered with an equal volume of 10 mM Tris-HCl, pH 7.5/5 mM MgCl₂/1% Nonidet P-40/0.8 M sucrose and centrifuged at $2700 \times g$ for 15 min in a Sorvall HB4 rotor. The pelleted nuclei were washed with 10 ml of 0.3 M sucrose/8 mM KCl/1.5 mM MgCl₂/15 mM Tris-HCl, pH 7.5, centrifuged at $430 \times g$ for 5 min, and washed with 10 ml of 100 mM NaCl/1 mM MgCl₂/10 mM Tris-HCl, pH 7.5. The purified nuclei were resuspended in 5 ml of 100 mM NaCl/1 mM MgCl₂/10 mM Tris-HCl, pH 8.5, and either extracted by shaking at 20°C for 1 hr or broken by sonication for 15 sec at setting 2 of a Branson Sonifier; the two methods gave comparable results. After clarification by centrifugation at $5300 \times g$ for 5 min, the nuclear supernatant was used immediately as a source of antigen.

Antibody was precipitated with Pansorbin (Calbiochem) as described by Kessler (9). In a typical experiment, 15 μl of IgG was incubated with the nuclear supernatant from 5×10^7 cells at 0°C for 15 min; 200 μl of Pansorbin was then added and the mixture was left on ice for 5 min. The complexes were pelleted by centrifugation and washed five times with NET2 (9). Al-

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Abbreviations: RNP, ribonucleoprotein; snRNP, small nuclear RNP; hnRNP, heterogeneous nuclear RNP; snRNA, small nuclear RNA; hnRNA, heterogeneous nuclear RNA; SLE, systemic lupus erythematosus.

ternatively, antigen-antibody complexes were adsorbed to columns of protein A-Sepharose (CL-4B, Pharmacia) and eluted with 0.1 M glycine (pH 3).

Proteins were concentrated by precipitation with 50 mM HCl in acetone and fractionated on 15% polyacrylamide gels (10).

RNAs prepared by phenol extraction were fractionated at room temperature on 10% polyacrylamide (27:1 acrylamide/bisacrylamide) gels in 7 M urea/45 mM Tris borate, pH 8.3/1.25 mM EDTA; bands were eluted electrophoretically. T1 and pancreatic RNase digests were fingerprinted by electrophoresis on Cellogel at pH 3.5 followed by homochromatography on thin layers of polyethyleneimine (Cel 300, Brinkmann) with homomix c (11); all resulting oligonucleotides were subsequently eluted and analyzed by digestion of T1 spots with pancreatic RNase and of pancreatic spots with T1 (11). Modified nucleotides were examined by two-dimensional chromatography (12) after digestion to completion with RNase P1.

RESULTS

Anti-RNP and Anti-Sm Antibodies Precipitate snRNAs.

Because the RNP and Sm antigens are located in mammalian cell nuclei, supernatants were prepared from purified nuclei of ^{32}P -labeled Ehrlich ascites (mouse) cells by either sonication or extraction at high pH, a procedure previously developed to isolate 30S heterogeneous nuclear ribonucleoprotein (hnRNP), the ribonucleoprotein particles that bind nascent transcripts in the nuclei of higher eukaryotic cells (13). In either case, the chromatin, nucleoli, and other debris were removed by centrifugation. Note in the gel of Fig. 1 that most of the small RNA species of total nuclei (lane 1) are present in such preparations (lanes 2 and 7); absent are U3 and 5.8S RNAs, both previously identified as nucleolar molecules (14, 15).

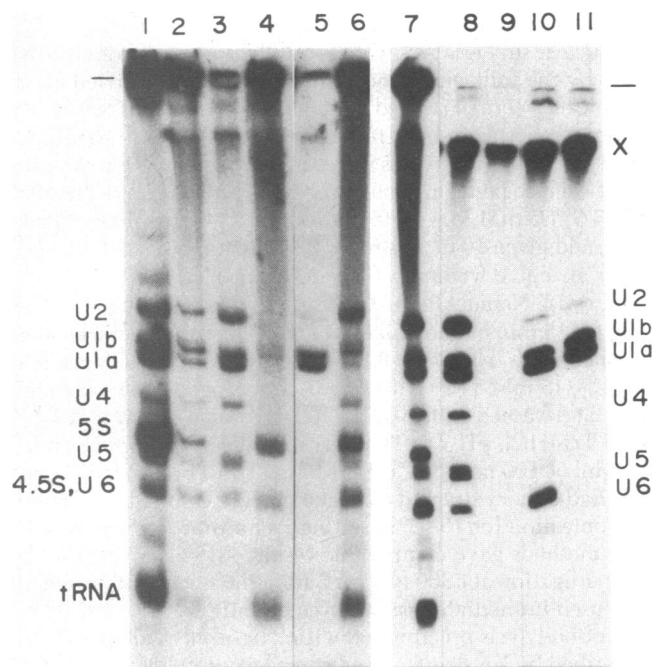


FIG. 1. Gel fractionation of snRNAs from nuclear preparations and immune precipitates. ^{32}P -Labeled RNAs were extracted with phenol from: lane 1, whole nuclei; lane 2, nuclear sonicate; lane 3, Pansorbin precipitate with anti-Sm serum; lane 4, remaining supernatant from lane 3; lane 5, Pansorbin precipitate with anti-RNP serum; lane 6, supernatant from lane 5; lane 7, nuclear sonicate; lane 8, anti-Sm-Pansorbin precipitate; lane 9, Pansorbin precipitate with normal serum; lane 10, Pansorbin precipitate with serum characterized as mostly RNP; lane 11, anti-RNP-Pansorbin precipitate. Lanes 1-6 and 7-11 represent two different experiments.

The sera from patients with SLE had previously been screened for anti-RNP and anti-Sm antibodies by immunodiffusion against standards at Scripps Clinic. Antigen-antibody complexes were obtained by mixing the IgG fraction from SLE serum with the ascites cell nuclear supernatant, followed by precipitation with Pansorbin [a commercial preparation of protein A-bearing *Staphylococcus aureus* (9)].

The polyacrylamide gel of Fig. 1 reveals that certain snRNA molecules selectively appear in the immunoprecipitates. The pattern is different for antiserum from different SLE patients. Anti-Sm serum (lane 8) precipitated six snRNAs, anti-RNP serum showed two of these RNAs (lane 11), and a serum characterized as mostly anti-RNP (lane 10) precipitated three bands. Many other SLE sera tested gave patterns similar to the three above, whereas normal sera (lane 9) and sera from a significant fraction of patients with clinically diagnosed SLE precipitated no snRNAs. In addition, all sera showed variable amounts (compare lanes 3 and 5 with 8-11) of a higher molecular weight band X, whose composition we do not yet know.

The three largest of the precipitated RNAs were labeled U1a, U1b, and U2 by comparison with published gel patterns of snRNAs from rat hepatoma (14) and HeLa (15) cells; these assignments are confirmed by fingerprint analysis below. We designate the three smaller bands U4, U5, and U6. Although U5 appears to be a doublet (see lanes 7 and 8), its fingerprint (Fig. 2) suggests that the multiple bands represent either conformers or two very closely related molecules.

Most importantly, Fig. 1 demonstrates that snRNA species U1-U6 are involved in specific RNA-protein complexes. First, all six RNAs are precipitated by anti-Sm serum (lane 8), previously deduced to be directed against a protein antigen (3, 4, 6). Second, antibodies against RNP, likewise characterized as having a protein component essential for antigenicity (3-5), precipitate a subset of these RNA molecules (lanes 10 and 11). Third, all three antibody preparations are unable to precipitate significant amounts of snRNAs after deproteinization by phenol extraction (S. Mount, personal communication).

The data of Fig. 1 further reveal that all molecules of U1-U6 are always complexed with protein, at least in the nuclear supernatant. Specifically, lane 3 shows that five RNAs can be quantitatively precipitated by anti-Sm serum (compare with lane 4, the remaining supernatant), whereas a comparison of lane 5 with lane 6 demonstrates nearly complete precipitation of U1a and U1b by anti-RNP serum. Because snRNA U6 usually comigrates with several nonantigenic RNA molecules (ref. 16, and see below), we can only speculate that it too is quantitatively precipitated.

Identification of Precipitated snRNAs. Fingerprint analysis confirmed that the mouse RNAs labeled U1a and U2 in Fig. 1 are identical to the previously studied molecules from Novikoff hepatoma (rat) cells (17, 18). All oligonucleotides in the T1 RNase (Fig. 2) and the pancreatic RNase (not shown) maps can be exactly aligned with the complete 171-nucleotide sequence of U1a (16) and the 196-residue sequence of U2 (18); the two 3' end spots (numbers 28 and 29) of U2 run too slowly in the first dimension (18) to be included on this particular fingerprint. Analyses of total P1 RNase digests (ref. 12; not shown) confirmed the existence of all expected modified nucleotides in these two RNAs.

U1b is a sequence variant of U1a, differing in only a few positions. Except for the absence of spots 6, 15, and 19 and the presence of three new spots labeled a, b, and c, the oligonucleotides in the T1 RNase fingerprints of U1a and U1b (Fig. 2) appear identical. Spot b in U1b, a heptamer containing (A-C,C,3U)G, may well replace oligonucleotide 15 in U1a (C-A_{me}-C-U-C-C-G) (17), and spot c (probably a 13-mer) may

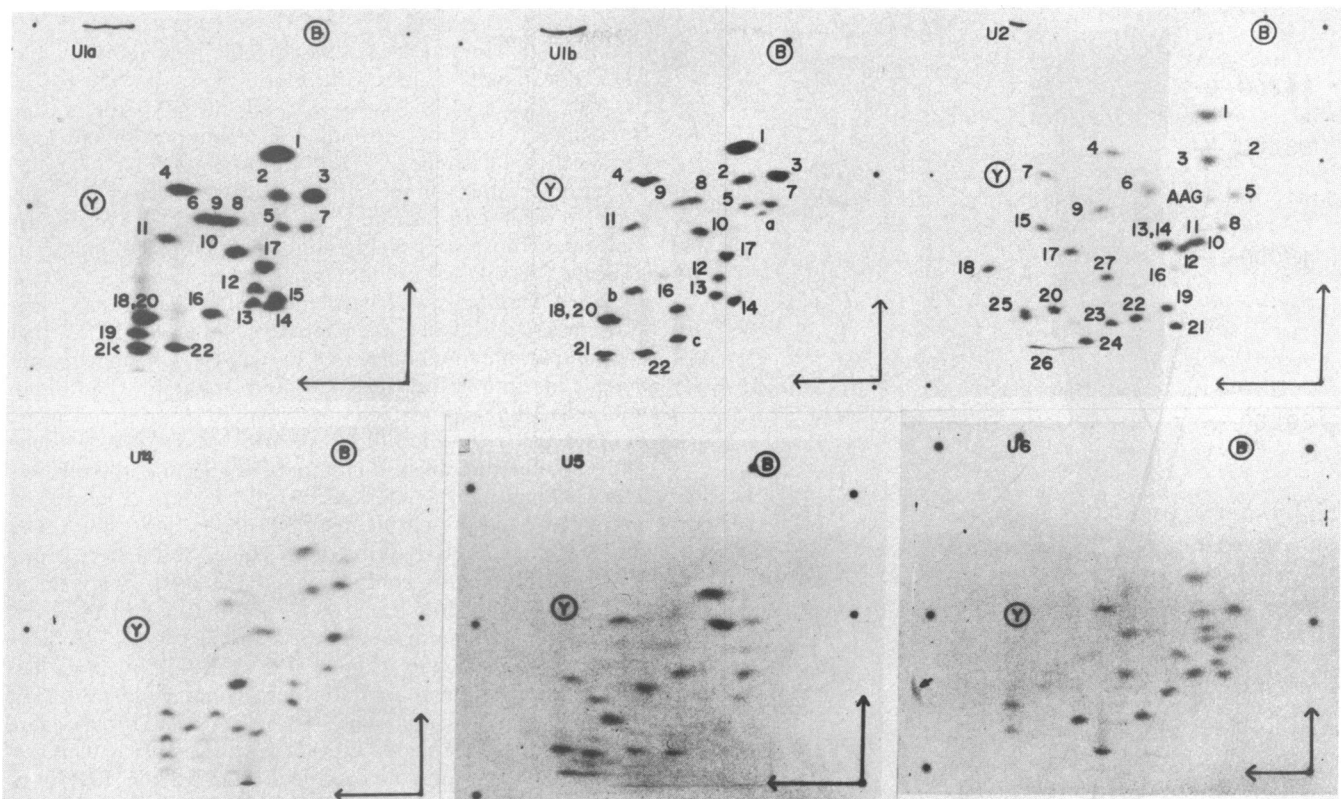


FIG. 2. T1 RNase fingerprints of snRNAs precipitated by SLE sera. The numbering system for U1a and U1b corresponds to that in ref. 16; that for U2 is from ref. 17. The small arrow designates the 5'-triphosphate moiety of U6. Circled B and Y indicate the positions of the blue and yellow dyes in the first (horizontal) and second (vertical) dimensions.

replace spot 19 (U-C-U-A-U-C-C-A-U-U-G) (17). Interestingly, both these and all detectable changes in the pancreatic RNase fingerprint of U1b (not shown) are clustered between positions 53 and 89 of the U1a sequence (17), suggesting that U1a and U1b are identical at both termini and differ only in the middle of the molecule.

The T1 RNase fingerprints of snRNAs U4, U5, and U6 (Fig. 2) and the corresponding pancreatic RNase patterns (not shown) convince us that these molecules have not been previously characterized by fingerprint analysis and that their nucleotide sequences have not been determined. Whereas fingerprints show that a 5S RNA of known sequence (19) and a 4.5S species (16) are not precipitated by our antibodies (see Fig. 1), the oligonucleotides of U4, U5, or U6 do not correlate with those of either a 5S variant (20) or another 4.5S RNA studied by Jelinek and Leinwand (ref. 21 and W. Jelinek, personal communication). Using the RNA species of known sequence as gel markers, we can assign lengths of about 145, 120, and 95 residues to U4, U5, and U6, respectively. All three molecules are uridine rich and contain some modified nucleotides (not shown). Only U6 possesses a 5'-triphosphate (indicated by arrow in Fig. 2); U4 and U5 (like the two U1 species and U2) are capped.

Precipitable snRNPs Contain Prominent Nuclear Proteins. To examine the protein components of snRNPs, we labeled Ehrlich ascites cells with [³⁵S]methionine and treated the nuclear supernatant with antibodies from the same patients as used in Fig. 1. Sodium dodecyl sulfate/polyacrylamide gel fractionation (10) shows that all three antibody systems bind the same seven bands in about the same amounts (Fig. 3, lanes 2-4). These correspond to polypeptides with molecular weights between 12,000 and 35,000. When a mixture of ¹⁴C-labeled amino acids was used to label cells, the same seven polypeptides ap-

peared in immunoprecipitates with anti-Sm serum (not shown). Normal sera gave essentially blank lanes; the appearance of variable amounts of higher molecular weight material (most obvious in lane 4) can be ascribed to nonspecific adsorption to the protein A-Sepharose column. Note that the seven immunoprecipitable bands are all prominent in lane 1, which displays the total ³⁵S-labeled proteins present in the nuclear supernatant. None of the bands comigrate with histones (Fig. 3).

DISCUSSION

We have established the biochemical identity of the nuclear antigens designated RNP and Sm in the rheumatic disease literature. When mixed with ³²P-labeled nuclear extracts from Erlich ascites cells, anti-RNP and anti-Sm antibodies selectively precipitate certain snRNA molecules. These include the previously studied and highly abundant snRNA species U1a, U1b, and U2 (14, 15) and three smaller RNAs.

The six snRNAs are recognized by virtue of their involvement in specific RNA-protein complexes (snRNPs) that apparently possess common antigenic determinants. Thus, anti-Sm (previously shown to be directed against a protein antigen) precipitates all six RNAs and approximately equal amounts of seven different polypeptides with molecular weights between 12,000 and 35,000. We have not established yet whether the Sm antigen is one or several of these proteins, although Powers *et al.* (22) have reported that Sm can be isolated as a homogeneous gel band. By contrast to anti-Sm, the two other antisera described here bind the same proteins in about the same amounts but precipitate distinct subclasses of the six snRNAs; anti-RNP precipitates exclusively the closely related U1a and U1b molecules (17), whereas a third distinctive serum precipitates U1a, U1b, and seemingly large amounts of U6. Douvas *et al.* (23) recently have found that affinity columns constructed

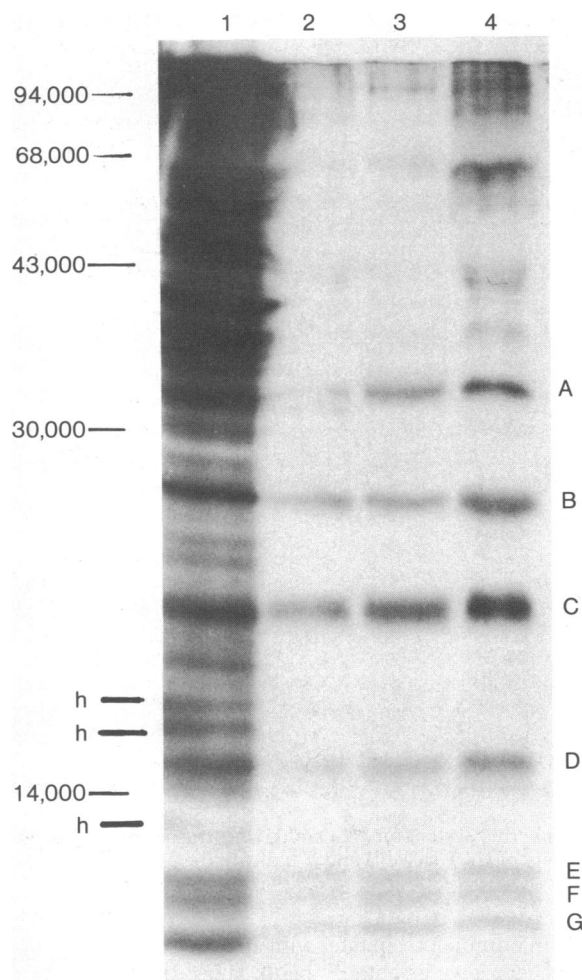


FIG. 3. Gel fractionation of proteins from a nuclear extract and immune complexes. A nuclear sonicate was prepared from ^{35}S -labeled cells, immune complexes were recovered from a protein A-Sepharose column, and the protein was analyzed on a 15% Laemmli gel. Lane 1, total nuclear extract; lane 2, immune complexes with anti-Sm serum; lane 3, immune complexes with anti-RNP serum; lane 4, immune complexes with the serum characterized as mostly RNP. The positions of marker proteins run in an adjacent lane are indicated. h, Histone.

from anti-RNP IgG adsorb two proteins of molecular weights 13,000 and 30,000 (probably equivalent to polypeptides B and D in Fig. 3), which they report are not precipitable by anti-Sm. However, our observations clearly demonstrate that the Sm antigenic determinant is present on particles reactive with anti-RNP sera.

Our results further argue, but do not rigorously prove, that each snRNA is contained in a separate snRNP complex. From the observation that anti-RNP serum precipitates only U1a and U1b (Fig. 1, lane 11), we can conclude that these two RNAs are not in the same physical particles as U2, U4, U5, or U6; moreover, in HeLa cells only one U1 species (U1a) is present and is alone precipitated by anti-RNP serum (J. Boyle and M. Lerner, unpublished observations). Thus, it seems reasonable to infer that each precipitable snRNA molecule represents a distinct snRNP complex. Also, it is somewhat unexpected that all three sera yield equivalent protein patterns (Fig. 3, lanes 2-4) although they precipitate different subsets of snRNAs. Here one possible interpretation is that each individual snRNP contains the same complement of seven proteins and that the antigenic site is determined by both RNA and protein. Based on the above assumptions, the combined molecular weight of the components

of an snRNP containing U1a or U1b would be about 175,000 (about 125,000 for one each of the proteins, plus 50,000 for one RNA), a value that agrees with prior estimates for the size of the RNP antigen (3, 4). Analysis of single snRNP particles after fractionation by gel electrophoretic techniques should soon resolve these questions.

Our observations on the antigenic snRNPs from mouse cells correlate well with previously reported properties of snRNA molecules from various higher eukaryotes. First, gel patterns indicate that snRNAs are highly conserved across species (14, 15, 24). Accordingly, we have demonstrated that anti-RNP and anti-Sm sera selectively precipitate five HeLa cell snRNAs that are comparable in mobility to U1a, U2, U4, U5, and U6, but appear in somewhat different relative amounts. The human antibodies also precipitate discrete small RNAs from the nuclei of chickens, frogs, and fall armyworms (M. Lerner, J. Boyle, and S. Wolin, unpublished observations). Second, the snRNAs are abundant nuclear molecules (14); U1 and U2 have been estimated to be present in about 10^6 copies per cell or about one-tenth the number of ribosomes. Hence, it is not surprising that the polypeptide components of the snRNPs appear as prominent bands in sodium dodecyl sulfate gel of total ^{35}S -labeled, nonchromatin, nuclear protein (Fig. 3). Third, Zieve and Penman (15), who assigned HeLa cell snRNAs to various cellular compartments but unfortunately did not identify their gel bands by fingerprint analysis, localized the most abundant small RNA SnD (probably U1a) in the nucleoplasm. This corresponds to the immunofluorescence localization of RNP (refs. 3 and 4 and our observations). Zieve and Penman (15) also found that SnD is released when isolated nuclei are warmed, consistent with our observation that the U1a and U1b snRNPs are readily recovered by extracting nuclei at high pH. Finally, several groups have previously reported finding various snRNAs in ribonucleoprotein complexes that sediment at 8-10 S (25, 26). Both Raj *et al.* (25) and Howard (26) determined a density of about 1.45 g/cm^3 for such complexes, consistent with our deductions that the snRNPs most likely contain about 25% RNA and 75% protein. For reasons we do not understand, the sizes reported by Raj *et al.* (25) for polypeptides associated with U1 and U2 are much larger than those we observe.

Now that the identity of the RNP antigen has been discovered, it should be possible to use anti-RNP and anti-Sm antibodies to elucidate the long-elusive function of snRNAs in nuclear processes. Potential roles include participation in DNA or RNA biosynthesis, for which *in vitro* assay systems (to which antibodies could be added) are rapidly becoming available (27, 28). Most intriguing is the possible involvement of snRNAs in the conversion of nuclear RNA precursors to mature cytoplasmic molecules. Here RNase P of *Escherichia coli*, which consists of a small (350-nucleotide) RNA molecule complexed with small protein(s) (29), sets a precedent. Further, it is tantalizing that sequences near the 5' terminus of the most abundant snRNAs, U1a and U1b (17), exhibit nearly total complementarity to the common sequence across splice junctions in heterogeneous nuclear RNAs (hnRNAs) (30): T-T-C-A-G↓-G-T. Thus, the snRNPs we have identified could be a group of closely related RNA processing enzymes.

Our work disproves the hypothesis (26, 31-33) that snRNAs are structural components of 30S hnRNP, the highly conserved protein particles that coat all rapidly labeled transcripts (hnRNA) in the nuclei of higher eukaryotic cells (13). Upon quantitative immunoprecipitation of the six snRNAs from nuclear supernatants that also contain 30S particles, the seven polypeptides observed in the immunoprecipitate are clearly not identical to the core 30S proteins, which have molecular weights of 30,000-40,000 (13, 34, 35). More likely, the snRNPs

cosediment with larger structures containing hnRNA (refs. 15, 26, and 31-33, and our observations) because of a functional association; the nature of this interaction remains to be elucidated.

An important question concerning rheumatic disease is whether a particular autoantibody spectrum can be correlated with the symptoms and prognosis of a patient's disease. For example, high titers of anti-RNP are associated with a form of SLE (often called mixed connective tissue disease) in which there is little renal involvement compared to patients with high titers of antibodies against DNA (1, 2, 36-38). Also, patients diagnosed as having either scleroderma or Sjorgens syndrome frequently possess high titers of antinucleolar antibodies (39). The techniques described here should be capable of precisely identifying all macromolecular assemblies against which patients with rheumatic disease produce antibodies.

Antibodies from patients with rheumatic disease are of obvious value in studying the structure and function of many important and highly conserved cellular macromolecules that otherwise may tend to be poor antigens. Other possible sources of antibodies are animals such as the F1 hybrids of NZB and NZW mice, which develop a disease sharing many characteristics with SLE in humans, including the production of anti-DNA, anti-Sm, and anti-RNP antibodies (40). Hybridomas constructed from the lymphocytes of such animals could be used to build a library of monoclonal antibodies for use in studying many aspects of the molecular biology of eukaryotic cells.

Note Added in Proof. We have recently learned that M. Medof, P. Billings, A. Eddie-Quartey, and T. Martin have partially purified a 10S RNP complex from mouse ascites cell nuclei by ion-exchange chromatography and density-gradient centrifugation. The preparations appear to form immune complexes with sera from patients characterized as having circulating "anti-RNP" antibodies; they contain three major snRNA species and seven to nine major polypeptides in the molecular weight range 15,000-45,000, which are not the major proteins of 30S hnRNP subcomplexes. (T. E. Martin, personal communication).

We thank Joan Weliky and Debbie Brooker for excellent technical assistance and John Hardin, Linda Kruse, and Norman Siegel for kind gifts of sera. We also thank John Hardin, Alan Weiner, Sherman Weissman, David Ward, John Boyle, and Steve Mount for advice and help. This work was supported by U.S. Public Health Service Training Grant GM 07205 to M.R.L. and National Science Foundation Grant PCM 74-01136 to J.A.S.

1. Notman, D. D., Kurata, N. & Tan, E. M. (1975) *Ann. Int. Med.* **83**, 464-469.
2. Provost, T. T. (1979) *J. Invest. Dermatol.* **72**, 110-113.
3. Mattioli, M. & Reichlin, M. (1971) *J. Immunol.* **107**, 1281-1290.
4. Northway, J. O. & Tan, E. M. (1972) *Clin. Immunol. Immunopathol.* **1**, 140-154.
5. Tan, E. M. & Kunkel, H. G. (1966) *J. Immunol.* **96**, 464-471.
6. Mattioli, M. & Reichlin, M. (1973) *J. Immunol.* **110**, 1318-1324.
7. Cohen, A. S., Reynolds, W. E., Franklin, E. C., Kulka, J. P., Ropes, M. W., Shulman, L. E. & Wallace, S. L. (1971) *Bull. Rheum. Dis.* **21**, 643-648.
8. Garvey, J. S., Cremer, N. E. & Susdorf, D. H. (1977) *Methods in Immunology* (W. A. Benjamin, Reading, MA), 3rd Ed.
9. Kessler, S. W. (1975) *J. Immunol.* **115**, 1617-1624.
10. Laemmli, U. K. (1970) *Nature (London)* **227**, 680-685.
11. Barrell, B. G. (1971) in *Procedures in Nucleic Acid Research*, eds. Cantoni, G. L. & Davies, D. R. (Harper, New York), Vol. 2, pp. 751-779.
12. Silberklang, M., Gillum, A. M. & RajBhandary, U. L. (1979) *Methods Enzymol.* **59**, 58-109.
13. Martin, T., Billings, P., Levey, A., Ozarslan, S. J., Quinlan, T., Swift, H., & Urbas, L. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **38**, 921-932.
14. Ro-Choi, T. S. & Busch, H. (1974) in *The Cell Nucleus*, ed. Busch, H. (Academic, New York), pp. 151-208.
15. Zieve, G. & Penman, S. (1976) *Cell* **8**, 19-31.
16. Ro-Choi, T. S., Reddy, R., Henning, D., Takano, T., Taylor, C. W. & Busch, H. (1972) *J. Biol. Chem.* **247**, 3205-3222.
17. Reddy, R., Ro-Choi, T. S., Henning, D. & Busch, H. (1974) *J. Biol. Chem.* **249**, 6486-6494.
18. Shibata, H., Ro-Choi, T. S., Reddy, R., Choi, Y. C., Henning, D. & Busch, H. (1975) *J. Biol. Chem.* **250**, 3909-3920.
19. Forget, B. G. & Weissman, S. M. (1968) *J. Biol. Chem.* **243**, 5709-5732.
20. Ro-Choi, T.-S., Reddy, R., Henning, D. & Busch, H. (1971) *Biochem. Biophys. Res. Commun.* **44**, 963-972.
21. Jelinek, W. & Leinwand, L. (1978) *Cell* **15**, 205-214.
22. Powers, R., Akizuki, M., Boehm-Truitt, M. J., Daly, V. & Holman, H. R. (1977) *Arthritis Rheum.* **20**, 131.
23. Douvas, A. S., Stumph, W. E., Reyes, P. & Tan, E. M. (1979) *J. Biol. Chem.* **254**, 3608-3616.
24. Hellung-Larsen, P. & Frederiksen, S. (1977) *Comp. Biochem. Physiol. B* **58**, 273-281.
25. Raj, N. B. K., Ro-Choi, T. S. & Busch, H. (1975) *Biochemistry* **14**, 4380-4385.
26. Howard, E. F. (1978) *Biochemistry* **17**, 3228-3236.
27. Mory, Y. & Geffer, M. (1978) *Nucleic Acids Res.* **5**, 3899-3912.
28. Bolden, A., Aucker, J. & Weissbach, A. (1975) *J. Virol.* **16**, 1584-1592.
29. Stark, B. C., Kole, R., Bowman, E. J. & Altman, S. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3717-3721.
30. Catterall, J. F., O'Malley, B. W., Robertson, M. A., Staden, R. Y., Tanaka, T. & Brownlee, G. G. (1978) *Nature (London)* **275**, 510-513.
31. Deimel, B., Louis, C. & Sekeris, C. E. (1977) *FEBS Lett.* **73**, 80-84.
32. Northemann, W., Scheurlen, M., Gross, V. & Heinrich, P. C. (1977) *Biochem. Biophys. Res. Commun.* **76**, 1130-1137.
33. Guimont-Ducamp, C., Sri-Widada, J. & Jeanteur, P. (1977) *Biochimie* **59**, 755-758.
34. Karn, J., Vidali, G., Boffa, L. C. & Allfrey, V. G. (1977) *J. Biol. Chem.* **252**, 7307-7322.
35. Beyer, A. L., Christensen, M. E., Walker, B. W. & LeSturgeon, W. M. (1977) *Cell* **11**, 127-138.
36. Sharp, G. C., Irvin, W. S., May, C. M., Holman, H. R., McDuffie, F. C., Hess, E. V. & Schmid, F. R. (1976) *N. Engl. J. Med.* **295**, 1149-1154.
37. Reichlin, M. (1976) *N. Engl. J. Med.* **295**, 1194-1195.
38. Parker, M. D. (1973) *J. Lab. Clin. Med.* **82**, 769-775.
39. Pinna, J. L., Northway, J. D. & Tan, E. M. (1973) *J. Immunol.* **111**, 996-1004.
40. Eisenberg, R. A., Tan, E. M. & Dixon, F. J. (1978) *J. Exp. Med.* **147**, 582-587.