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Human U1 and U2 Small Nuclear Ribonucleoproteins Contain Common and Unique Polypeptides

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Immunoprecipitation of human small nuclear ribonucleoproteins (snRNPs) containing the small nuclear RNAs U1, U2, U4, U5, and U6 with two antibodies produced in certain patients suffering from systemic lupus erythematosus was used to identify the polypeptides present on human U1 and U2 snRNPs. U1 and U2 snRNPs contain both common and unique polypeptides; visualization of the differences was possible through the use of non-methionine protein labeling and partial fractionation of snRNP populations. To facilitate comparisons with results from other laboratories, we have designated the snRNP polypeptides by their molecular weights. Four small polypeptides, P8, P9, P10, and P12, of 8,000 to 12,000 daltons, are each present in equal amounts on both U1 and U2 snRNPs. U1 snRNPs also contain a unique 30,000-dalton polypeptide, P30, whereas U2 snRNPs contain a unique 27,000-dalton, methionine-deficient polypeptide, P27. A closely migrating pair of polypeptides, P23 and P22, of 23,000 and 21,500 daltons, respectively, is present on both snRNPs; U2 snRNPs are enriched in the former, and U1 snRNPs are enriched in the latter.

Nuclei of higher eucaryotic cells contain a family of related small RNAs which exist in the form of small ribonucleoprotein complexes (snRNPs) (8). Certain patients suffering from systemic lupus erythematosus (SLE) produce antibodies directed against snRNPs (6). SLE anti-RNP antibodies selectively immunoprecipitate U1 snRNPs. SLE anti-Sm antibodies define and immunoprecipitate the entire family of U1, U2, U4, U5, and U6 snRNPs. When [^{35}S]methionine-labeled antigen is used, both antibodies immunoprecipitate the same set of polypeptides from murine cells (6). Individual snRNP populations have not been sufficiently fractionated to assign individual polypeptides to individual snRNPs, although several polypeptides have been implicated as the actual SLE antigens (2, 11, 13).

It has been suggested that U1 snRNPs provide an adaptor function for RNA splicing through hybridization of U1 RNA sequences complementary to consensus intron sequences at splice junctions (5, 9). The function of the other U snRNPs is unclear, although U2 RNA also appears to contain sequences complementary to at least several exon sequences proximal to splice junctions (7). Both SLE antibodies inhibit the production of mature mRNA in in vitro nuclei, supporting a role for these complexes in RNA biosynthesis (14). Here we present evidence that all human U small nuclear RNAs (snRNAs) are not complexed with the same polypeptides. Instead, there are both common and unique polypeptides associated with at least the two most abundant U snRNAs, U1 and U2, indicating enough dissimilarity in snRNP structure to suggest related but different functions.

MATERIALS AND METHODS

Preparation of labeled nuclear extracts. HeLa cells grown in suspension culture were labeled for 15 h with $^{32}P_i$ (50 mCi/10^8 cells) or [^{3}H]leucine (2 mCi/10^8 cells) or for 5 h with [^{35}S]methionine (5 mCi/10^8 cells). Nuclei were prepared and washed as described by Lerner and Steitz (6). Low-salt nuclear extracts were prepared by incubating nuclei for 30 min at 20°C in 0.01 M Tris-hydrochloride (pH 8.1)–0.1 M NaCl–1.0 mM $MgCl_2$. High-salt extracts were prepared by incubating similar nuclei for 20 min at 0°C in 0.01 M Tris-hydrochloride (pH 8.1)–0.40 M NaCl–1.0 mM $MgCl_2$. After removal of nuclei, the extracts were immediately loaded onto 15 to 30% sucrose gradients in 0.01 M Tris-hydrochloride (pH 7.9)–0.10 M NaCl–1.0 mM $MgCl_2$–0.05% Nonidet P-40 or immunoprecipitated.

Immunoprecipitations. Labeled extracts were immunoprecipitated with control or selected SLE antibodies by collection on Pansorbin (Calbiochem). Control antisera were from laboratory personnel. SLE antibodies were obtained from patients at the Baylor College of Medicine Section of Rheumatology through the generosity of M. D. Lidskey. Sera were classified as anti-RNP or anti-Sm depending upon their ability to immunoprecipitate U1 snRNA or U1–U6 snRNAs, respectively. A high-titer, anti-Sm-only antibody was used for the majority of the immunoprecipitations discussed below and was the very generous gift of

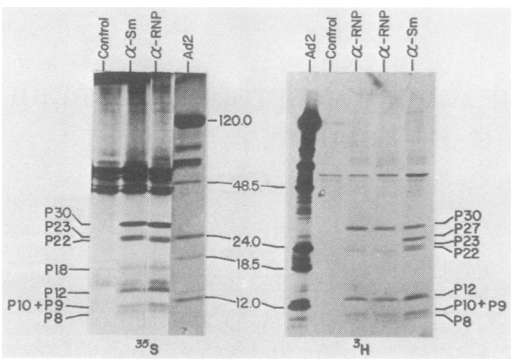

FIG. 1. Immunoprecipitation of [^{35}S]methionine- and [^{3}H]leucine-labeled human snRNP polypeptides with SLE antibodies. Nuclear extracts were immunoprecipitated with anti-RNP, anti-Sm, or control sera. Marker proteins were from adenovirus type 2 virus. The gels were 7.5 to 17.5% gradient acrylamide gels containing SDS. Gels were prepared for fluorography and exposed for 1 week (^{35}S) or 2 weeks (^{3}H). The ^{35}S-labeled extract was prepared in 0.1 M NaCl; the ^{3}H-labeled extract was prepared in 0.40 M NaCl.

E. M. Tan. Immunoprecipitations used to determine polypeptide ratios were performed with sufficient antibody to quantitatively immunoprecipitate all snRNPs.

Calculations of snRNP polypeptide ratios. Values were calculated by using snRNP polypeptide molecular weight determinations on acrylamide gels containing 0.1% sodium dodecyl sulfate (SDS) and 7 M urea. Intensities of individual bands were determined by scanning autoradiograms prepared so as to yield a linear relationship between band intensity and radioactivity (4). The polypeptide ratios of U1 snRNPs were calculated from anti-RNP immunoprecipitation data by assuming one copy of P8 per assembly. Calculations made by assuming one copy of P22 or P23 per assembly yielded U1 snRNP molecular weights too large to be consistent with either previous estimates (3, 11) or sedimentation analysis (see Fig. 2). Anti-Sm antibodies immunoprecipitated equal amounts of $^{32}P_i$-labeled U1 and U2 RNAs from crude extracts; anti-RNP and anti-Sm antibodies immunoprecipitated equal amounts of U1 RNA. Therefore, anti-Sm immunoprecipitations displayed a protein pattern consisting of equal contributions of U1 and U2 snRNPs. U2 polypeptide ratios were determined from anti-Sm immunoprecipitations after the contribution from the immunoprecipitation of U1 snRNPs was subtracted and by assuming one copy of P8 per assembly.

RESULTS

To define the polypeptides in human snRNPs, [^{35}S]methionine- and [^{3}H]leucine-labeled nuclear extracts were immunoprecipitated with SLE anti-RNP or anti-Sm antibodies (Fig. 1 and Table 1). In agreement with similar experiments with murine cells (6), both types of antibodies immunoprecipitated the same group of polypeptides from [^{35}S]methionine-labeled extracts. An additional polypeptide, however, was immunoprecipitated from [^{3}H]leucine-labeled extracts by anti-Sm antibodies. This Sm-specific polypeptide must contain little or no methionine and thus has escaped previous detection. [^{35}S]methionine- and [^{3}H]leucine-labeled immunoprecipitates were subjected to polyacrylamide gel electrophoresis under a variety of conditions to estimate the molecular weights of individual snRNP polypeptides (Table 1). Resolution of the three smallest polypeptides required either gradient acrylamide gels or gels containing 7 M urea in addition to SDS. We have designated the SLE antibody-immunoprecipitable polypeptides as P30, P27, P23, P22, P18, P12, P10, P9, and P8 based upon the molecular weight estimates

TABLE 1. Immunoprecipitable snRNP polypeptides

Polypeptide	Immunoprecipitation[a] with:				Mol[b]	Mol[c]	Sm/RNP ± SD[d]
	Anti-RNP		Anti-Sm				
	Met	Leu	Met	Leu			
P30	+	+	+	+	31,000	30,000	1.01 ± 0.08
P27	−	−	−	+	27,000	27,000	
P23	+	+	+	+	25,000	23,000	6.36 ± 0.86
P22	+	+	+	+	24,000	21,500	1.54 ± 0.63
P18	+	−	+	−	18,400	17,500	
P12	+	+	+	+	13,300	12,300	2.09 ± 0.42
P10	+	+	+	+	11,500	10,200	2.21 ± 0.31
P9	+	+	+	+	11,000	9,100	
P8	+	+	+	+	10,300	8,500	2.02 ± 0.05

[a] Polypeptides were precipitated with anti-RNP or anti-Sm antibodies in [^{35}S]methionine (Met)- or [^{3}H]leucine (Leu)-labeled extracts. +, Immunoprecipitable; −, not immunoprecipitable.

[b] From 7.5 to 17.5% gradient polyacrylamide gels containing 0.1% SDS.

[c] From 15% polyacrylamide gels containing 7 M urea and 0.1% SDS.

[d] Ratio of anti-Sm to anti-RNP immunoprecipitability from unfractionated nuclear extracts as determined from densitometer tracings of autoradiograms. Values are average estimates of five immunoprecipitations of [^{35}S]methionine- and [^{3}H]leucine-labeled extracts. P10 and P9 were considered as a unit.

shown in Table 1. Minor bands of higher molecular weight were also observed. These were present in less than unit levels and were not considered here, but they may represent loosely associated polypeptides, polypeptides present in minor fractions of snRNPs, or polypeptides recognized by minor serum activities. Polypeptide P18 was not observed in immunoprecipitations of [^{3}H]leucine-labeled extracts. Its abundance varied with different sera in a manner which could not be correlated with the RNP or Sm character of the serum. For these reasons it was not included in any of the calculations.

Autoradiograms of gels of immunoprecipitates such as those shown in Fig. 1 were traced and integrated by densitometry. These data were used to estimate the relative intensities of individual bands to establish the distribution and relative amounts of individual polypeptides in different snRNP populations. Unfractionated nuclear extracts contain equal amounts of U1 and U2 RNAs and 5% each of U4, U5, and U6 RNAs (7). In the absence of contradictory data, we estimate the abundance of each type of snRNP to be equal to that of its constitutive snRNA. Immunoprecipitation of labeled extracts with an anti-RNP serum should indicate the relative amount of U1 snRNP polypeptides. Use of anti-Sm antibodies should yield information concerning the cumulative amounts of U1 and U2 snRNP polypeptides, assuming that U4, U5, and U6 snRNPs are in insufficient abundance to influence the observed immunoprecipitation pattern. Table 1 indicates the ratio of anti-Sm to anti-RNP immunoprecipitability for each polypeptide from leucine- or methionine-labeled unfractionated nuclear extracts. This ratio provided consistent information about the distribution of each polypeptide in U1 versus U2 snRNPs. Polypeptide P30 exhibited an Sm/RNP ratio of 1.0 in all precipitations, indicating that it is a U1-specific polypeptide. Polypeptide P27 was only observed in anti-Sm immunoprecipitations, indicating that it is U2 specific. Polypeptide P23 exhibited an Sm/RNP ratio of 5 to 6, suggesting that it is highly enriched in U2 snRNPs relative to U1 snRNPs. Polypeptide P22 had an Sm/RNP ratio of less than 2.0, suggesting that it might be enriched in U1 versus U2 snRNPs. Polypeptides P12, P10, P9, and P8 exhibited Sm/RNP ratios of 2.0, suggesting that they are constituents of both types of RNPs and present in roughly equal ratios in both classes.

Estimates of the relative content of each polypeptide in U1 and U2 snRNPs were made from leucine-labeled immunoprecipitates (assuming proportional leucine content) and from molecular weight estimates from gel electrophoresis (Table 2). Alternate relative levels of U1 and U2 polypeptides were calculated by choosing a different polypeptide to represent unity (see above). The assumption that P8 represented unity yielded values best fitted to whole integers and produced U1 snRNP molecular weights consistent both with previous estimates (3, 8, 11) and with sucrose density centrifugation (Fig. 2). Under this assumption, several polypeptides were present in more than one copy per assembly, with polypeptide P12 being the most abundant. Polypeptides P23 and P22 were present individually in U1 snRNPs in less than unit amounts. Cumulatively, however, P22 and P23 were present in unit levels, suggesting either that there are subpopulations of U1 snRNPs or that these two bands represent alternate forms of the same polypeptide. Polypeptide P23 was present in higher relative quantities than polypeptide P22 in U2 immunoprecipitations, and polypeptide P22 was present in higher levels than polypeptide P23 in U1 immunoprecipitations, indicating that if P23 and P22 are two forms of the same polypeptide, then one form predominates in U2 snRNPs and the other in U1 snRNPs. Polypeptides P12, P10, P9, and P8 appeared in equal ratios in both classes. The snRNP values in Table 2 yield minimum RNP molecular weights of 210,000 and 235,000 for U1 and U2 snRNPs, respectively.

TABLE 2. Relative levels of snRNP polypeptides in U1 and U2 snRNPs

Polypeptide	Relative level ± SD in:		Predicted[c] Sm/RNP
	U1 snRNPs[a]	U2 snRNPs[b]	
P30	2.13 ± 0.22	0	1.0
P27	0	2.02 ± 0.25	
P23	0.17 ± 0.04	1.51 ± 0.39	9.8
P22	0.70 ± 0.18	0.32 ± 0.03	1.5
P12	3.91 ± 0.46	4.16 ± 0.75	2.0
P10 + P9	2.38 ± 0.17	2.37 ± 0.29	2.0
P8	1.00 ± 0.08	1.00 ± 0.12	2.0

[a] Average of three immunoprecipitations. Two of these were from unfractionated snRNP extracts; one was from the fractionation experiment shown in Fig. 4.

[b] The observed U2 polypeptide ratios were also consistent with a U2 assembly containing two RNAs per complex and twice the indicated polypeptide content.

[c] Ratio of anti-Sm to anti-RNP immunoprecipitability from an unfractionated nuclear extract as predicted by the indicated values.

Sucrose gradient centrifugation of nuclear extracts was performed to obtain fractions enriched in individual snRNP populations to confirm the results of experiments with unfractionated extracts (Fig. 2). By using $^{32}P_i$-labeled extracts, three subpopulations of snRNPs defined by distinct sedimentation behavior were observed: U1 snRNPs (10S); U2, U4, and U6 snRNPs (15S); and U5 snRNPs (sedimented

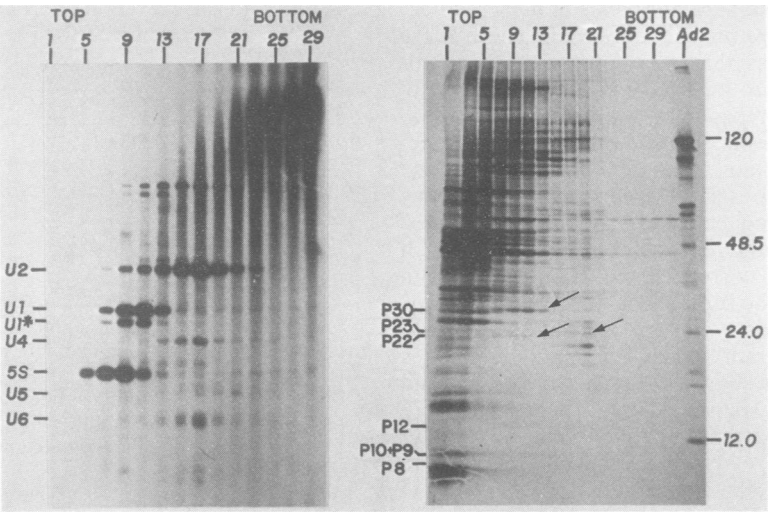

FIG. 2. Sucrose gradient fractionation of $^{32}P_i$- and [^{35}S]methionine-labeled human snRNPs. RNA was prepared from alternate fractions of the phosphate-labeled gradient and subjected to electrophoresis on 10% polyacrylamide gels containing 7 M urea. Protein was precipitated from alternate fractions of the methionine-labeled gradient and subjected to electrophoresis on 7.5 to 17.5% SDS-acrylamide gels. Exposure was for 1 day (RNA) or 6 weeks (protein). Suspected snRNP polypeptides are indicated by arrows. A cosedimented phage P22 tail sedimentation marker (9S) sedimented in fractions 8 and 9. Extractions of all populations subjected to sucrose gradient centrifugation were performed in 0.10 M NaCl.

throughout the lower half of the gradient). Observation of three sedimenting populations was dependent upon the ionic strength used. The gradient and extraction conditions used in the experiment shown in Fig. 2 were identical to those used in the immunoprecipitation experiments. Thus, ignoring possible sedimentation effects, the snRNPs in Fig. 2 would be predicted to contain the polypeptides defined by immunoprecipitation. Parallel gradients of [^{35}S]methionine-labeled extracts were also analyzed for polypeptides. The snRNP polypeptides, as defined by immunoprecipitation, were present in one or more regions of the gradient. Polypeptide P30 sedimented exclusively in the 10S region of the gradient containing U1 snRNA. The majority of polypeptide P22 also sedimented in this region of the gradient, whereas the majority of polypeptide P23 sedimented in the U2-rich region of the gradient. Polypeptides P12, P10, P9, and P8 were difficult to visualize in Fig. 2; the use of unlabeled nuclear extracts indicated that polypeptides P12, P10, P9, and P8 appeared in both regions. No snRNP polypeptides could be discerned in the broad region of the gradient containing U5 snRNPs, presumably because of the low amounts of material in any given fraction. The observed sedimenting populations of [^{35}S]methionine-labeled polypeptides agreed with the immunoprecipitation designations.

[^{35}S]methionine-labeled gradient samples were also analyzed by immunoprecipitation. All of the RNA in a given fraction could be immunoprecipitated, indicating that all resided in ribonucleoprotein assemblies. The intensities of individual polypeptides varied according to the particular fraction tested and the antibody used (Fig. 3). The observed Sm/RNP ratios for each polypeptide, however, correlated with the ratio of U2 plus U1 RNA to U1 RNA and agreed with the assignments made from immunoprecipitation of unfractionated nuclear extracts.

[^{3}H]leucine- and $^{32}P_i$-labeled sucrose gradient fractions were also immunoprecipitated with the two SLE antibodies (Fig. 4). Two fractions of the gradients were chosen for immunoprecipitation. The first, fraction 5, had a U1/U2 ratio of 10:1. The second, fraction 7 (RNA) or 8 (protein) had a U2/U1 ratio of 10:1; the amount of U2 in fraction 8 was roughly equivalent to the amount of U1 in fraction 5 (Fig. 4A). All of the U RNA in fractions 5 and 7 was immunoprecipitable by the appropriate antibody, indicating quantitative immunoprecipitation (Fig. 4B). Immunoprecipitates of the [^{3}H]leucine-labeled U1-rich fraction contained P30, P22, P12, P10, P9, P8, traces of P23, and no P27 (Fig. 4C). Each appeared in equal intensity in anti-RNP and anti-Sm immunoprecipitates, reflecting the lack of other snRNPs in this gradient fraction. Anti-RNP immunoprecipitation of the U2-rich gradi-

ent fraction produced little signal, reflecting the lack of U1 snRNPs in this fraction. Anti-Sm immunoprecipitates contained no P30 but did contain P27, P23, P12, P10, P9, P8, and some P22. Polypeptides P12, P10, P9, and P8 were present in equal intensity in the anti-RNP-immunoprecipitated U1-rich fraction and in the anti-Sm-immunoprecipitated U2-rich fraction, which is consistent with their presence in both classes of snRNPs in roughly equal amounts. Densitometer tracings of fractions 5 and 8 indicated the same level for each snRNP polypeptide as calculated (Table 2) from data obtained by using unfractionated nuclear extracts. Thus, gradient centrifugation confirmed the exclusive presence of P30 and P27 in U1 and U2 snRNPs, respectively, and the enrichment of P23 in U2 snRNPs.

DISCUSSION

Previous experiments with [^{35}S]methionine-labeled extracts and the SLE anti-RNP and anti-Sm antibodies had suggested that the five snRNAs immunoprecipitated by one or both of these antibodies were immunoprecipitated with an identical set of seven polypeptides. Because two of these RNAs, U1 and U2, are present in 10-fold higher abundance than the other three and because no serum selectively reacts with the latter three, this similarity at best concerned U1 and U2 snRNPs. Both U1 and U2 snRNPs have been postulated to provide similar adaptor functions for RNA splicing (6, 9). It therefore was of interest to determine which polypeptides were associated with each of the five snRNP snRNAs. We report here that U1 and U2 snRNPs do not contain the same set of polypeptides; instead, each class contains common and unique polypeptides. To facilitate comparisons of data with that of other laboratories, we have designated these polypeptides by their molecular weights.

Four small polypeptides, P12, P10, P9, and P8, appeared to be associated with both U1 and U2 snRNPs. Both anti-RNP and anti-Sm SLE antibodies immunoprecipitated all four polypeptides. The use of anti-Sm antibodies to immunoprecipitate an unfractionated nuclear extract in which U1 and U2 snRNPs were in roughly equal abundance produced a polypeptide signal for each of these four polypeptides which was twice that produced by using anti-RNP antibodies. Immunoprecipitation of fractionated U1 and U2 snRNPs confirmed the presence of these four polypeptides in equal amounts on both snRNPs. Polypeptides P10, P9, and P8 appeared to be present in one copy per snRNP assembly; polypeptide P12 was present in multiple copies based on tracing and integration of bands in protein gels of immunoprecipitates labeled with leucine

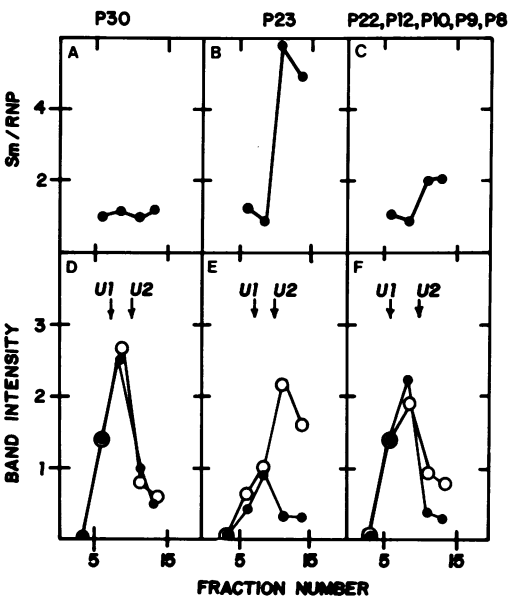

FIG. 3. Immunoprecipitation by SLE antibodies of [^{35}S]methionine-labeled, sucrose gradient-fractionated snRNPs. Intensities of individual bands determined from scanned autoradiograms were plotted with respect to gradient fraction. Immunoprecipitation of polypeptides with respect to anti-RNP serum (●) and anti-Sm serum (○) fell into three patterns represented in panels D, E, and F. The ratio of intensity of each band immunoprecipitated by anti-Sm serum to that immunoprecipitated by anti-RNP serum is indicated in panels A, B, and C. The positions of peak U1 and U2 RNA sedimentation in parallel $^{32}P_i$-labeled gradients are indicated by arrows.

and assuming proportional leucine content. The four smallest snRNP polypeptides migrated differently in different gel systems. Therefore, with the exception of P12, which can be identified by its relative abundance, the identification of P10, P9, and P8 in any given system will require purification of individual polypeptides.

The largest snRNP polypeptide, P30, appeared to be U1 snRNP specific. It was immunoprecipitated from unfractionated extracts by anti-RNP antibodies and by anti-Sm antibodies so as to produce bands of equal intensity. Separation of U1 and U2 snRNPs on sucrose gradients indicated that P30 could be visualized and immunoprecipitated from only the U1 snRNP portion of the gradient. Two copies of P30 were estimated to be present on each U1 snRNP.

Polypeptide P27 could be visualized in anti-Sm immunoprecipitations but not in anti-RNP immunoprecipitations, suggesting that it resides on U2 snRNPs. This assignment was confirmed by showing that P27 could be immunoprecipitated from only the U2 snRNP region of a sucrose gradient. Polypeptide P27 was barely detectable

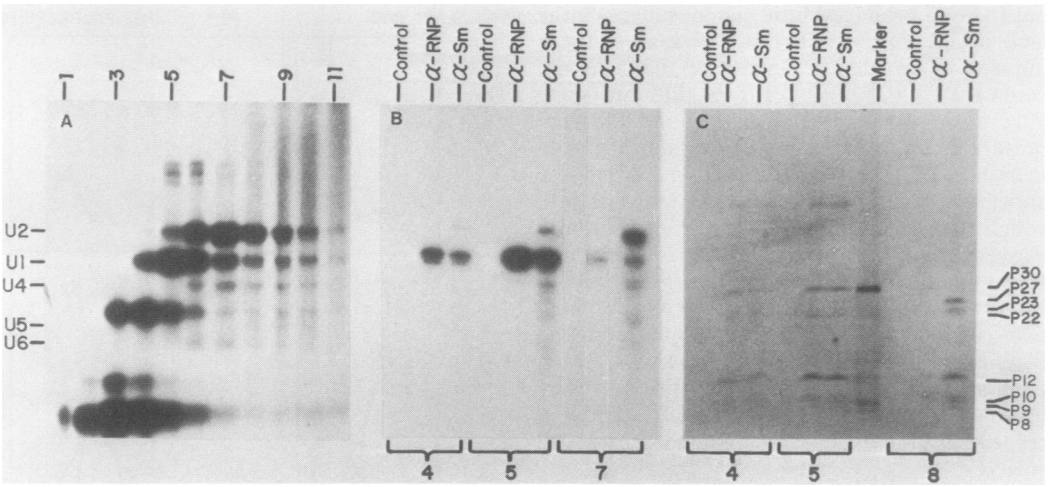

FIG. 4. Immunoprecipitation by SLE antibodies of $^{32}P_i$- or [^{3}H]leucine-labeled, sucrose gradient-fractionated snRNPs. Labeled extracts were applied to parallel sucrose gradients. (A) RNA present in the $^{32}P_i$-labeled gradient fractions. (B) RNA immunoprecipitated by control, anti-RNP, or anti-Sm antibodies from fractions 4, 5, and 7 of the gradient in (A). (C) Polypeptides immunoprecipitated by control, anti-RNP, or anti-Sm antibodies from fractions 4, 5, and 8 of a parallel gradient of [^{3}H]leucine-labeled material. Equivalent fractions in the two gradients had identical refractive indices.

in experiments utilizing methionine-labeled snRNPs but was readily apparent with leucine-labeled snRNPs. The deficiency of methionine in P27 presumably explains why it has not been previously detected. Each U2 snRNP was estimated to have two copies of P27.

Two polypeptides of similar molecular weight, P23 and P22, were present on both U1 and U2 snRNPs. U1 snRNPs were enriched in P22; U2 snRNPs were enriched in P23. Although quantitation of these two polypeptides was difficult because of their close migration in gels, separation of U1 and U2 snRNPs on sucrose gradients clearly demonstrated a bias of each snRNP for one of these two polypeptides. Because neither P22 or P23 is present in U1 snRNPs in unit level, but their combined values equal one, and because they have similar molecular weights, it is possible that they represent alternate forms of the same polypeptide. If they do represent altered or modified forms of a single polypeptide, then their modification must be snRNP specific.

The antibodies used in these studies were from human patients diagnosed as suffering from SLE. It is possible that one or more of the polypeptides which we observed is not a component of snRNPs but is a protein immunoprecipitated by some other antibody present in the sera. We have tested over 20 anti-RNP and anti-Sm sera and found results identical to those shown here. Furthermore, the invariance of snRNP polypeptide stoichiometry in fractionated snRNPs and unfractionated extracts strongly supports the contention that the polypeptides that we have characterized are indeed snRNP proteins.

The calculations presented in Table 2 depend upon representative leucine content in each snRNP polypeptide. Some of the snRNP polypeptides were quite small and might contain abnormally low or high amounts of leucine, causing an incorrect estimation. We chose the smallest observed molecular weights for polypeptides P10, P9, and P8 from polyacrylamide gels containing urea and SDS. If higher molecular weights are correct, however, then the values indicated in Table 2 underestimate the relative abundance of the larger snRNP polypeptides.

Because snRNPs are macromolecular assemblies, the possibility exists that we lost snRNP constituents during immunoprecipitation. We have never observed non-immunoprecipitable snRNAs, but it is impossible to comment on the presence of non-immunoprecipitable snRNP polypeptides in such crude extracts. Thus, the assessments in Table 2 are minimum values and are only valid for those snRNP constituents which remain associated throughout immunoprecipitation.

Polypeptides with molecular weights similar to those observed here have been reported by others (1, 6, 12). The smaller snRNP polypeptides, our P12, P10, P9, and P8, are the proteins which remain most tenaciously associated with U RNAs during purification attempts (2, 10, 11). Several laboratories have reported antigenic activity of polypeptides in this size range from immunoaffinity-purified RNP and Sm antigens

(2, 11, 12). By protein gel blotting, White and Hoch (13) have identified a lapine 13,000-dalton polypeptide which reacts with anti-Sm antibodies; this is presumably the lapine equivalent of the human polypeptide P12 found on U1 and U2 snRNPs. Anti-RNP antibodies recognize lapine 40,000- and 70,000-dalton polypeptides and a bovine 30,000-dalton polypeptide; the 30,000- to 40,000-dalton anti-RNP antigen is presumably equivalent to the U1-specific P30 described in this paper. We observed a large U1-specific polypeptide (Fig. 4) of 60,000 to 70,000 daltons, but its immunoprecipitation was neither reproducible nor quantitative. Its relationship to the structure of snRNPs is, therefore, unclear at this time.

The assignment and relative ratios of snRNP polypeptides in U1 and U2 snRNPs suggested that although each contained unique polypeptides, the ribonucleoproteins were very similar assemblies of 200,000 daltons. Sucrose density centrifugation, however, indicated a very different S value for the two populations in 0.1 M NaCl. Immunoprecipitation of snRNPs after such sucrose gradient centrifugation demonstrated that the differentially sedimenting snRNPs contained the same polypeptides in the same relative amounts as they did before fractionation. Thus, the sedimentation differences shown in Fig. 2 for U1 and U2 snRNPs are not caused by differential snRNP stability. At a salt concentration of 0.40 M, which is reported to release snRNPs from heterogeneous nuclear RNP (15), differential snRNP sedimentation behavior disappeared, and all snRNPs sedimented at 10S (data not shown). Immunoprecipitation experiments with labeled extracts prepared in 0.40 M NaCl gave results identical to those observed with antigen prepared at a lower ionic strength. Thus, the change in sedimentation behavior of U2, U4, U5, and U6 snRNPs in high salt is also not caused by loss of snRNP constituents.

Two explanations for the different sedimentation behavior of U1 versus U2, U4, and U6 snRNPs seem plausible. The relative amounts of snRNP constituents for the latter class of snRNPs (Table 2) are consistent either with an assembly of minimum molecular weight of 235,000 containing one RNA per snRNP or with a particle of twice this molecular weight containing two RNAs. Sedimentation differences in high salt may reflect a monomer-dimer transition. Alternatively, the high S values for U2, U4, U6, and especially U5 snRNPs could reflect associations with other nuclear components (notably heterogeneous nuclear RNP) in a salt-sensitive fashion.

Because of the presence of the Sm antigen on all snRNPs and the different abundances of U2, U4, U5, and U6 snRNPs, immunoprecipitations are ineffectual in indicating much about the structure of the latter three snRNPs. Preliminary purification attempts (Kinlaw and Berget, unpublished data) have fractionated U2 snRNPs away from U4 and U6 snRNPs. The U2 snRNP polypeptide composition after several steps of purification agreed with the results discussed herein. U5 snRNPs consistently fractionated away from the other snRNPs in purification experiments, and P12, P10, P9, and P8 were the only snRNP polypeptides found in these fractions. Further purification will be required to unambiguously define the structure of these very interesting ribonucleoproteins.

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ADDENDUM

Recently we obtained fractions of U1 and U2 snRNPs substantially free of other snRNPs and contaminating cellular proteins. Both populations contain the common and unique polypeptides described in this paper. Estimations of polypeptide ratios from Coomassie blue staining of these purified fractions agreed with those shown in Table 2, with the exception of P27 which was reduced to one copy per U2 snRNP.

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