Fractionation and Characterization of Human Small Nuclear Ribonucleoproteins Containing U1 and U2 RNAs*

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Human small nuclear ribonucleoproteins (snRNPs) containing U1 and U2 snRNAs have been isolated from cultured cells by nonimmunological methods. The U1 snRNP population remained immunoprecipitable by systemic lupus erythematosus anti-RNP and anti-Sm antibodies throughout fractionation and contained polypeptides of molecular weights corresponding to those defined as U1 snRNP polypeptides by immunoprecipitation of crude extracts. The purified assemblies contained U1 RNA and nine snRNP polypeptides of molecular weights 67,000 (P67), 30,000 (P30), 23,000 (P23), 21,500 (P22), 17,500 (P18), 12,300 (P12), 10,200 (P10), 9,100 (P9), and 8,500 (P8). P67, P30, and P18 were unique to U1 snRNPs. The U2 snRNP population remained immunoprecipitable by the systemic lupus erythematosus anti-Sm antibody throughout fractionation. The purified U2 assemblies contained six polypeptides of molecular weights corresponding to those defined by immunoprecipitation to be common to U1 and U2 snRNPs including P23, P22, P12, P10, P9, and P8. In addition, U2 snRNPs contained a unique polypeptide of 27,000 Da.

Eukaryotic cells contain large amounts of several snRNAs which are assembled into snRNPs (1-3). The snRNAs (U1, U2, U4, U5, and U6) in one class of these ribonucleoproteins are of potential importance in eukaryotic gene expression in that one or more of them may play a role in RNA splicing (2-5). Sequence comparisons have indicated that U1 RNA contains sequences complementary to consensus sequences at the termini of intervening sequences (3, 4). Such comparisons have also indicated that U2 RNA has sequences complementary to at least some exon sequences proximal to splice junctions (5). It has been postulated that U1 and U2 snRNAs, in the form of their snRNPs, act as adapters of splicing by juxtaposing exon sequences through RNA-RNA hybridization (3-5). Antibodies to snRNPs have been shown to inhibit the production of mature spliced RNA in in vitro nuclear subunit-saturating snRNP involvement in some step in RNA biosynthesis (6). Direct proof of the involvement of snRNPs in splicing, however, has not been reported.

Because of the sedimentation behavior of snRNPs through sucrose gradients, it has been suggested that each snRNA resides in a separate ribonucleoprotein complex (3). This has been demonstrated for U1 snRNPs through the use of two types of antibodies produced by certain patients suffering from SLE (7-13). One class of SLE antibody, anti-RNP, selectively recognizes U1 snRNPs. Immunoprecipitation with anti-RNP antibodies defines U1 snRNPs as assemblies of U1 snRNA and eight polypeptides of molecular weights 30,0, 23,0, 21.5, 17.5, 12.3, 10.2, 9.1, and 8.5 \times 10^3 which we have designated P30, P23, P22, P18, P12, P10, P9, and P8, respectively (10). The second type of SLE antibody, anti-Sm, immunoprecipitates and defines the entire class of U1, U2, U4, U5, and U6 snRNPs. Anti-Sm antibodies immunoprecipitate the same group of proteins as do anti-RNP antibodies plus one additional polypeptide of 27,000 Da (7, 10). Because of the paucity of anti-Sm-specific polypeptides, it was originally postulated that U1, U2, U4, U5, and U6 snRNPs were assemblies of very similar structure (7).

Because U1 and U2 snRNPs are 10-fold more abundant than U4, U5, and U6 snRNPs, immunoprecipitation experiments yield little information about the structure of the assemblies containing these RNAs other than they must contain Sm antigen. Polypeptides of molecular weights similar to the snRNP polypeptides P23, P22, and P12 are recognized by various anti-Sm sera in experiments utilizing protein-blotting techniques (12, 13). Thus, each snRNA must reside in a complex containing one or more of these polypeptides.

An assessment of snRNP structure and function will require purification of snRNP subpopulation and development of in vitro assays of their activity. Their purification is complicated by several factors. Because no assay for snRNPs other than immunoreactivity has been developed, their presence during purification must be monitored by observation of known snRNP constituents. Furthermore, snRNPs are related multicompartment assemblies of unknown complexity and stability. Any successful purification protocol must fractionate individual snRNP subpopulations without destroying the integrity of the snRNP assembly. Most published fractionation attempts have utilized SLE antibodies and immunofluorescence chromatography (8, 12). Although such an approach has been used successfully to purify antigenic components, it has not resulted in the isolation of intact snRNP assemblies or in the fractionation of U2, U4, U5, and U6 snRNPs. Furthermore, it has never been established that the SLE antibodies immunoprecipitate the entire set of snRNP constituents or if some constituents disassociate during immunoprecipitation. Immunoprecipitation of nuclear extracts with the SLE antibodies, however, does define a minimum set of polypeptides associated with U1 and U2 RNAs. These polypeptides should be present in the final fraction from a successful isolation.
Ul and U2 snRNP Isolation

scheme and include the species P30, P23, P22, P18, P12, P10, P9, and P8 (7-13) for U1; and P27, P23, P22, P12, P10, P9, and P8 for U2. An additional polypeptide of 67,000 Da has been observed using protein-blotting techniques with the anti-RNP antibody and might also be U1-snRNP-associated (9, 11, 13). If all of these polypeptides were to reside in single populations of U1 and U2 snRNPs, a purified U1 preparation would therefore contain one RNA and nine polypeptides, and a purified U2 preparation would contain one RNA and seven polypeptides. Because some of these proteins are present in more than one snRNP, a successful purification must document the absence of other snRNP assemblies and their components in each final fraction. We have developed an isolation scheme for human snRNPs relying on ion exchange chromatography to separate snRNPs both from each other and from the bulk of cellular protein. Here we report the application of this protocol to the isolation of U1 and U2 snRNPs.

EXPERIMENTAL PROCEDURES

Fractionation of Human snRNPs—An isolation scheme for human U1, U2, U4, U5, and U6 snRNPs has been designed to permit both fractionation of snRNP subpopulations and maintenance of the integrity of each snRNP assembly. The steps in this protocol used to isolate U1 and U2 snRNPs are diagrammed in Fig. 1. A detailed description of the various fractionation steps is included in the Miniprint.

Because no functional assay for snRNPs has been developed, fractionation steps were monitored for snRNP RNA and protein components by gel electrophoresis. U1 and U2 RNAs are very abundant, and the RNA content of even crude fractions was easily visualized on 10% polyacrylamide gels (see Fig. 2). U1 and U2 snRNP polypeptides, however, did not become directly visible on SDS polyacrylamide gels until after heparin agarose chromatography. At this stage, protein components were identified by comparison of their migration in 7.5-17.5% gradient SDS polyacrylamide gels to that of radiolabeled U1 and U2 snRNP polypeptides which had been immunoprecipitated from crude extracts by the SLE antibodies. Such immunoprecipitations are included in Fig. 11 to aid the reader in making similar comparisons. All protein polyacrylamide gels shown in this paper are of the same composition to facilitate comparison.

The initial steps in the isolation scheme involved extraction of isolated nuclei and subsequent dialysis before chromatography on DEAE (see the Miniprint). The extraction conditions employed were those which have been used previously to provide nuclear extracts for immunoprecipitation experiments (7, 10). Nuclear extracts were dialyzed in preparation for chromatography. During dialysis, an insoluble pellet containing large

3The "Experimental Procedures" are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-3176, cite the authors, and include a check or money order for $1.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
the pellet from dialysis 3 was used in order to better visualize the U5 RNA in this fraction. The very top of the gel is carrier P22 DNA. Equal portions of each dialysis supernatant-precipitate pair were loaded for comparison except for nuclear extract; from dialysis 2. Some U1 RNA (varying from 5-25%) was lost in this pellet and has not been further characterized. Chromatography of the dialyzed nuclear extract on DEAE afforded initial DEAE-fractions due to the presence of significant quantities of contaminating cellular proteins at this stage of purification. It was not possible to identify snRNP polypeptides in the DEAE-fractions due to the presence of significant quantities of contaminating cellular proteins at this stage of purification. Because U1 and U2 snRNPs are multicomponent assemblies of uncertain stability, it was necessary to demonstrate that the bulk of snRNP polypeptides were co-purifying with U1 and U2 RNAs during the early stages of purification when they were not directly observable. To monitor snRNP polypeptides during DEAE-chromatography, the protocol was repeated utilizing extracts labeled in vivo with [35S]methionine. Parallel DEAE-columns were run and fractions of the same ionic strength from both columns were analyzed for snRNP RNAs or proteins by immunoprecipitation with SLE antibodies. An autoradiogram of the protein gel from this experiment is depicted in Fig. 4. Four fractions, including a U1-rich (fraction 9) and a U2-rich (fraction 13) fraction, were immunoprecipitated with control, anti-RNP, and anti-Sm antibodies. The snRNP polypeptides P67, P30, P27, P23, P22, P12, P10, P9, and P8 could be immunoprecipitated only from fractions also containing U1 or U2 RNAs (Fig. 4 and data not shown). The U1-specific polypeptide P18 does not label with leucine and could not be observed in this experiment (a leucine label was chosen to maximize the appearance of the U2-specific, methionine-deficient P27). The fraction containing the maximal signal of anti-RNP immunoprecipitable U1-specific P30 corresponded to the fraction containing the maximal signal of U1 RNA (fraction 9). Furthermore, this fraction also contained the highest level of total anti-RNP immunoprecipitable protein. The fraction containing the maximal signal of anti-Sm-immunoprecipitable U2-specific P27 (P27 is indicated with an arrow) corresponded to the fraction containing the maximal signal of U2 RNA and considerable protein formed and was removed (Fig. 2). Some U1 RNA (varying from 5-25%) was lost in this pellet and has not been further characterized. Chromatography of the dialyzed nuclear extract on DEAE afforded initial fractionation of snRNP subpopulations (Fig. 3). At this stage of the isolation, elution of individual snRNPs was monitored by the snRNA content of column fractions. Early eluting fractions (27-33) eluted at 0.15 M NH4Cl; peak U2-containing fractions (36-42) eluted at 0.30 M NH4Cl. Because U1 and U2 snRNPs are multicomponent assemblies of uncertain stability, it was necessary to demonstrate that the bulk of snRNP polypeptides were co-purifying with U1 and U2 RNAs during the early stages of purification when they were not directly observable. To monitor snRNP polypeptides during DEAE-chromatography, the protocol was repeated utilizing extracts labeled in vivo with [35S]methionine. Parallel DEAE-columns were run and fractions of the same ionic strength from both columns were analyzed for snRNP RNAs or proteins by immunoprecipitation with SLE antibodies. An autoradiogram of the protein gel from this experiment is depicted in Fig. 4. Four fractions, including a U1-rich (fraction 9) and a U2-rich (fraction 13) fraction, were immunoprecipitated with control, anti-RNP, and anti-Sm antibodies. The snRNP polypeptides P67, P30,
RNA (fraction 13). As expected, both of the fractions containing maximal U1 and U2 RNA contained high levels of total anti-5S immunoprecipitable protein (fractions 9 and 13). Thus, the fractions defined as rich in U1 or U2 RNA corresponded to those containing maximal amounts of U1 or U2-specific immunoprecipitable protein. By this criterion, therefore, the defined U1 and U2 RNA-associated polypeptides (especially P30 and P27) fractionated during DEAE-chromatography as single and distinct populations which correlated with their respective snRNAs.

Isolation of U1 snRNPs—Peak U1 RNA-containing fractions from DEAE-chromatography were subjected to chromatography on heparin agarose. Fractions of the DEAE-column were chosen such that U1 RNA was the major RNA present. U1 snRNPs bound tightly to heparin agarose (Fig. 5). Any contaminating U2, U4, U5, and U6 snRNPs from later DEAE-fractions also bound to heparin agarose but eluted at much lower ionic strength than did U1 snRNPs (compare Figs. 5 and 7). Thus, following DEAE and heparin agarose chromatography, U1 snRNPs were homogeneous with respect to their RNA content. Polypeptides of the correct mobility to be the U1-associated, anti-RNP immunoprecipitable P67, P30, P23, P22, P12, P10, P9, and P8 first became visible at this stage in the isolation.

As a last step in the isolation of U1 snRNPs, the peak fractions from heparin agarose were subjected to chromatography on Cibacron blue agarose (Fig. 6). The applied snRNPs eluted as a single population containing both U1 RNA and snRNP polypeptides of the correct mobility to be P30, P23, P22, P12, P10, P9, and P8 (Fig. 6, fractions 17–23). Several polypeptides of 65–70,000 Da, one of which was major, copurified with U1 RNA on both heparin agarose and Cibacron
blue agarose. One of these is presumably the U1-specific P67 which reacts with the anti-RNP antibody in protein blots (13). A few polypeptides not observed in immunoprecipitations of crude extracts with anti-RNP antibodies were also present in the peak fractions. Their significance is unclear. It is possible that one or more of these additional polypeptides are actual U1 snRNP constituents which do not remain tightly associated with the snRNP assembly during immunoprecipitation.

Isolation of U2 snRNPs—After DEAE-chromatography (Fig. 3), a fraction enriched in U2, U4, and U6 snRNPs was obtained. Chromatography of this fraction on heparin agarose separated these snRNPs into two populations and removed contaminating U1 snRNPs (Fig. 7). Elution with a salt gradient released U4 and U6 snRNAs early (fractions 32–36) and U2 snRNA late (fractions 36–44) (Fig. 7, left). Some contaminating U5 snRNA was also visible in the fractions between the peak U2 and U4 fractions. Contaminating U1 snRNPs, present in the applied sample, bound tightly to heparin agarose and were not eluted under the salt conditions shown in Fig. 7. Analysis of the polypeptides present in various column fractions (Fig. 7, right) indicated that polypeptides of the correct mobility to be the snRNP proteins P23, P22, P12, P10, P9, and P8 appeared in both the U2 and U4-rich regions of the column. A polypeptide of the correct mobility to be the anti-Sm-specific P27 appeared only in the U2-rich region. Although well separated from other contaminating snRNPs at this stage of purification, the U2 population still contained substantial amounts of contaminating non-snRNP polypeptides. Pooled U2-rich fractions from heparin agarose chromatography were applied to ω-aminopentyl agarose and eluted with an NH₄Cl gradient; U2 snRNPs eluted as a single peak at a salt concentration of 0.70 M (data not shown). The eluted fractions still contained contaminating cellular proteins; U2 snRNPs were further fractionated on Cibacron blue agarose (Fig. 8) resulting in a better separation of putative snRNP polypeptides from other cellular proteins. A variable amount of degradation of U2 RNA occurred during this step as is evident in comparison of Figs. 7 and 8. The only major polypeptides co-purifying with the U2 snRNA (Fig. 8, fractions 6–12) following this step were the putative snRNP polypeptides P27, P23, P22, P12, P10, P9, and P8 and one larger polypeptide of 62,000 Da.

In an attempt to assess both the yield and purity afforded
by our fractionation scheme, the total amounts of U1 and U2 RNAs in various fractions were calculated (Table I). Such an analysis was possible because of the abundance of U1 and U2 RNAs and the ability to identify and quantitate them in ethidium bromide-stained gels of crude samples (see Fig. 2). By such calculations, the total yield of U1 and U2 RNAs was 17 and 24%, respectively. Most of the losses occurred during dialysis of the nuclear extract and DEAE-chromatography (the DEAE early eluting U1-rich fractions contained 50–70% of the applied U1 RNA). Thus, although the final fractions contained all of the polypeptides defined as U1 and U2 snRNPs constituents by immunoprecipitation, it is unambiguously proven that the isolated snRNPs populations are representative of total cellular U1 and U2 snRNPs.

Characterization of snRNPs—To assess the integrity of the snRNPs in the final fractions, various column fractions were immunoprecipitated with anti-Sm, anti-RNP, or control antisera. RNA present in both the immunoprecipitate and the supernatant from immunoprecipitation were subjected to gel electrophoresis to detect U1 and U2 RNAs. Fig. 9 shows the results from such experiments using U1 and U2 snRNPs from Cibacron blue agarose chromatography. U1 RNA (Fig. 9A) was quantitatively immunoprecipitated with both the anti-RNP and the anti-Sm antibodies; U2 RNA (Fig. 9B) was quantitatively immunoprecipitated with the anti-Sm antibody, indicating that all of the RNA in the final U1 and U2 fractions resided in immunoreactive complexes. No U2 RNA could be detected in anti-RNP immunoprecipitations in agreement with results using crude extracts. Thus, all of the U1 RNA was simultaneously associated with the RNP and Sm antigens, and all of the U2 RNA in the final fraction was associated with the Sm antigen(s) but not with the RNP antigen(s).

The final fractions, however, were dialyzed to a final salt concentration of 0.10 M before immunoprecipitation. We do not know if some reassembly of partially disassembled snRNP complexes occurred during dialysis.

Direct immunoprecipitation of the polypeptides in the purified U1 and U2 fractions was not useful because of the high backgrounds associated with the collection of immune complexes with Pansorbin. In an attempt to visualize the immunoprecipitable proteins present in our final fractions, U1 and U2 snRNPs were incubated with SLE antibodies and the resulting immune complexes were collected on Staph A Sepharose. Following elution with strong denaturants, the proteins present in both the Staph A Sepharose eluate and flow-through fractions were subjected to gel electrophoresis. U1 polypeptides identified as P30, P12, P10, P9, and P8 bound to the RNP antibodies and were detected in the eluate from Staph A Sepharose (Fig. 10A). Thus, the identification of these polypeptides as U1 snRNP polypeptides is based on their relative gel mobility and not on their reaction with the Sm antigen(s) but not with the RNP antigen(s). Observation of P67, P23, and P22 was obscured by the presence of IgG released under the stringent conditions required to disrupt snRNP-antibody complexes. The presence of IgG in the eluate precluded analysis of all but the low molecular weight U2 snRNP polypeptides P12, P10, P9, and P8. These proteins, however, remained immunoreactive in the final U2 fraction (Fig. 10B). Therefore, both the proteins of these molecular weights in stained gels of isolated U2 snRNPs were correctly identified as snRNP proteins.

Application of the fractionation protocol described here resulted in considerable purification of U1 and U2 snRNPs.

**Table 1**

| Fraction                  | U1 Yield (%) | U2 Yield (%) |
|---------------------------|--------------|--------------|
| Nuclei                    | 100          | 100          |
| Nuclear extract           | 77           | 86           |
| DEAE-pool                 | 18           | 34           |
| Heparin pool              | 20           | 31           |
| &-Aminopentyl pool        | 16           | 30           |
| Cibacron blue pool        | 17           | 24           |

*The amount of RNA in an aliquot of each fraction was determined by densitometer tracing and integration of an acrylamide gel stained with ethidium bromide. These values were compared to a standard curve created for 5S RNA which had been subjected to electrophoresis and stained on the same gel as the samples. 5S RNA was chosen as a standard because it is of comparable size and secondary structure as U1 RNA.

*Before dialysis.*

![Fig. 9. Immunoprecipitation of U1 and U2 snRNPs](http://www.jbc.org/2020/03/24/immunoprecipitation.png)

Aliquots of pooled U1 (A) and U2 (B) Cibacron blue fractions were dialyzed against TMN to reduce the salt concentration. Dialyzed material was immunoprecipitated with control antibody, SLE anti-RNP antibody, or SLE anti-Sm antibody as described in the miniprint. RNA was extracted from both supernatant and precipitate fractions and subjected to gel electrophoresis in a denaturing gel with 5 S RNA marker. The brightly staining material in the precipitate lanes is from the Pansorbin used to collect the immunoprecipitates (see the no antibody lane).
**Fig. 10. Immunoreactivity of purified U1 and U2 snRNP proteins.** U1 (A) and U2 (B) snRNPs from pooled Cibacron blue agarose fractions were dialyzed against TMN to reduce the salt concentration and were then incubated with SLE or control antibodies. Immune complexes were selected on Staph A Sepharose and eluted with 7 M urea, 0.1% SDS following extensive washing. Fractions binding and not binding to Staph A Sepharose were analyzed for protein content. Flow-through and successive eluted fractions are indicated at the top of the gels. The antibody used in each incubation is indicated at the bottom of the gels. The flow-through fractions contained proteins not bound to antibodies; the eluate fractions contained antibodies and those proteins bound to the antibodies. Successive eluate fractions were collected to distribute the eluted IgG. A small fraction of the IgG did not bind to the Staph A Sepharose and can be detected in the flow-through lanes. The proteins present in the U1 and U2 samples before immunoprecipitation are also shown in the lanes marked U1 and U2 snRNP, respectively. U1 and U2 snRNP polypeptides are indicated to the sides of the gels. The gels were stained with silver. 67K, M, = 67,000.

Fig. 11 compares the low molecular weight polypeptides present in the final U2 snRNP fraction to those in U1 snRNPs as visualized by Coomassie staining. Several snRNP polypeptides stained poorly or appeared light in color with silver reagents and thus appear under-represented in black and white photographs. In particular, P12 and the U2-specific P27 appeared white to light brown in silver stained gels but stained brightly with Coomassie (compare Figs. 6, 8, and 11). The U1-specific polypeptide P18 only became visible when gels of purified U1 snRNPs were stained with Coomassie blue (compare Figs. 6 and 11). Inspection of one-dimensional gels indicated that the snRNP polypeptides P23, P22, P12, P10, P9, and P8 appeared in both snRNP populations. The relative amount of P23 and P22 in gradient SDS gels varied for U1 versus U2 snRNP subpopulations. U1 snRNPs contained more P22 than P23, and U2 snRNPs contained more P23 than P22. This variation was consistent in different gels and in different snRNP preparations and was observed previously in immunoprecipitation experiments with partially fractionated [3H]leucine-labeled snRNPs (10).

Fig. 11 (left) also depicts the proteins immunoprecipitated by anti-RNP and anti-Sm antibodies from partially fractionated [3H]leucine-labeled U1 and U2 snRNPs (10) to serve as a comparison for the Coomassie-stained protein gels of purified fractions. The pattern of snRNP proteins in immunoprecipitated and purified U1 and U2 snRNP populations shown in Fig. 11 were similar with respect to both the number of proteins in each and the relative amount of these proteins. Polypeptides P30, P18, and P67 (data not shown) were only found in purified U1 fractions and anti-RNP immunoprecipitates and were, therefore, designated U1-specific polypeptides. Anti-Sm immunoprecipitable P27 was found only in the purified U2 population designating it as a U2-specific polypeptide.

We had previously estimated the relative stoichiometry of snRNP constituent polypeptides by quantitating the amount of each polypeptide present in anti-RNP and anti-Sm immunoprecipitates of leucine-labeled nuclear extracts (10). To estimate if purification resulted in any observable loss of polypeptide constituents and to reevaluate stoichiometry us-
those derived for leucine-labeled immunoprecipitated sn-
estimates derived for purified material agreed very well with
ulative amounts of each U1 and U2 snRNP polypeptide in the
The U1-specific polypeptide P18 does not label with
in each snRNP polypeptide band was determined by integra-
the U1 fraction also contained U4 and U6 snRNPs (less than 10%
snRNPs can be estimated from the amount of P30 visible on the
by anti-RNP immunoprecipitations for U1 and anti-Sm immunopre-
stante in 7.5-17.5% gradient polyacrylamide gels containing 1% SDS
fractions of purified U1 and U2 snRNPs and subjected to electropho-
U6 (10) and subtracting the U1 contribution.

**DISCUSSION**

Immunoochemical experiments with the SLE anti-RNP and
anti-Sm antibodies have been previously used to identify the
polypeptides associated with U1 snRNA and collectively with
U2, U4, U5, and U6 snRNAs (7-13). Because of the lack of a
specific antibody for any of the latter four snRNPs, immuno-
chemical experiments have not yielded much information
about their structure. Furthermore, such experiments cannot
be used to address the existence of possible subpopulations of
a given snRNP type. Thus we have undertaken the task of
fractionation of snRNP populations.

Several laboratories have successfully purified snRNPs
populations through immunological methods (8, 9, 12). The sting-
ent conditions required to release antibody-antigen com-
plexes, however, have resulted in final fractions which lacked
some of the polypeptides indicated as snRNP components by
immunoprecipitation. Furthermore, biochemical isolations in-
volving high salt treatments (involving ammonium sulfate
precipitations or CsCl gradients) have also resulted in com-
xplexes lacking putative snRNP polypeptides (14). Thus we have
designed a biochemical protocol involving several types of
ion exchange chromatography performed at moderate ionic
strength to attempt to isolate intact snRNP assemblies.

We and others have isolated U1 snRNPs by a multi-step
nonimmunological purification scheme through which they
remained immunoprecipitable and contained the nine snRNP
polypeptides P67, P30, P23, P22, P18, P12, P10, P9, and P8
(Hinterberger, Pettersson, and Steitz, personal communica-
) U2 snRNPs have been isolated by a 6-step protocol
involving multiple ion exchange chromatography steps. The
final fraction contained U2 RNA and seven snRNP polypep-
dies, P27, P23, P22, P12, P10, P9, and P8. The latter six
were polypeptides also present on purified U1 snRNPs; P27
was unique to U2 snRNPs. One additional major polypeptide
of 60,000 Da was also present in the isolated material (Fig.
A protein of this molecular weight has never been detected
in immunoprecipitation experiments. This polypeptide may
represent a previously unidentified U2 snRNP constituent
which does not remain snRNP-associated during immuno-
precipitation. Polypeptides P67, P30, and P18 appeared only in
U1 snRNP fractions and anti-RNP immunoprecipitates and
are thus U1 specific.

The isolation scheme detailed in this paper permitted suc-
cessful fractionation of U1 and U2 snRNPs. Both the DEAE-
and heparin agarose columns aided in the separation. No
other snRNAs or proteins specific for other assemblies (not-
tably the U1-specific P67, P30, and P18 and the U2-specific
P27) were observable in the respective fractions after these
two chromatography steps.

The isolated assemblies appeared intact as judged by (a)
the presence of all of the appropriate SLE antibody immu-
 noprecipitable components in the final material, (b) the quan-
titative immunoprecipitability of the final complex, and (c)
the maintenance of the same relative amounts of various
snRNPs from crude extracts (with the exception that P27 and
P23 appeared under-represented in the purified U2). Thus,
by this criterion, there was no evidence for substantial loss of
snRNP polypeptides during purification. It should be noted
that by both estimates polypeptides P23 and P22 were present
in non-unit stoichiometry and polypeptide P12 was present
in multiple copies/assembly. A U1 snRNP containing the
constituents listed in Table II plus one copy of U1 RNA, P18,
and P67 would have a minimum molecular weight of 290,000.
This is in rough agreement with the previously observed 10 S
sedimentation value for U1 snRNPs (7).
what is observed in immunoprecipitated snRNPs from crude extracts.

It is assumed that the final fractions represent single populations of ribonucleoproteins. The RNA and proteins present in the final fractions did co-elute as a single peak from Cibacron blue agarose. Furthermore, this elution required high salt suggesting the presence of a single stable assembly. Some evidence, however, for the presence of subpopulations was obtained during isolation attempts. Late eluting U1 snRNPs from DEAE-chromatography behaved identically with the earlier peak eluting fractions upon subsequent chromatography on heparin agarose. Analysis of the proteins in this fraction, however, indicated the absence of a band at the position of P18. It is not clear if this absence reflects loss of P18 during isolation attempts or an in vivo subpopulation of U1 snRNPs lacking this protein.

The isolated snRNPs appeared to be quite stable. Several of the utilized elution conditions involved relatively high ionic strengths. Dialysis was always employed before either further isolation steps or characterization. We do not know if the salt treatment caused partial snRNP disassembly which was subsequently reversed during dialysis. The dialed snRNPs behaved as a single population with correct constituent stoichiometry during any further analysis suggesting that no irreversible dissociation had occurred. It should be noted, however, that the isolation protocol was specifically designed to involve no high salt treatment until snRNPs had been fractionated into RNA-specific subpopulations.

Purified U1 and U2 snRNPs contained both common and unique polypeptides. The four smallest common polypeptides, P12, P10, P9, and P8 were found in approximately the same relative amount in U1 and U2 snRNPs when estimated by quantitation of Coomassie staining of purified material or [3H]leucine label of immunoprecipitates of crude extracts. Polypeptides P23 and P22 were also common to both U1 and U2 snRNPs although there was a striking difference in the relative amount of each polypeptide in the two snRNPs. Polypeptides P23, P22, and P12, have all been shown to be recognized by the SLE anti-Sm antibodies, including an anti-Sm monoclonal antibody, in protein-blotting experiments suggesting that P23, P22, and P12 may be related proteins. If they are related by proteolysis, such alteration presumably occurs in the cell prior to isolation because the presence or absence of a variety of proteolysis inhibitors during purification did not alter the obtained pattern of proteins.

Polypeptide P30 is the major U1-specific polypeptide and appeared to be present in two copies/assembly. Polypeptides of molecular weights similar to those of P30 and P67 have been identified as the anti-RNP antigen(s) by protein-blotting techniques, using both SLE patient serum (9, 11, 13) and a monoclonal antibody, in protein-blotting experiments utilizing two-dimensional gel electrophoresis of purified U1 assemblies are in progress to resolve questions concerning the identity of the 67,000-Da U1 snRNP polypeptides.

U1 snRNPs contain one constituent of 15,000-18,000 Da (P18) with an unusual amino acid content. It was observed to be extremely rich in methionine or cysteine and deficient in leucine (10). In analysis of [35S]labeled snRNPs it appeared to be a U1-specific constituent (10). It stained poorly with either Coomassie blue or silver. It was insensitive to RNase, sensitive to protease, and contained no phosphate. It could be detected in our purified fractions by Coomassie-staining methods but only when gels were overloaded with respect to other snRNP polypeptides. Therefore, for lack of contradictory evidence, we deduce that P18 is a U1-specific, sulfur-rich, leucine-deficient polypeptide.

U2 snRNPs contained one polypeptide not found in U1 snRNPs, P27. Preliminary purification of U4, U5, or U6 snRNPs from nuclear extracts indicated no polypeptide P27 in these snRNPs. Therefore, P27 appears to be a U2-specific polypeptide. P27 is methionine deficient (5, 10) and has therefore often been missed in immunoprecipitation studies using [35S]methionine-labeled snRNPs.

A comparison of the polypeptide constituents of U1, U2, U4, and U6 snRNPs indicates a large number of common polypeptides. Five of these are very small polypeptides. Three of the five behave as quite basic on two-dimensional gels. These small polypeptides are those which remain associated following multiple cycles of isopycnic centrifugation (14) and may constitute a snRNP core. Further investigation of this possibility is in progress.

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