Nuclear Exchange of the U1 and U2 snRNP-specific Proteins

Robert J. Feeney and Gary W. Zieve

Department of Anatomical Sciences and Program in Cellular and Developmental Biology, State University of New York at Stony Brook, Stony Brook, New York 11794

Abstract. The snRNP particles include a set of common core snRNP proteins and snRNP specific proteins. In rodent cells the common core proteins are the B, D, D', E, F and G proteins in a suggested stoichiometry of $B_2D'_2D_2EFG$. The additional U1- and U2-specific proteins are the 70-kD, A and C proteins and the A' and B'' proteins, respectively. Previous cell fractionation and kinetic analysis demonstrated the snRNP core proteins are stored in the cytoplasm in large partially assembled snRNA-free intermediates that assemble with newly synthesized snRNAs during their transient appearance in the cytoplasm (Sauterer, R. A., R. J. Feeney, and G. W. Zieve. 1988. *Exp. Cell Res.* 176:344–359). This report investigates the assembly and intracellular distribution of the U1 and U2 snRNP-specific proteins. Cell enucleation and aqueous cell fractionation are used to prepare nuclear and cytoplasmic fractions and the U1- and U2-specific proteins are identified by isotopic labeling and immunoprecipitation or by immunoblotting with specific autoimmune antisera. The A, C, and A' proteins are found both assembled into mature nuclear snRNP particles and in unassembled pools in the nucleus that exchange with the assembled snRNP particles. The unassembled proteins leak from isolated nuclei prepared by detergent extraction. The unassembled A' protein sediments at 4S-6S in structures that may be multimers. The 70-kD and B'' proteins are fully assembled with snRNP particles which do not leak from isolated nuclei. The kinetic studies suggest that the B'' protein assembles with the U2 particle in the cytoplasm before it enters the nucleus.

THE snRNP particles are a family of ribonucleoprotein particles that contain an snRNA and from 6 to 10 proteins. In the nucleus, the snRNP particles function in RNA processing, including splicing and 3' end formation of pre-mRNA and rRNA maturation. During the processing events, the snRNPs assemble into complexes with their substrates, which often include several different snRNP particles. Some of the snRNAs provide sequence specificity by basepairing with conserved sequence motifs in their substrates (for review, see Maniatis and Reed, 1987; Sharp, 1987; Steitz et al., 1988; Zieve and Sauterer, 1990).

With limited exceptions, the snRNP particles have seven common proteins in addition to several snRNP-specific proteins. The common core of snRNP protein in rodent cells consists of the B, D, D', E, F and G proteins in a suggested stoichiometry of $B_2D'_2D_2EFG$. There are species and tissue specific variants of the B protein including a B' variant present in primate cells but not rodent cells and an N protein present in neuronal tissue (Lelay-Taha et al., 1986; Feeney et al., 1989; McAllister et al., 1989; Van Dam et al., 1989). The U1 snRNP has three specific proteins, A, C, and 70-kD and the U2 snRNP has two, A' and B''. This generates snRNP particles that are approximately 85% protein. (For review see Brunel et al., 1985; Reddy and Busch, 1988; Zieve and Sauterer, 1990.) Autoimmune antisera from patients with

systemic lupus erythematosus (SLE)[1] recognize both the common core proteins and the U1- and U2-specific proteins.

The snRNAs are a family of stable 4–6S nuclear RNAs characterized by their unique 5' trimethylguanosine caps and their transient appearance in the cytoplasm immediately after transcription. The two most abundant snRNAs, U1 and U2, are present in $\sim 1 \times 10^6$ copies per cell, the U3–U6 snRNAs are 10-fold less abundant and the U7–U13 snRNAs are present in below 25,000 copies per cell nucleus (Reddy et al., 1985; Kramer, 1987; Montzka and Steitz, 1988). While in the cytoplasm the nucleoplasmic snRNAs assemble with the snRNP core proteins and undergo a variety of processing steps, including hypermethylation of the 5' cap and internal nucleotides and 3' end processing (Eliceiri, 1974, 1980; Madore et al., 1984; Zieve and Penman, 1976; Zieve et al., 1988). SnRNP core assembly is directed by a conserved sequence motif of $PuA(U)_nGPu$ ($n > 3$) present in a single stranded region of the snRNA (for review see Parry, 1988; Zieve and Feeney, 1989).

SnRNP core proteins are present in the cytoplasm in large pools of partially assembled RNA-free intermediates. The D,

---

1. *Abbreviations used in this paper*: NLS, nuclear localization signal; SLE, systemic lupus erythromatosus; SMEM, suspension minimal essential medium.

E, F, and G proteins preassemble into a 6S particle of D_2EFG, which is the first intermediate to assemble with the snRNA followed by the D' and B protein. The B protein is present in the cytoplasm in a heterogeneous set of structures that may be homooligomers and the D' protein is present in 20S particles (Fisher et al., 1985; Sauterer et al., 1988, 1990). Estimates from immunoblots of cytoplasts and karyoplasts obtained by enucleation of proliferating murine fibroblasts indicate that the snRNP B protein is present in the cytoplasm in a 10-fold excess over the amount of snRNA undergoing processing and assembly (Sauterer et al., 1988). The presence of large pools of snRNP core proteins in the cytoplasm is consistent with the observation that snRNA transcription and snRNP particle assembly continues for over an hour after the inhibition of protein synthesis (Chandrasekharappa et al., 1983; Zieve, 1987).

In this report we describe the assembly and dynamic behavior of the U1 and U2 snRNP-specific proteins. Cell enucleation allows the preparation of a bona fide cytoplasmic fraction uncontaminated by nuclear material. These data suggest that in contrast to the snRNP core proteins, the U1 and U2 snRNP-specific proteins are restricted to the nucleus. The U1-specific A and C proteins and the U2-specific A' protein are present in unassembled pools in the nucleus that exchange with the mature snRNP particles and are prone to leak from nuclei prepared by aqueous cell fractionation. In contrast, the 70-kD and B" proteins are not found in unassembled pools. The possible implications of this dynamic behavior for the functions of the mature snRNP particles in nuclear pre-mRNA processing are discussed.

Materials and Methods

Cell Culture and Labeling

L929 mouse fibroblast cells and HeLa cells were grown in suspension culture in minimal essential medium (SMEM; Gibco Laboratories, Grand Island, NY) supplemented with 5% calf serum (Hyclone Laboratories, Logan, UT) and 5% Serum Plus (Hazelton Biologicals, Lenexa, KS). Cells were maintained at a concentration between 4×10^5 and 6×10^5 cells per ml by daily feeding.

For labeling times of 3 h or less, cells were concentrated to 5×10^6/ml in SMEM with reduced methionine, leucine, arginine, or phosphate content and supplemented with 3 mM glutamine and 25 mM Hepes, pH 7.1. [^{35}S]Methionine (New England Nuclear, Boston, MA), [^{3}H]leucine (ICN Biomedicals, Irvine, CA), [^{3}H]arginine (ICN Biomedicals) and [^{32}P]orthophosphate (ICN Biomedicals) are added as indicated. For labeling times of over 3 h, cells were labeled at a concentration of 5×10^5 cells/ml in medium with reduced methionine, leucine, or phosphate content as indicated. [^{3}H]Uridine and [^{3}H]adenosine (ICN Biomedicals) were added to the cultures in normal growth medium.

For inhibitor treatments, cells were treated as described with 10 μg/ml Actinomycin D (Sigma Chemical Co., St. Louis, MO) from a 200× stock in ethanol (Zieve, 1987).

Cell Fractionation

Aqueous cytoplasmic and nuclear fractions were obtained by a two step fractionation procedure (Feeney et al., 1989). Between 5×10^7 and 1×10^8 cells were extracted in 1 ml cytoskeletal extract buffer (100 mM NaCl, 10 mM Pipes, pH 6.8, 3 mM $MgCl_2$, and 0.5% Triton X-100) with 1% aprotinin, 1 mM PMSF, and 1 ug/ml leupeptin (all from Sigma Chemical Co.) and 2.5 mM vanodyl ribonucleoside complex (Berger and Birkenmeirer, 1979) added to inhibit proteases and RNases, respectively. Nuclei and associated insoluble skeletal frameworks were pelleted by centrifugation for 3 min at 2,000 g, and the supernatant was collected. The pellet was then extracted again in CSK buffer with 1% Tween 40 and 0.5% DOC and the nuclei were pelleted at 2,000 g for 3 min. The supernatants from the

two extractions are pooled and represent the cytoplasmic fraction. This fraction is clarified by centrifugation at 5,000 g before use to remove any contaminating nuclei that are inadvertently picked up in the soluble fractions. Nuclear extracts are prepared for immunoprecipitation by extensive sonication of the residual pellet in 1 ml cytoskeletal extract, with inhibitors added, then clarified by centrifugation for 5 min at 12,500 g in a microcentrifuge.

For experiments requiring bona fide cytoplasmic fractions, L929 cells were treated with 10 μg/ml cytochalasin B and enucleated in suspension on Ficoll gradients as previously described (Zieve et al., 1988). Cytoplast fractions were solubilized by extraction in cytoskeletal extract buffer and insoluble cytoskeletal frameworks were removed by centrifugation at 2,000 g for 3 min.

Antisera

Hybridomas secreting mAbs of the anti-Sm and anti-(U1)RNP serotype were grown in culture and the tissue culture supernatants, containing 2-4 μg/ml IgG, were used for immunoprecipitations. The Y12 anti-Sm hybridoma (Lerner et al., 1981) was a gift of Dr. J. Steitz (Yale University) and recognizes primarily the B protein on immunoblots (Pettersson et al., 1984), whereas the (U1)RNP hybridoma that recognizes the 70-kD U1-specific protein in immunoblots was the gift of Dr. S. Hoch (Agouron Institute) (Billings et al., 1982). The human autoimmune (U1)RNP antiserum recognizes all three of the U1 specific proteins, 70-kD, A and C in immunoblots and was obtained from the Department of Pathology, SUNY Stony Brook. The human anti-U2 serum was a generous gift of Dr. J. Craft (Yale University) (Craft et al., 1988). The antiserum specifically immunoprecipitates the U2 snRNP and recognizes the A' and B" proteins in immunoblots of purified snRNP particles. However, the background in immunoblots of whole cell fractions obscures the U2-specific snRNP proteins.

Immunoprecipitation and Immunoblots

0.1 mg preswelled protein A-Sepharose (Sigma Chemical Co.) per immunoprecipitate was prebound for 24-72 h before use at 4°C with 5 ml of tissue culture supernatant or 15 μl patient serum, then was washed three times with CSK-ext just before use. Cell extracts or sucrose gradient fractions were added to the prebound antibodies and rotated at 4°C for 2-3 h. For analysis of the 70-kD U1 specific proteins, cell extracts were precleared by mock immunoprecipitation with protein-A Sepharose bound to a mouse mAb to a Drosophila-specific nuclear envelope antigen, which was the generous gift of Dr. P. Fisher (SUNY Stony Brook). This significantly reduces the background in the high-molecular weight region.

After incubation with antibodies, samples were washed five times in NP-40 IP wash (150 mM NaCl, 10 mM Tris-HCl, pH 7.5, and 1% NP-40). Samples were transferred to new Eppendorf tubes at the end of the second wash to eliminate proteins nonspecifically adsorbed to the walls of the tube. After the washes, the pellets were rinsed in IP wash without detergents and the samples were drained and boiled in protein sample buffer.

For immunoblots, proteins were transferred from polyacrylamide gels to nitrocellulose by electrophoresis at 15 V/cm for 4 h in 25 mM Tris, 192 mM glycine, 20% methanol. Nitrocellulose was then blocked by incubation in 1% nonfat dried milk in TBS (5 mM Tris pH 7.4, 150 mM NaCl), then rinsed three times in TBS 0.2% Tween 20 for 30 min, then air dried and maintained at 4°C until needed. For analysis, nitrocellulose strips were incubated with antisera, either tissue culture supernatant containing mouse mAbs or a 1/100 dilution of patient serum with TBS, for 1 h at 37°C. Strips were then rinsed extensively in 0.2% Tween 20 in TBS and incubated in a second antibody (either goat anti-mouse IgG or goat anti-human IgG) conjugated to alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, IN) for 1 h at 37°C. After extensive rinsing, strips were developed with 0.2 mg/ml p-NBT chloride and 0.2 mg/ml 5-bromo-4-chloro-3-indoyl phosphate (U.S. Biochemical Corp., Cleveland, OH) in 100 mM Tris, pH 9.7.

Electrophoresis

Electrophoretic analysis of proteins in one dimension used either 13 or 6-12% acrylamide running gels as previously described (Feeney et al., 1989). TCA precipitated cell fractions or immunoprecipitates were boiled in protein sample buffer (2% SDS, 10% glycerol, 100 mM DTT, 80 mM Tris-HCl, pH 6.8, 2 mM EDTA, and 0.01% bromphenol blue) and run for 1050 V-h. Two-dimensional gel electrophoresis using NEPHGE was performed as previously described (Feeney et al., 1989).

RNAs were analyzed using 6-15% nondenaturing gradient gels (Zieve and Penman, 1976). RNA samples in sample buffer (50 mM Tris-HCl, pH

6.8, 1% SDS, 10% glycerol, and 0.01% each of bromphenol blue and xylene cyanol) were run for 1200 V-h.

Gels were then treated with Enhance (New England Nuclear, Boston, MA) according to product instructions and vacuum dried for fluorography using preflashed X-Omat AR film (Eastman Kodak Co., Rochester, NY). Typical exposures were from 2 to 10 d. Scanning and quantitation of fluorographs were performed using an LKB Ultroscan (LKB Instruments, Gaithersburg, MD) and associated software for an IBM XT computer. Autoradiographic images were maintained between 0.4 and 2.5 O.D. units, within the linear range of the x-ray film and laser densitometer.

Results

Identification of U1 and U2 snRNP-specific Proteins

In addition to the snRNP core proteins shared by all the nucleoplasmic snRNP particles, there are snRNP-specific proteins. The specific proteins of the U1 and U2 snRNP particles, the two most abundant snRNP particles, are the A, C, and 70-kD proteins and the A' and B″ proteins, respectively. The cDNAs of all the U1 and U2 specific snRNP proteins are cloned and sequenced and autoimmune sera are available that specifically recognize these proteins (Thiessen et al., 1986; Habets et al., 1987; Sillekens et al., 1987; Yamamoto et al., 1988; Sillekens et al., 1988; Query et al., 1989; Fresco and Keene, 1989). Fig. 1 shows the profile of immunoprecipitated snRNP particles labeled with [³H]leucine, [³⁵S]methionine, [³H]arginine, or [³²P]orthophosphate. Leucine labeling is best for identifying the U2 specific A' protein because sequence data indicate it is low in methionine; however, the U1-specific C protein is low in leucine. The U1-specific 70-kD protein is especially rich in arginine and is the only snRNP phosphoprotein.

The Sm antiserum found in patients with SLE recognizes the common core snRNP proteins and with limited exceptions, immunoprecipitates the entire family of snRNP particles including U1 and U2 (Fig. 1, lanes 2, 3, and 5). The B, D, E, F, and G snRNP core proteins all label with either leucine, methionine or arginine and the B and D core proteins are the most heavily labeled proteins in Sm immunoprecipitates. The D snRNP protein can be resolved into a pair of closely spaced D and D' proteins in gels containing urea, however in this system they migrate as one wide band and are labeled as D (Bringmann and Luhrmann, 1986; J. Andersen, R. J. Feeney, and G. W. Zieve, manuscript in preparation). In addition, the E and F proteins often comigrate in this gel system. The U1-specific 70-kD, A, and C proteins and the U2-specific A' and B″ proteins are identifiable in the Sm immunoprecipitates and this demonstrates they are assembled into snRNP particles with the snRNP core proteins. The A and C and B″ proteins label well with methionine (lanes 3 and 4), the A, A', and B″ proteins label well with leucine (lanes 1 and 2) and the 70-kD protein labels especially well with arginine (lanes 5 and 6). The 70-kD protein is the only major snRNP protein that is phosphorylated (lane 7). The specific proteins of the less abundant snRNP particles are not identifiable in these preparations at this level of resolution. The availability of specific antisera to the U1 and U2 proteins and two-dimensional gel electrophoresis helps confirm the identities of the U1- and U2-specific proteins.

Immunoprecipitates of leucine-labeled cell extracts with a U2 specific serum specifically precipitates the A' and B″ U2 specific proteins along with the core snRNP proteins (lane 1, Mimori et al., 1984; Craft et al., 1988). The mobility of
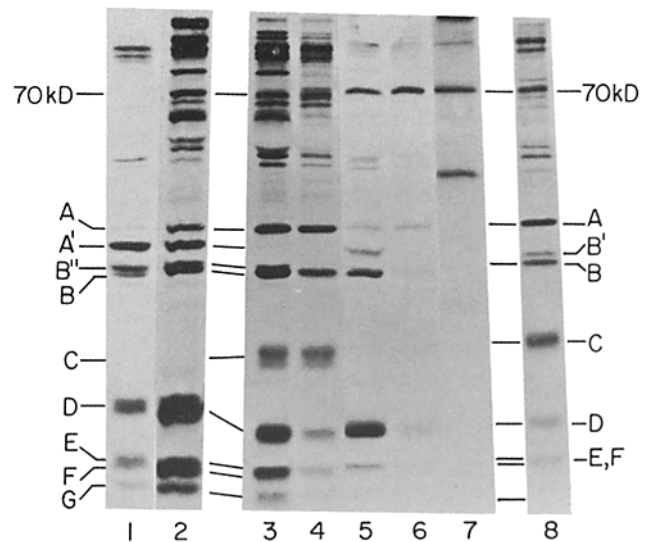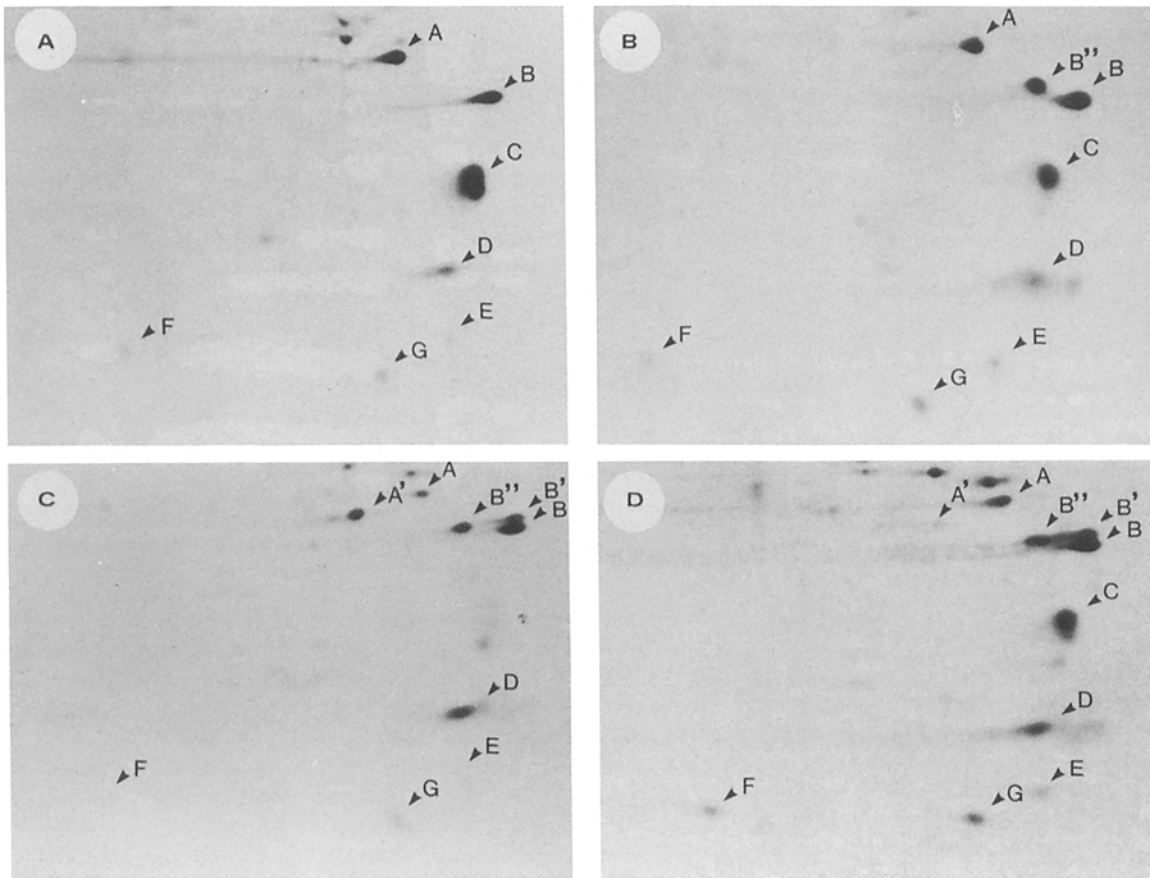

Figure 1. Immunoprecipitation of snRNP proteins with SLE autoimmune antisera. L929 cells were labeled with either [³H]leucine (lanes 1 and 2), [³⁵S]methionine (lanes 3 and 4), [³H]arginine (lanes 5 and 6), or [³²P]orthophosphate (lane 7). HeLa cells were labeled with [³⁵S]methionine (lane 8) as described in Materials and Methods and nuclear fractions were immunoprecipitated with the Y12 anti-Sm monoclonal antisera (lanes 2, 3, and 5), the anti-U2 autoimmune antiserum (lane 1) or the 70-kD-specific anti-U1 (RNP) monoclonal antisera (lanes 4, 6, 7, and 8).

the U2-specific protein B″ is slightly slower than the B protein. This is also the mobility of the B' core protein found in primate cells, which is closely related to the B protein and may differ by either post translational modification or differential RNA splicing (Reuter et al., 1987; Van Dam et al., 1989). The B' core protein is visible in the U1-specific immunoprecipitates of the human HeLa cell line (lane 8). However, the B' core protein is absent in rodent cells. This is seen in the two-dimensional gels in Fig. 2. In HeLa cells the B protein is a closely spaced doublet with the 1-kD larger protein designated as B' (Fig. 2, C and D). The B' protein is missing in the rodent cells and therefore the B″ protein can be analyzed directly on one-dimensional gels (Fig. 2, A and B). The B″ protein is more acidic than the B and B' proteins and is separated from them in two-dimensional gels (Fig. 2, B and D). The U2-specific A' protein has only 2 methionines but over 30 leucines and is easily identified in one- or two-dimensional gels of cells labeled with [³H]leucine (Fig. 2 C).

The U1-specific A and C proteins are easily identifiable in immunoprecipitates with either Sm- or U1-specific sera of methionine labeled extracts (Fig. 1, lanes 3 and 4). They are basic proteins with pI's similar to that of the B and D core proteins. The 70-kD U1-specific protein is difficult to identify in methionine-labeled cells because of the high background in the 70-kD region, but is readily apparent in cells labeled with either arginine or phosphate (Fig. 1, lanes 5, 6, and 7). The yields of the snRNP core proteins are occasionally low in immunoprecipitates with the 70-kD-specific anti-(U1)RNP monoclonal antiserum, which suggests the association of the 70-kD protein with the U1 particles can be disrupted during immunoprecipitation (lane 6).

The 70-kD protein is the only snRNP protein that is phos-

Figure 2. Identification of the U1 and U2 snRNP proteins in two-dimensional gel electrophoresis. (*A*) Immunoprecipitate with the (U1)RNP human antiserum of nuclear extracts of L929 cells labeled for 16 h in 80% methionine-free SMEM with 5 μCi/ml [^{35}S]methionine analyzed by nonequilibrium two-dimensional gel electrophoresis as described in Materials and Methods. In this and all other two-dimensional gels, the basic side of the gel is on the right and arrowheads mark the identified snRNP proteins. (*B*) Immunoprecipitate with the anti-Sm Y12 mAb of nuclear extracts of L929 cells labeled for 16 h in 80% methionine-free SMEM with 5 μCi/ml [^{35}S]methionine analyzed by nonequilibrium two-dimensional electrophoresis as described in Materials and Methods. (*C*) Immunoprecipitate with the anti-U2 human serum of nuclear extracts of HeLa cells labeled for 16 h in 80% leucine-free SMEM with 10 μCi/ml [^{3}H]leucine analyzed by nonequilibrium two-dimensional electrophoresis as described in Materials and Methods. (*D*) Immunoprecipitates using the anti-Sm Y12 antibody of nuclear extracts of HeLa cells labeled for 16 h in 80% methionine-free SMEM with 5 μCi/ml [^{35}S]methionine were analyzed by nonequilibrium two-dimensional electrophoresis as described in Materials and Methods.
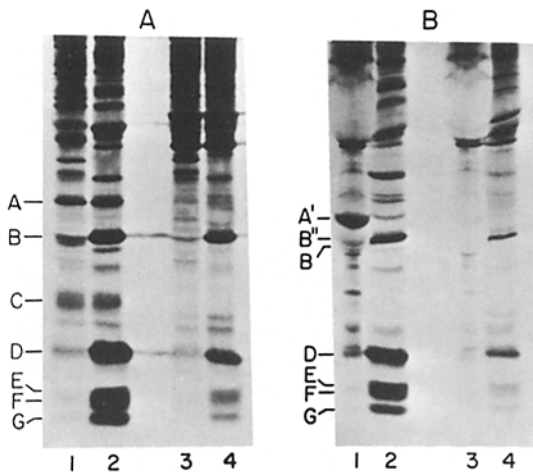
phorylated and it is clearly identifiable in immunoprecipitates of phosphate labeled cells (lane 7; Wooley et al., 1983). There is a phosphoprotein of ∼50 kD in the U1(RNP) immunoprecipitates of unknown origins. The identity of the 70-kD phosphorylated protein is confirmed by immunoblotting of phosphate labeled immunoprecipitates with a 70-kD-specific mAb (Billings et al., 1982; Fig. 3). Because the immunoprecipitates and the immunoblots used mouse monoclonal antisera the alkaline phosphatase–coupled anti–mouse antibody used as a second antibody in the immunoblots also recognized the IgG heavy and light chain present in the immunoprecipitates. The 70-kD U1-specific phosphoprotein is immunoprecipitated in the nucleus by both the Sm and U1(RNP) antisera, demonstrating it is assembled into snRNP particles (Fig. 3, lanes *1* and *3*). As will be discussed later, the 70-kD protein is absent from cytoplasmic fractions (Fig. 3, lane *2*).

The phosphate-labeled 70-kD protein provides a marker to identify the 70-kD protein in cells labeled with amino acids. Because of the large number of nonspecific proteins immunoprecipitated in the 70-kD region by either the Sm or U1(RNP) antisera, it is necessary to preclear nuclear extracts by a mock immunoprecipitation with a nonspecific antibody to reduce the background to help identify the 70-kD U1-specific protein. The 70-kD protein is ∼20% arginine and is especially well labeled with [^{3}H]arginine (Fig. 1, lanes *5* and *6*). The methionine-labeled 70-kD protein is more highly labeled in U1(RNP) immunoprecipitates of HeLa cells than in L929 cells, where it is still difficult to identify in one-dimensional gels even after preclearing (lanes *4* and *8*). An immunoprecipitation of the arginine-labeled nuclear extract with the monoclonal antisera to the 70-kD protein identifies a large amount of the 70-kD protein in the L929 cells but very few snRNP core particles (lane *6*). This suggests that the U1 particles were disrupted during the immunoprecipitation or that there is a significant fraction of the 70-kD protein not assembled into snRNP particles.
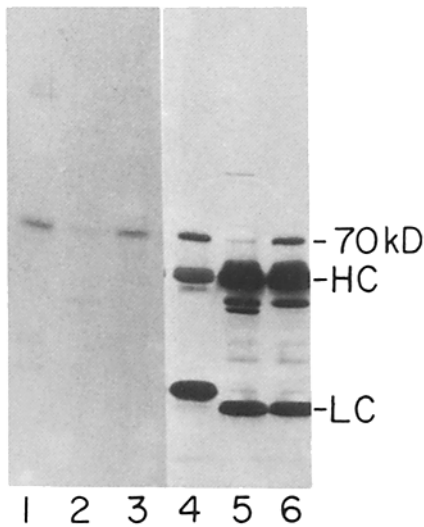
Figure 3. Identification of the 70-kD U1-specific protein. 1×10^8 L929 cells were labeled for 3 h with 50 μCi/ml 32P in 95% phosphate-free SMEM then fractionated into cytoplasm (lane 2 and 5) and nucleus (lane *1, 3, 4,* and *6*) by aqueous cell fractionation. Fractions were immunoprecipitated with the Y12 anti-Sm (lane *1*) or anti(U1)RNP monoclonal antisera (lanes 2 and 3) and analyzed by gel electrophoresis and then transferred to nitrocellulose. The blot was then probed with the anti-(U1)RNP monoclonal antisera (lanes *4, 5,* and *6*) and the immunoblot was exposed to film to identify the immunoprecipitated radiolabeled 70-kD protein (lanes *1, 2,* and *3*). The IgG heavy chain (HC, 50,000 kD) and light chain (LC, ~25,000 kD) in the immunoprecipitates is recognized by the alkaline phosphatase–coupled goat-anti–mouse immunoglobulin used as a second antibody in the immunoblots.

Intracellular Distribution of the snRNP-specific Proteins

Previous cell fractionation studies using cell enucleation to prepare bona fide cytoplasmic fractions identified large pools of the snRNP core proteins stored in the cytoplasm of the L929 cells (Sauterer et al., 1988). Because of the high abundance of the snRNP proteins in the nucleus, it was necessary to eliminate the possibility of nuclear leakage during aqueous cell fractionation. In Figs. 4 and 5 the intracellular distribution of the U1- and U2-specific snRNP proteins is investigated using both cell enucleation and aqueous cell fractionation. The snRNP proteins are identified by both immunoprecipitation (Fig. 4) and immunoblotting (Fig. 5). The data suggest that in contrast to the snRNP core proteins, the U1- and U2-specific proteins are all localized in the nucleus however the dynamic behavior varies. The A, C, and A' proteins, but not the B" and 70-kD proteins, are present in large unassembled pools that are prone to leak from nuclei generated by aqueous cell fractionation.

In Fig. 4 aqueous cytoplasmic fractions (lanes *1* and *2*) and cytoplast fractions prepared by cell enucleation (lanes *3* and *4*) are immunoprecipitated with anti-Sm (lanes 2 and *4*) and anti-(U1)RNP (*A*, lanes *1* and *3*) and anti-U2 (*B*, lanes *1* and *3*) sera. Cells are labeled with [^{35}S]methionine for the analysis of the U1 specific proteins (*A*) and [^{3}H]leucine for the U2-specific proteins (*B*). The anti-Sm sera immunoprecipitates the full set of core proteins from both the aqueous cytoplasm and cytoplast fraction (*A* and *B*, lanes 2 and *4*). The signal is lower from the cytoplasts because this fraction
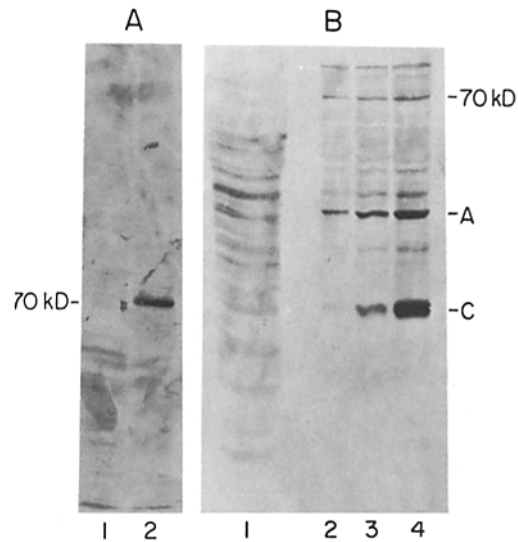

Figure 4. Distribution of the U1 and U2 snRNP-specific proteins by immunoprecipitation in cytoplasmic fractions prepared by both aqueous fractionation and cell enucleation. 5×10^7 L929 cells were labeled for 30 min with 80 μCi/ml [^{35}S]methionine in 100% methionine-free medium (*A*) or with 80 μCi/ml [^{3}H]leucine in 100% leucine-free medium (*B*). Half the cells were enucleated and half were fractionated by aqueous cell fractionation. Aqueous cytoplasm (lanes *1* and *2*) and cytoplast fractions (lanes *3* and *4*) were immunoprecipitated with the Y12 anti-Sm monoclonal antisera (*A* and *B*, lanes 2 and *4*) or the anti-(U1)RNP human serum (*A*, lanes *1* and *3*) or the anti-U2 human serum (*B*, lanes *1* and *3*).

Figure 5. Distribution of the U1-specific proteins in bona fide nuclear and cytoplasmic fractions by immunoblotting. L929 cells were enucleated and cytoplast and karyoplast fractions were immunoblotted with the anti-(U1)RNP mAb (*A*) or the anti-(U1)RNP human serum (*B*). (*A*) Cytoplasts (lane *1*) and karyoplasts (lane 2) from 5×10^6 L929 cells were analyzed on 6–12% gradient gels and immunoblotted with the anti-(U1)RNP monoclonal antiserum that recognizes the 70-kD U1-specific protein. (*B*) 2×10^7 cytoplasts (lane *1*) and 2.5×10^6 (lane 2), 5×10^6 (lane 3), and 1×10^7 karyoplasts (lane *4*) were immunoblotted with the anti-(U1)RNP human serum that recognizes the A, C, and 70-kD U1-specific proteins.

contains less material. In contrast, where the U1 specific sera immunoprecipitates the A and C proteins from an aqueous cytoplasm (*A*, lane *1*), they are totally absent from a cytoplast fraction (*A*, lane *3*). The anti-Sm antiserum also immunoprecipitates the U1-specific A and C protein from the aqueous cytoplasm (lane *2*) but not the cytoplast fraction (lane *4*). Recognition of the U1-specific A and C proteins by anti-Sm sera has been reported previously and the A protein has been suggested to contain an Sm epitope (Sillekens et al., 1987; Sauterer et al., 1990).

Similarly to the U1-specific A and C proteins the A′ protein which is precipitated by the anti-U2 sera from an aqueous cytoplasm (*B*, lane *1*) is totally lacking from a cytoplast fraction (*B*, lane *3*). Substantial amounts of the U2-specific B″ protein and the U1-specific 70-kD protein are found only in nuclear fractions (Fig. 3; Feeney et al., 1989). These data suggest that the A, C, and A′ proteins appear in the aqueous cytoplasm because they leak from the isolated nuclei. This localization is also confirmed by immunoblotting.

In Fig. 5, cytoplast and karyoplast fractions are immunoblotted with the U1-specific mAb and human autoimmune sera. The mAb is specific for the 70-kD protein and it identifies the 70-kD protein only in the nuclear fraction (*A*, lanes *1* and *2*). Aqueous cell fractionation and immunoprecipitation with the 70-kD specific mAb also identifies the phosphate labeled 70-kD protein only in the nuclear fraction (Fig. 3, lanes *2* and *3*), indicating that it does not leak from isolated nuclei. The human autoimmune serum that recognizes all three of the U1 specific proteins was also immunoblotted against karyoplast and cytoplast fractions to evaluate the distribution of the A, C, and 70-kD proteins (Fig. 5 *B*). Immunoblotting of dilutions of the karyoplast fraction indicate that the intensity of the immunoblot reaction is linearly related to the abundance of the individual proteins (Fig. 5 *B*, lanes *2–4*). Although there are some nonspecific bands identified in the region of A and C, the immunoblots fail to identify the A, C, or 70-kD U1-specific snRNP proteins in the cytoplast fractions. This suggests that all the U1 specific snRNP proteins are restricted to the nucleus in vivo and that the A and C proteins, but not the 70-kD protein, leak from nuclei prepared by aqueous cell fractionation. Analogous experiments with the U2-specific autoimmune serum were not successful. Although the U2 serum specifically immunoprecipitates the U2 snRNP and recognizes the U2-specific proteins in immunoblots of purified snRNPs, the nonspecific background in immunoblots of whole cell fractions obscures the U2-specific proteins.

Sedimentation Distribution of the U2-specific A′ Protein

The sedimentation distribution of the A and C U1-specific proteins that leak from isolated nuclei was evaluated in a previous report (Sauterer et al., 1990). The C protein sediments at ∼6S and the A protein at 18–20S. Neither of the proteins that leak into the cytoplasm is assembled with 11–15S snRNP particles. In Fig. 6 the sedimentation distribution of the A′ protein that leaks from the nucleus was investigated by sucrose gradient sedimentation.

L929 cells were pulse-labeled for 2 h with [³H]leucine to label the A′ protein and aqueous cytoplasmic fractions were resolved by sucrose gradient sedimentation. Parallel gradients were immunoprecipitated with the anti-Sm monoclonal
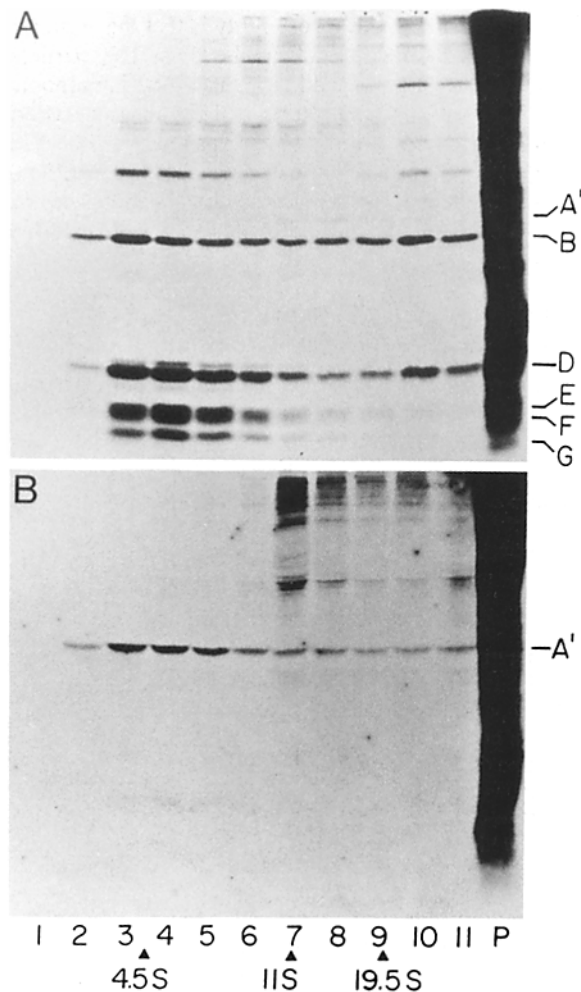

Figure 6. Sedimentation distribution of the A′ protein in the cytoplasm. 2 × 10⁸ L929 cells were labeled for 2 h in 95% leucine-free SMEM with 80 μCi/ml [³H]leucine. Cytoplasmic fractions were fractionated on 7.5–25% sucrose gradients and the gradient fractions and the pellet (*P*) were immunoprecipitated with the anti-Sm mAb Y12 (*A*) or the anti-U2 human serum (*B*) as described in Materials and Methods. Locations of the marker proteins from parallel gradients is indicated.

antiserum that recognizes the snRNP core proteins, and the anti-U2 antiserum. The immunoprecipitated snRNP core proteins have the characteristic sedimentation distribution reported earlier (Sauterer et al., 1990). The newly synthesized B protein sediments from 4–20S, D protein sediments at 6 and 20S and the E, F, and G proteins are at 6S. A faint amount of the A′ protein is immunoprecipitated by the Sm antiserum in the 11–15S region of the gradient where fully assembled mature snRNP particles sediment. The precipitation of A′ by the anti-Sm sera indicates the A′ protein in the 11–15S structures is assembled into snRNP particles that are either newly assembled in the cytoplasm or which have leaked from the nucleus during cell fractionation.

In contrast to the sedimentation of Sm precipitated proteins, the A′ protein precipitated by anti-U2 sera sediments primarily at 4–8S (*B*). This is distinct from the 11–15S assembled snRNP particles and suggests that the A′ that leaks into the cytoplasmic fraction is not assembled into snRNPs.

An alternative but less likely possibility is that the A' protein in the 4–8S structures is eluted from the U2 snRNP particles during cell fractionation. Small amounts of the immunoprecipitated A' protein are in structures of 11S and larger, which include protein assembled into snRNP particles as observed previously in the Sm immunoprecipitates. No U2-specific B″ protein is seen in the cytoplasmic fractions, which is consistent with the cell fractionation data that show it is localized in the nucleus and does not leak from isolated nuclei.

Assembly Kinetics of the U1 and U2 snRNP-specific Proteins

To determine if the U2-specific proteins require de novo snRNP particle assembly to enter the nucleus, the nuclear accumulation of newly synthesized A' and B″ proteins was monitored in the presence and absence of snRNP assembly. SnRNP particle assembly was halted by pretreating the L929 cells with actinomycin D to arrest snRNA synthesis. Preexisting snRNAs assemble into snRNPs in the cytoplasm and move into the nucleus with a half-life of ~20 min and this results in cells with no snRNPs assembling in the cytoplasm (Zieve, 1987; Feeney et al., 1989). In Fig. 7, cytoplasmic and nuclear fractions of [³H]leucine–labeled control and actinomycin D-treated cells are immunoprecipitated with both anti-Sm and anti-U2 sera after a pulse label and after a chase. The Sm antisera will identify the U2-specific protein only if they are assembled into mature snRNP particles and the U2-specific serum immunoprecipitates both assembled and unassembled A' and B″ proteins.

In the control cells immunoprecipitated with anti-Sm sera, large amounts of snRNP core proteins are immunoprecipitated from cytoplasmic fractions (Fig. 7 A, lanes 1 and 3) and the movement of the snRNP core proteins into the nuclear fraction, indicative of de novo snRNP particle assembly, is observed after a chase (Fig. 7 A, lane 4). The A, A', and B″ proteins are also precipitated from the chased nuclear fractions indicating that assembled U1 and U2 snRNPs develop during the chase. With prior actinomycin D treatment, the movement of the snRNP core proteins into the nucleus is halted (Fig. 7 A, lane 8). However, the U1- and U2-specific A, A', and B″ proteins still appear in mature nuclear snRNPs (Fig. 7 A, lane 8). The immunoprecipitation of these proteins in the nucleus by the Sm antiserum in the presence of actinomycin D suggests that they enter the nucleus independently of cytoplasmic snRNP assembly and associate with preexisting U1 and U2 snRNP particles.

Immunoprecipitation of an identical set of cell fractions with the U2-specific antisera identifies both the assembled and unassembled pools of the U2-specific proteins. Immunoprecipitation of cytoplasmic and nuclear fractions from pulse and chased control cells with U2-specific antiserum identifies a large pool of A' protein in the cytoplasm and ~15% of the total immunoprecipitated A' protein appears in the nuclear fraction during a chase (Fig. 7 B, lane 4). The actinomycin D pretreatment blocks the movement of the snRNP core proteins into the nucleus as expected but it has no effect on the distribution of the A' protein (Fig. 7 B, lane 8). The results discussed above suggest that the 85% of the pulse-labeled A' protein immunoprecipitated from the cytoplasmic fraction is in a form that has leaked out of the nuclei during the detergent fractionation. The inability to immunoprecipitate the majority of this protein with the Sm antiserum
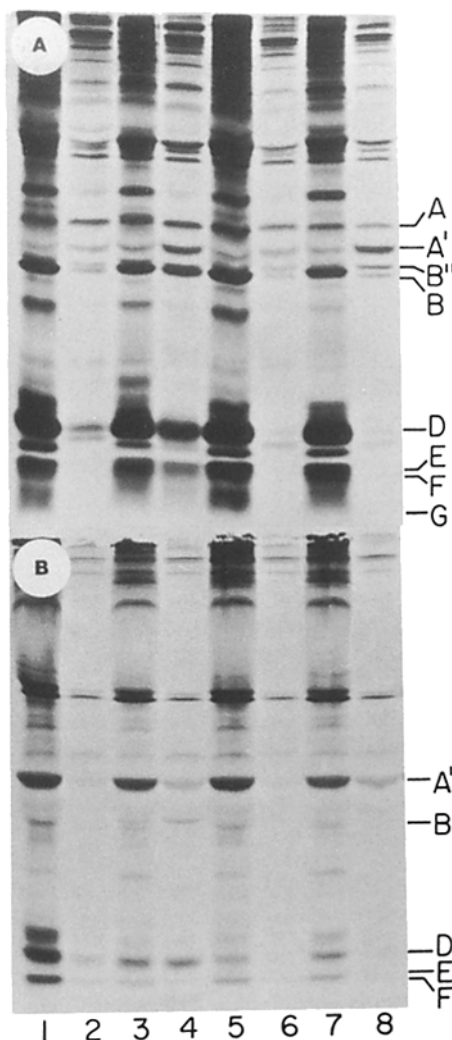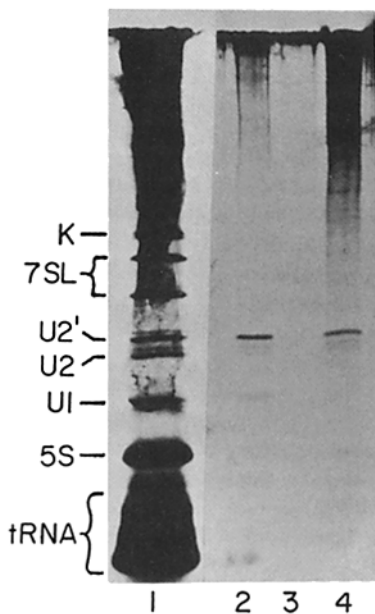

Figure 7. Nuclear accumulation of the U2-specific A' protein does not require snRNP assembly. 1.5×10^8 L929 cells were labeled for 30 min in 100% leucine-free SMEM with 100 μCi/ml [³H]leucine (lanes 1, 2, 5, and 6) and chased in normal SMEM (lanes 3, 4, 7, and 8) for 90 min. Lanes 1–4 are controls and lanes 5–8 are cells pretreated with 10 μg/ml actinomycin D for 30 min before labeling. Cells were fractionated into aqueous cytoplasm (1, 3, 5, and 7) and nucleus (2, 4, 6, and 8) and immunoprecipitated with either the anti-Sm Y12 mAb (A) or the anti-U2 human autoimmune serum (B) as described in Materials and Methods.

indicates it is not assembled into snRNP particles (Fig. 7 A, lanes 1, 3, 5, and 7). This suggests the majority of the newly synthesized protein is not assembled with the U2 snRNPs in the nucleus and is free to leak from the nucleus during cell fractionation. Alternatively, this A' protein is stripped from the U2 snRNP particles by the extraction conditions. This seems less likely because the U2 snRNPs are stable under more stringent buffer conditions containing up to 0.5 M NaCl. It is difficult to identify the U2-specific B″ protein in the immunoprecipitates with the U2-specific serum.
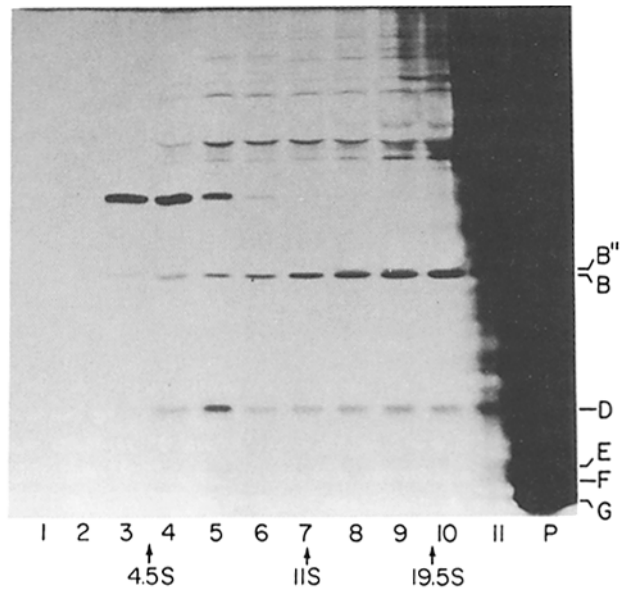
The observation that the arrest of snRNP assembly by depletion of the snRNAs does not halt the nuclear accumulation of the U2-specific A' or B″ proteins suggests these proteins can move into the nucleus independently and enter a pool of

protein that associates with preexisting snRNP particles. In previous experiments, it was demonstrated that the U1 A and C proteins show a similar behavior (Feeney et al., 1989). However, it does not determine whether the newly assembled snRNP particles in the cytoplasm can bind newly translated snRNP-specific protein in the cytoplasm before entering the nucleus or whether assembly occurs only after the particles enter the nucleus.

To determine if the snRNP-specific proteins can assemble with newly formed snRNPs in the cytoplasm, newly synthesized snRNAs were immunoprecipitated from bona fide cytoplasmic fractions by the specific antisera. In Fig. 8, pulse-labeled snRNAs were immunoprecipitated from cytoplasts with specific antisera. The anti-Sm sera precipitates the major snRNAs as expected because snRNP core assembly occurs in the cytoplasm (lane 2). The U2 snRNP is also precipitated by the anti-U2 serum (lane 4) but U1 snRNA in the cytoplasm is not precipitated with the anti–70-kD mAb (lane 3). No U1 snRNA was detected in the (U1)RNP immunoprecipitates even in exposures where the other lanes were significantly overexposed. This suggests that indeed some of the U2-specific proteins do assemble into snRNP particles in the cytoplasm but not the U1-specific 70-kD protein. To investigate the possible assembly of the U2-specific proteins with the U2 snRNP particles in the cytoplasm, the sedimentation distribution of snRNP proteins was investigated in bona fide cytoplasmic fractions generated by cell enucleation (Fig. 9). The snRNP core proteins exhibit the sedimentation distribution described previously (Fig. 6). However,

Figure 9. Sedimentation distribution of U2-specific B″ protein in assembled snRNP particles in cytoplasts. 2×10^8 L929 cells were labeled for 1 h in 95% methionine-free SMEM with 100 μCi/ml [^{35}S]methionine. Cells were enucleated and the cytoplasts were extracted with cytoskeletal extract buffer and 0.5% Triton-X-100 as described in Materials and Methods and the detergent soluble fractions were analyzed by sucrose gradient sedimentation as described in the legend to Fig. 6. The gradient fractions were immunoprecipitated with Y12 anti-Sm monoclonal antiserum.

Figure 8. SnRNP assembly in the cytoplasm. 7.5×10^7 L929 cells were pulse-labeled for 45 min with 100 μCi/ml [^{3}H]uridine and 100 μCi/ml [^{3}H]adenosine in normal SMEM and enucleated as described in Materials and Methods. The cytoplast fraction was extracted in CSK-ext and the solubilized cytoplasm was immunoprecipitated with the anti-Sm monoclonal antisera (lane 2), anti-(U1)RNP monoclonal antisera (lane 3), and anti-U2 human sera (lane 4). Marker RNA of a total cytoplasmic fraction is illustrated in lane 1. RNA gel electrophoresis was performed as previously described.

a small amount of the U2-specific B″ protein, but not the other U1 or U2 specific proteins, is found sedimenting in the 11–13S region where assembled U2 snRNP particles sediment (Sauterer et al., 1990). This strongly suggests that the B″ protein can assemble with the U2 snRNP particles while they are in the cytoplasm.

Discussion

With limited exceptions, snRNP particles share a common core of proteins, with the snRNA component and several specific proteins differing in the individual snRNP particles. In this report we use autoimmune antisera that recognize the snRNP core proteins and the U1 and U2 snRNP-specific proteins to analyze the synthesis and assembly of the U1 and U2 snRNP particles. Cell fractionation by cell enucleation, to eliminate nuclear leakage, and by conventional aqueous extraction demonstrate that in contrast to the snRNP core proteins, present in snRNA-free pools in the cytoplasm, the U1 and U2 snRNP-specific proteins are localized almost quantitatively in the interphase nucleus. The three U1-specific proteins, A, C, and 70-kD, and the U2-specific A′ and B″ proteins, display two types of behavior. The A, C, and A′ snRNP proteins are present both assembled into snRNP particles and in unassembled pools in the nucleus while the B″ and 70-kD snRNP proteins are present only in assembled snRNP particles (Fig. 10). The unassembled pools of the A, C, and A′ proteins leak from isolated nuclei prepared by detergent extraction procedures but are absent from bona fide cytoplasmic fractions prepared by cell enucleation.
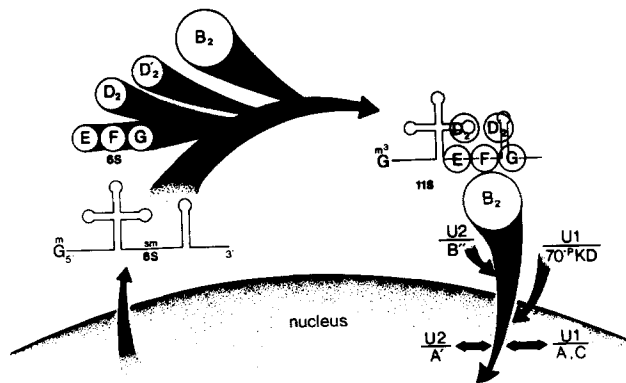
Figure 10. Cartoon model of snRNP particle assembly. The assembly pathway of the snRNP core particle in the cytoplasm and of the U1 and U2 snRNP specific proteins is based on the data in this manuscript and in Feeney et al. (1989) and Sauterer et al. (1990).

Previous studies demonstrated that snRNP core proteins are stored in the cytoplasm in large pools of partially assembled intermediates (Sauterer et al., 1988; Feeney et al., 1989). SnRNP core assembly occurs when newly synthesized snRNAs appear in the cytoplasm immediately after synthesis before returning permanently to the interphase nucleus (Fig. 10). This suggests that some of the snRNP-specific proteins may also assemble with the snRNP particles in the cytoplasm immediately after translation and move back into the nucleus with the newly assembled snRNP core particles. Several results suggest that this is possible for some but not all of the snRNP-specific proteins.

The A, C, and A' proteins move into the nucleus independently of snRNP assembly. If snRNP particle assembly is inhibited by blocking the synthesis of new snRNAs with actinomycin D, newly synthesized A, C, and A' proteins continue to move into the nucleus and assemble with preexisting snRNP particles (Feeney et al., 1989). This demonstrates that these proteins can enter the nucleus independently and that the nuclear pools are in a dynamic equilibrium with the existing stable nuclear snRNPs. However, it does not eliminate the possibility that some of these proteins can also associate with newly assembled snRNPs that they encounter in the cytoplasm.

The nuclear accumulation of the snRNP-specific proteins in the absence of snRNP assembly suggests that these proteins have independent nuclear localization signals. A variety of studies have demonstrated that the nuclear accumulation of specific proteins is the result of a short sequence motif known as a nuclear localization signal (NLS) (Dingwall and Laskey, 1986). Studies on a variety of cellular and viral proteins indicate that NLS are heterogeneous although they are usually a combination of basic and hydrophobic residues and several additional factors affect its activity including localization in the protein and cooperation between multiple NLS (Roberts et al., 1987; Dingwall et al., 1988). The SV-40 large T antigen NLS is considered a canonical NLS and has a minimum sequence of pro-lys-lys-lys-arg-lys-val (Dingwall and Laskey, 1986). Analysis of the primary sequences of the U1- and U2-specific proteins deduced from cDNA cloning identifies sequence motifs that are putative NLS in the U1-specific A (arg$_{107}$ glu lys arg lys pro lys) and

70-kD protein (arg$_{288}$ lys arg arg ser ser arg ser arg) and in the U2-specific A' (lys$_{191}$ lys lys gly gly pro ser pro) and B" (lys$_{103}$ lys lys glu lys lys lys ala lys thr val) proteins. The B" NLS is the closest to that of SV-40 large T antigen. Genetic analysis of the proteins will be required to determine if these are functional NLS.

The newly assembled U2 snRNP is immunoprecipitated from cytoplasts by an antiserum that recognizes the U2-specific A' and B" proteins and by antiserum directed against the core proteins. The precipitated particles include newly assembled snRNPs containing the U2 snRNA precursor U2', which is ~12 nt larger than U2 at the 3' end. The extra nucleotides are removed in the cytoplasm after snRNP assembly (Zieve et al., 1988). A small fraction of the B" protein is also found sedimenting in 11-13S snRNP particles in the cytoplast fraction. This demonstrates that at least one of the U2-specific proteins, the B" protein, assembles with the U2 snRNP particle in the cytoplasm early in the maturation sequence. Although both immunoprecipitation analysis and direct analysis on two-dimensional gels demonstrates that the vast majority of B" protein is assembled into snRNP particles in the nucleus, small amounts of pulse-labeled B" are found in cytoplasmic fractions (Sauterer et al., 1990; Feeney et al., 1989). This is consistent with the hypothesis that the B" protein assembles with the U2 snRNP in the cytoplasm immediately after translation. The absence of an unassembled pool of this protein is also consistent with the observation that the maturation and nuclear accumulation of the U2 snRNP is more sensitive to the inhibition of protein synthesis than the U1 snRNP particle. After a 1-h inhibition of protein synthesis the U2 snRNA accumulates in the cytoplasm as U2' while U1 snRNP assembly and nuclear accumulation continues (Zieve, 1987). If the arrest of U2 maturation is correlated with the absence of the B" protein, this suggests that B" is involved in the final maturation of the U2 snRNA. Analysis of mutant U2 snRNAs that do not bind the U2 specific proteins in Xenopus oocytes suggests the U2 specific proteins are not necessary for either the hypermethylation of the 5' cap or the nuclear accumulation of the U2 snRNP (Mattaj and De Robertis, 1985; Mattaj, 1986). However, in yeast, mutations in the U2-specific protein binding sites resulted in U2 RNAs with improperly terminated 3' ends and significantly lowered growth rates (Shuster and Guthrie, 1988).

Both immunoprecipitates of the [^{32}P]phosphate-labeled 70-kD U1-specific protein and immunoblots fail to detect any 70-kD protein in cytoplasmic fractions prepared by aqueous fractionation or cell enucleation. In addition, newly synthesized U1 snRNA can be immunoprecipitated from the cytoplasm by antisera to the core proteins but not by a monoclonal antiserum to the 70-kD protein. This suggests the 70-kD U1-specific protein enters the nucleus independently of U1 snRNP assembly.

The presence of the A, C, and A' snRNP proteins in forms that are not immunoprecipitated with the snRNP particles suggests that they are either in unassembled pools in vivo or have been eluted from the snRNP particles during the isolation procedure. Several results suggest that they are not from disassembled particles. The immunoprecipitated snRNP particles are stable and retain the identical protein composition in buffers up to 0.5 M NaCl (Sauterer et al., 1990). The stoichiometry of these particles, calculated from ratios

of incorporated radioactivity corrected for the amino acid composition of the proteins, suggests there are two copies of the A and C proteins per U1 snRNP (Feeney et al., 1989). It appears unlikely that the substantial pools of A, C, and A' proteins are all quantitatively assembled into snRNP particles. Although there are possible differences in the immunoprecipitation efficiencies of unassembled proteins in cytoplasmic fractions and assembled particles in nuclear fractions, comparison of immunoprecipitated cytoplasmic and nuclear fractions and two-dimensional gels of total cytoplasmic and nuclear fractions suggest that while the unassembled pools of the U1-specific A and C proteins are of almost equal size to the assembled pools, the unassembled pool of the U2-specific A' protein is substantially larger than the assembled pool (Feeney et al., 1989).

The available sequence data have identified two different functional domains in the U1- and U2-specific proteins. The 70-kD, A, and B″ proteins (but not the C or A' proteins) have a sequence motif identified in a large family of proteins that bind RNA or single-stranded DNA known as the RNA binding domain. This domain is often 80–90 amino acids in length and included in it is an eight amino acid motif, the RNP consensus, which is more highly conserved (Swanson et al., 1987; Mattaj, 1989; Bell et al., 1988). The A and B″ snRNP proteins both have two copies of the RNA binding domain. In fact, there is extensive homology between the U1-specific A and the U2-specific B″ proteins and several antisera cross-react with the two proteins. However, our data indicate these proteins have different assembly strategies with the A protein but not the B″ protein present in unassembled pools. The 70-kD protein has only one RNA binding domain and deletion analysis suggests that almost the entire domain is necessary for the specific interaction of the 70-kD protein with the first stem loop of the U1 snRNA (Query et al., 1989). However, the 70-kD protein also has a sequence motif found in two Drosophila proteins that regulate alternative pre-mRNA splicing (Bell et al., 1988; Query et al., 1989; Mattaj, 1989). This motif is a highly charged set of amino acids near the COOH terminus of the 70-kD protein. The Drosophila proteins also contain the RNP consensus sequence in addition to the highly charged region and it is postulated that the charged regions function through protein-protein interactions (Mattaj, 1989; Bingham et al., 1988). The similarity between the Drosophila proteins and the 70-kD protein suggests that the 70-kD protein may also have a role in the regulation of pre-mRNA processing. Further studies will be needed to determine the contributions of these motifs to the sequence specific interaction of the snRNP proteins with the snRNAs and possibly with their processing substrates.

The snRNP particles are stable components of the interphase nucleus. While the U1 snRNP particle is as stable as ribosomes the U2 snRNP turns over with a half-life of about two cell generations (Sauterer et al., 1988). Within the nucleus the particles function in repeated rounds of pre-mRNA splicing. The observation that the U1 specific A and C and the U2-specific A' proteins are in pools that exchange with stable snRNPs, and that the U1-specific 70-kD protein has homologies to other proteins that regulate pre-mRNA splicing, opens the possibility that these proteins can regulate the function of the U1 and U2 snRNPs in nuclear pre-mRNA splicing. Experiments are underway to test the hypothesis that the A, C, and A' proteins dissociate from the snRNP particles during pre-mRNA splicing.

References

Bell, L. R., E. M. Maine, P. Schedl, and T. W. Cline. 1988. Sex-lethal, a drosophila sex determination switch gene, exhibits sex-specific RNA splicing and sequence similarity to RNA binding proteins. *Cell.* 55:1037–1046.

Berger, S. L., and C. S. Birkenmeirer. 1979. Inhibition of intractable nucleases with ribonucleoside-vanadyl complexes. *Biochemistry.* 18:5143–5149.

Billings, P. B., R. W. Allen, F. C. Jensen, and S. O. Hoch. 1982. Anti-RNP monoclonal antibodies derived from a mouse strain with lupus-like autoimmunity. *J. Immunol.* 128:1176–1180.

Bingham, P. M., T.-B. Chou, I. Mims, and Z. Zachar. 1988. On off regulation of gene expression at the level of splicing. *Trends Genet.* 4:134–138.

Bringmann, P., and R. Luhrmann. 1986. Purification of the individual snRNPs U1, U2, U5, and U4/U6 from HeLa cells and characterization of their protein constituents. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:3509–3516.

Brunel, C., J. Sri-Widada, and P. Jeanteur. 1985. SnRNPs and scRNPs in eukaryotic cells. *Prog. Mol. Cell. Biol.* 9:1–52.

Chandrasekharappa, S. C., J. H. Smith, and G. L. Eliceiri. 1983. Biosynthesis of small nuclear RNAs in human cells. *J. Cell. Physiol.* 117:169–174.

Craft, J., T. Mimori, T. L. Olsen, and J. A. Hardin. 1988. The U2 small nuclear ribonucleoprotein particle as an autoantigen. *J. Clin. Invest.* 81:1716–1724.

Dingwall, C., and R. A. Laskey. 1986. Protein import into the cell nucleus. *Annu. Rev. Cell Biol.* 2:367–390.

Dingwall, C., J. Robbins, S. M. Dilworth, B. Roberts, and W. D. Richardson. 1988. The nucleoplasmin nuclear localization sequence is larger and more complex than that of SV-40 large T antigen. *J. Cell Biol.* 107:841–849.

Eliceiri, G. L. 1974. Short-lived, small RNAs in the cytoplasm of HeLa cells. *Cell.* 3:11–14.

Eliceiri, G. L. 1980. Formation of low molecular weight RNA species in HeLa cells. *J. Cell. Physiol.* 102:199–207.

Feeney, R. J., R. A. Sauterer, J. L. Feeney, and G. W. Zieve. 1989. Cytoplasmic assembly and nuclear accumulation of mature snRNP particles. *J. Biol. Chem.* 264:5776–5783.

Fisher, D. E., G. E. Conner, N. H. Reeves, R. Wisniewolski, and G. Blobel. 1985. Small nuclear ribonucleoprotein particle assembly in vivo: demonstration of a 6S RNA-free core precursor and posttranslational modification. *Cell.* 42:751–758.

Habets, W. J., P. T. G. Sillekens, M. H. Hoet, J. A. Schalken, A. J. M. Roebroek, J. A. M. Leunissen, W. J. M. van de Ven, and W. J. van Venrooij. 1987. Analysis of a cDNA clone expressing a human autoimmune antigen: full-length sequence of the U2 small nuclear RNA-associated B″ antigen. *Proc. Natl. Acad. Sci. USA.* 84:2421–2425.

Kramer, A. 1987. Fractionation of HeLa cell nuclear extracts reveals minor small nuclear ribonucleoprotein particles. *Proc. Natl. Acad. Sci. USA.* 84:8408–8412.

Lelay-Taha, M.-N., I. Reveillaud, J. Sri-Widada, C. Brunel, and P. Jeanteur. 1986. RNA-protein organization of U1, U5, and U4-U6 small nuclear ribonucleoproteins in HeLa cells. *J. Mol. Biol.* 189:519–532.

Lerner, E. A., M. R. Lerner, C. A. Janeway, Jr., and J. A. Steitz. 1981. Monoclonal antibodies to nucleic acid-containing cellular constituents: probes for molecular biology and autoimmune disease. *Proc. Natl. Acad. Sci. USA.* 78:2737–2741.

Madore, S. J., E. D. Wieben, and T. Pederson. 1984. Intracellular site of U1 small nuclear RNA: processing and ribonucleotide assembly. *J. Cell Biol.* 98:188–192.

Maniatis, T., and R. Reed. 1987. The role of small nuclear ribonucleoprotein particles in pre-mRNA splicing. *Nature (Lond.).* 325:673–678.

Mattaj, I. W. 1986. Cap trimethylation of U snRNA is cytoplasmic and dependent on U snRNP protein binding. *Cell.* 46:905–911.

Mattaj, I. W. 1989. A binding consensus: RNA-protein interactions in splicing, snRNPs and sex. *Cell.* 57:1–3.

Mattaj, I. W., and E. M. De Robertis. 1985. Nuclear segregation of U2 snRNA requires binding of specific snRNP proteins. *Cell.* 40:111–118.

McAllister, G., A. Roby-Shemkovitz, S. G. Amara, and M. R. Lerner. 1989. cDNA sequence of the rat U snRNP-associated protein N: description of a potential Sm epitope. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:1177–1181.

Mimori, T., M. Hinterberger, T. Pettersson, and J. A. Steitz. 1984. Autoantibodies to the U2 small nuclear ribonucleoprotein in a patient with scleroderma-polymyositis overlap syndrome. *J. Biol. Chem.* 259:560–565.

Montzka, K. A., and J. A. Steitz. 1988. Additional low-abundance human small nuclear ribonucleoproteins: U11, U12, etc. *Proc. Natl. Acad. Sci. USA.* 85:8885–8889.

Parry, H. D., D. Scherly, and I. W. Mattaj. 1989. Snurpogenesis: The tran-

scription and assembly of U snRNP components. *Trends Biochem. Sci.* 14:12–16.

Pettersson, I., M. Hinterberger, T. Mimori, E. Gottlieb, and J. A. Steitz. 1984. The structure of mammalian small nuclear ribonucleoproteins: identification of multiple protein components reactive with anti-(U1)RNP and anti-Sm autoantibodies. *J. Biol. Chem.* 259:5907–5914.

Query, C. C., R. C. Bentley, and J. D. Keene. 1989. A common RNA recognition motif identified within a defined U1 RNA binding domain of the 70 K U1 snRNP proteins. *Cell.* 57:89–101.

Reddy, R., and H. Busch. 1988. Small nuclear RNAs: RNA sequences, structure and modification. *In* Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles. M. L. Birnstiel, editor. Springer-Verlag, New York. 1–37.

Reddy, R., D. Henning, and H. Busch. 1985. Primary and secondary structure of U8 small nuclear RNA. *J. Biol. Chem.* 260:10930–10935.

Reuter, R., S. Rothe, and R. Luhrmann. 1987. Molecular relationships between U snRNP proteins as investigated by rabbit antisera and peptide mapping. *Nucleic Acids Res.* 15:4021–4034.

Roberts, B. L., W. D. Richardson, and A. E. Smith. 1987. The effect of protein context on nuclear location signal function. *J. Cell Biol.* 50:465–475.

Sauterer, R. A., R. J. Feeney, and G. W. Zieve. 1988. Cytoplasmic assembly of snRNP particles from stored proteins and newly transcribed snRNAs in L929 mouse fibroblasts. *Exp. Cell Res.* 176:344–359.

Sauterer, R. A., A. Goyal, and G. W. Zieve. 1990. Cytoplasmic assembly of small nuclear ribonucleoprotein particles from 6S and 20S RNA-free intermediates in L929 mouse fibroblasts. *J. Biol. Chem.* 265:1048–1058.

Sharp, P. A. 1987. Splicing of messenger RNA precursors. *Science (Wash. DC).* 235:766–771.

Shuster, E. O., and C. Guthrie. 1988. Two conserved domains of yeast U2 snRNA are separated by 945 nonessential nucleotides. *Cell.* 55:41–48.

Sillekens, P. T., W. J. Habets, R. P. Beijer, and W. J. van Venrooij. 1987. cDNA cloning of the human U1 snRNA-associated A protein: extensive homology between U1 and U2 snRNP-specific proteins. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3841–3848.

Sillekens, P. T., R. P. Beijer, W. J. Habets, and W. J. van Venrooij. 1988. Human U1 snRNP-specific C protein: complete cDNA and protein sequence and identification of a multigene family in mammals. *Nucleic Acids Res.* 16:8307–8321.

Sillekens, P. T., R. P. Beijer, W. J. Habets, and W. J. van Venrooij. 1989. Molecular cloning of the cDNA specific for the human U2 sRNA-specific A' protein. *Nucleic Acids Res.* 17:1893–1906.

Steitz, J. A., D. L. Black, V. Gerke, K. A. Parker, A. Kramer, D. Frendewey, and W. Keller. 1988. Functions of the abundant U-snRNPs. *In* Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles. M. L. Birnstiel, editor. Springer-Verlag, New York. 115–154.

Swanson, M. S., T. Y. Nakagawa, K. LeVan, and G. Dreyfuss. 1987. Primary structure of human nuclear ribonucleoprotein particle C proteins: conservation of sequence and domain structures in heterogeneous nuclear RNA, mRNA and pre-rRNA-binding proteins. *Mol. Cell. Biol.* 7:1731–1739.

Theissen, H., M. Etzerodt, R. Reuter, C. Schneider, F. Lottspeich, P. Argos, R. Luhrmann, and L. Philipson. 1986. Cloning of the human cDNA for the U1 RNA-associated 70K protein. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:3209–3217.

Van Dam, A., I. Winkel, R. Smeents, and H. J. Cuypers. 1989. Cloned human snRNP B and B' differ only in their carboxy-terminal part. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:3853–3860.

Wooley, J. C., L. R. Zukerberg, and S. Y. Chung. 1983. Polypeptide components of human small nuclear ribonucleoproteins. *Proc. Natl. Acad. Sci. USA.* 80:5208–5212.

Yamamoto, K., H. Miura, Y. Moroi, S. Yoshinoya, M. Goto, K. Nishioka, and R. Miyamoto. 1988. Isolation and characterization of a complementary DNA expressing human U1 small nuclear ribonucleoprotein C polypeptide. *J. Immunol.* 140:311–317.

Zieve, G. W. 1987. Cytoplasmic maturation of the snRNAs. *J. Cell. Physiol.* 131:247–254.

Zieve, G. W., and S. Penman. 1976. Small RNA species of the HeLa cell: Metabolism and subcellular localization. *Cell.* 8:19–31.

Zieve, G. W., and R. J. Feeney. 1989. Cytoplasmic assembly and nuclear transport of the snRNP particles. *Prog. Mol. Subcell. Biol.* 11:51–85.

Zieve, G. W., and R. A. Sauterer. 1990. Cell biology of the snRNP particles. *CRC Cirt. Rev. Biochem. Mol. Biol.* 25:1–46.

Zieve, G. W., R. A. Sauterer, and R. J. Feeney. 1988. Newly synthesized snRNAs appear transiently in the cytoplasm. *J. Mol. Biol.* 199:259–267.