Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 9784–9788, November 1991
Biochemistry

Two-step affinity purification of U7 small nuclear ribonucleoprotein particles using complementary biotinylated 2′-*O*-methyl oligoribonucleotides

(RNA processing/small nuclear RNA/molecular weight/core proteins)

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ABSTRACT **U7 snRNP is a low-abundance small nuclear ribonucleoprotein particle essential for 3′ processing of replication-dependent histone pre-mRNA. We have developed a two-step purification of the particle from TB21 mouse mastocytoma cell nuclear extracts, with about a 20% overall yield, using affinity binding to 2′-*O*-methyl oligoribonucleotides. The purified particle is homogeneous with respect to RNA content. SDS/PAGE of the U7 snRNP proteins revealed a full complement of the standard core proteins (B, DD′, E, F, and G) found in the majority of snRNPs. In addition, two U7-specific polypeptides of 14 kDa and 50 kDa were identified. Summation of the molecular masses of the identified components of the U7 particle yields a particle mass of 249 kDa, in approximate agreement with estimates from sucrose gradient sedimentation (261 kDa) and nondenaturing gradient PAGE (217 kDa).**

The nuclei of eukaryotic cells contain large amounts of several small RNAs (snRNAs) that associate with proteins to form ribonucleoprotein particles (snRNPs) that participate in a variety of pre-mRNA splicing and processing reactions (1). Six high-abundance mammalian snRNPs, U1–U6 (10^5 to 10^6 copies per cell) have been purified and are well characterized. The U1, U2, U4, U5, and U6 snRNPs are involved in pre-mRNA splicing and contain in common a set of six core proteins: B, and a variant B′ in primates (29 kDa), D (18 kDa), D′ (16 kDa), E (13 kDa), F (12 kDa), and G (11 kDa) (1). In addition, each particle contains one or more specific proteins; for example, U2 snRNP contains protein A′ (32 kDa) and B″ (28 kDa). U3 snRNP contains six specific proteins and none of the core proteins. Seven low-abundance ($<5 \times 10^4$ copies) snRNPs, U7–U13, have been identified but are poorly characterized. U8 and U13 snRNPs are found in the nucleolus and participate in pre-rRNA processing (1–4). U11 snRNP is a component of the polyadenylylation complex that processes the 3′ end of most mRNAs (5). U7 snRNP is an essential cofactor in histone pre-mRNA 3′ processing (6). All of the minor snRNPs are immunoprecipitable by anti-Sm antibody, indicating that they contain one or more of the core proteins (1).

The histone pre-mRNA processing reaction mediated by U7 snRNP has been studied using mammalian nuclear extracts (6) and in *Xenopus* oocytes (7). Site-directed mutagenesis of the 3′ region of the pre-mRNA and oligonucleotide competition studies indicate that a hairpin structure and a downstream purine-rich consensus sequence are required for recognition and cleavage of the pre-mRNA adjacent to and 3′ to the hairpin (8, 9). The reaction requires U7 snRNP and a heat-labile protein, a fraction of which can be obtained free of U7 particles (10). A hairpin-binding protein has been identified that appears to be an integral part of the U7 particle (8). U7 snRNP has not yet been purified, primarily because of its low abundance (800–3000 copies per mammalian cell). Mouse U7 RNA is 63 nucleotides long and has the standard m_3GpppA cap found in nearly all of the snRNPs (Fig. 1) (6). One or more proteins homologous to the core proteins are presumably present because of the anti-Sm reactivity. Literature estimates for the molecular mass of the particle range from 115 kDa (11) to >300 kDa (10).

In this work we have used ribonuclease-resistant 2′-*O*-methyl oligoribonucleotides (12–14) complementary to the 5′ end of the U7 RNA to affinity purify the particle and we have analyzed its protein content. U2 snRNP was purified in parallel to provide core protein standards.

MATERIALS AND METHODS

Buffers. Buffer D contains 20% (vol/vol) glycerol, 20 mM Hepes (pH 7.9), 100 mM KCl, 0.25 mM EDTA, and 0.5 mM dithiothreitol. Buffer DT100 is buffer D containing 0.02% Tween 20. Buffers DT200, DT300, and DT1000 are buffer DT100 containing 200, 300, and 1000 mM KCl, respectively. Buffer ET0 contains 20% glycerol, 1 mM Hepes (pH 7.9), 0.25 mM EDTA, 0.5 mM dithiothreitol, and 0.02% Tween 20.

Biotinylated 2′-*O*-Methyl Oligoribonucleotides. The 2′-*O*-methyl oligoribonucleotides 5′-HO-UGUAACACUU-NH_2 (10-mer, oligo I), 5′-HO-AGAGCUGUAA-NH_2 (10-mer, oligo II), and 5′-HO-CUAAAAGAGCUGUAACACU-NH_2 (19-mer, oligo III), complementary to sequences at the 5′ end of the U7 RNA (Fig. 1), and 5′-HO-GAUACUACAC-NH_2 (10-mer) and 5′-HO-CAGAUACUACAC-NH_2 (12-mer), complementary to sequences in the 5′ region of the U2 RNA, were synthesized as described (14). Biotinylation of the 10-mers (25–50 nmol) was carried out in 0.25 M sodium bicarbonate buffer at pH 9 (13) with NHS-LC-biotin (Pierce). To remove free biotin the reaction mixture was diluted with an equal volume of water and loaded onto a 0.25-ml bed of DEAE-Sepharose CL-6B (Pharmacia) in a 2-ml disposable Bio-Rad column. After washing with eight 0.5-ml aliquots of TE150 (10 mM Tris·HCl, pH 8/1 mM EDTA/150 mM NaCl), the biotinylated oligonucleotide was eluted with TE1000 buffer. Essentially 100% of the oligonucleotide was recovered in a volume of ≈0.6 ml. Nonbiotinylated oligonucleotide was removed by chromatography on a 2-ml monomeric avidin-Sepharose (Sigma) column as described (15). Free biotin introduced by the elution from the monomeric avidin was removed by DEAE-Sepharose chromatography as above. Typically, about 50% of the original oligonucleotide was recovered in the biotinylated form.

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Abbreviation: snRNP, small nuclear ribonucleoprotein particle.

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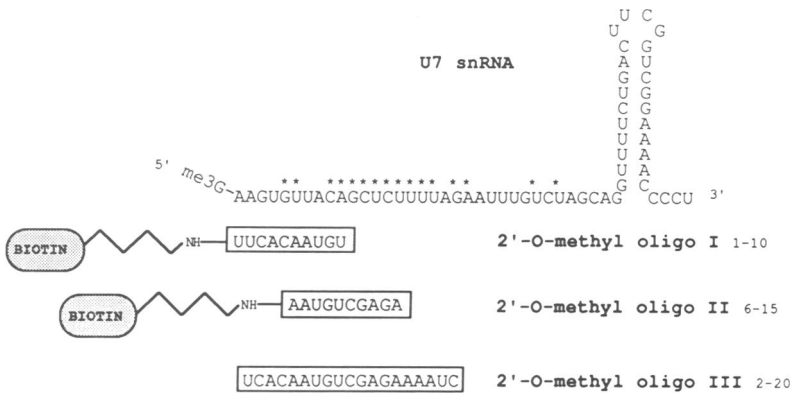

FIG. 1. The U7 RNA nucleotide sequence and the U7-specific complementary biotinylated 2'-*O*-methyl oligoribonucleotides used in this work. Stars indicate nucleotides complementary to the downstream conserved 3' sequence in the histone pre-mRNA.

Cell Culture and Nuclear Extracts. Nuclear extracts of TB21 mouse mastocytoma cells (16, 17) and mouse hybridoma EBI cells (18) were prepared as described (19).

Gel Retardation Assays. The 19-mer (11 pmol) was 5'-^{32}P-labeled in a reaction mixture (10 μl) containing 50 mM Tris·HCl (pH 7.6), 10 mM $MgCl_2$, 5 mM dithiothreitol, 0.1 mM EDTA, 5% polyethylene glycol 6000 (Sigma) (20), 0.1 mM spermidine, 11 units of T4 kinase (Boehringer Mannheim), and 20–50 μCi of [γ-^{32}P]ATP (5000 Ci/mmol; 1 Ci = 37 GBq). After 30 min at 37°C, 100 μl of TE150 was added, the mixture was extracted once with phenol/chloroform, and the aqueous phase was chromatographed on DEAE-Sepharose as described above to remove labeled ATP. The oligonucleotide was precipitated in the presence of 20 μg of glycogen with 2.5 volumes of ethanol and redissolved in 100 μl of distilled water; 1 μl of the final solution contained about 95 fmol of oligonucleotide and 20–100 $\times$ 10^3 Cerenkov cpm. For the retardation assay, 10 μl of U7 snRNP fraction in buffer D containing 0.1% Tween 20 (to inhibit aggregation) was mixed with 1 μl each of heparin sulfate (50 mg/ml), *Escherichia coli* tRNA (40 mg/ml), and labeled 19-mer, and the mixture was incubated for 45 min at 37°C. Tracking dye (1 μl) was added and the reaction mixture was loaded into a 1-cm-wide slot of a 1-mm-thick 3.5% polyacrylamide (80:1 acrylamide/*N,N'*-methylenebisacrylamide weight ratio)/1% agarose gel (21) containing Tris acetate/EDTA buffer (22), 10% glycerol, and 0.1% Tween 20. Electrophoresis was at 23°C and 8 V/cm for 90 min. The gel was picked up on DE81 paper (Whatman), dried, and autoradiographed. Labeled 19-mer associated with U7 snRNP migrated above the xylene cyanol marker and free 19-mer migrated near the bromphenol blue marker. Heparin and tRNA inhibit binding of basic proteins to the oligonucleotide (21), and tRNA helps to prevent removal of 5' label by phosphatases. U7 snRNP was quantitated from the Cerenkov cpm per excised band and the specific activity of labeled 19-mer. U2 snRNP retardation assays were done similarly using labeled complementary 12-mer.

Purification of U2 and U7 snRNPs from Nuclear Extracts. TB21 nuclear extract (30 ml), dialyzed against buffer D, and 660 pmol of biotinylated U7-complementary oligo II were incubated at 30°C for 30 min to form oligo/U7 RNA hybrids. A 0.3-ml packed bed volume of streptavidin-agarose (BRL), washed twice with 10 vol of buffer D, was added to absorb the biotinylated complex. The mixture was rotated in two 15-ml plastic centrifuge tubes for 18 hr at 4°C. The gel was pelleted at 3000 $\times$ *g* for 1 min in a swinging-bucket rotor. The supernatant was saved for U2 snRNP purification (see below). The gel was given four 15-min washes on the rotator with 15 ml of cold DT300 buffer per tube to remove nonbound extract components. The gel was then transferred to two 1.5-ml Eppendorf centrifuge tubes and each half was washed twice at 4°C with 1 ml of ET0 buffer (no KCl), lowering the KCl concentration to ≈6 mM. The gel in each tube was then suspended in 0.5 ml of ET0 buffer, lowering the KCl concentration below 2 mM, and incubated at 37°C for 5 min. After centrifugation for 5 sec at 14,000 rpm in an Eppendorf centrifuge, the supernatant was recovered and the gel was eluted once more with 0.5 ml of ET0 at 37°C. Most of the U7 snRNP was eluted in the first 37°C fraction. The melting point of the oligo II/U7 hybrid in 300 mM KCl is about 55°C (23) and is calculated to be below 30°C in 2 mM KCl (24).

A second step of purification and concentration was carried out using Q-Sepharose (Pharmacia). A 50-μl bed of Q-Sepharose packed on top of a plug of glass wool in a 1-ml Eppendorf micropipet tip was equilibrated with DT100 buffer. The two 1-ml 37°C elution fractions were adjusted to 100 mM KCl and 20 mM Hepes (pH 7.9) and passed slowly through the column by gravity (≈1 hr). The column was sequentially washed with DT100, DT200, and DT300 (100 μl each). The U7 snRNP was then recovered by two 100-μl DT1000 elutions. KCl was removed from the 100-μl U7 fractions by microdialysis against ET0 for 1–2 hr. Fractions were stored at −20°C.

An alternative second step of purification and concentration used oligo I for a second round of affinity purification. The two 37°C elutions were pooled and adjusted to 100 mM KCl and 20 mM Hepes (pH 7.9). Biotinylated oligo I (≈200 pmol) was added and the mixture was incubated at 30°C for 30 min. Streptavidin-agarose (25 μl) was added and the mixture was rotated slowly overnight at 4°C. The gel was then washed twice with 1 ml of DT300 buffer, twice with 200 μl of ET0 at 4°C, and twice with 100 μl of ET0 at 37°C.

U2 snRNP was isolated from the nuclear extract supernatant of the U7 extraction by addition of 1500 pmol of biotinylated U2-complementary 10-mer, as described above.

SDS/PAGE. Proteins were prepared in sample buffer and electrophoresed in 15% discontinuous polyacrylamide gels (25) as described (22). Proteins were stained with colloidal Coomassie brilliant blue G250 (26).

Nondenaturing Gradient PAGE. The molecular weight of native snRNPs was determined by electrophoresis in a 1-mm-thick by 7-cm-long nondenaturing (4–16%) polyacrylamide (30:1) linear gradient gel (27, 28). A superimposed 10–30% glycerol gradient stabilized the acrylamide gradient during preparation of the gel. The gel also contained Tris acetate/EDTA buffer and 0.1% Tween 20 and the running buffer was Tris acetate/EDTA. Samples of nuclear extract (10 μl) were incubated with labeled complementary oligonucleotides as in the retardation assay prior to loading. Native protein standards (Sigma) were thyroglobulin (669 kDa), apoferritin (443 kDa), and β-amylase (200 kDa). Electrophoresis was at 23°C for 21 hr at 100 V with continual buffer circulation. At termination, the portion of the gel containing labeled samples was removed, dried, and autoradiographed. Protein standards were stained with colloidal Coomassie blue.

RNA Extraction and Analysis. Samples were digested with proteinase K (1 mg/ml) in the presence of 1% SDS for 30 min

at 56°C, followed by phenol/chloroform extraction, addition of 20 ug of glycogen, and ethanol precipitation. The pellets were dissolved in 10 μl of distilled water. The RNA was 3′-^{32}P-labeled using pCp and RNA ligase (29). RNA was analyzed in 10% polyacrylamide sequencing gels (22).

RESULTS

Gel Retardation Assay of U7 snRNP Particles in Nuclear Extracts. The "oligonucleotide decoration" assay of Melin *et al.* (34) was used to characterize the U7 snRNPs. When labeled complementary oligoribonucleotide was hybridized to U7 snRNP in nuclear extracts, a fraction of the label representing particle-bound oligonucleotide migrated at about a quarter the rate of the free oligonucleotide during electrophoresis in a nondenaturing 3.5% polyacrylamide/1% agarose gel (Fig. 2*A*). No retardation of label was observed with a nonsense oligonucleotide lacking complementarity to the U7 RNA (data not shown). The amount (fmol) of particle-bound oligo was determined from the radioactivity in the U7 snRNP band and from the known specific activity of the oligonucleotide. Approximate saturation of the particles was achieved with >10-fold molar excesses of complementary oligonucleotide (Fig. 2*B*). In our hands, TB21 cells yield about twice as much U7 snRNP as EBI cells (Fig. 2*B*).

Molecular Mass of U7 snRNP. A retardation assay was run in a nondenaturing 4–16% gradient gel against native protein standards. U2 snRNP was electrophoresed in parallel for comparison. The relative position of bands after 21 hr (bromphenol blue exited the gel at about 1 hr and xylene cyanol at about 2 hr) is shown in Fig. 3. The distances migrated by the standards varied almost linearly with molecular weight (results not shown). Retarded U2-specific oligo migrated to a position corresponding to about 290 kDa. The calculated mass from addition of component masses is 290 kDa (162 kDa for the $B_2D_2D_2^1EFG$ core plus 60 kDa for the specific proteins A′ and B″ plus 68 kDa for the 189-nucleotide U2 RNA). About 80% of the retarded U7-specific oligonucleotide migrated at 217 kDa. In a sucrose gradient sedimentation velocity experiment (data not shown), two peaks containing U7 RNA were seen, the first near the top of the gradient, perhaps consisting of incomplete particles or free RNA, and the second positioned one tube further than the β-amylase standard (200 kDa) and three fractions in front of the apoferritin peak (443 kDa). Assuming spherical particles and using no correction for protein or particle densities, we obtained another estimate for the U7 snRNP mass of 261 kDa.

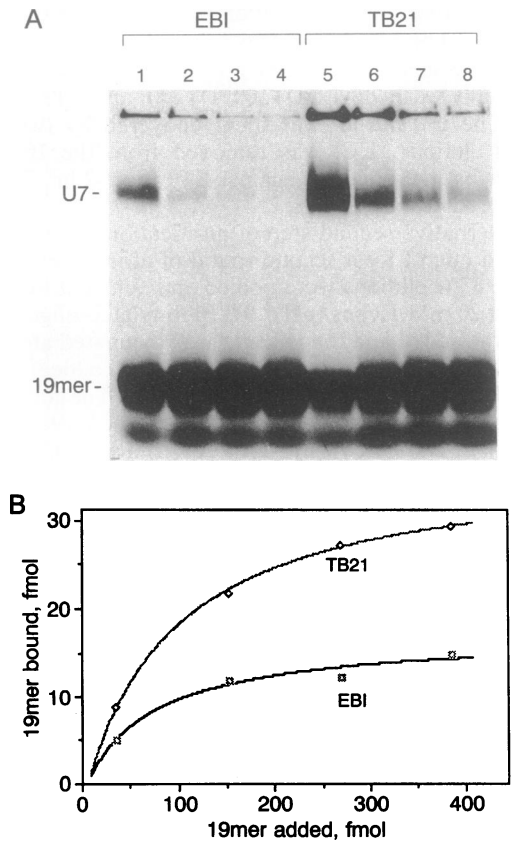

FIG. 2. Gel retardation saturation assays showing the effects of increasing amounts of unlabeled 19-mer on the extent of binding of labeled 19-mer. (*A*) EBI or TB21 nuclear extract (10 μl) was incubated with 27 fmol of labeled 19-mer plus 0 fmol (lanes 1 and 5), 117 fmol (lanes 2 and 6), 234 fmol (lanes 3 and 7), or 351 fmol (lanes 4 and 8) of unlabeled 19-mer. Samples were then electrophoresed in a polyacrylamide/agarose gel. (*B*) Amounts of bound 19-mer plotted against total 19-mer (data from *A*). An ≈10-fold molar excess of free 19-mer was required to quantitatively saturate the particle. The total binding capacity of the TB21 extract was nearly double that of the EBI extract.

Purification of U7 snRNP by Affinity Chromatography with Biotinylated Complementary Oligoribonucleotides. Biotinylated oligo II (Fig. 1) was hybridized to the complementary region of the 5′ end of U7 RNA in nuclear extract (fraction I, Table 1), and the complexes were then bound to streptavidin-agarose. The gel was thoroughly washed with high-salt buffer and then with low-salt buffer to lower the melting point of the duplex RNA so that the U7 snRNP could be released by brief incubation at 37°C. About 50–60% of the U7 particles in the extract were removed at this step as determined by gel retardation assay of the extract before and after absorption (Fig. 4). A fraction of the bound particles were not released by the melting treatment, so that total recovery in the first step (fraction II) was ≈33% (Table 1). The concentration of U7 particles in fraction II was too low for satisfactory analysis. We used two different second steps, Q-Sepharose chromatography or oligo I binding, for additional purification and concentration (Table 1). With Q-Sepharose, ≈15% of the U7 eluted at 0.3 M KCl, but most of the U7 was recovered in the 1.0 M KCl step. About 14 pmol of particles (3.1 μg) was obtained in 100 μl (19% final yield). With the oligo I affinity procedure, 13 pmol (2.8 μg) was obtained (17% yield).

Analysis of Purified U7 snRNP. RNA was extracted from an aliquot of fraction IIIB (Table 1), 3′-end-labeled using radioactive pCp and RNA ligase, and analyzed in a 10% polyacrylamide/urea gel. Compared with the starting material, a single RNA band was visible at the expected position for intact U7 RNA (Fig. 5). Samples of fractions IIIA and IIIB were analyzed by SDS/PAGE. Fraction IIIA (Fig. 6*A*) showed all of the characteristic core protein bands as compared with the U2 snRNP lane. In addition, a band was present at 14 kDa. We have seen this band in the same relative amount in four independent purifications. A band was also consistently found at 50 kDa. Four weakly stained bands were seen between 31 and 45 kDa. These and a weak series of bands at the top of the gel are presumed to be contaminants (see *Discussion*). When

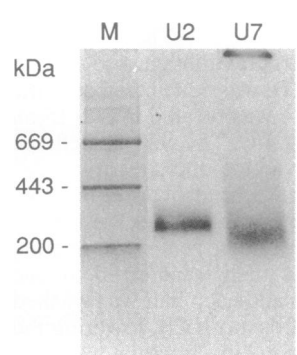

FIG. 3. Determination of the molecular masses of U2 and U7 snRNPs by native gradient PAGE. TB21 nuclear extract (10 μl) was incubated with labeled U7-specific 19-mer or U2-specific 12-mer and then electrophoresed for 21 hr at room temperature in a 4–16% linear gradient polyacrylamide gel. Complexes were detected by autoradiography. Lane M, Coomassie blue-stained marker proteins.

Table 1. Summary of U7 purification

Fraction	Volume, ml	U7 snRNP, pmol	Protein, μg	Yield, %
I (nuclear extract)	30.0	75	180,000*	100
II (oligo II binding)	2.0	25.0†	5.3‡	33
IIIA (Q-Sepharose)	0.1	14.4	3.1‡	19
IIIB (oligo I binding)	0.1	12.8	2.8‡	17

*Total proteins, determined by Bradford assay (30).
†Forty-two picomoles of U7 snRNP bound to gel, but only 25 pmol was released by 37°C melting.
‡Micrograms of U7 snRNP calculated by assuming pure particles of 220 kDa.

fractions IIIA and IIIB were analyzed without reduction with 2-mercaptoethanol (Fig. 6*B*) the banding patterns were similar to those of the reduced samples except for the core proteins D and D′ at 16 kDa, which migrated as individual bands in both the U2 and the U7 lanes. We conclude that the 14- and 50-kDa bands represent specific U7 proteins. Assuming a complete complement of core proteins plus these two specific proteins plus the U7 RNA (23 kDa), the predicted mass for U7 snRNP is 249 kDa, in reasonable agreement with our determinations (217–261 kDa).

DISCUSSION

Considerable knowledge of the U7 snRNP-mediated 3′ histone pre-mRNA processing reaction has been obtained from nuclear extract studies (32). A purified system has not been described. With such a system, studies of the roles of U7 RNA and the individual snRNP proteins in the reaction and structural studies of the particle and its complexes with pre-mRNA would become feasible. A major impediment to developing such a system has been the relatively small amount of U7 per cell. In TB21 nuclear extract, we found 75 pmol of U7 per 1.5×10^{10} rapidly dividing cells, or 3×10^3 U7 particles per cell. This compares with $0.2–1 \times 10^6$ per cell for each of the abundant snRNPs. Because only a few micrograms of U7 snRNP is present in a relatively large amount of extract and because the general chromatographic properties of U7 snRNP are similar to those of the abundant snRNPs, an affinity purification procedure specific for U7 seemed mandatory. Recent work has shown that U1 and U2 snRNPs can be selectively removed from extracts in one step

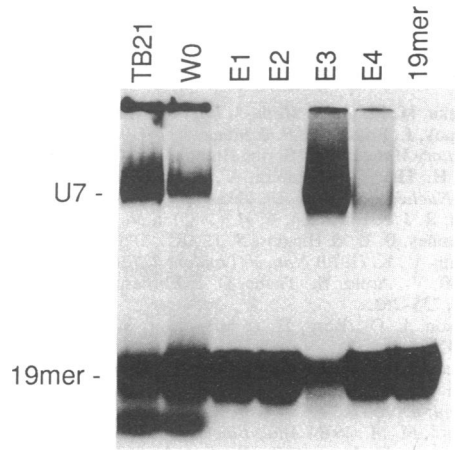

FIG. 4. Gel retardation assays of the wash and elution fractions from the affinity purification with oligo II (fraction II; Table 1). Measurement of the counts in the U7 snRNP band (U7) for TB21 nuclear extract and for the first gel supernatant fraction (W0) indicated that 56% of the hybridizable U7 was removed from the nuclear extract. Fractions E1 and E2 were low-salt washes at 4°C, and E3 and E4 were low-salt 37°C elutions. Lane 19-mer shows free oligo III.

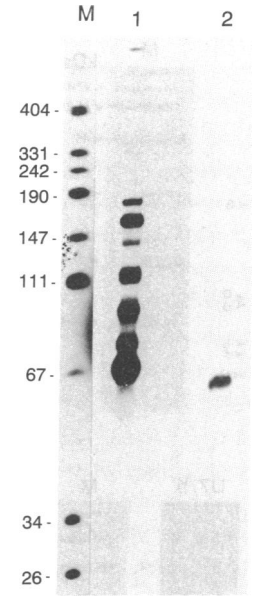

FIG. 5. RNA from a TB21 nuclear extract (20 μl; lane 1) and purified U7 snRNP (fraction IIIB; 5 μl; lane 2) was extracted and pCp-labeled. The RNA was analyzed in a 10% polyacrylamide sequencing gel (21). Labeled pUC19 *Hpa* II restriction fragment markers (lane M) were run in parallel. Sizes are in nucleotides.

using streptavidin-bound biotinylated complementary 2′-*O*-methyl oligoribonucleotides (12) that are highly resistant to ribonucleases (13). Relatively long (20 to 25 nucleotide) oligonucleotides were used in that work and the particles were released from the solid support only by vigorous extraction procedures. We quickly found that the melting point of long oligonucleotides was too high for efficient release of the intact bound particles. With 10-mers, however, we found that the specificity to uniquely bind either U2 or U7 was retained and the particles could be released under relatively mild conditions.

The overall recovery of a little less than 20% of the U7 from the extract is accounted for by the inefficiency of the initial hybridization reaction and by losses due to nonspecific binding to the gel matrix. We used a 9-fold excess of 10-mer for the initial affinity binding step. We have shown with retardation assays that >10-fold excesses are generally required for saturation with 19-mer under similar reaction conditions. However, we feared that greater oligonucleotide excesses might lead to excessive contamination with various nucleic acid-binding proteins. Furthermore, larger amounts of streptavidin-agarose gel would then be required with consequently greater nonspecific losses. We also found in working with only microgram quantities of U7 that we consistently suffered a loss of about a third of our U7 at each gel binding step, whether with streptavidin-agarose or with Q-Sepharose. A variety of other matrixes (Cibacron Blue-Sepharose, heparin-Sepharose, and S-Sepharose) proved no better than Q-Sepharose. A second round of oligonucleotide affinity purification appeared slightly superior to Q-Sepharose in terms of final purity and avoided the need for microdialysis.

Fraction III is essentially homogeneous by the criterion of RNA purity and it is also sufficiently free of contaminating proteins to identify the major protein components of the U7 particle. SDS/PAGE analysis reveals bands at the positions of the standard core proteins B, DD′, E, F, and G found in the majority of snRNPs. That these bands actually represent the core proteins is based on at least three arguments. (*i*) That U7 snRNP is precipitable by anti-Sm antibody implies that one or more core proteins are present. (*ii*) The bands consistently migrate identically to the U2 core proteins whether denatured with SDS or with SDS and 2-mercaptoethanol. In fact, the single DD′ band differentiates into two bands in both the U2 and U7 preparations in the absence of 2-mercaptoethanol. Apparently, D and D′ contain intramolecular disulfide bridges

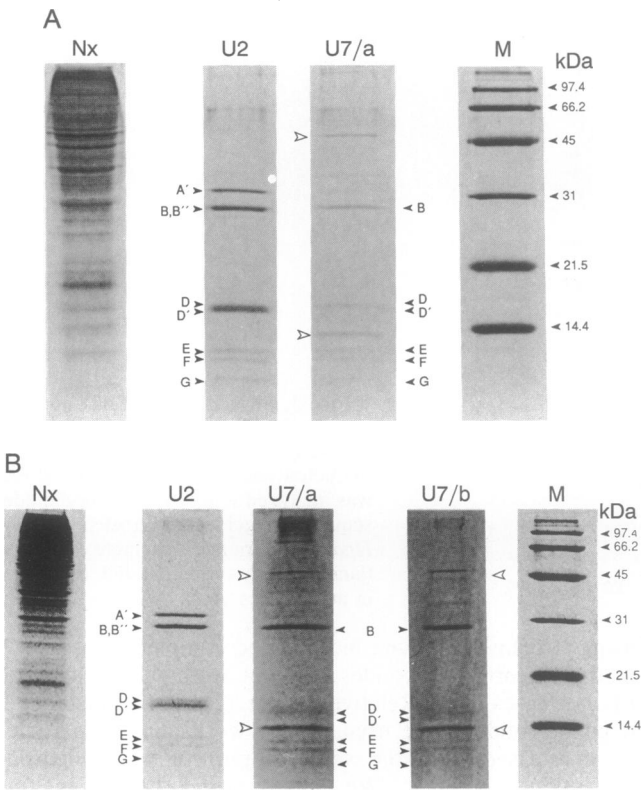

FIG. 6. SDS/PAGE of purified snRNPs. (*A*) Nuclear extract (Nx), purified U2 snRNP (U2), and fraction IIIA (U7/a) were treated with 1% SDS/2% 2-mercaptoethanol at 95°C for 4 min and then electrophoresed in parallel with molecular mass marker proteins (M). The cluster of bands at 55–65 kDa was also present in loading-buffer control lanes and represents the so-called mercaptan artifact (31). (*B*) Nuclear extract (Nx), purified U2 snRNP (U2), and fractions IIIA (U7/a) and IIIB (U7/b) were treated only with 1% SDS prior to analysis. The mercaptan artifact is not visible. The core proteins and the U2-specific A′ and B″ bands are identified (solid arrowheads) as well as the putative U7-specific species (open arrowheads) at 14 kDa and 50 kDa.

that affect their migration. (*iii*) The relative band intensities appear comparable in U7 and U2, with the possible exception of DD′, which may be underrepresented in the isolated U7. We have identified bands at 14 kDa and 50 kDa as U7-specific because (*a*) they have been consistently seen in several independent purifications using the U7-specific 10-mer but not when the U2-specific 10-mer is used and (*b*) they are retained nearly equally during the second stage of purification whether by Q-Sepharose or by oligonucleotide affinity. A few putative contaminant bands can also be seen. The barely visible series of bands at the top of the gel (Fig. 6) probably represents proteins that enter the agarose gel during the long 4°C incubation and do not completely diffuse out of the gel during the shorter wash procedure. In fact, we observed that insufficient washing led to greater contamination with high molecular weight proteins. We cannot rule out the possibility that one or more of the weakly stained bands at 31–45 kDa are U7-specific proteins. However, when these bands are included, the calculated particle mass exceeds our best experimental values.

Literature estimates for the size of U7 snRNP are quite inconsistent. Kramer (11) reported a size of 115 kDa based on gel chromatography. However, the only identification of the particle was by RNA size (65 nucleotides). Further, since several steps of purification were involved, the particle may have lost some of its proteins. Gick *et al.* (10) estimated the size of particles active in processing to be about 300 kDa by gel chromatography. We have used native gradient gel electrophoresis and sucrose gradient sedimentation to obtain estimates of 217 kDa and 261 kDa for the particle in nuclear extracts. Our confidence in the gradient gel determination was strengthened by the finding that U2 snRNP migrated to a final position very closely corresponding to its calculated size. If we assume that the shapes of U2 and U7 are similar (they share the same core proteins), then the value for U7 may be reasonably accurate, if the particle is still fully intact.

The starting nuclear extract showed histone pre-mRNA processing activity (33) but fraction II and later fractions were inactive, suggesting either that an essential factor had been removed or that some denaturation or loss of particle components had occurred. Further work will be needed to determine the cause of the activity loss.

We thank Lisa Ballow and Stephen Phillips for reviewing the manuscript. H.O.S. is an American Cancer Society Research Professor.

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