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Additional low-abundance human small nuclear ribonucleoproteins: U11, U12, etc.

(RNA processing/small RNA/polyadenylylation/autoantibodies)

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ABSTRACT **Two-dimensional gel fractionation has revealed the existence of a number (≥8) of additional species of HeLa cell small RNAs that have 5′ trimethylguanosine cap structures and are bound by proteins containing Sm epitopes. Therefore, these low-abundance (10^3–10^4 per cell) RNAs belong to the Sm class of small nuclear ribonucleoproteins (snRNPs), whose best-known members are the four highly abundant (≈10^6 per cell) particles required for pre-mRNA splicing. The complexity of Sm snRNPs in mammalian cells is thus not greatly different from that previously established for lower eukaryotes. Two of the new RNAs, designated U11 (131 nucleotides) and U12 (150 nucleotides), have been sequenced. The U11 and U12 snRNPs have been characterized further by examining their nuclease sensitivity and their possible interactions with other snRNPs. Potential roles for the low-abundance snRNPs in aspects of pre-mRNA processing are discussed.**

In mammalian cells there exists a set of highly abundant small nuclear ribonucleoprotein particles (snRNPs) containing U1, U2, U4, U5, and U6 RNAs (reviewed in ref. 1). Each of these particles is present in the nucleoplasm at 10^5–10^6 copies per cell and contains at least one small nuclear RNA (snRNA) and a set of specific proteins. The snRNAs are very stable, and all except U6 have at their 5′ ends a unique 2,2,7-trimethylguanosine (m_3G)-cap structure (see Fig. 2). Anti-Sm antibodies from patients with autoimmune disorders recognize a common epitope present in one or more proteins of the U1, U2, U4/U6, and U5 snRNPs (2). Therefore, snRNPs of the Sm family are defined by their ability to be immunoprecipitated with both anti-Sm antibodies and antibodies directed against the m_3G-cap structure.

The Sm snRNPs whose functions are known have been assigned roles in RNA processing. The highly abundant U1, U2, U4/U6, and U5 particles are essential components of the pre-mRNA splicing machinery (reviewed in refs. 3 and 4). The U7 particle, the first low-abundance Sm snRNP to be identified, is required for correct 3′ end formation of histone pre-mRNAs (reviewed in ref. 5). In addition, Sm snRNPs have been implicated but not yet demonstrated to be involved in polyadenylylation (reviewed in ref. 6).

Sm snRNPs precipitable by anti-m_3G and anti-Sm antibodies are also present in fungi (7–9), plant (10–12), and parasitic (13) organisms but in much lower abundance than their mammalian counterparts. Furthermore, the number of distinct snRNA species identified in yeast and plant cells [about 20–30 (7, 9)] is much greater than in HeLa cells. This larger number may represent the actual complexity of snRNAs in all eukaryotic cell nuclei. Indeed, several low-abundance HeLa snRNAs, U7–U11, have already been identified (14–18).

Here, we have used two-dimensional gel fractionation techniques to assess the true complexity of HeLa cell Sm snRNPs. We have uncovered the existence of a number of additional low-abundance particles. We present the sequences of the RNA components of two of these and the partial characterization of the snRNPs in which they reside.*

MATERIALS AND METHODS

Preparation of Labeled RNA. HeLa whole-cell extracts were immunoprecipitated (19) with monoclonal anti-Sm antibodies (Y12) (20). The recovered RNAs were 3′-end-labeled with ^{32}pCp and T4 RNA ligase (Pharmacia) (21) followed by immunoprecipitation with anti-m_3G rabbit antiserum (22). Alternatively, to prepare uniformly labeled RNA, HeLa cells were labeled with [^{32}P]orthophosphate (23) and immunoprecipitated as above with anti-Sm antibodies only.

Two-Dimensional Gels. Two-dimensional gels were run as described by Tollervey (9).

RNA Sequencing. 3′-End-labeled RNAs eluted from two-dimensional gels were further purified on a 15% sequencing gel. Digests using the base-specific ribonucleases T1, U2, Phy M, *Bacillus cereus*, and CL3 (Pharmacia) (24, 25) were fractionated on a 20% sequencing gel. Purified 3′-end-labeled RNA was also analyzed by wandering spot analysis (26), and the 3′-terminal nucleotide was determined by TLC of a complete ribonuclease T2 digest (26).

Primer Extension and cDNA Sequencing. Deoxyoligonucleotide primers used for reverse transcription of U11 or U12 included 10b (complementary to nucleotides 110–129 of U11), 10e (complementary to nucleotides 62–81 of U11), 10P-T (T_{15}AAGGGCGCCG, complementary to nucleotides 122–131 of U11), 9c (complementary to nucleotides 53–71 of U12), and 9P-2 (complementary to nucleotides 136–150 of U12). Templates were either RNA prepared by anti-Sm immunoprecipitation from an unlabeled whole-cell sonicate as above (for oligonucleotides 10b, 10e, 10P-2, and 9c) or immunoprecipitated RNA tailed by *Escherichia coli* poly(A) polymerase (Pharmacia) (27) (for 10P-T). Prior to the extension reactions, 5′-^{32}P-labeled oligonucleotide primers were added in ≈10-fold excess over U11 or U12 RNA and hybridized in 40 mM Hepes, pH 7.6/5 mM boric acid/0.1 M KCl at 80°C for 10 min and 42°C for 15 min. Extension was initiated by adding (final concentrations) 120 mM Tris·HCl, pH 8.4, 9 mM $MgCl_2$, 18 mM 2-mercaptoethanol, 18 μg of bovine serum albumin per ml, 350 μM (each) dNTPs, and 1.5 units of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) and incubating at 42°C for 45 min. Primer extension was terminated by addition of EDTA to 100 mM, followed by extraction with PCA [50:50:1 (vol/vol) phenol/chloroform/isoamyl alcohol] and precipitation with

Abbreviations: snRNA, small nuclear ribonucleic acid; snRNP, small nuclear ribonucleoprotein; m_3G, trimethyl guanosine.

*The sequences reported in this paper are being deposited in the EMBL/GenBank data base (accession no. J04118 for U11 and J04119 for U12 RNAs).

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ethanol. Sequencing ladders were generated by including dideoxynucleotides in the extension reactions at a final concentration of 53 μM. Primer-extension products were analyzed on 8% polyacrylamide sequencing gels. Alternatively, full-length primer-extended products were eluted from gels and sequenced by chemical cleavage (28).

RNase Digestion. RNase H digestion of U11 and U12 was carried out in nuclear extracts as described (29). Micrococcal nuclease digestion of U11 and U12 was examined in nuclear extracts or whole-cell extracts as described (22) with the following modifications: micrococcal nuclease (Cooper Biomedical, Malvern, PA) concentrations were 50, 100, 250, 500, 1000, 5000, and 10,000 units/ml; and 50 μg of carrier RNA was added per 40 μl of reaction mixture. RNAs were analyzed by blot hybridization (Northern blots).

Glycerol Gradients. Glycerol gradients (10–30%) were run by the protocol of D. L. Black and J.A.S. (unpublished results). Nuclear extracts were preincubated in 2.2 μM $MgCl_2$/0.5 mM ATP/20 mM creatine phosphate/1 unit of RNase inhibitor (Boehringer Mannheim) at 30°C for 60 min and then were loaded on a 12-ml 10–30% glycerol gradient containing 100 mM KCl, 1 mM $MgCl_2$, and 20 mM Hepes (pH 7.9), followed by centrifugation at 198,000 × *g* for 13 hr at 4°C. The gradients were fractionated into ≈30 samples, extracted with PCA, and precipitated with ethanol. RNAs were analyzed on Northern blots.

Northern Blot Analyses. RNA samples were fractionated on 10% polyacrylamide/7 M urea gels and were transferred by electroblotting to GeneScreen*Plus* membrane (duPont) according to the manufacturer's protocol. To detect U11 and U12, blots were probed with a mixture of 5′-^{32}P-labeled deoxyoligonucleotides (Table 1): 10z, 10g, 10e, and 10b for U11, and 9b, 9e, 9g, and 9l for U12. U1, U2, U4, U5, and U6 RNAs were probed with either SP6 polymerase antisense transcripts (D. L. Black and J.A.S., unpublished data) or with oligonucleotides supplied by D. Black [U1a (29), U2-L15 (29), U4-C18 (30), U5-baseS (30), and U6-18a (31)] as described by the GeneScreen*Plus* manufacturer.

RESULTS

Two-Dimensional Gel Fractionation Reveals Many Additional HeLa Sm RNAs. snRNAs were isolated from HeLa cells by serial immunoprecipitation with anti-Sm and anti-m_3G antibodies. Specifically, after selecting RNPs possessing Sm epitopes from a crude extract, RNAs were extracted and labeled at their 3′ ends with ^{32}pCp. Since RNA ligase utilizes only RNAs with 3′ OH groups as acceptors (21), degraded RNAs that possess 3′ phosphate termini created by nucleases were not labeled. Subsequent selection of RNAs with anti-m_3G antibodies likewise discriminated in favor of full-length rather than degraded RNAs.

Table 1. Oligonucleotide sequences and effect on RNase H digestion

	Oligonucleotide			Digestion	
RNA	Name	Length, nt	Complementary to bases	RNA	Particle
U11	10a	12	120–131	−	−
	10b	20	110–129	++	−
	10c	17	30–46	−	−
	10d	17	11–27	+++	+/−
	10e	20	62–81	+++	−
	10f	24	16–39	+++	−
	10g	20	38–57	+	−
	10l	20	52–71	+++	−
	10p	26	93–118	+	−
	10z	15	2–16	+	−
U12	9b	22	2–23	+++	+++
	9c	19	53–71	+++	−
	9d	21	16–36	+++	−
	9e	22	37–58	+++	−
	9f	23	83–105	+++	−
	9g	23	109–131	++	−
	9p	26	122–147	+++	−

+++, All cut; ++, most cut; +, some cut; +/−, very small amount of cut product; −, no cut product; nt, nucleotides.

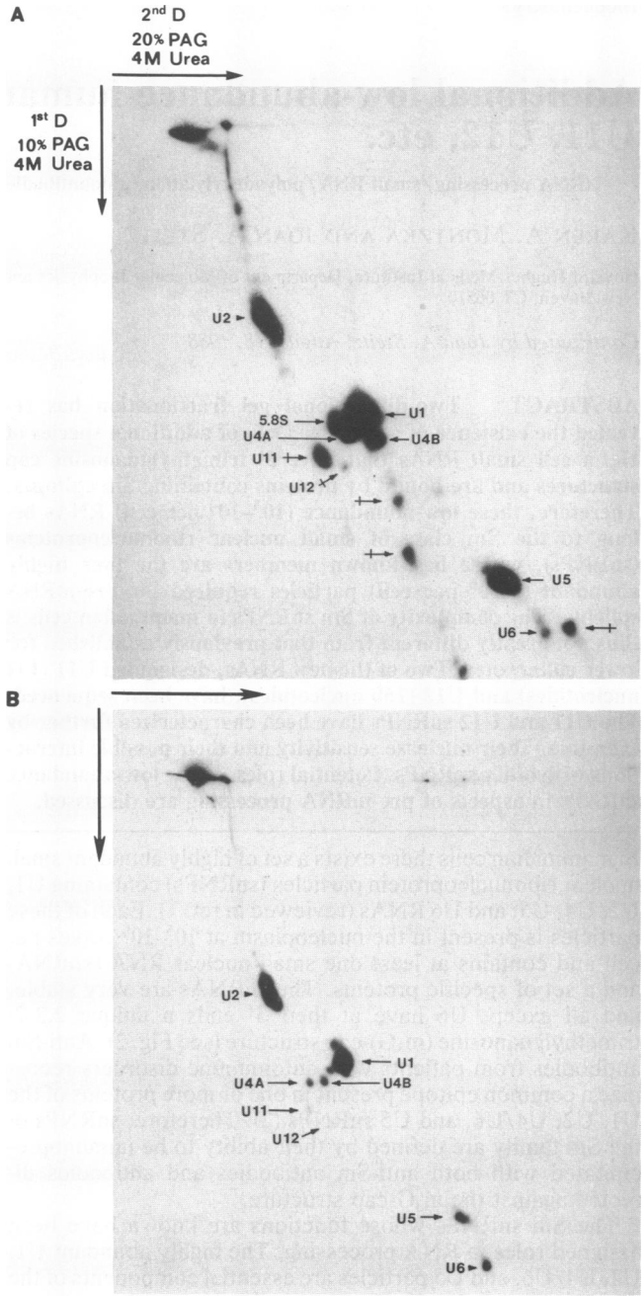

FIG. 1. Two-dimensional gel electrophoresis of Sm RNAs from HeLa Cells. (*A*) 3′-End-labeled RNA. (*B*) Uniformly labeled RNA. The highly abundant U1–U6 snRNA species are designated (note that U6 RNA does not contain a m_3G cap and therefore is underrepresented in *A*) as well as the U11 and U12 snRNAs. Although U11 migrates as two spots, no differences were detected by sequence analysis. Three additional RNA species (indicated in *A* by crossed arrows) were examined by partial sequence analysis.

Fig. 1*A* shows a typical pattern of 3′-end-labeled HeLa cell Sm RNAs fractionated by two-dimensional gel electropho-

resis under partially denaturing conditions (4 M urea). In addition to the highly abundant U1, U2, U4, and U5 snRNAs, many lower abundance RNAs are apparent. Several of these were eluted and further purified by one-dimensional electrophoresis under fully denaturing conditions (7 M urea). To determine whether they were novel species rather than breakdown products of the abundant snRNAs or contaminating rRNAs, the 3′-end-labeled RNAs were analyzed by partial digestion with a set of base-specific ribonucleases. Comparison of the 3′-end sequences generated (≈30 nucleotides in each case) with known snRNA and rRNA sequences revealed that at least five of the low-abundance RNAs (indicated by crossed arrows in Fig. 1*A*) are indeed previously undescribed RNAs. One of these is the U11 RNA initially defined on the basis of partial nuclease digestion by A. Kramer (15); another we shall designate U12.

Since labeling with ^{32}pCp can seriously misrepresent the relative abundances of RNAs, we quantitated U11 and U12 RNAs by labeling HeLa cells *in vivo* with [^{32}P]orthophosphate. The uniformly labeled snRNAs were isolated by anti-Sm precipitation and then fractionated by two-dimensional gel electrophoresis as above (Fig. 1*B*). The abundances of U11 and U12 were compared with that of U1 RNA by Cherenkov counting of gel slices. U11 was estimated to be 1/100th as abundant as U1 (≈10^4 copies per cell); and U12, about 1/500th as abundant as U1 (≈2×10^3 copies per cell).

Sequence Analysis of U11 and U12 snRNAs. To obtain full-length sequences for U11 and U12 RNAs, 5′-end-labeled deoxyoligonucleotides complementary to their 3′ ends were used as primers for reverse transcription. Primer extension analyses in the presence of dideoxynucleotides and chemical cleavage of the full-length cDNAs (28) allowed elucidation of complete sequences. In addition, sequences near the 3′ ends were confirmed by wandering spot analysis, while 3′ terminal nucleotides were determined by TLC of digests of the ^{32}pCp-labeled RNAs (26). Computer modeling was used to predict secondary structures for U11 and U12. The sequences of U11 and U12 depicted in possible secondary structures are shown in Fig. 2.

Susceptibility of U11 and U12 snRNPs to Nuclease Degradation. Since snRNPs often use their RNA moieties to engage in base-pairing with other RNAs, we asked what regions of the U11 and U12 snRNPs might be available for such interactions. Deoxyoligonucleotides complementary to various regions of the U11 and U12 RNAs (Table 1) were synthesized and incubated with HeLa cell nuclear extract or with RNAs isolated from a nuclear extract in the presence of RNase H. The extent of cleavage of U11 and U12 was then analyzed on Northern blots. Although 11 different oligonucleotides complementary to the whole of the U11 sequence were tested, no significant degradation of the U11 particle was detected. By contrast, complete degradation of U12 in its snRNP was observed by using one oligonucleotide (9b) that is complementary to bases 2–23 (Fig. 3*A*, lane 2). Six other oligonucleotides were unable to target cleavage of the U12 snRNP. However, note from Table 1 that many oligonucleotides promoted degradation of deproteinized U11 or U12.

The RNAs in the U11 and U12 particles were also tested for their sensitivity to micrococcal nuclease. Nuclear extract or cell sonicate was incubated with increasing concentrations of the enzyme, and, after addition of EGTA, any remaining RNA was extracted and analyzed on Northern blots. Because of the large quantity of rRNA present in cell sonicates, the amount of nuclease previously reported to be required for significant digestion of the abundant snRNPs (22) was much higher than we observed for nuclear extract. Therefore, in the experiment in Fig. 3*B*, additional carrier RNA was added to the nuclear extract prior to digestion, making the concentration of nuclease required for degradation the same as in cell

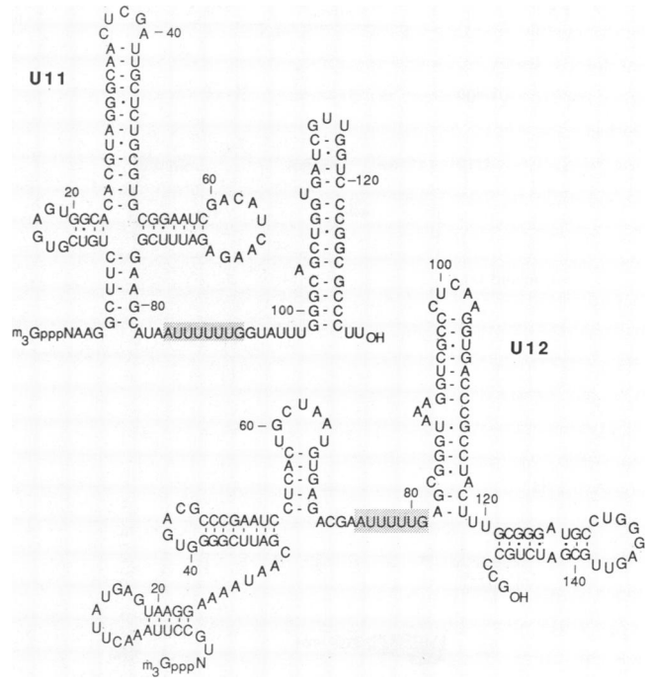

FIG. 2. Sequences of human U11 and U12 snRNAs. U11 (131 nucleotides) and U12 (150 nucleotides) RNAs are shown in possible secondary structures generated by using the Fold Program of the University of Wisconsin Genetics Computer Group. The putative Sm binding sites (32) are shaded.

sonicate (not shown). We see that moderate levels of micrococcal nuclease produced a U11 snRNA shortened by a few nucleotides ("U11s"; Fig. 3*B*, lanes 2–4), which then remained stable to much higher levels of the enzyme (Fig. 3*B*, lanes 5–7). On the other hand, U12 snRNA was nearly completely degraded by low levels of micrococcal nuclease (Fig. 3*B*, lane 2). Compared with the abundant Sm snRNPs, the shortened form of U11 (which could conceivably still be functional) was even more resistant than U5 [which had previously been shown to be the most resistant of the splicing snRNPs (22)], while U12 was slightly more susceptible to degradation than U2 [which is moderately sensitive (22)].

Higher Order Complexes of U11 and U12 snRNPs in Glycerol Gradients. The sedimentation behavior of the U11 and U12 snRNPs was examined to ask whether either of these particles might interact with other cellular components to form larger complexes. Nuclear extract was incubated in the presence of ATP under splicing conditions (29) and fractionated in a 10–30% glycerol gradient; the RNA isolated from each fraction was analyzed on a Northern blot. The positions of U11 and U12 RNAs are compared with those of U1, U2, U4, U5, and U6 RNAs in Fig. 4. Based on previous analyses (D. L. Black and J.A.S., unpublished results; K. Tyc and J.A.S., unpublished results), the peaks of the abundant snRNAs have been assigned as follows: U2 snRNP, fractions 14 and 20 (the predominant form varies with salt); U1 snRNP, fraction 20; U4/U6 snRNP, fraction 15; U5 snRNP, fraction 12; U6 particles, fractions 19 and 22; and U4/U5/U6 snRNP complex, fraction 7.

Both U11 and U12 seem to exist in at least two populations of particles. The major peak of U11 is in fraction 22, with a second peak migrating quite rapidly around fraction 12. The U12 particle has a much broader profile, with one peak in fraction 19 and another also centered around fraction 12. Whereas the slower migrating peak in each case is consistent with a monoparticle, it is noteworthy that the heavier forms of U11 and U12 appear to cosediment with each other and with the major peak of U5 RNA.

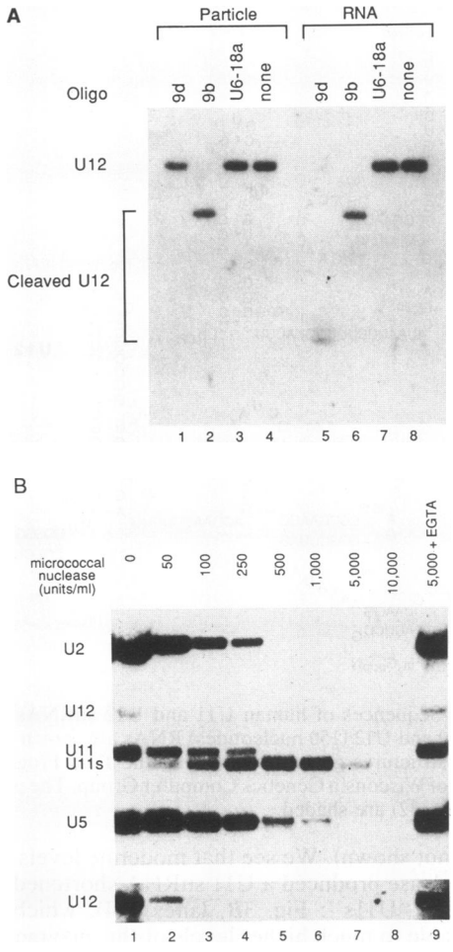

FIG. 3. Nuclease degradation of U11 and U12 snRNPs. (*A*) Oligonucleotide-directed RNase H degradation of the U12 snRNP. Nuclear extract (lanes 1–4) or RNA isolated from nuclear extract (lanes 5–8) was incubated in a 25-μl reaction mixture containing 0.8 mM ATP, 20 mM creatine phosphate, 4.4 mM $MgCl_2$, 1 unit of RNase H, and 2 μg of oligonucleotide 9d (lanes 1 and 5), oligonucleotide 9b (lanes 2 and 6), oligonucleotide U6-18a (lanes 3 and 7), or no oligonucleotide (lanes 4 and 8). The Northern blot was probed for U12 RNA by using a mixture of 5′-end-labeled U12 oligonucleotides (9b, 9e, 9g, and 9l); hence, digested forms as well as full-length U12 RNA are visualized. (*B*) Micrococcal nuclease sensitivity of U11, U12, U2, and U5 snRNPs. Nuclear extract was incubated for 15 min at 30°C in 40-μl reaction mixtures containing 1 μM $CaCl_2$ and micrococcal nuclease at 0 (lane 1), 50 (lane 2), 100 (lane 3), 250 (lane 4), 500 (lane 5), 1,000 (lane 6), 5,000 (lanes 7 and 9), or 10,000 (lane 8) units/ml. Lane 9 also contained 4.5 μM EGTA. The membrane was probed with a mixture of U12 oligonucleotides (9b, 9e, 9g, and 9l), a mixture of U11 oligonucleotides (10z, 10g, 10b, and 10e), a U2 oligonucleotide (U2-L15), and a U5 oligonucleotide (U5-baseS). *B Lower* shows a darker exposure of the U12 region of the same Northern blot. "U11s" (lanes 2–7) is a digestion product of U11.

DISCUSSION

Fractionation of HeLa cell small RNAs by two-dimensional gel electrophoresis has revealed many species of low abundance (ranging from 1/100th to 1/1000th that of U1) that fit the criteria of Sm snRNAs: they bind proteins with Sm epitopes and possess a m_3G cap. U11 and U12 have been most thoroughly studied, but partial sequence data for three others (indicated by crossed-arrows in Fig. 1*A*) demonstrate that they are previously undescribed Sm RNAs. Human U7 RNA (65 nucleotides) (16) would not have been retained in our gels because of its small size; and none of the five additional RNAs appear to be the human equivalent of rat U8

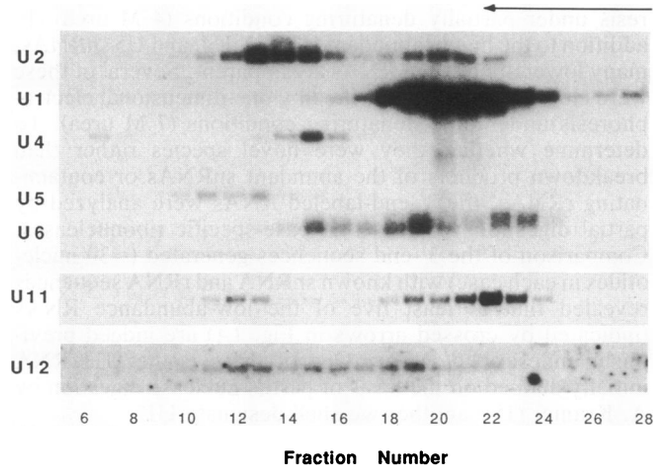

FIG. 4. Sedimentation of Sm snRNPs in glycerol gradients. Nuclear extract preincubated with ATP was run on a 10–30% glycerol gradient as described; 28 fractions (1 being the bottom fraction) were collected and analyzed by Northern blotting. (*Top*) The membrane was first probed for U1–U6 with SP6 transcripts. (*Middle*) After the signal had decayed significantly, the blot was reprobed for U11 by using a mixture of 5′-end-labeled U11 oligonucleotides (10z, 10g, 10e, and 10b). (*Bottom*) The membrane was then stripped by boiling in 0.15 M NaCl/0.015 M sodium citrate/1% sodium dodecyl sulfate and reprobed for U12 by using a mixture of 5′-end-labeled U12 oligonucleotides (9b, 9e, 9g, and 9l).

(14), the only other low-abundance mammalian Sm RNA whose sequence has been reported. Three additional spots are also candidates for Sm RNAs based on their migration patterns in two-dimensional gels. Therefore, at least eight low-abundance Sm RNAs are likely to exist in HeLa cell nuclei at $\approx 10^3$–10^4 copies per cell. These eight, plus the seven already sequenced RNAs [U1, U2, and U4–U8 (33)], bring the complexity of mammalian Sm RNAs closer to the numbers (20–30) reported for m_3G-capped RNAs in lower eukaryotes (7, 9). [Also recall that some RNAs that possess a m_3G cap are not bound by Sm proteins (e.g., U3) (33).] It is possible that further HeLa Sm RNAs are present at $<10^3$ copies per cell, but such species would be even more overshadowed by the highly abundant Sm RNAs and low levels of rRNA breakdown products.

The discovery of a large number of additional low-abundance mammalian Sm RNAs poses the question of their functions. The highly abundant Sm snRNPs and the low-abundance U7 snRNP are all involved in pre-mRNA processing reactions occurring in the nucleus (reviewed in ref. 1). We presume, but cannot be certain, that the low-abundance particles are likewise located in the nucleoplasm based on their efficient recovery from nuclear extracts compared to whole-cell extracts (unpublished data). Therefore, it seems likely that they also will be involved in some aspect of pre-mRNA processing; possibilities include events to which snRNPs have not yet been assigned, such as polyadenylylation, regulated splicing, and trans splicing (the last being purely hypothetical in mammalian cells). Since the highly abundant U1, U2, U4/U6, and U5 snRNPs are necessary for splicing of all substrates tested, they are considered to be part of the general splicing machinery. By contrast, the U7 snRNP participates in processing only histone messages, which represent about 1/100th of the total mammalian mRNA population; U7's low abundance ($\approx$1/500th of U1 abundance) (16–18) is therefore reasonable when one assumes that an average pre-mRNA contains about 10 introns. Similarly, processing events such as tissue-specific splicing or trans splicing might be expected to affect only a small percentage of mRNAs, suggesting that any snRNP involved might be

quite low in abundance. Since most mammalian mRNAs acquire poly(A) tails, a polyadenylylation snRNP should probably be more abundant than such putative substrate-specific snRNPs (but less numerous than the splicing snRNPs).

Our ability to target the U12 snRNP for degradation with RNase H provides an invaluable tool for assigning its function. Nuclear extracts lacking U12 have already been examined for their activity in splicing the first intron of the adenovirus major late transcript: no change in the time course or pattern of product formation was observed (unpublished data). Therefore, the U12 snRNP is probably not a component of the general splicing machinery but could be required for some substrate-specific splicing event. A requirement for U12 in polyadenylylation remains to be tested.

The resistance of the U11 snRNP to specific degradation with RNase H means that it will be much more difficult to prove its function using *in vitro* processing systems. However, fractionation of nuclear components necessary for polyadenylylation by three different methods has already revealed that: (*i*) the cleavage reaction is sensitive to both micrococcal nuclease and depletion with anti-Sm antibodies (34); (*ii*) U11 copurifies with a cleavage and polyadenylylation factor described by Christofori and Keller (35); and (*iii*) a fraction of U11 is present in a partially purified activity required for specific polyadenylylation and cleavage (36) (Y. Takagaki and J. L. Manley, personal communication). U11 is also the most abundant of the low-abundance Sm RNAs we have characterized, making it likely that it participates in a relatively prevalent nuclear process. It was previously proposed that a snRNP might interact with the conserved AAUAAA signal located upstream of the polyadenylylation cleavage site (37); no complementary sequence is found in the U11 RNA. Alternatively, crosslinking studies have indicated that a protein [of 64 (38, 39) or 155 kDa (38)] binds to the AAUAAA signal. Similar protein recognition has been suggested for the 3′ splice site, involving a 70- (40) or 100-kDa (41) polypeptide that appears to be loosely associated with the U5 snRNP. Therefore, tantalizing parallels can be drawn in that the AAUAAA recognition protein is also reported to be associated with an Sm antigen (39) and the U11 particle mimics the U5 snRNP in its relative insensitivity to both RNase H (D. L. Black and J.A.S., unpublished results) and micrococcal nuclease (22) digestion. The latter property of the U11 snRNP might explain conflicting reports of the sensitivity of *in vitro* polyadenylylation systems to pretreatment with micrococcal nuclease (34, 37, 42).

As an alternative approach for assigning functions to low-abundance snRNPs, we have asked whether they might interact with snRNPs of known function. Glycerol gradients have proven useful for characterizing multi-snRNP assemblies such as the U4–U5–U6 complex (D. L. Black and J.A.S., unpublished results). It is intriguing that the heavier forms of U11 and U12 cosediment with U5 upon fractionation of a nuclear extract (see Fig. 4). Thus, we have looked for short stretches of complementarity between the U11, U12, and U5 sequences (in regions that might be single-stranded). Among those identified are 5 base pairs between U5 nucleotides 37–41 and U11 nucleotides 2–6, 7 base pairs between U5 nucleotides 68–74 and U12 nucleotides 133–139, and 7 base pairs between U11 nucleotides 32–38 and U12 nucleotides 134–140. Establishing whether such snRNP–snRNP interactions actually do occur and what their functional significance might be will require more detailed analyses.

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