Purification of the major UsnRNPs from broad bean nuclear extracts and characterization of their protein constituents

Zsófia Pálfi, Montserrat Bach¹⁺, Ferenc Solymosy and Reinhard Lührmann¹⁺

Institute of Plant Physiology, Biological Research Center, Hungarian Academy of Sciences, Szeged, PO Box 521, 6701, Hungary and ¹Max-Planck-Institut für Molekulare Genetik, Otto-Warburg-Laboratorium, D-1000 Berlin 33, FRG

Received December 8, 1988; Revised and Accepted January 17, 1989

ABSTRACT

Small nuclear ribonucleoprotein particles containing the five major nucleoplasmic snRNAs U1, U2, U4, U5 and U6 as well as two smaller sized snRNAs were purified from broad bean nuclear extracts by anti-m³G, monoclonal antibody, immunoaffinity chromatography. We have so far defined 13 polypeptides of approximate mol. wts. of 11 kd, 11.5 kd, 12.5 kd, 16 kd, 17 kd, 17.5 kd, 18.5 kd, 25 kd (double band), 30 kd, 31 kd, 35 kd, 36 kd and 54 kd. Upon fractionation of the UsnRNPs by anion exchange chromatography, essentially pure U5 snRNPs were obtained, containing the 11 kd, 11.5 kd, 12.5 kd, 16 kd, 17 kd, 17.5 kd, 35 kd and 36 kd polypeptides. These may therefore represent the common snRNP polypeptides and which may also be present in the other snRNPs. By immunoblotting studies, using anti-Sm sera and mouse monoclonal antibodies we show that the 35 kd and 36 kd proteins are immunologically related to the mammalian common B/B' proteins. The broad bean 16 kd and 17 kd proteins appear to share structural elements with the mammalian D protein. The three proteins of mol. wts. 11 kd, 11.5 kd and 12.5 kd probably represent the broad bean polypeptides E, F, and G. Cross-reactivity of proteins of mol. wts of 30 kd and 31 kd with Anti-(U1/U2)RNP antibodies suggests that they may represent the broad bean A and B'' polypeptides. The 54 kd protein and the 18.5 kd protein could be candidates for the U1 specific 70 k and C polypeptides. Our results demonstrate a strong similarity between the overall structure of broad bean and mammalian snRNPs.

INTRODUCTION

All eucaryotic cells contain a number of small nuclear RNAs termed UsnRNAs, which are characterized by the presence of an m³G cap at their 5'termini, and which are organized in ribonucleoprotein particles (reviewed in 1). The major snRNPs U1, U2, U5 and U4/U6 (snRNAs U4 and U6 are base-paired in the U4/U6 RNP particle; (2,3) participate in pre-mRNA splicing and are components of spliceosomes, both in mammalian and in yeast (4) in vitro splicing systems (for reviews see ref. 5, 6). One of the functions of the major snRNPs appears to be the recognition of an intron's signal structures such as the 5' and 3' splice sites and the branch point (reviewed in 7). For some snRNPs, such as U1, the specificity of the interaction derives at least in part from basepairing of the snRNAs with specific pre-mRNA sequences (8). Significantly, snRNP proteins are also required for the particles to exert their function during splicing (9-11). The protein composition of the individual snRNPs has been investigated so far best in

mammalian cells (reviewed in 12). Seven polypeptides of approximate mol. wts. 29 kd (B'), 28 kd (B), 16 kd (D), 15.5 kd (D'), 12 kd (E), 11 kd (F) and 9 kd (G) are found in each of the snRNPs U1, U2, U5 and U4/U6. In addition to the common proteins, U1 RNPs contain three unique polypeptides of mol.wts. of 70 kd, 34 kd (A) and 22 kd (C). U2 RNPs are characterized by the presence of a 33 kd and a 28.5 kd protein, denoted A' and B''. snRNP proteins of mol. wts. 100 kd (10) or 70 kd (11) are candidates for U5 specific polypeptides.

Sera from patients suffering from connective tissue diseases that contain antibodies against snRNP polypeptides (13, reviewed in 14) have considerably aided the elucidation of structure and function of UsnRNPs. Anti-RNP antibodies react selectively with the U1 RNP specific proteins 70 k, A and C, while anti-Sm antibodies are directed predominantly against the common proteins B', B and D. Two further antibody specificities have been described which react with polypeptide A' (15) or cross-react with proteins A and B'' (anti-(U1/U2) antibodies)(16).

In plants, much information has been accumulated during the last five years with respect to the occurrence and structure of the UsnRNA molecules. The major plant snRNAs U1, U2, U4 and U5 are practically of the same size as their metazoan counterparts, are also m³G capped and contain all those evolutionarily highly conserved primary and secondary structural elements (17-24) to which a role has been attributed during pre-mRNA splicing already in the case of mammalian (reviewed in 7) and yeast snRNAs (25,26). Little is known, however, concerning the protein content of plant UsnRNPs, except that they are also precipitable by anti-Sm containing antisera, which indicates evolutionary conservation of some Sm antigenic regions between plant and mammalian snRNP polypeptides (27,28).

We report here the isolation and partial fractionation of individual snRNP particles U1, U2, U5 and U4/U6 from broad bean nuclear extracts by anti-m³G immunoaffinity chromatography. We have identified the polypeptides associated with the snRNAs. Furthermore, by the use of human and mouse anti-snRNP autoantibodies, we have defined those broad bean snRNP proteins which most likely represent counterparts of the mammalian snRNP polypeptides A, B, B', B'' and D.

MATERIALS AND METHODS

Plant material

Fully expanded leaves of 15 to 20-day-old broad bean (*Vicia faba* L.) plants, grown under ordinary greenhouse conditions at 25 °C and 16 h light period, were collected freshly and used as starting material for the isolation of nuclei.

Antibodies

Rabbit anti-m³G antibodies were obtained as described previously (29). The monoclonal anti-m³G antibody H-20 was isolated from hybridoma supernatants by chromatography on m⁷G-AH Sepharose columns (30). Monoclonal antibody H-57, which selectively reacts

with snRNP proteins B and B' was isolated from a mouse immunized with snRNPs U1 (Daser, A., Reuter, R. and Lührmann, R., unpublished). The anti-D specific mAb 7.13 was a generous gift of S. Hoch (La Jolla). Anti-Sm, anti-RNP and anti-(U1/U2) RNP autoimmune sera from patients suffering from mixed connective tissue disease or systemic lupus erythematosus were obtained from the Universities of Freiburg and Nijmegen through the kind help of H.H. Peter and W. v. Venrooij, respectively.

Isolation of nuclei from broad bean leaves and preparation of nuclear extracts

Isolation of nuclei and their purification by Percoll density gradient centrifugation were carried out according to Method II of Kiss et al. (18), except that the glycerol concentration in the isolation buffer was reduced to 30 % (v/v).

Purified nuclei were resuspended at a density of 1×10^8 nuclei/ml in a buffer containing 40 mM Tris-HCl, pH 8.0, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTE, 0.5 mM PMSF and agitated gently on a magnetic stirrer (twice for 30 min each time). Nuclear extracts were centrifuged at $800 \times g$ for 5 min and the resulting supernatants (supernatant 1 and 2) were pooled and freed from high-molecular-weight nuclear structures by a further centrifugation at $10,000 \times g$ for 10 min in a Sorvall RC-5C centrifuge equipped with an HB-4 rotor. The high-speed supernatant was used directly for the immunoprecipitation assays and for the isolation of snRNPs on anti-m3G columns.

Affinity chromatography of UsnRNPs

The broad bean nuclear extracts were applied to an anti-m3G affinity column (bed volume 2 ml) carrying 10 mg mAb H-20 (30) covalently bound to CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's protocol.

For isolation of snRNPs from unlabelled plant material, 10 ml nuclear extract was passed over the column at a flow rate of 2 ml/h, which was followed by washing the column with 20 ml TMN 300 buffer (20 mM Tris-HCl, pH 8.0, 1.5 mM $MgCl_2$, 300 mM NH_4Cl , 0.2 mM EDTA, 1 mM DTE, 1 mM PMSF). The antibody-bound snRNPs were eluted with 3 ml TMN300 containing 12 mM m^7G . After a 10 ml wash with TMN300 the affinity column was regenerated by washing with 5 ml 6M urea/TMN300 and 20 ml TMN300 + 0.02 % (w/v) NaN_3 . Ten 1-ml fractions were collected. The snRNP-containing fractions (with a total yield of 60 μg protein) were pooled and kept frozen at $-80^\circ C$.

Fractionation of the m^7G -eluate by anion exchange chromatography

A 1 ml Mono Q column connected to a Pharmacia FPLC (Fast Protein Liquid Chromatography) system was equilibrated with buffer A (20 mM Tris-HCl, pH 7, 15 mM $MgCl_2$, 0.5 mM PMSF, 0.5 mM DTE) containing 50 mM KCl. The m^7G -eluate (5 ml, 20 μg of protein/ml) of the anti-m3G column was diluted 1:1 with buffer A and loaded onto the Mono Q column at 2ml/min. Then the column was washed with 4 bed volumes of buffer A + 50 mM KCl (2 ml/min). Elution of bound components from the anion-exchanger was achieved with a linear salt gradient of 50-1000 mM KCl in buffer A at a flow rate of 1 ml/min. Four-ml fractions were collected during loading and washing and

1-ml fractions during elution. Absorbance at 280 nm was measured. Protein-containing fractions eluted in distinct peaks were pooled separately and analyzed.

Immunoprecipitation assays

Precipitation of antigen-antibody complexes was performed following the procedures described by Matter et al. (31) and Habets et al. (16). RNA, purified from immunoprecipitates, was 3'-end labelled by incubation with 10 μ Ci of [5'-³²P]pCp (3000 Ci/mmol, Amersham) overnight at 4 °C according to the procedure of England and Uhlenbeck (32).

RNA and protein analysis

After PCA extraction of the samples, the RNAs were precipitated from the aqueous phase with 2.5 volumes of ethanol, and the proteins were recovered from the organic phase by precipitation with 5 volumes of acetone.

Fractionation of RNAs was performed on 10 % polyacrylamide gels containing 7 M urea, 100 mM Tris-borate, pH 8.3 and 2 mM EDTA. RNA bands were stained with silver as described by Merrill et al. (33).

Proteins were pelleted by centrifugation (15 min at 16,000 g), washed twice with 80 % ethanol and dissolved in SDS-sample buffer [50 mM Tris-HCl, pH 6.8, 2 % SDS, 5 % 2-mercaptoethanol, 0.005 % bromphenol blue, 10 % (v/v) glycerol]. Analysis of proteins was carried out on 15 % polyacrylamide gels (34). Protein bands were visualized by staining with Coomassie brilliant blue R250 which was followed by the more sensitive silver staining. The protein concentration of the samples was determined by the method of Bearden (35) using BSA as a standard.

Immunoblotting

Immunologically reactive proteins of anti-m3G purified plant snRNPs were identified by the immunoblotting procedure of Towbin et al. (36). snRNP proteins were dissolved in SDS gel sample buffer and loaded on preparative 15 % polyacrylamide gels (10 μ g protein/cm of sample well) along with marker proteins. After electrophoresis, the proteins were transferred onto nitrocellulose in a Bio-Rad transblot cell at 60 V/0.3 A for 1 h in 25 mM Tris/192 mM glycine buffer (pH 8.3) containing 20 % methanol and 0.1 % SDS. The blots were cut into longitudinal strips of about 5 mm. The strip bearing marker proteins was directly stained with Ponceau S. Visualization of antigenic proteins on the other filter strips was achieved essentially as described by Zeller et al. (37).

RESULTS

Characterization of broad bean nuclear extracts with respect to the presence of UsnRNAs and UsnRNPs

Nuclear extracts prepared from broad bean leaf cells were pooled and the combined extract was divided into five aliquots. These were precipitated with rabbit non-immune serum, rabbit anti-m3G antibodies, human non-immune serum, human anti-Sm antiserum and human anti-(U1)RNP antiserum, respectively. From all these precipitates

Fig 1

Polyacrylamide gel fractionation of UsnRNAs immunoprecipitated from broad bean nuclear extracts.

Immunoprecipitation assays were performed as described in Materials and Methods. RNAs were recovered from the precipitates by phenol extraction, 3' end-labelled and separated on a 10 % polyacrylamide gel containing 7M urea. The autoradiogram shows the RNAs present in immunoprecipitates prepared with: IgGs from a rabbit pre-immune serum (lane 2), rabbit anti-m3G IgGs (lane 3), IgGs from a normal human serum (lane 4), human anti-Sm/(U1)RNP IgGs (lane 5), and anti-(U1)RNP IgGs (lane 6). HeLa UsnRNAs are marked on the left, and broad bean UsnRNAs on the right side of the figure. Lane 1 shows anti-m3G affinity-purified HeLa UsnRNAs.

RNAs were phenol-extracted, 3'end-labelled and subjected to polyacrylamide gel electrophoresis. As a standard, 3'end-labelled HeLa snRNAs were used.

A comparison of the gel electrophoretic mobilities of the antibody-precipitated plant snRNAs and HeLa snRNAs indicates that anti-m3G and anti-Sm antibodies precipitated broad bean snRNAs U1, U2 and U5 (Fig. 1, lanes 3 and 5), whereas anti-RNP antibodies reacted only with U1RNPs (Fig. 1, lane 6). Since both rabbit and human non-immune sera precipitated one or two plant RNA species (Fig. 1, lanes 2 and 4) - the smaller one being present in all other immunoprecipitates as well -, these were not further analyzed. Since the mobility of broad bean U4 RNA in the polyacrylamide gel electrophoretic system used is very close to that of broad bean U1 RNA (22), it seems likely that broad

Fig.2

Polyacrylamide gel fractionation of UsnRNAs extracted from broad bean UsnRNPs purified by chromatography with anti-m³G antibodies.

Affinity chromatography was carried out as described in Materials and Methods, using a 2 ml column of monoclonal anti-m³G IgG (H-20) covalently bound to CNBr-Sepharose. Following phenol extraction of the crude nuclear extract (lane 1), the flow-through fraction (lane 2) and the m⁷G-eluate (lane 3), the RNAs were precipitated from the aqueous phase, separated on a 10 % polyacrylamide-7M urea gel and stained with silver. The positions of the major RNAs are marked. The asterisks indicate two minor m³G-capped RNA species.

bean U4 RNA was also present in the immunoprecipitates obtained with both anti-m³G and anti-Sm antibodies. This assumption is supported by the fact that in the gel electrophoretograms the "U1 region" shows a more complex pattern in the anti-m³G (Fig. 1, lane 3) and anti-Sm (Fig. 1, lane 5) precipitates, expected to contain both U1 and U4 RNAs, than in the anti-(U1)RNP (Fig. 1, lane 6) precipitate, expected to contain only U1 RNA. The lack of detection of broad bean U6 RNA in immunoprecipitates obtained with anti-m³G (Fig. 1, lane 3) and anti-Sm (Fig. 1, lane 5) antibodies is most probably due to the very low efficiency of 3'-end-labelling of this particular plant UsnRNA species (19).

The major conclusion from the above experiments is that (i) broad bean nuclear extracts

are an appropriate starting material for studying the structure of plant U-snRNPs and that (ii) in accordance with the results of Tollervey and Mattaj (28) plant and mammalian UsnRNPs have at least some evolutionarily highly conserved epitopes in common that are recognized by human antisera.

Purification of broad bean UsnRNPs

To analyze the polypeptide composition of broad bean UsnRNPs, nuclear extracts were subjected to anti-m³G affinity chromatography. The snRNPs U1, U2, U4, U5 and U6 were preferentially enriched in the m⁷G-eluate of the affinity column as compared to the crude nuclear extract (Fig. 2). Some additional bands, one below that containing U5 RNA and two others of lower mol.wt. were also visible in the m⁷G eluate (Fig. 2, lane 3). These RNAs have not been characterized further. It is important to note that the strong RNA band in the 5S RNA region in the affinity-purified sample contains 5S RNA-sized m³G-capped RNA molecules, as demonstrated by our finding that after extraction from this region of the gel the RNAs could be quantitatively precipitated by anti-m³G IgG (not shown).

Polypeptides from purified snRNPs were displayed by SDS-polyacrylamide gel electrophoresis and successive Coomassie blue and silver staining (Fig. 3). At least 13 polypeptides of approximate mol. wts. of 11 kd, 11.5 kd, 12.5 kd, 16 kd, 17 kd, 17.5 kd, 18.5 kd, 25 kd (double band), 30 kd, 31 kd, 35 kd, 36 kd and 54 kd were reproducibly found in the broad bean snRNP preparations. The abundance of a protein of mol. wt. of 14.4 kd varied considerably from one snRNP preparation to another and thus may be associated with snRNPs by nonspecific interactions. The dark bands marked by arrow heads in lane 3 of Fig. 3B only become visible by the silver stain. Since these bands were RNase sensitive (not shown) they most probably represent contaminating free RNA molecules or SDS-resistant snRNA protein complexes. Such SDS-resistant RNP complexes have been noticed previously in mammalian snRNPs (38).

Fractionation of broad bean snRNPs

An attempt was made to partially separate individual snRNPs by ion exchange chromatography on a Mono Q column under FPLC conditions (Fig. 4). A considerable amount of pure U5 snRNP was eluted from the Mono Q column at about 0.4 M KCl (fractions 42-45) as a single peak, whereas the remaining snRNPs (U1, U2 and U4/U6) exhibited a quite heterogeneous distribution in the fractions eluted at salt concentrations above 0.45 M (fractions 50-60). U5 snRNP behaved in a particular way in that it reappeared at a higher KCl concentration (0.5 M) during fractionation (Fig. 4A and B). In most of the fractions the protein concentration was too low to allow visualization of individual protein bands in SDS polyacrylamide gels, with the exception of the U5 RNP containing fractions 42 to 45. Figure 4C shows that broad bean U5 RNPs contain at least eight prominent polypeptides of approximate mol. wts. 11 kd, 11.5 kd, 12.5 kd, 16 kd, 17 kd, 17.5 kd, 35 kd and 36 kd.

Fig. 3

Polyacrylamide gel fractionation of polypeptides from purified broad bean UsnRNPs. Following phenol extraction of the crude nuclear extract (lanes 1), the flow-through fraction (lanes 2) and the m^7G eluate (lanes 3) the proteins were recovered from the organic phase by precipitation with 5 volumes of acetone, separated on a 15 % polyacrylamide-0.1 % SDS gel and visualized by staining with Coomassie brilliant blue alone (Panel A) or by successive staining with Coomassie brilliant blue and silver (Panel B). Lane M shows marker proteins. Note the presence of additional sharp RNA bands in the double-stained gel (Panel B marked by arrowheads) compared to the Coomassie-stained gel (Panel A).

Identification of broad bean snRNP proteins reacting with human and mouse anti-snRNPAutoantibodies

The antigenic activity of the proteins from purified broad bean snRNPs was investigated by the protein blotting technique first using anti-Sm sera from patients with systemic lupus erythematosus (Fig. 5). Sera 2 to 7 which all contained antibodies against proteins B, B' and D on HeLa snRNP blots, though to varying extent, consistently showed reaction with plant proteins of mol. wts. 35 kd, 36 kd, and somewhat weaker with the lower band of the 25 kd double band as well as with one or two proteins in the 16 to 17 kd region, i.e. with D-sized proteins (Fig. 6). Serum 7, which contained high-titered antibodies against HeLa snRNP proteins C and A in addition to anti-Sm antibodies, also stained two proteins of mol. wts. 18.5 kd and 31 kd (Fig. 5, lane 7). The latter protein also reacts with two anti-(U1/U2)RNP sera (Fig 5, lanes 8 and 9), suggesting that the 31

Fig. 4

Separation by Mono Q anion exchange chromatography of broad bean UsnRNPs purified on anti-m3G immunoaffinity column and analysis of the protein-containing fractions.

The m⁷G-eluate of the anti-m3G column was applied onto Mono Q and the UsnRNPs were eluted with a linear salt gradient (50 to 1000 mM KCl). In Panel A only the protein-containing fractions are shown. RNAs were then extracted from fractions eluted in distinct peaks, run separately on denaturing 10 % polyacrylamide gel and stained with silver (Panel B). UsnRNAs present in anti-m3G-purified HeLa nuclear extracts (lane 1), as well as in Mono Q-fractions 42 to 45 (lane 2), 50 to 53 (lane 3), 55 to 58 (lane 4) and 59+60 (lane 5) of anti-m3G-purified broad bean nuclear extracts. HeLa UsnRNAs are marked on the left, and broad bean UsnRNAs on the right side of the figure. The pooled fraction 42 to 45 which contained only U5 RNA (lane 2 in Panel B) was further analyzed for protein composition on a 15 % SDS-polyacrylamide gel after staining with Coomassie brilliant blue (Panel C). Lane 1, proteins of anti-m3G-purified HeLa UsnRNPs; lane 2, proteins of broad bean U5 snRNP particles present in fractions 42 to 45; lane 3, the same as lane 2 after additional staining with silver; lane M, marker proteins of 66kd, 45kd, 36kd, 29kd, 24kd, 20kd and 14kd.

Fig. 5

Immunoblot analysis of broad bean snRNP proteins using human autoimmune sera specific for Sm, U1RNP or U1/U2 RNP antigens.

Proteins extracted from anti-m³G purified snRNPs of broad bean cells were fractionated by electrophoresis on 15 % SDS-polyacrylamide gels, electroblotted onto nitrocellulose sheets and immunostained with patient' antisera (diluted 1:25). Immunoreactive proteins were detected with alkaline phosphatase-conjugated anti-human IgGs (diluted 1:4000). Individual strips of nitrocellulose blots were probed with a normal human serum (lane 1), various anti-Sm/RNP sera (lanes 2 to 7) and anti-(U1/U2)RNP sera (lanes 8 and 9). Marker proteins are indicated on the right.

kd protein may represent the broad bean A polypeptide. Serum 8 stains a second protein of mol. wt. 30 kd in addition to the 31 kd polypeptide. Thus this may be an analogue of the mammalian B'' polypeptide. The lack of reactivity of the anti-(U1/U2) RNP serum 9 with the 30 kd polypeptide could simply be due to the lower antibody titer of this serum as compared with serum 8. Staining of broad bean snRNP polypeptides in the mol. wt. range 65 to 70 k by some of the sera appears to be nonspecific, since a control human serum which does not react with human snRNP polypeptides also reacts with these proteins on broad bean immunoblots Fig. 5, lane 1). It is important to note that we could not detect specific reactions of anti-70 k antibody containing patient sera, i.e. sera 3, 5, 6 and 7, with a particular broad bean snRNP polypeptide (Fig. 5).

The immunological relatedness between particular broad bean and human snRNP polypeptides was further clarified by the use of mAbs. Monoclonal antibody H-57 which reacts selectively with mammalian snRNP polypeptides B and B', strongly reacts with broad bean snRNP proteins of mol. wts. 35 kd and 36 kd and weakly reacts with the upper band of the 25 kd double band (Fig. 6A, lane 3). Interestingly, anti-B/B'

Fig. 6

Reactivity of broad bean UsnRNP proteins with mAbs raised against individual HeLa UsnRNP proteins. Polypeptides prepared from anti-m3G purified UsnRNPs of broad bean nuclei were separated on SDS-polyacrylamide gels, blotted onto nitrocellulose filters and probed with undiluted hybridoma supernatants containing antibodies of defined specificity or with unfractionated autoimmune sera. Panel A shows a broad bean U-snRNP protein immunoblot processed with mAbs developed against an animal ribosomal protein (lane 1), B/B' proteins of HeLa U-snRNPs (lane 2) and an additional filter strip treated with a patient's serum containing anti-B/B' antibodies. Panel B displays immunostaining of a broad bean U-snRNP protein blot with a non-immune human serum (lane 1), an anti-Sm/(U1)RNP autoimmune serum (lane 2), mAbs directed against an animal ribosomal protein (lane 3), and mAb 7.13 specific for HeLa UsnRNP protein D. Marker proteins are indicated.

containing anti-Sm sera stain the lower band of the 25 kd double band (see Fig. 5 and 6A, lane 2, for comparison). The monoclonal anti-D specific antibody 7.13 reacts selectively with a protein of mol. wt. 16 kd (Fig. 6B, lane 4). Staining of a protein of mol. wt. 26 kd appears to be nonspecific since this protein is also stained by a mAb directed against a protein not related to snRNP proteins (Fig. 6B, lane 3).

DISCUSSION

In this report, we describe the purification by anti-m3G immunoaffinity chromatography of the major snRNPs U1, U2, U5 and U4/U6 from broad bean nuclear extracts. At least 13 polypeptides of approximate mol. wts. 11 kd, 11.5 kd, 12.5 kd, 16 kd, 17 kd, 17.5 kd, 18.5 kd, 25 kd (double band), 30 kd, 31 kd, 35 kd, 36 kd and 54 kd have been found

consistently in our snRNP preparations. Of these, eight polypeptides with mol. wts. 11 kd, 11.5 kd, 12.5 kd, 16 kd, 17 kd, 17.5 kd, 35 kd and 36 kd were also constituents of purified U5 snRNPs. By analogy to the situation in mammalian snRNPs (see Introduction) it is reasonable to assume that the latter group constitutes the common proteins of broad bean snRNPs which are probably contained in every major nucleoplasmic snRNP particle. How does this set of proteins relate to the mammalian core proteins B', B, D, D', E, F and G. Our findings, that the 35 kd and 36 kd polypeptides strongly react with anti-B/B' containing anti-Sm sera and with mAb H-57, which is directed against human B/B' polypeptides, suggests that these polypeptides represent equivalents of the mammalian B/B' proteins. It is interesting to note then, that the plant B/B' polypeptides display a mol. wt. about 30 % higher as compared to their mammalian counterparts.

The proteins of mol. wt. 25 kd (double band) also share epitopes with the 35 kd and 36 kd proteins, since they weakly react with anti-Sm sera and with mAb H-57. More data are needed, however, to clarify whether they are variants of polypeptides B/B' which would be expressed simultaneously in broad bean leave cells. A precedent for the expression of variant B/B' polypeptides has recently been described in rat cells (39). It should be noted, however, that the 25 kd polypeptides were not detected in purified broad bean U5 RNPs which would be expected for a common polypeptide. This suggests either that they are only loosely associated with U5 RNPs and lost during anion exchange chromatography, or that they are found only in one of the other broad bean snRNPs.

There are three D-sized proteins present in purified U5 snRNPs (Fig. 4C). The 16 kd protein reacts with a monoclonal anti-D antibody as well as with anti-D containing sera 2, 5 and 6, demonstrating immunological relatedness with the mammalian D protein. The 17 kd protein appears to share anti-D epitopes since it is stained on the immunoblot by most of the anti-D antibody containing anti-Sm sera except serum 5 (Fig. 5). This could indicate that Sm epitopes which reside on a single D protein from mammalian snRNPs may be found on related but distinct D proteins in plant snRNPs. The third D-sized protein of mol. wt. 17.5 kd present in U5 RNPs, does not react with any of the autoimmune antisera, neither does it react with monoclonal anti-D antibody. This could be a counterpart of the snRNP protein D' of vertebrates (38), which likewise has not been found so far to react with anti-Sm antibodies (our unpublished observations). The presence of the three low mol. wt. proteins (12.5 kd, 11.5 kd and 11 kd) in broad bean U5 RNPs, their similarity in size with mammalian polypeptides E, F and G, as well as their lack of reactivity with anti-Sm antibodies suggest that they represent the plant analogues of protein E, F and G of mammalian snRNPs.

With respect to the U1- and U2- specific polypeptides we have immunological evidence for the presence of analogues of the A and B'' polypeptides in broad bean snRNPs (Fig. 5). An analogue of the metazoan U1 specific 70 k protein could not be detected by immunoblotting. However, the protein of approximate mol. wt. 54 kd which was found

in total snRNPs and which was lacking from purified U5 RNPs could be a candidate for this. A candidate for a C protein could be the 18.5 kd protein in total snRNPs, which again was lacking from U5 RNPs, and which on immunoblots, reacted with antiserum 7. This serum contained a high titer of anti-C antibodies.

Taken together, our results suggest that the overall structure of snRNPs from broad bean and mammalian cells is quite similar and it will be interesting to see whether isolated plant snRNPs may also functionally complement splicing extracts from human cells which have been depleted of their endogenous snRNPs.

ACKNOWLEDGEMENTS

We thank S.O. Hoch, H. H. Peter and W. v. Venrooij for the monoclonal anti-Sm antibody and sera from patients with rheumatoid diseases. We are grateful to P. Bringmann for valuable advice during the experimental work. Thanks are due to Mrs. M. Doktor and to V. Buckow for typing the manuscript. This work was supported by grants from the Deutsche Forschungsgemeinschaft (to R.L.) and OTKA 564/86 from the Hungarian Academy of Sciences (to F.S.). Z.P. was supported by a short-term fellowship from EMBO and M.B. by a fellowship from the Alexander-von-Humboldt-Stiftung.

*Present address: Institut für Molekularbiologie und Tumorforschung, Emil-Mannkopff-Straße 2, D-3550 Marburg, FRG

REFERENCES

- (1) Reddy, R. and Busch, H. (1988) In: Structure and function of major and minor small nuclear ribonucleoprotein particles, Birnstiel, M.L. (ed), Springer-Verlag Berlin, pp. 1-37.
- (2) Bringmann, P., Appel, B., Rinke, J., Reuter, R., Theissen, H. and Lüthmann, R. (1984) EMBO J. 3: 1357-1363.
- (3) Hashimoto, C. and Steitz, J.A. (1984) Nucleic Acids Res. 12: 3283-3293.
- (4) Brody, E. and Abelson, J. (1985) Science 228: 963-967.
- (5) Maniatis, T. and Reed, R. (1987) Nature 325: 673-678.
- (6) Sharp, P.A. (1987) Science 235: 766-771.
- (7) Steitz, J.A., Black, D.L., Gerke, V., Parker, K.A., Krämer, A., Frendewey, D. and Keller, W. (1988) In: Structure and function of major and minor small nuclear ribonucleoprotein particles, Birnstiel, M.L. (ed), Springer-Verlag Berlin, pp 115-154.
- (8) Zhuang, Y. and Weiner, A.W. (1986) Cell 46: 827-835.
- (9) Mount, M.S., Pettersson, I., Hinterberger, M., Karmas, A. and Steitz, J.A. (1983) Cell 33: 509-518.
- (10) Tazi, J., Alibert, C., Tamsamani, J., Reveillaud, J., Cathala, G., Brunel, C. and Jeanteur, Ph. (1986) Cell 47: 755-766.
- (11) Gerke, V. and Steitz, J.A. (1986) Cell 47: 973-984.
- (12) Lüthmann, R. (1988) In: Structure and function of major and minor small nuclear ribonucleoprotein particles, Birnstiel, M.L. (ed), Springer-Verlag Berlin, pp. 71-99.
- (13) Lerner, M.R. and Steitz, J.A. (1979) Natl. Acad. Sci. USA 76: 5495-5499.
- (14) Tan, E.M. (1982) Adv. Immunol. 33: 167-240.
- (15) Mimori, T., Hinterberger, M., Pettersson, I. and Steitz, J.A. (1984) J. Biol. Chem. 259: 560-565.
- (16) Habets, W., Hoet, M., Bringmann, P., Lüthmann, R. and van Venrooij, W. (1985) EMBO J 4: 1545-1550.

- (17) Krol, A., Ebel, J.-P., Rinke, J. and Lührmann, R. (1983). *Nucleic Acids Res.* 11: 8583-8594.
- (18) Kiss, T., Tóth, M. and Solymosy, F. (1985) *Eur. J. Biochem.* 152: 259-266.
- (19) Kiss, T., Antal, M. and Solymosy, F. (1987a) *Nucleic Acids Res.* 15: 543-560.
- (20) Kiss, T., Antal, M. and Solymosy, F., (1987b) *Nucleic Acids Res.* 15: 1332.
- (21) Kiss, T., Antal, M., Hegyi, H. and Solymosy, F. (1988a) *Nucleic Acids Res.* 16: 2734.
- (22) Kiss, T., Jakab, G., Antal, M., Pálfi, Z., Hegyi, H., Kis, M. and Solymosy, F. (1988b) *Nucleic Acids Res.* 16, 5407-5426..
- (23) van Santen, V.L. and Spritz, R.A. (1987) *Proc. Natl. Acad. Sci. USA* 84: 9094-9098.
- (24) Vankan, P. and Filipowicz, W. (1988) *EMBO J.* 7: 791-799.
- (25) Siliciano, P.G., Jones, M.H. and Guthrie C. (1987) *Science* 237: 1484-1487.
- (26) Parker, R., Siliciano, P.G. and Guthrie, C. (1987) *Cell* 49: 229-239.
- (27) Riedel, N., Wolin, S. and Guthrie, C. (1987) *Science* 235: 328-331.
- (28) Tollervey, D. and Mattaj, I.W. (1987) *EMBO J.* 6: 469-476.
- (29) Lührmann, R., Appel, B., Bringmann, P., Rinke, J., Reuter, R., Rothe, S., Bald, R. (1982) *Nucl. Acids Res.* 10: 7103-7113.
- (30) Bochnig, P., Reuter, R., Bringmann, P. and Lührmann, R. (1987) *Eur. J. Biochem.* 168: 461-467.
- (31) Matter, L., Schopfer, K., Wilhelm, J.A., Nyffenegger, T., Parisot, R.F. and De Robertis, E.M. (1982) *Arthritis Rheum.* 25: 1278-1283.
- (32) England, T.E. and Uhlenbeck, O.C. (1978) *Nature (London)* 275: 560-561.
- (33) Merril, C.R., Goldman, D., Sedman, S.A. and Ebert, M.H. (1981) *Science (Washington)* 211: 1437-1438.
- (34) Laemmli, U.K. (1970) *Nature (London)* 227: 680-685.
- (35) Bearden, J.C. (1978) *Biochim. Biophys. Acta* 533: 525-529.
- (36) Towbin, H., Staehelin, T. and Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* 76: 4350-4354.
- (37) Zeller, R., Nyffenegger, T. and De Robertis, E.M. (1983) *Cell* 32: 42.
- (38) Bringmann, P. and Lührmann, R. (1986) *EMBO J.* 5: 3509-3516.
- (39) McAllister, G., Amara, S.G. and Lerner, M.R. (1988) *Proc. Natl. Acad. Sci. USA* 85: 5296-5300.