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20S small nuclear ribonucleoprotein U5 shows a surprisingly complex protein composition

(splicing/systemic lupus erythematosus/autoantibodies)

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ABSTRACT **U5 small nuclear ribonucleoprotein (snRNP), purified from HeLa nuclear extracts (splicing extracts), shows a complex protein composition. In addition to the snRNP proteins B′, B, D, D′, E, F, and G, which are present in each of the major snRNPs U1, U2, U4/U6, and U5, U5 snRNP contains a number of unique proteins characterized by apparent molecular masses of 40, 52, 100, 102, 116, and 200 (mostly a double band) kDa. The latter set of proteins may be regarded as U5-specific for the following reasons. They are not only eluted specifically, together with snRNP particles, from anti-2,2,7-trimethylguanosine immunoaffinity columns by 7-methylguanosine, they also cofractionate with U5 snRNP during chromatography and, most importantly, in glycerol gradient centrifugation. These U5 snRNP particles show a high sedimentation constant of about 20S. U5 snRNPs that lack the U5-specific proteins are also found in nuclear extracts but have (in comparison) a lower sedimentation value of only 8–10S. Autoimmune sera from patients with systemic lupus erythematosus were identified that, on immunoblots with purified U5 snRNP proteins, reacted selectively with the 100- or 200-kDa proteins. This indicates that at least the high molecular mass U5-specific proteins are structurally distinct and not derived one from the other by proteolytic degradation. The existence of so many unique proteins in the U5 snRNP suggests that this snRNP particle may exert its function during splicing mainly by virtue of its protein components.**

U5 small nuclear ribonucleoprotein (snRNP) is one of the major snRNP particles found in the nucleoplasm of all eukaryotic cells investigated so far. It is presumed to participate in the splicing of nuclear pre-mRNA molecules (reviewed in refs. 1–3). In particular, it has been suggested that U5 snRNP might be involved in the recognition of the 3′ end of an intron. This was inferred indirectly from nuclease protection experiments, which revealed the association of an snRNP-containing particle with the 3′ splice site. This particle was highly resistant to treatment with nucleases, a property that, of the abundant snRNPs, is only shown by U5 snRNP (4). Further evidence for the involvement of U5 snRNP in the splicing reaction was adduced by the demonstration that it is part of the spliceosome (5, 6). It seems most probable that the U5 snRNP particle does not enter the spliceosome as such, but instead, in an ATP-requiring process, associates with U4/U6 snRNPs. This association results in the formation of a multi-snRNP complex, which then becomes integrated in the splicing complex (7, 8). The interaction of U5 with U4/U6 snRNP appears to be of dynamic nature, since the multi-snRNP complex dissociates again as the splicing reaction proceeds. While U4 snRNP is released during splicing, U5 snRNP and U6 small nuclear RNA (snRNA), and probably U2 snRNP, remain associated with the excised intron (8, 9). Comparison of mammalian and yeast *in vitro* splicing systems reveals apparently similar behavior of U5 snRNP as part of the spliceosome (10, 11).

In contrast to the detailed information on the sequence of events leading to the integration of the U5 snRNP into and its dissociation from the spliceosome, the structure of the U5 snRNP and in particular its protein composition remain enigmatic. It is established that U5 snRNP possesses the set of seven proteins, B′, B, D, D′, E, F, and G, that are also contained in the other major snRNPs U1, U2, and U4/U6 (refs. 12–14; reviewed in ref. 15). However, when it comes to putative mammalian U5-specific proteins, the situation is far from being clear. Thus, Lelay–Taha *et al.* (13) identified a 25-kDa protein by methionine-labeling *in vivo*. This protein cofractionated with U5 RNA during various purification steps, which included CsCl gradient centrifugation. Two further polypeptides have been described as candidates for U5 snRNP proteins; these share the ability to recognize the 3′ end of an intron but differ in their molecular masses—70 kDa (16) and 100 kDa (17). Interestingly, they both associate with snRNPs at low Mg^{2+} concentrations only (<10 mM) (16, 17), which might explain the fact that they were not discovered in snRNPs prepared at higher Mg^{2+} concentrations (12, 14). The most direct evidence for a U5 snRNA-associated specific protein has been provided by Lossky *et al.*, working on yeast (18). These workers were able to demonstrate that rabbit antibodies raised against the yeast RNA 8 product, a 260-kDa protein, can precipitate the yeast U5 snRNA equivalents, snR7L and snR7S (19), from nuclear extracts. Moreover, they were able to show that this protein was also involved in an ATP-dependent association with two other small nuclear RNAs, snR14 and snR6 [the counterparts of mammalian U4 and U6 RNA (20)].

The observations described above make clear the increasing need for a comparative analysis of the protein composition of U5 snRNPs isolated from HeLa nuclear extracts prepared under various salt conditions. We demonstrate that a fraction of U5 snRNPs purified from splicing extracts by anti-2,2,7-trimethylguanosine (Me_3Gua) affinity chromatography exhibits a complex protein composition containing at least six U5-specific polypeptides in the range of 40–200 kDa.

MATERIALS AND METHODS

Cells and Anti-Sera. HeLa cells (S3) were grown in suspension culture as described (21). Sera from patients diagnosed as having systemic lupus erythematosus (SLE) or mixed connective tissue disease (MCTD) were used as a

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Abbreviations: snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein; Me_3Gua, 2,2,7-trimethylguanosine; SLE, systemic lupus erythematosus; MCTD, mixed connective tissue disease; mAb, monoclonal antibody.

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source of antibodies reacting with the U5 snRNP-specific proteins; these were obtained through the help of H. A. Peter (Freiburg, F.R.G.). Anti-Sm-type monoclonal antibody (mAb) Y12 (22) was a gift of J. A. Steitz.

Preparation of Nuclear Extracts. For the preparation of NX-50 extracts, HeLa cells were first fractionated into cytoplasm and nuclei essentially as described by Zieve and Penman (23) except that the DNase digestion step was omitted. Splicing extracts were prepared from HeLa nuclei as described by Dignam *et al.* (24).

Anti-Me_3Gua Immunoaffinity Chromatography of snRNPs. Affinity purification of snRNPs U1–U6 from NX-50 extracts was performed by using mAb H-20 bound covalently to CNBr-activated Sepharose 4B, essentially as described (25).

When snRNPs were to be isolated from splicing extracts, the anti-Me_3Gua affinity column was equilibrated with a buffer containing 20 mM Hepes adjusted with KOH to pH 7.9/420 mM NaCl/1.5 mM $MgCl_2$/0.2 mM EDTA adjusted with NaOH to pH 8/0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride/5% glycerol (buffer C-5). Splicing extracts from 5×10^9 cells (i.e., about 15 ml) were thawed and centrifuged at 16,500 × *g* for 10 min. The supernatant was diluted by addition of 4 volumes of buffer C (the same as C-5 but lacking glycerol) and then passed over the anti-Me_3Gua affinity column (5-ml bed volume) overnight. Washing of the column and specific desorption of antibody-bound snRNPs by elution with 7-methylguanosine were carried out essentially as described (14) except that buffer C-5 was used in all steps.

Mono Q Chromatography of snRNPs U1–U6. A mixture of about 10 mg of snRNPs U1–U6 isolated from splicing extracts in ≈30 ml of the 7-methylguanosine elution buffer used in the anti-Me_3Gua affinity chromatography (containing 420 mM NaCl; see above) was diluted with 2 volumes of Mono Q 0 buffer [20 mM Tris·HCl (pH 7)/1.5 mM $MgCl_2$/0.5 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride/2 μg of leupeptin per ml] and allowed to adsorb to a Mono Q column (1-ml bed volume). All steps were carried out at 4°C. The snRNPs were then fractionated by elution in Mono Q–O buffer containing added KCl at a concentration that rose stepwise from 50 mM to 1 M (see the profile in Figs. 2 and 3) at a flow rate of 1 ml/min. The fractionation of snRNPs U1–U6 isolated by anti-Me_3Gua affinity chromatography from NX-50 followed the same protocol except that the Mono Q buffer contained 15 mM $MgCl_2$ instead of 1.5 mM. The RNA and protein contact of fractions containing snRNPs was determined as described (21).

Glycerol Gradient Centrifugation. Mono Q fractions containing U5 snRNPs prepared as described above were layered onto a linear 5–20% (vol/vol) glycerol gradient in a buffer containing 20 mM Tris·HCl (pH 7.0) (or Hepes adjusted with KOH to pH 7.9), 150 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, and 2 μg of leupeptin per ml. The gradients were centrifuged in a Beckman SW 60 Ti rotor at 84,000 × *g* for 20 hr.

Immunoblot. Total protein of 20S U5 snRNPs purified by glycerol gradient centrifugation was separated on a sodium dodecyl sulfate (SDS)/polyacrylamide gradient (4–20%) gel and transferred electrophoretically onto nitrocellulose (26). Probing of snRNP proteins with anti-Sm or anti-RNP autoimmune sera or with mAbs was carried out essentially as described (27).

RESULTS

Protein Composition of snRNPs U1–U6 Isolated from Splicing Extracts Versus Extracts Prepared at High Mg^{2+} Concentrations. Two kinds of nuclear extracts from HeLa cells were used as starting material for affinity chromatography of U snRNPs with immobilized anti-Me_3Gua antibodies. In the first procedure, nuclei from HeLa cells were extracted with a buffer containing 0.5 M NaCl and 50 mM $MgCl_2$. This extract will be termed NX-50 (for nuclear extract with 50 mM $MgCl_2$). In the second procedure, nuclear extracts capable of splicing pre-mRNA molecules *in vitro* were prepared (splicing extracts).

An example of the RNA and polypeptide composition of the mixture of snRNPs U1–U6 obtained by anti-Me_3Gua affinity chromatography from both extracts is shown in Fig. 1. The most striking difference between the two snRNP preparations becomes apparent when their protein compositions are compared. Although the snRNP polypeptides 70-kDa, A, A′, B′, B, B″, C, D, D′, E, F, and G are present in both snRNP preparations, several proteins characterized by molecular masses of about 100, 102, 116, and 200 kDa were found only in the snRNPs isolated from splicing extracts (Fig. 1*B*, lanes 1 and 2). It should be noted that the 200-kDa protein sometimes migrates as a broad band (as in Fig. 1*B*) or separates into two bands of similar masses (e.g., see Fig. 5). Owing to the lack of appropriate markers, the molecular mass estimation of the latter protein(s) must be regarded as rather tentative.

Cofractionation of High Molecular Mass Proteins with U5 snRNP on Mono Q Columns. Next, we investigated whether the high molecular mass proteins partitioned among the various snRNP species or whether they were associated with only one of them. For this purpose the mixture of snRNPs U1–U6 isolated from splicing extracts was first subjected to ion-exchange fast protein liquid chromatography on a Mono Q column. The fractionation conditions were similar to those described by Krämer *et al.* (28) for total nuclear extracts. The analysis of protein and RNA composition of the main fractions from the various RNP peaks obtained along the salt gradient is displayed in Fig. 2. The greater part of the high molecular mass proteins is contained in the first major RNP peak, fractions 18–22 (eluted with 0.37 M salt; Fig. 2*C*, lane 1). This peak contains predominantly U1 snRNPs and a

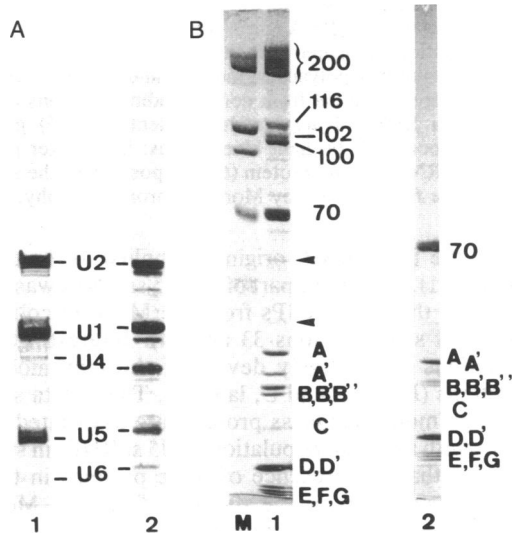

FIG. 1. Purification of snRNPs U1–U6 from NX-50 and splicing extracts by anti-Me_3Gua immunoaffinity chromatography. (*A*) Silver-stained RNAs after fractionation in denaturing polyacrylamide gels extracted from 7-methylguanosine eluate from splicing extracts (lane 1) and from NX-50 (lane 2). The two RNA preparations were separated on distinct polyacrylamide gels. (*B*) Coomassie brilliant blue-stained proteins extracted from purified snRNPs isolated from splicing extracts (lane 1) or NX-50 (lane 2) after separation on SDS/polyacrylamide gradient (4–20%) gels. Lane M shows marker proteins (200, 116.25, 92.5, 66.2, and 45 kDa from top to bottom). Arrows in lane 1 indicate the position of the 40- and 52-kDa U5 snRNP proteins.

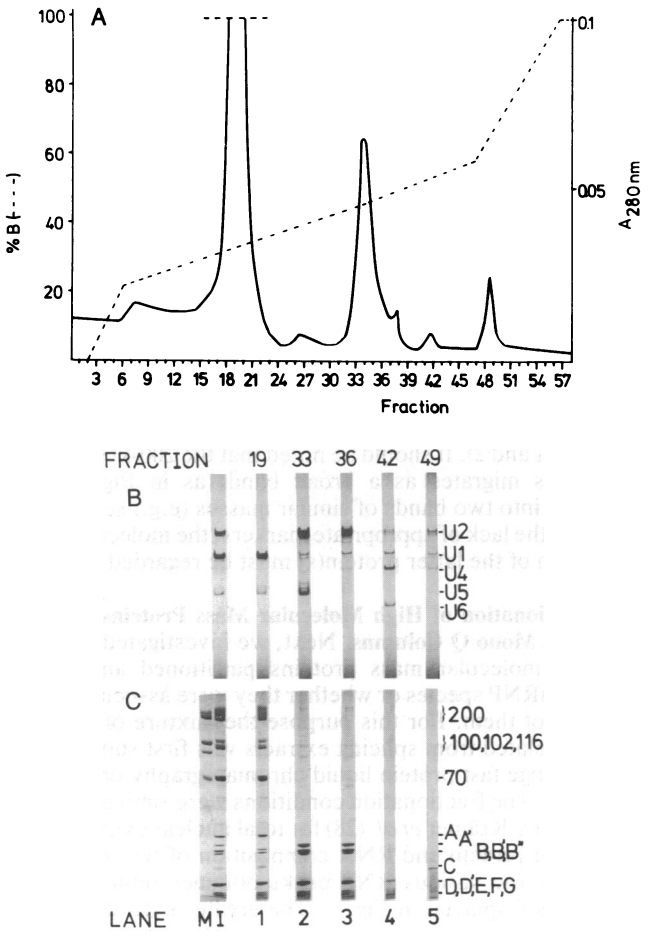

FIG. 2. Fractionation by Mono Q chromatography of snRNPs U1–U6 isolated from splicing extracts by anti-Me_3Gua affinity chromatography. (*A*) "B" is the Mono Q-1000 buffer with a KCl concentration rising from zero to 1 M. - - -, Salt gradient profile; —, absorbance at 280 nm. (*B*) RNA corresponding to 15 μg of the protein mass of the fractions indicated at the top of the lanes was extracted, run on a denaturing 10% polyacrylamide gel, and stained with silver. (*C*) Proteins were extracted from corresponding fractions as in *B*, separated on a SDS/polyacrylamide gradient (4–20%) gel, and stained with Coomassie brilliant blue. Lanes: M, marker proteins (see Fig. 1); I, RNA (*B*) and protein (*C*) composition of the snRNPs subjected to the fractionation by Mono Q chromatography.

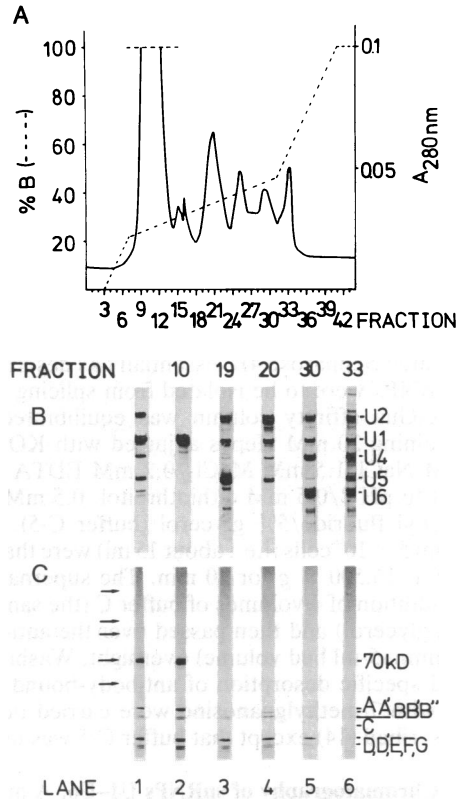

FIG. 3. Fractionation by Mono Q chromatography of snRNPs U1–U6 isolated by anti-Me_3Gua affinity chromatography from NX-50 extracts. (*A*) "B" is the Mono Q-1000 buffer with a KCl concentration rising from zero to 1 M. ---, Salt gradient profile; —, absorbance at 280 nm. (*B*) RNA corresponding to 15 μg of the protein mass of the fractions from the Mono Q gradient as indicated at the top of the lanes was extracted, run on a denaturing 10% polyacrylamide gel, and stained with silver. (*C*) Proteins were extracted from corresponding fractions as in *B*, separated on a SDS/polyacrylamide gradient (4–20%) gel, and stained with Coomassie brilliant blue. Arrows show the position of the molecular weight markers (see Fig. 1). Lanes 1 show the RNA (*B*) and protein (*C*) composition of the mixture of snRNPs U1–U6 subjected to Mono Q chromatography.

fraction of the U5 snRNPs originally applied to the column (Fig. 2*B*, lane 1). The major part of the U5 snRNPs was eluted together with the U2 snRNPs from the Mono Q column at about 0.48 M salt (fractions 33 and 34). Interestingly, the latter fractions were largely devoid of the high molecular mass proteins (Fig. 2 *B* and *C*, lanes 2). These data suggest that the high molecular mass proteins are associated either with U1 or with a certain population of U5 snRNPs in splicing extracts and that the presence of these proteins in the U5 snRNP causes elution of the particles from the Mono Q column at lower salt concentrations.

The latter contention is supported indirectly by the following findings. When snRNPs U1–U6 isolated from NX-50 extracts (which lack the high molecular mass proteins) were fractionated on the Mono Q column under the same conditions as described above, U5 snRNPs were completely absent from the U1 snRNP-containing peak, which is eluted at 0.29 M salt. In this case, all U5 snRNP particles fractionate together with U2 snRNP in 0.4 M salt (Fig. 3, lanes 2 and 3, respectively).

Cosedimentation of High Molecular Mass Proteins with U5 snRNPs on Glycerol Gradients. More direct experimental evidence for the association of the high molecular mass proteins with U5 snRNPs was obtained by sedimentation analysis in glycerol gradients of the mixture of U5 and U1 snRNPs eluted from the Mono Q column in 0.37 M salt (fraction 19 from Fig. 2). A major fraction of the U5 snRNP sediments with a high sedimentation coefficient of about 20 S (Fig. 4*A*). Most important, the high molecular mass proteins cosediment with this fraction of U5 snRNP (Fig. 4*B*). The 200-kDa protein appeared to migrate in a bimodal fashion. The second peak at fraction 12 (Fig. 4*B*) may possibly represent free 200-kDa protein. U5 snRNP particles isolated from NX-50, when centrifuged under the same conditions, sedimented at an S value of 8–10 only (Fig. 4*C*), which is in agreement with our previous observations (29). Taken together, these data indicate that there is a physical association of the high molecular mass proteins with U5 snRNPs from splicing extracts that causes a dramatic shift of the sedimentation constant of the U5 snRNPs from 8–10S to about 20S.

The residual amounts of U1 snRNPs contained in the U5 snRNP fraction appear to peak at an S value of 8–10. The decreased S value is most probably because these U1 snRNPs represent a fraction of U1 snRNP particles that have lost all of the U1-specific proteins 70 kDa, A, and C (see Fig. 3*C*). U1 snRNPs from NX-50, which are composed of a full complement of U1 snRNPs (e.g., Fig. 3 *B* and *C*, lane 2) sediment at 10–12S (29).

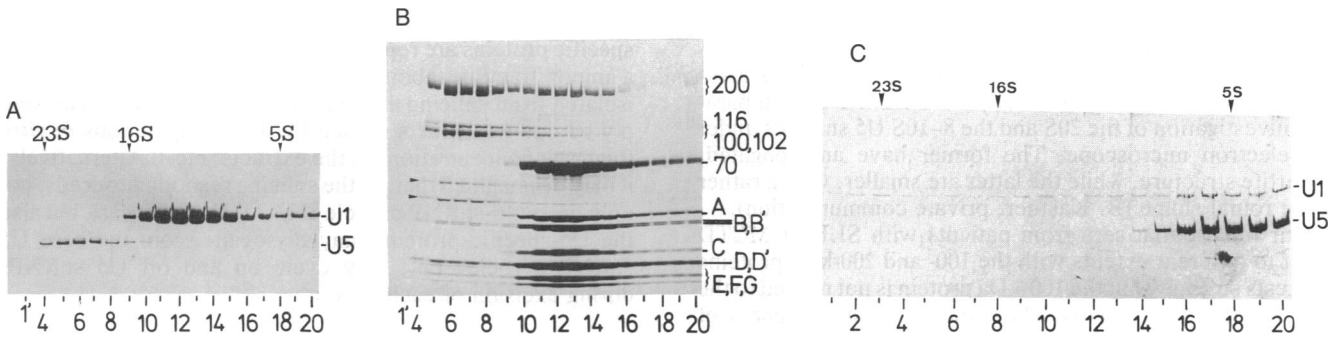

FIG. 4. Sedimentation in glycerol gradients of U5 snRNP particles isolated by Mono Q chromatography from splicing extracts or NX-50 extracts. The RNP particles present in 300 μl of fraction 19 of the Mono Q chromatography of snRNPs U1–U6 from splicing extracts (Fig. 2) and of fraction 18 of the Mono Q chromatography of snRNPs U1–U6 from NX-50 (Fig. 3), were subjected to glycerol gradient centrifugation. (*A*) Gel fractionated RNAs obtained by phenol extraction of RNPs contained in the glycerol gradient of the fraction 19. Staining of RNAs was performed with silver. (*B*) Proteins extracted from the same fractions of the glycerol gradient (corresponding to 70 μl of the glycerol gradient fraction) as in *A* after separation on a SDS/polyacrylamide gradient (4–20%) gel. Arrows in lane 4 indicate the position of the 40- and 52-kDa U5 snRNP proteins. Staining of proteins was with Coomassie brilliant blue. Lanes 1' in *A* and *B* correspond to 70 μl of pooled fractions 1–3 from the gradient. (*C*) Experiment identical with that of *A* with purified U5 snRNP from NX-50 extracts (fraction 18, Fig. 3). S values for 5S, 16S, and 23S *Escherichia coli* rRNAs, respectively, used as standards are indicated in *A* and *C*.

Apart from the common proteins B'–G and the high molecular mass proteins, two further polypeptides of apparent molecular masses of 40 and 52 kDa were also found in the 20S U5 snRNP particles (Fig. 4*B*). Therefore, they may be considered as further candidates for U5 snRNP-specific proteins.

Differential Reactivity of U5 snRNP-Specific Proteins with Autoimmune Sera. It has been demonstrated during recent years that patients suffering from connective tissue diseases such as SLE or MCTD can produce autoantibodies against every snRNP protein described so far (for review, see ref. 30). Therefore, we investigated whether these sera might also contain antibodies reacting with the high molecular mass U5 snRNPs.

Among 15 sera that had been selected from our serum collection by reason of their high titer in anti-Sm or anti-RNP, we indeed found a number that reacted in an immunoblotting test (see Fig. 5 for selected examples). Total proteins from

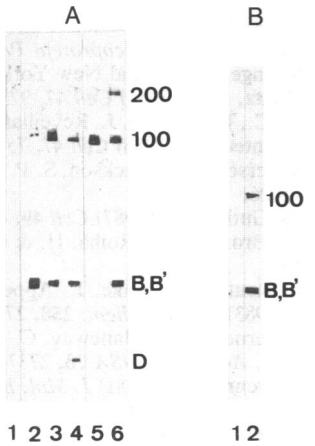

FIG. 5. Reactivity of U5 snRNP-specific polypeptides with anti-Sm and anti-RNP sera. (*A*) Immobilized proteins from 20S U5 snRNPs (purified by glycerol gradient centrifugation as described in Fig. 4) probed with normal human control serum (lane 1) and with sera from patients suffering from SLE and MCTD (lanes 2–6). Sera used in lanes 2, 3, 4, and 6 were characterized as being anti-Sm by the criteria that they precipitated all snRNPs U1–U6 from nuclear extracts and contained antibodies against the common snRNP proteins B'/B (lanes 2, 3, and 6) or B'/B and D (lane 4). In lane 5 an anti-RNP antiserum was used that precipitated U1 snRNP only and contained antibodies against the U1 snRNP-specific proteins 70 kDa and A. (*B*) The same immobilized proteins probed with a mAb not related to snRNP proteins (lane 1) and with mAb Y12 (lane 2).

20S U5 snRNP particles purified by glycerol gradient centrifugation were used as antigenic material. Most of the sera that contained antibodies against a specific U5 snRNP reacted with the 100-kDa protein. Sera with high titers against this protein frequently showed a reaction with the 102-kDa protein as well (e.g., Fig. 5*A*, lane 3). We have found so far two sera that react (albeit weakly) with the proteins in the 200-kDa region (Fig. 5*A*, lanes 5 and 6). In contrast, none of the sera tested until now have reacted with the 116-kDa or the 40- and 52-kDa proteins.

Most of the sera that react with the 100-kDa protein show at the same time a reaction with proteins B and B'. This could indicate the presence of Sm epitopes on the 100-kDa protein, a hypothesis that is supported by our observation that the monoclonal anti-Sm antibody Y12 also reacts with the 100-kDa protein (Fig. 5*B*, lane 2). Depending on the concentration of the 200-kDa proteins on the nitrocellulose, Y12 also weakly reacts with these proteins, a result that could be confirmed by ELISA (not shown). Thus, the 200-kDa proteins may also bear Sm-related epitopes. It is interesting to notice that the 100-kDa protein shows not only Sm-related epitopes but also specific autoreactive epitopes. In Fig. 5*A*, lane 5 shows an anti-RNP antiserum that reacts with the 100-kDa protein but has no anti-Sm titer.

DISCUSSION

In this paper we have described several proteins with apparent molecular masses of 200 (usually seen on the gel as a double band), 116, 102, 100, 52, and 40 kDa. The following evidence supports their consideration as candidates for U5-specific proteins.

(*i*) The proteins are not only eluted specifically, together with the snRNP particles, from anti-Me_3Gua columns by the use of 7-methylguanosine, but also they cofractionate with U5 snRNP in successive chromatographic steps on Mono Q and in glycerol gradient centrifugation.

(*ii*) The physical properties of U5 snRNP particles (containing these proteins) differ from those of U5 snRNPs containing only the common proteins B'–G. The former are eluted from Mono Q columns by a lower salt concentration (0.37 M instead of 0.48 M) and have a markedly higher S value in glycerol gradient centrifugation (20S instead of 8–10S). A sedimentation constant at about 20S has been reported previously for U5 snRNPs in experiments where nuclear extracts from HeLa cells were subjected to centrifugation in glycerol or sucrose gradients (8, 31). The precise reason for

the dissociation of the U5-specific proteins from U5 snRNPs in the NX-50 extraction is not clear.

(*iii*) The physical association of the high molecular mass proteins with U5 snRNPs is supported further by comparative investigation of the 20S and the 8–10S U5 snRNPs under the electron microscope. The former have an elongated, tripartite structure, while the latter are smaller, with a rather more round shape (B. Kastner, private communication).

Our finding that sera from patients with SLE or MCTD react to different extents with the 100- and 200-kDa proteins suggests strongly that the 100-kDa protein is not related to the 116- or 200-kDa proteins. The structural distinctiveness of these proteins is supported further by a preliminary comparative protease digestion experiment carried out with the 200-, 116-, and 100-kDa proteins (not shown). As yet, we have no direct experimental evidence to extend this notion to the 40- and 52-kDa proteins that comigrate with the 20S U5 snRNPs, except that their abundance in U5 particles does not depend on the use of protease inhibitors during the purification of the snRNPs.

How do our data relate to other published results on putative U5 snRNP-specific proteins (see the Introduction)? The 25-kDa protein observed by Lelay-Taha *et al.* (13) was not found in the 20S U5 snRNPs, whose purification is in this paper. A further polypeptide has been detected by the same group and proposed as a candidate for a U5-specific protein; it was characterized by a molecular weight of about 100-kDa, reactivity with anti-Sm antibodies, and the ability to recognize the polypyrimidine-rich 3′ end of an intron (17). The first two criteria apply also to the 100-kDa protein detected in our U5 snRNP preparation, which could indicate structural homology between the two proteins. While the 100-kDa protein recognizes intron-containing pre-mRNA substrates in RNA immunoblots, we have as yet no evidence that the 20S U5 snRNP particle by itself interacts directly with the 3′ end of an intron.

A protein that also recognized specifically the 3′ end of an intron and showed anti-Sm antigenicity but has a molecular weight of 70 kDa was described as a possible U5-specific polypeptide by Gerke and Steitz (16). While we failed to detect such a protein in our 20S U5 snRNPs purified by glycerol gradient centrifugation (see Figs. 4 and 5), we sometimes have observed a 70-kDa protein in the mixture of snRNPs U1–U6 obtained from splicing extracts by anti-Me_3Gua affinity chromatography. This protein reacts on immunoblots with monoclonal anti-Sm antibody Y12 (unpublished observation). Apparently, this protein is lost from the snRNPs upon further fractionation on Mono Q columns. It remains to be seen whether there is a structural relationship between this protein and any of the U5-specific high molecular mass proteins described in this paper.

The most intriguing question concerns the possible structural and functional relationships between the 200-kDa protein(s) found in the HeLa 20S U5 snRNP preparation and the yeast RNA 8 product, a protein of 260 kDa that is found in close association with the U5 RNA equivalent snR7 and snR7S (Lossky *et al.*, ref. 18). Our preliminary investigations indicate at least an immunological relationship between the HeLa 200-kDa protein(s) and the yeast RNA 8 product (G. J. Anderson, J. Beggs, M.B., and R.L., unpublished data). Since the 260-kDa protein in yeast is also involved in an ATP-dependent association with snRNAs snR14 and snR6 (the counterparts of mammalian snRNAs U4 and U6), there should be an investigation into whether the U5-specific high molecular weight proteins detected in the 20S U5 snRNP in this paper are also involved in the interaction of this particle with purified snRNPs U4/U6 *in vitro*, and whether additional factors present in nuclear extracts are needed that might interact only transiently with the two snRNPs.

A final question concerns our finding that the U5 snRNP-specific proteins are reproducibly found associated with only a minor fraction (about 15–20%) of the total U5 snRNPs isolated from splicing extracts. This heterogeneity may simply be due to artifacts caused by the manipulations *in vitro* (high salt concentrations in the extracts, etc.). Alternatively, it may be imagined that, as the splicing reaction proceeds, not only the multi-snRNP particle U4/U5/U6 falls apart, but also the U5-specific proteins may dissociate from the core U5 snRNP particle—i.e., they cycle on and off U5 snRNPs during excision of each intron.

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1. Sharp, P. A. (1987) *Science* **235,** 766–771.
2. Maniatis, T. & Reed, R. (1987) *Nature (London)* **325,** 673–678.
3. Steitz, J. A., Black, D. L., Gerke, V., Parker, K. A., Krämer, A., Frendewey, D. & Keller, W. (1988) in *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*, ed. Birnstiel, M. L. (Springer, Berlin and New York), pp. 115–154.
4. Chabot, B., Black, D. L., LeMaster, D. M. & Steitz, J. A. (1985) *Science* **230,** 1344–1349.
5. Grabowski, P. J. & Sharp, P. A. (1986) *Science* **233,** 1294–1299.
6. Bindereif, A. & Green, M. R. (1987) *EMBO J.* **6,** 2415–2424.
7. Grabowski, P. J., Seiler, S. R. & Sharp, P. A. (1985) *Cell* **42,** 345–353.
8. Konarska, M. M. & Sharp, P. A. (1987) *Cell* **49,** 763–774.
9. Lamond, A. I., Konarska, M. M., Grabowski, P. J. & Sharp, P. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 411–415.
10. Pikielny, C. W., Rymond, B. C. & Rosbash, M. (1986) *Nature (London)* **324,** 341–345.
11. Cheng, S.-C. & Abelson, J. (1987) *Genes Dev.* **1,** 1014–1027.
12. Hinterberger, M., Pettersson, I. & Steitz, J. A. (1983) *J. Biol. Chem.* **258,** 2604–2613.
13. Lelay-Taha, M. N., Reveillaud, I., Sri-Widada, J., Brunel, C. & Jeanteur, P. (1986) *J. Mol. Biol.* **189,** 519–532.
14. Bringmann, P. & Lührmann, R. (1986) *EMBO J.* **5,** 3509–3516.
15. Lührmann, R. (1988) in *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*, ed. Birnstiel, M. L. (Springer, Berlin and New York), pp. 71–99.
16. Gerke, V. & Steitz, J. A. (1986) *Cell* **47,** 973–984.
17. Tazi, J., Alibert, C., Temsamani, J., Reveillaud, I., Cathala, G., Brunel, C. & Jeanteur, P. (1986) *Cell* **47,** 755–766.
18. Lossky, M., Anderson, G. J., Jackson, S. P. & Beggs, J. (1987) *Cell* **51,** 1019–1026.
19. Patterson, B. & Guthrie, C. (1987) *Cell* **49,** 613–624.
20. Siliciano, P. G., Brow, D. A., Roiha, H. & Guthrie, C. (1987) *Cell* **50,** 585–592.
21. Bringmann, P., Reuter, R., Rinke, J., Appel, B., Bald, R. & Lührmann, R. (1983) *J. Biol. Chem.* **258,** 2745–2747.
22. Lerner, E. A., Lerner, M. R., Janeway, C. A. & Steitz, J. A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2737–2741.
23. Zieve, G. W. & Penman, S. (1981) *J. Mol. Biol.* **145,** 501–523.
24. Dignam, J. D., Lebovitz, R. M. & Roeder, R. D. (1983) *Nucleic Acids Res.* **11,** 1475–1489.
25. Bochnig, P., Reuter, R., Bringmann, P. & Lührmann, R. (1987) *Eur. J. Biochem.* **168,** 461–467.
26. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
27. Reuter, R. & Lührmann, R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8689–8693.
28. Krämer, A., Frick, M. & Keller, W. (1987) *J. Biol. Chem.* **262,** 17630–17642.
29. Bringmann, P., Appel, B., Rinke, J., Reuter, R., Theissen, H. & Lührmann, R. (1984) *EMBO J.* **1,** 1259–1265.
30. Tan, E. M. (1982) *Adv. Immunol.* **33,** 167–240.
31. Kinlaw, C. S., Dusing-Swartz, S. K. & Berget, S. M. (1982) *Mol. Cell. Biol.* **2,** 1159–1166.