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Molecular relationships between U snRNP proteins as investigated by rabbit antisera and peptide mapping

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ABSTRACT

Each of the major U snRNP polypeptides from human cells was purified by electroelution from SDS-polyacrylamide gels. Rabbit antisera could be obtained against the individual proteins 70K, A, B', B and D, although rabbits failed to elicit antibodies against E, F and G. A strong structural homology was found between proteins B' and B, against which patients with connective tissue diseases produce predominantly anti-Sm autoantibodies. Thus, rabbit antisera against B' strongly crossreact with B and *vice versa*. Peptide patterns of the proteins B' and B obtained with chymotrypsin are identical with the exception of one fragment in each case. Polypeptide D, the third major Sm-antigenic protein, is structurally distinct from B' and B, as evidenced by the failure of anti-D antisera to crossreact with B' or B and *vice versa*, as well as by the different peptide patterns observed for proteins D and B' or B. The U1 specific polypeptide A and the U2 specific polypeptide B" share homologous regions, as indicated by the crossreactivity of anti-A antisera with protein B", and the occurrence of common fragments in the peptide patterns of the two proteins. Further homologies between other snRNP protein pairs were not detected.

INTRODUCTION

Eucaryotic cells synthesize a group of abundant small nuclear ribonucleoprotein particles containing the snRNAs U1, U2, U4, U5 and U6 (1,2). While the snRNAs U1, U2, and U5 are organized as separate RNP particles, the snRNAs U4 and U6 reside in one and the same ribonucleoprotein complex (3-6). The protein constituents of the isolated snRNPs from human cells have recently been characterized (7-12). Seven proteins of approximate mol. wts. 29 kDa (B'), 28 kDa (B), 16 kDa (D), 15.5 kDa (D'), 12 kDa (E), 11 kDa (F) and 9 kDa (G) are present in each of the individual snRNPs U1, U2, U5 and U4/U6. In addition to the common proteins, U1 RNPs contain three unique polypeptides of mol. wts. 70 kDa, 34 kDa (A) and 22 kDa (C). U2 RNPs are characterized by the presence of a 33 kDa and a 28.5 kDa protein, denoted A' and B".

In the cell the snRNPs are associated with hnRNP particles, the complex ribonucleoprotein structures where pre-mRNA processing takes place (see ref 13 for a review). In accordance with this physical linkage it has been demonstrated during the last three years that all the major snRNPs U1 to U6 contribute to the splicing of nuclear pre-mRNAs (14-20). Besides the intact snRNA molecules, the protein components of snRNPs are also important for the

function of the snRNP particles in pre-mRNA splicing (21), a contention which is supported by the fact that anti-RNP and anti-Sm autoantibodies inhibit the *in vitro* splicing reaction (14).

The snRNP proteins are further interesting from a clinical immunological point of view. Patients with systemic lupus erythematosus or related connective tissue diseases often develop autoantibodies reacting with selected snRNP proteins (3, see ref. 22 for a review). Anti-RNP autoantibodies react with the U1 RNP characteristic polypeptides 70 kDa, A and C, and consequently precipitate only U1 snRNPs. In contrast, anti-Sm autoantibodies precipitate all nucleoplasmic snRNPs U1 to U6. (7-10,23,24) The major immunoreactive Sm proteins are B', B and D. Autoantibodies against the common proteins E, F or G also occur in some patient sera but are less abundant (9,25, Reuter et al., to be published). Finally, two types of autoantibodies have recently been detected, which react with the U2 RNP specific polypeptides A' and B" respectively (11,26).

Immunoblotting studies revealed that most individual patient sera simultaneously contained autoantibodies against multiple snRNP proteins. Of particular interest in this respect is the observation that autoantibodies against some of the polypeptides, such as B, B' and D or B" and A, often occur together in the patient sera, raising the possibility that the respective snRNP polypeptides are structurally related (9,26-28). The sharing of at least one epitope between the members of the above groups of antigens has been formally proved by the isolation of monoclonal antibodies that crossreact with B', B, D (9,29-31) and B", A, respectively (30,32).

In this report we investigate the extent of the immunological and structural relationships between the human snRNP polypeptides in more detail, using polyclonal antibodies raised in rabbits against individual snRNP polypeptides, as well as by comparative partial protease digestion of the snRNP proteins.

MATERIALS AND METHODS

Cell growth

HeLa S3 cells were grown in suspension culture at 37°C in Eagles minimum essential medium (Flow Laboratories) - 5 % newborn calf serum (Biochrome Berlin) supplemented with penicillin at 50 μg/ml and streptomycin at 100 μg/ml. Cells were kept in logarithmic growth by sequential dilution every 24 hrs, keeping the cell concentration between 2.5 x 10^5 and 6 x 10^5 cells per ml.

Purification of U snRNPs and electroelution of proteins

snRNPs U1, U2, U5 and U4/U6 were purified from nuclear extracts of HeLa S3 cells by immunoaffinity chromatography with rabbit anti-m_3G as described (24). For the isolation of pure U1 snRNPs, affinity purified snRNPs U1 to U6 were chromatographed on DEAE-Sepharose columns. For the preparation of pure snRNP protein 70K, A, B', B, C, D, E, F and

G, a mixture of total proteins from purified U1 RNPs was subjected to preparative electrophoresis in a 15 % SDS polyacrylamide gel. Coomassie blue stained proteins were cut out from the gel, washed with water for 1 hr and soaked in solution buffer (50 mM NH_4HCO_3, 0.1 % (w/v) SDS) for 5 min. Electroelution was performed in an apparatus and under conditions identical to those described by Hunkapiller et al. (33).

Protein A' was prepared by electroelution using total proteins from snRNPs U1 to U6 as starting material for the SDS polyacrylamide gel electrophoresis. For the preparation of the U2 specific protein B" a mixture of anti-m_3G affinity purified snRNPs U1 to U6 (about 1 mg) in a total volume of 20 ml TMN300 buffer (20 mM Tris-HCl, pH 7.4, 1 mM MgCl, 300 mM NH_4Cl) was treated with 0.5 mg RNAse A for 30 min at 37°C. The mixture was passed over a 0.5 ml Sepharose affinity column carrying 5 mg of covalently bound monoclonal antibody D-5 at a flow rate of 0.1 ml/min. Monoclonal antibody D-5 reacts with the snRNP proteins B" and A (32). After washing the column with 10 ml of TMN buffer, the antibody bound snRNP proteins were desorbed by elution with 1 ml of 0.2 mM glycine buffer, pH 2.5. The eluate was immediately neutralized by the addition of 1 mM Tris. The eluate contained essentially the proteins B" and A. Pure B" was recovered by electroelution after electrophoretic separation of the proteins in a 15 % polyacrylamide gel.

Immunization of rabbits with purified snRNP proteins

Prior to being used for immunization, electroeluted snRNP proteins were extracted with a mixture of acetone triethylamine and acetic acid as described by Konigsberg and Henderson (34) in order to remove excess SDS. Rabbits were immunized subcutaneously on days 1, 14 and 28 with 20 µg each of purified snRNP proteins 70K (R1338), A (R1329), B' (R1341), B (R1194), D (R1340), E (R1369), F (R1370) and G (R1353), emulsified in complete (day 1) or incomplete Freund's adjuvant. Except for rabbit R1194 (which died on day 40) all other rabbits received at least two booster injections of 20 µg snRNP protein each on days 42 and 56. Blood was drawn prior to immunization on day 35 and seven days after each booster injection.

Immunoblotting

For blotting analysis of the rabbit antisera, total proteins of purified snRNPs U1 to U6 were used. 150 µg of protein was dissolved in 2 % SDS containing sample buffer and loaded over the entire width of a 15 % polyacrylamide gel (35). After electrophoresis, the gels were blotted for 4 hrs onto nitrocellulose in a Bio-Rad transblot cell at 60V/0.3A in 192 mM glycine, 25 mM Tris-HCl pH 8.3 and 20 % methanol and 0.1 % SDS (36). The blots were dried and cut from top to bottom into strips of about 5 mm. Individual strips were saturated with 3 % BSA and incubated with serum as described (36). Antigen-bound antibody specificities were visualized with alkaline phosphatase conjugated second antibody which catalyzed the colour reaction of 5-bromo-4-chloro-indolyl-phosphate and nitro tetrazolium blue.

ELISA assay

Purified snRNPs U1 or U1 to U6 were adsorbed to the wells of polystyrol multiwell microtiter plates (100 ng/well) by incubation for 16 hrs at 4°C. Alternatively, isolated single snRNP proteins (10 ng/well) were adsorbed to the wells by drying the plates at 37°C for twelve hrs. The wells were then washed three times with 0.15 M sodium chloride / 0.02 M sodium phosphate, pH 7.4 (PBS), and nonspecific binding sites on the polystyrol surface were blocked by incubating the plates with 1 % bovine serum albumin in PBS for 3 hrs at room temperature, after which the plates were washed twice with PBS. Appropriate dilutions of rabbit antisera in 80 µl of PBS containing 1 % BSA, 5 % fetal calf serum and 0.1 % Tween 20 (BSATF) were added and the mixture was incubated for 16 hrs at 4°C. After five washes of the plates with PBS containing 0.1 % Tween 20 (PBST), goat anti-rabbit Ig phosphatase conjugate (Dianova Hamburg) diluted in PBST was added and the mixture was incubated for 4 hrs at room temperature. The colour reaction of p-nitrophenylphosphate was developed for 1 hr (3 hrs with single proteins as antigen) at room temperature before the optical density at 405 nm was measured in the respective microtiter wells by an automated ELISA reader (Dynatech, USA).

Protease mapping procedure

Comparative partial digests of snRNP proteins with selected proteases were made according to Cleveland et al. (37) with some modifications. Briefly electroeluted snRNP proteins (in general 4 µg each) were lyophilized and dissolved in cleavage buffer (125 mM Tris-HCl pH 6.8, 0.5 % w/v SDS, 10 % v/v glycerol) at a concentration of about 1 µg/µl and heated for 2 min. at 100°C. After cooling the mixture on ice, the protease dissolved in cleavage buffer was added in the amounts indicated in the Figure legends, followed by incubation at 37°C for 30 min. The reaction mixture was placed on ice. SDS and β-mercaptoethanol concentrations were raised to 2 % (w/v) and 5 % (v/v) respectively, and the samples were boiled for 2 min. at 100°C and analyzed by SDS polyacrylamide gel electrophoresis (35). The peptide fragments produced were visualized by silver staining (38).

RESULTS

Isolation of the individual snRNP polypeptides

Purified snRNPs were used as starting material for the isolation of individual snRNP polypeptides by electroelution of the proteins after separation on preparative SDS polyacrylamide gels. For this purpose total nucleoplasmic snRNPs U1 to U6 were isolated from nuclear extracts of HeLa cells by affinity chromatography with antibodies specific for 2,2,7-trimethylguanosine (m_3G), which is part of the snRNAs' 5'-terminal cap structure. U1 RNPs were separated from U2 to U6 RNPs by fractionation of the anti-m_3G affinity purified snRNPs on DEAE-Sepharose. The purified U1 RNPs were used as a source of the polypeptides 70 kDa, A, B', B, C, D, D', E, F and G. The U2 specific polypeptide A' was

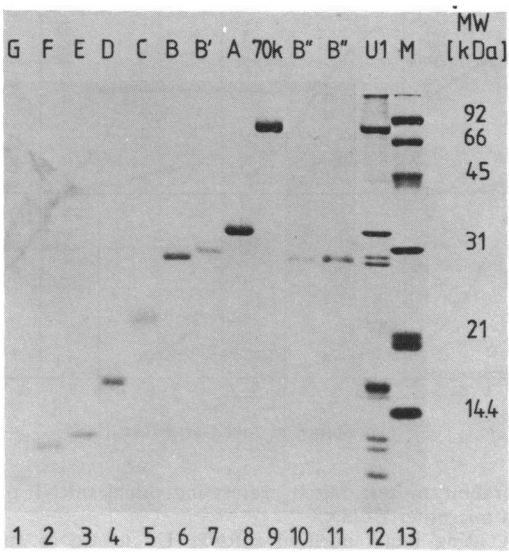

Fig. 1 One dimensional gel electrophoresis analysis of electroeluted snRNP proteins. Individual snRNP protein bands were cut out from one-dimensional SDS polyacrylamide gels and electroeluted from the gel as described under Materials and Methods. Re-electrophoresis in 15 % SDS-polyacrylamide gels shows that this procedure yields pure protein species. Slots 1-9 represent the snRNP proteins G to B, B', A, and 70K; slots 10 and 11 represent 0.3 and 1 μg of B", respectively. Slot 12 displays proteins of purified U1 snRNP (U1) and slot 13 molecular weight marker proteins (M).

obtained by electroelution after separation on SDS polyacrylamide gels of the total proteins in the fractions containing U2 to U6 RNP from the DEAE-Sepharose column.

Since the U2 specific protein B" cannot be separated sufficiently well from the polypeptides B' and B on one-dimensional polyacrylamide gels to allow its direct isolation from a U2 RNP protein mixture, a different procedure had to be adopted in this case. A mixture of partially purified snRNPs U1 to U6 was treated with ribonuclease A, so as to guarantee that the U1 and U2 specific proteins dissociate from the core RNP structures and exist as individual soluble proteins. This RNase A treated RNP mixture was passed over an affinity column which contained immobilized anti-(U1,U2) monoclonal antibody D-5. mAb

Table 1 Recoveries of individual snRNP proteins after electroelution

snRNP protein	70K	A	A'	B'	B	B"	C	D	D'	E	F	G
Yields[a] (μg)	220	260	30	120	120	20	30	300	15	80	80	60

[a] Recoveries are expressed in μg snRNP protein obtained by electroelution of individual polypeptides when 3 mg of purified snRNPs U1 to U6 were used as starting material.

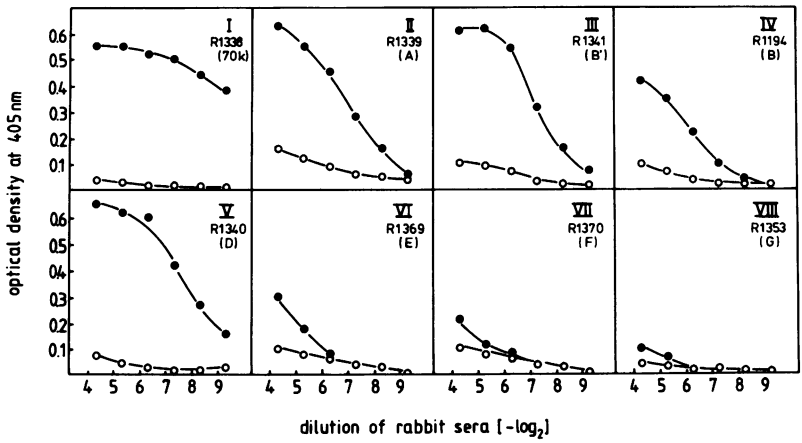

Fig. 2 Reactivity of rabbit antisera raised against individual snRNP proteins with purified snRNPs U1 to U6 in a microtiter ELISA.
The microtiter ELISA using intact purified snRNPs U1 to U6 as antigenic material was performed as described under Materials and Methods with serial dilutions (1:20, 1:40 etc.) of the first immune serum of the indicated rabbits. The respective snRNP protein used as immunogen is given in parentheses in each panel. o-o, preimmune sera; o-o immune sera.

D-5 recognizes an epitope shared by the U2 specific protein B" and the U1 specific polypeptide A (32). The proteins A and B" were selectively retained on the D-5 column. Polypeptide B" was recovered by electroelution, after dissociation of the proteins from the affinity column and separation on an SDS polyacrylamide gel. Re-electrophoresis of the various isolated snRNP proteins shows that the procedures used yield pure protein species (Fig. 1), with the exception of protein D' which was still contaminated by protein D (not shown). Typical recoveries of individual snRNP proteins obtained from 3 mg snRNPs U1 to U6 are given in Table 1.

Immunological relationships between snRNP polypeptides as determined by rabbit antisera against individual proteins

Rabbits were immunized with the purified individual proteins 70 kDa (R1338), A (R1339), B' (R1341), B (R1194), D (R134O), E (R1369), F (R1370) and G (R1353) using one animal for each protein. (The yields of isolated proteins A', B", C and D' were too low to allow immunization studies). Antibody production against the proteins was first followed by a microtiter ELISA using intact anti-m_3G affinity purified snRNPs U1 to U6 as an antigen. As shown in Figure 2, sera from rabbits immunized with proteins 70 kDa, A, B', B and D were positive for the snRNPs, although the antibody titers were not very high, ranging between 1:9.000 for the 70K antisera and 1:300 for the B protein (Figure 2). Only weak or no reaction at all with the snRNPs of the sera from rabbits immunized with polypeptides E, F and G was observed (Figure 2). The sera also failed to react with purified single proteins E, F and G,

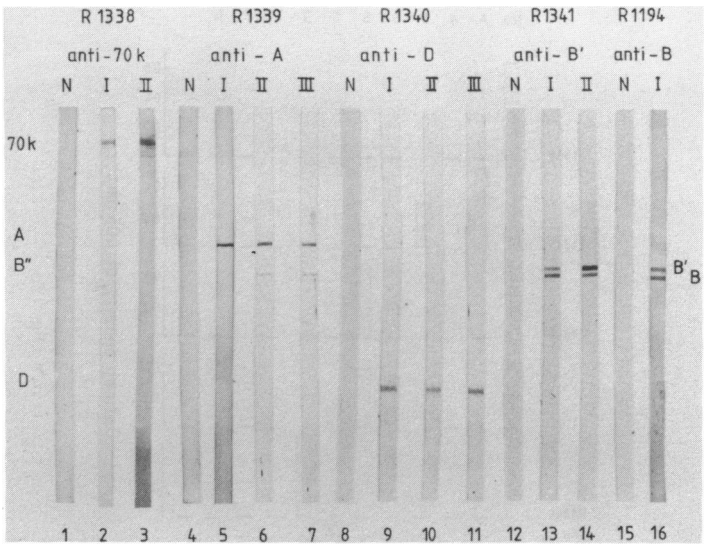

Fig. 3 Immunoblot analysis of rabbit antisera raised against individual snRNP proteins. The nitrocellulose blot strips contained total proteins from purified HeLa snRNPs U1 to U6, transferred after separation on 15 % SDS-polyacrylamide gels as described under Materials and Methods. Each strip was incubated with a rabbit serum at a 1:50 dilution. N: preimmune serum; I, II, III represent rabbit antisera obtained on day 35 (I) or after serial booster injections (II + III).

thus excluding the possibility that antibodies were produced by the rabbits that reacted with these proteins only in a denatured state, but not when they were part of intact snRNPs (not shown).

The immune positive rabbit antisera were further characterized by immunoblotting with total proteins from the mixture of purified snRNPs U1 to U6 and by ELISA where individual proteins were coated to the microtiter plates. Except for the anti-B rabbit (R1194), multiple blood drawings were obtained from the other animals after repeated booster injections. As a control the preimmune sera from all rabbits used were shown to be negative with respect to antibodies against snRNP proteins (Figure 3). Antisera raised against the 70 kDa protein reacted only with the 70 kDa protein on immunoblots (Figure 3), and no crossreaction could be observed with any of the other snRNP proteins, a result which could be confirmed by the more sensitive microtiter-ELISA (Figure 4). (The faint bands observed in the immunoblots in the molecular weight range 30 kDa to 70 kDa (Figure 3) are due to reaction of the antibodies with degradation products of the 70 kDa protein).

The change in the specificity of the anti-A immune sera after various booster injections is interesting. While the first immune serum only reacts with the A protein on immunoblots, the hyperimmune sera react in addition with one of the proteins in the B region (Figure 3).

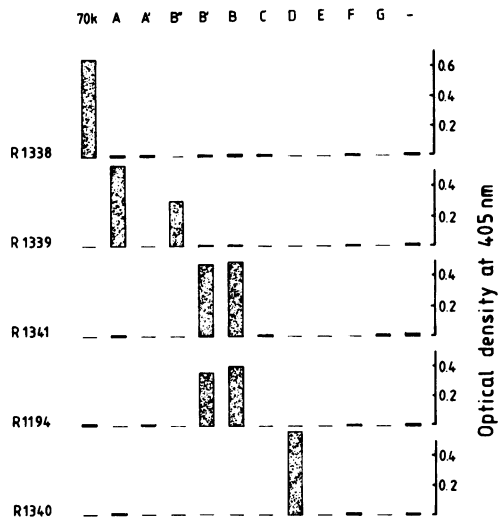

Fig. 4 Characterization of rabbit antisera by ELISA using single snRNP proteins as antigens. Microtiter ELISA was performed as described under Materials and Methods. The wells were coated with single snRNP proteins (70K to G) or with bovine serum albumin only (-), and were incubated with a 1:400 dilution of the immune sera of the rabbits as indicated. The indicated optical densities at 405 nm are mean values of four independent experiments. Standard deviations were smaller than 0.003 OD_{405}.

The results obtained by the microtiter ELISA show that it is the U2 specific B" protein which crossreacts with antibodies raised against purified A protein (Figure 4).

The results obtained with the antisera against the proteins B', B and D were surprising. Anti-B' sera strongly cross-reacted with polypeptide B, while antibodies raised against protein B cross-reacted to the same extent with protein B'. Neither reacted with protein D, however (Figures 3 and 4). These results indicate strong immunological relationships between the polypeptides B' and B but no apparent relatedness of protein D with polypeptides B' or B. This supposition was confirmed by our finding that antisera raised against protein D only reacted with the immunogen but not with the polypeptides B' or B (Figures 3 and 4). This was true even for the immune sera obtained from rabbit R1341 after various booster injections with isolated protein D (Figure 3).

Comparative partial protease digestion of snRNP polypeptides

In order to study further the extent of sequence homology between selected snRNP polypeptides, comparative protease digestion experiments were carried out with the electroeluted purified proteins, and the products were analyzed by SDS-polyacrylamide gel electrophoresis.

A striking primary sequence homology between the proteins B' and B is indicated by the

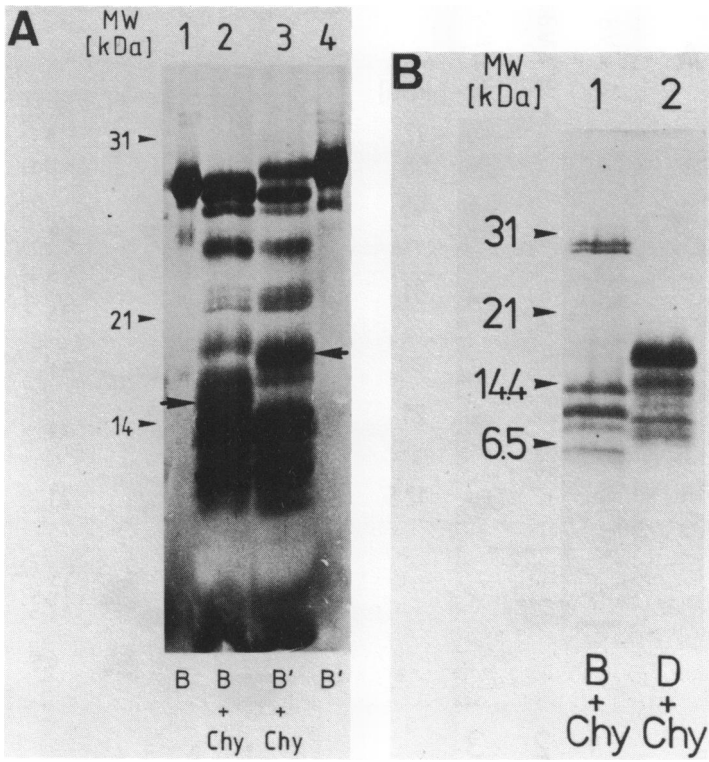

Fig. 5 Peptide maps of proteins B', B and D with chymotrypsin.
Partial chymotrypsin digestion of the proteins B', B and D and fractionation of cleavage products in 15 % SDS-polyacrylamide gels was performed as described under Materials and Methods. Panel A shows comparative partial chymotrypsin digests of proteins B (lane 2) and B' (lane 3) at a mass ratio of chymotrypsin to snRNP proteins of 1:75. Lanes 1 and 4 display undigested proteins B and B', respectively, for comparison. Arrows indicate fragments unique to proteins B and B'. Panel B shows partial chymotrypsin digests of proteins B and D. Experimental conditions were as described above, except that, in this case, the mass ratio of protease to snRNP proteins was 1:15. Mol. wts. of marker proteins used are given on the left of each panel.

results shown in Figure 5A. When the two proteins were treated with chymotrypsin, the resulting cleavage patterns were identical except for two fragments (indicated by arrows in Figure 5A). This is in good agreement with our finding above that rabbit antisera against B' strongly crossreact with B and *vice versa*.

A comparative peptide mapping of proteins B' or B with D was complicated by the fact that the D protein was highly resistant to digestion with various proteases. V8 protease and elastase did not cleave the SDS denatured protein at all. The best result was obtained with chymotrypsin, where some fragments were produced from the protein at high protease/protein

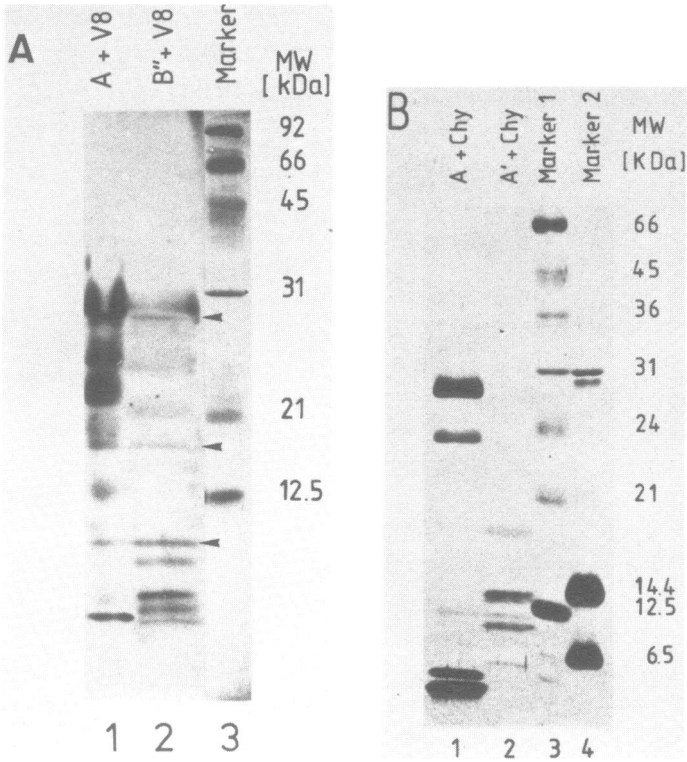

Fig. 6 Peptide maps of proteins A, B" and A' with *Staphylococcus aureus* V8 protease and chymotrypsin.
Partial protease digestion of snRNP proteins was performed as described in Figure 5 and Materials and Methods. Panel A shows comparative peptide patterns of proteins A (lane 1) and B" (lane 2) with *Staphylococcus aureus* V8 protease at a mass ratio of protease to polypeptides of 1:15. In this case protease digestion proceeded within the stacking gel (37). Panel B shows comparative peptide patterns of proteins A (lane 1) and A' (lane 2) with chymotrypsin at a mass ratio of protease to polypeptide of 1:15. Arrowheads in panel A indicate fragments of polypeptides A and B" displaying the same apparent mol. wts.. Mol. wts. of marker proteins used are given on the right of each panel.

concentrations. The peptide patterns obtained from the two proteins are clearly distinct (Figure 5B). The same result was obtained when proteins B and D were compared (not shown). These data indicate that there are no strong structural relationships between proteins B'/B and D.

Figure 6A shows the peptide patterns obtained by digestion with V8 protease of the U1 specific polypeptide A and the U2 specific polypeptide B". It is clear from these results that the two proteins are distinct. However, the occurrence of three fragments of the same molecular weights in the two peptide patterns may indicate that the two proteins share a similar structural domain (Figure 6A).

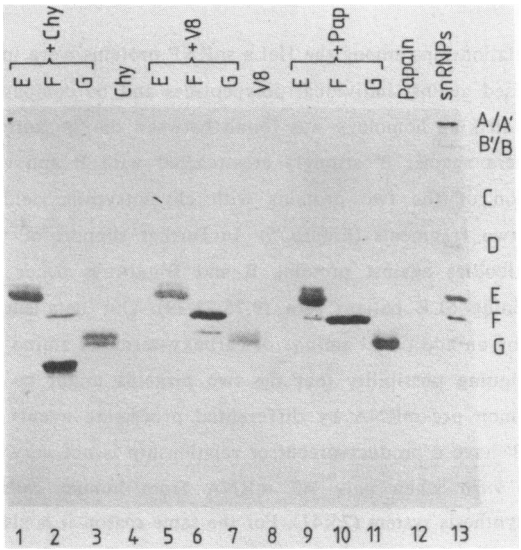

Fig. 7 Partial digest with various proteases of snRNP polypeptides E, F and G.
snRNP polypeptides E, F and G each were digested with proteases chymotrypsin (lanes 1-3), *Staphylococcus aureus* V8 protease (lanes 5 to 7) and papain (lanes 9-11) at mass ratios of protease to polypeptide of 1:15, essentially as described under Materials and Methods. Protein digests were separated in 20 % SDS polyacrylamide gels. In lanes 4, 8 and 12 the same amounts of chymotrypsin (lane 4), *Staphylococcus aureus* V8 protease (lane 8) and papain (lane 12) as were used for the digestion experiments were subjected to gel electrophoresis after incubation at 37°C for 30 min. Lane 13 represents undigested snRNP polypeptides as marker proteins.

No structural relationship was observed between the polypeptides A and A' , as indicated by the distinct peptide patterns observed for the two proteins after digestion with chymotrypsin (Figure. 6B).

Figure 7 shows partial digests of snRNP proteins E, F and G with the proteases chymotrypsin, *Staphylococcus aureus* V8 protease and papain. Protein G proved to be highly resistant to digestion by any of the three enzymes. Only chymotrypsin appears to be able to cleave a short fragment from either the N- or C-terminus of this polypeptide (Figure 7, lane 3). Protein E was cleaved by papain (Figure 7, lane 9) while being resistant to the action of the other two proteases (Figure 7). Only protein F was sensitive to cleavage by two proteases, viz. chymotrypsin and *Staphylococcus aureus* V8 protease (Figure 7, lanes 2 and 6). Since in each case only one or a few fragments were produced, no definite conclusions as to possible structural relationships between the proteins E, F and G could be drawn from these results. However, the mere fact that polypeptides E, F and G display differential resistance to the proteases used indicates at least that the three proteins must be structurally distinct.

DISCUSSION

The structural relationships among the HeLa snRNP proteins were investigated by the use of rabbit antisera raised against individual polypeptides and by comparative partial protease digestions. The most striking homology was found between the Sm antigenic proteins B' and B. Thus rabbit antisera against B' strongly crossreacted with B and *vice versa* (Figure 3). Furthermore, digestion of the two proteins with chymotrypsin yielded identical peptide patterns except for two fragments (Figure 5). In further support of this homology is the finding that autoantibodies against proteins B and B' always occur together in anti-Sm autoantibodies containing SLE patient sera (9,26,28,39). Our data suggest that protein B' differs from B only by an additional amino- or carboxy-terminal amino acid sequence, which would raise the intriguing possibility that the two proteins might be encoded by mRNAs derived from a common pre-mRNA by differential processing events. The possibility that polypeptides B and B' have a product-precursor relationship is not very likely, since they are both synthesized *in vitro* when poly A^+ mRNA from human cells is translated in a reticulocyte protein synthesis system (25,41). For the same reason it is also not very likely that B' simply represents a post-translationally modified version of protein B.

A comparison of proteins B/B' with polypeptide D, the third major Sm antigen, demonstrated that the latter is clearly structurally distinct from the proteins B/B'. This is evidenced by our findings that rabbit antisera against B or B' did not crossreact with D and *vice versa*. Secondly, the peptide patterns obtained by digestion of proteins B and D did not show significant overlaps, which precludes the possibility of product-precursor relationships between proteins D and B/B'. The distinctiveness of proteins B/B' and D is also supported by the existence of anti-Sm SLE patient sera that recognize only polypeptides B/B' but not D (27,28,39), and by monoclonal antibodies selectively reacting with either proteins B/B' or D (31,40). On the other hand, monoclonal antibodies could be isolated that crossreact with all three Sm proteins simultaneously (9,29-31). Autoantibodies crossreacting with B, B' and D have also been observed in patient sera (26). These results, together with the data presented in the present report, suggest that the relatedness of polypeptide D with the B/B' proteins may just be restricted to the sharing of one or a few epitopes. The failure of the rabbits to elicit antibodies which crossreacted with the common epitope(s) of the proteins D and B/B' could be explained by a low immunogenicity of the respective determinants when being presented to the immune system as part of the SDS-denatured polypeptides.

The possible sharing of larger homologous regions (and not just an epitope) between proteins A and B" is indicated by our findings that the rabbit antiserum against polypeptide A crossreacts on immunoblots with protein B" (Figure 3), and by the occurrence of peptides with the same mol. wts. in the respective peptide patterns of the two proteins after *Staphylococcus aureus* V8 protease digestion (Figure 6). These data are in good agreement with previous observations that anti-(U1/U2) autoantibodies occurring in sera of patients with connective

tissue diseases (26), as well as monoclonal anti-(U1/U2) antibodies, recognize proteins A and B" simultaneously. The apparent structural relatedness between proteins A and B" is interesting insofar that the two proteins reside on distinct snRNP particles (U1 and U2, respectively), and could indicate that the homologous region(s) of A and B" may exert similar functions in the different snRNP species.

Besides the crossreactivities discussed above, no reaction of the rabbit antisera raised against proteins 70kDa, A, B', B and D with one of the snRNP proteins A', C, E, F and G was observed (Figure 3), suggesting that there are no strong immunological relationships between the proteins of the first group and one of the latter polypeptides.

Since rabbits failed to elicit antibodies against the proteins E, F and G, we were not able to study the immunological relatedness of these proteins. Comparative peptide mapping experiments revealed a differential resistance of the proteins E, F and G towards digestion by various proteases, indicating that the three proteins are structurally distinct. This notion is supported by our recent observation that patients with connective tissue diseases in rare cases produce autoantibodies against proteins E, F and G differentially, which suggests that the three proteins carry at least one unique epitope each (R. Reuter et al., to be published).

In a recent immunization study of mice with intact purified U1 snRNPs from human cells, we noticed that the animals preferentially produced antibodies against the proteins 70kDa, A, B', B and D (30). No immune response against proteins E, F and G was elicited. It should be noted that the same differential immune response of the various snRNP proteins was observed in the present report when the proteins were immunized individually. This is of theoretical interest in view of the fact that patients with connective tissue diseases also spontaneously produce autoantibodies predominantly against the proteins 70kDa, A, B', B, C and D but only in rare cases against E, F or G. Taken together, these data suggest the possibility that the pattern of autoantibodies produced against the snRNPs is due to the intrinsic immunogenic structural features of the snRNP polypeptides.

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