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Evidence that the 60-kDa protein of 17S U2 small nuclear ribonucleoprotein is immunologically and functionally related to the yeast PRP9 splicing factor and is required for the efficient formation of prespliceosomes

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ABSTRACT **Small nuclear ribonucleoprotein (snRNP) U2 functions in the splicing of mRNA by recognizing the branch site of unspliced mRNA. The binding of U2 snRNP and other components to pre-mRNA leads to the formation of a stable prespliceosome. In HeLa nuclear extracts, U2 snRNP exists either as a 17S form (under low salt conditions) or a 12S form (at higher salt concentrations). We have recently shown that the purified 17S U2 snRNP contains nine proteins with apparent molecular masses of 35, 53, 60, 66, 92, 110, 120, 150, and 160 kDa in addition to the common snRNP proteins and the U2 proteins A′ and B″ that are found in the 12S U2 snRNP form. By using antibodies against the PRP9 protein from *Saccharomyces cerevisiae* (a protein required for the addition of U2 to prespliceosomes in yeast), we have shown that the 60-kDa protein specific to human U2 snRNP particles is structurally related to the yeast PRP9 protein. Interestingly, anti-PRP9 antibodies strongly inhibit prespliceosome formation in HeLa nuclear splicing extracts, resulting in a complete inhibition of the mRNA splicing reaction *in vitro*. This indicates that the U2 60-kDa protein may also be functionally related to its yeast counterpart PRP9. Most importantly, the addition of purified 17S U2 snRNPs, but not of 12S U2 snRNPs, to HeLa splicing extracts in which the endogeneous U2 snRNPs have been functionally neutralized with anti-PRP9 antibodies fully restores the mRNA-splicing activity of the extracts. These data suggest further that the 17S form is the functionally active form of U2 snRNP in the spliceosome.**

The catalysis of intron removal from eukaryotic nuclear pre-mRNA molecules requires the activity of four abundant nuclear U small nuclear ribonucleoprotein (snRNP) species U1, U2, U4/U6, and U5 (for reviews, see refs. 1–3). These assemble with an as yet unestablished number of auxiliary factors and the pre-mRNA substrate into a dynamic RNP complex, termed the spliceosome (for reviews, see refs. 4 and 5). Like the constitutive splicing mechanism, the pathway of spliceosomal assembly has been substantially conserved between yeast and humans (4, 6, 7).

As an important early step during spliceosome formation, the U2 snRNP binds to the branch site of the intron (8–10). This interaction, which involves base pairing between sequences near the 5′ end of the U2 snRNA with the branch point sequence (11–14) depends on the hydrolysis of ATP and leads to the formation of a stable prespliceosomal complex (15–17).

The requirements for U2 snRNP addition to the pre-mRNA have been studied to some extent: In *Saccharomyces cerevisiae*, several lines of evidence indicate an ATP-independent interaction of the U1 snRNP with the 5′ splice site and the 3′-terminal region of the intron, which commits the pre-mRNA substrate to the splicing reaction, as an essential prerequisite for the assembly of U2 snRNP (18–21). The situation in mammalian systems appears to be similar (22–24). Stable binding of U2 snRNP to pre-mRNA requires, apart from U1 snRNP, additional protein factors such as U2AF and SF3 (25, 26). Mature spliceosomes are formed upon binding of a 25S [U4/U6·U5]tri-snRNP complex to the spliceosome (15–17).

It is not yet clear whether U2 snRNP proteins are also important for the addition of U2 snRNP to the spliceosome. In HeLa cell nuclear extracts, two forms of U2 snRNP exist. At salt concentrations >300 mM, a 12S form predominates, and under splicing conditions (at ≈100 mM salt), the majority of the U2 snRNPs exhibits a sedimentation coefficient of ≈17 S (16, 27). The 12S U2 snRNP contains in addition to U2 RNA the common proteins B′/B, D1, D2, D3, E, F, and G and the U2-specific proteins A′ and B″ (for a review, see ref. 2). Recently, it has been shown that the 17S U2 snRNP has a strikingly complex protein composition. Besides the proteins present in the 12S U2 snRNP, the 17S form contains at least nine additional proteins with respective apparent molecular masses of about 35, 53, 60, 66, 92, 110, 120, 150, and 160 kDa (28). In nuclear extracts, the latter group of proteins associates with U2 RNP only at low salt concentrations permissive to the *in vitro* splicing reaction and the majority of the proteins appears to bind to the 5′-terminal region of U2 RNA (28), which is involved in base-pair interactions with the intron branch point and U6 RNA in the spliceosome (29–32). These findings suggested that at least some of the 17S U2 proteins could be important for the function of U2 RNA in mRNA splicing.

Although the protein composition of U2 snRNP in the yeast *S. cerevisiae* has not been analyzed in detail, there is evidence that the yeast PRP9 splicing factor protein may be structurally and functionally related to U2 snRNP. It was shown previously that the 63-kDa PRP9 protein is an essential splicing factor and that it functions in an early step of mRNA splicing (33). More recently, Abovich *et al.* (34) showed that PRP9 is important for the addition of U2 snRNP to the spliceosome and that in yeast extracts PRP9 may associate with U2 snRNP at low salt concentrations. In view of these interesting correlations between the association behavior of PRP9 with yeast U2 snRNP on the one hand and the 17S U2 proteins with human U2 snRNP on the other, we investigated whether one of the 17S U2 proteins might represent a human counterpart of the yeast PRP9 protein. We found that antibodies raised in rabbits against recombinant yeast PRP9 protein reacted specifically with the 60-kDa protein of human 17S U2 snRNP, indicating that the structure

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Abbreviations: snRNP, small nuclear ribonucleoprotein; mAb, monoclonal antibody.

of this protein is evolutionarily conserved between yeast and humans. Interestingly, anti-PRP9 antibodies completely inhibit the splicing of mRNA in a HeLa splicing system *in vitro*. Moreover, we show that the 60-kDa protein of the 17S U2 snRNP, like its yeast counterpart, is required for the efficient formation of prespliceosomes.

MATERIALS AND METHODS

Expression and Purification of and Immunization with Recombinant PRP9. The PRP9 subclone FG98 (35, 36), containing aa 25–530, was expressed in *Escherichia coli* BL21 DE3 by using the pET-3c expression system of Studier and Moffat (37). Purification of FG98 and the immunization procedure were as described by Galisson and Legrain (38). For the inhibition/complementation experiments, antibodies from rabbit sera or hybridoma supernatants were prepared as pure IgG fractions, by using protein A-Sepharose for the purification of the antibodies (39). Western blot analysis was carried out essentially as described (40). Immunoprecipitation and ELISA studies were performed according to Behrens and Lührmann (41).

Purification of U snRNPs. U1 and 12S U2 snRNPs were purified by Mono Q ion-exchange chromatography as described in detail by Bach *et al.* (42). 17S U2 snRNPs were purified under low-salt conditions from splicing extracts as described by Behrens *et al.* (28). If necessary, the U snRNPs were concentrated by centrifugation in a TL-100 ultracentrifuge (Beckman) for 3 h at 70,000 rpm in a TLA 100.3 rotor at 4°C.

Splicing Reactions and Assays. Nuclear extracts were prepared from freshly grown HeLa cells as described by Dignam *et al.* (43) and dialyzed to 20 mM Hepes·KOH, pH 8/100 mM KCl/1.5 mM $MgCl_2$/0.5 mM dithioerythritol/0.5 mM phenylmethylsulfonyl fluoride/5% (vol/vol) glycerol. The extracts were active in splicing at dilutions between 30% and 80% per assay (see below). As pre-mRNA substrate, ^{32}P (Amersham)-labeled run-off T7 transcripts of the rabbit β-globin gene were used. These contained the first two exons and the first intron of the gene (44). The transcripts were capped with guanosine(5')triphospho(5')guanosine (Pharmacia) and had a specific activity of 4×10^6 cpm/pmol. Standard splicing reactions were carried out on the basis of the protocols of Krainer *et al.* (45), as follows. The reaction was performed in a total volume of 50 μl for 90 min at 30°C. Standard assays of 50 μl used for inhibition/complementation experiments contained 20 μl of nuclear extract [40% (vol/vol), see above], 2 μl of 50 mM $MgCl_2$ [concentrations per assay (cpa), 3 mM], 2 μl of 40 mM rATP (cpa, 1.7 mM), 1 μl of RNasin (Promega; cpa, 1 unit/μl), 2.5 μl of 500 mM creatine phosphate (cpa, 25 mM), 0.5 μl of 100 mM dithioerythritol (cpa, 1 mM), 2 μl of transcript (2×10^4 cpm per assay), and 10 μl of water. The remaining 10 μl was used for the inhibition and/or complementation agents as antibodies (in water) and U snRNPs (in buffer G), respectively (see below). The splicing reaction was stopped by the addition of the same volume of 2× PK buffer (300 mM NaCl/100 mM Tris Cl, pH 7.5/1% SDS) containing proteinase K at 4 μg/100 μl. Thereafter, the mixture was incubated for a further 30 min. Splicing products were extracted by perchloric acid and analyzed on 10% polyacrylamide/Tris borate gels (TBE = 50 mM Tris borate, pH 8.3/1 mM EDTA) containing 7.5 M urea (see also ref. 44).

Electrophoretic Analysis of Splicing Complexes. The splicing reaction was carried out essentially as described above. At the times indicated, 10 μl was removed, 2 μl of heparin (5 mg/ml) and 2 μl of glycerol (87%) were added to stop the reaction, and the mixture was stored at room temperature until the time course was finished. The samples were loaded on a native composite gel containing 0.3× TBE, 3.5% polyacrylamide [acrylamide/*N,N'*-methylene bisacrylamide, 80:1 (wt/wt)], 0.5% agarose, and 10% glycerol. Running buffer was 0.3× TBE, and running conditions were exactly as described by Nelson and Green (46).

RESULTS

The 60-kDa Protein of Human 17S U2 snRNPs Is Immunologically Related to the Yeast PRP9 Protein. Antibodies specific for the PRP9 protein were raised by immunization of rabbits with a recombinant PRP9 fusion protein that encoded aa 25–530 of the yeast PRP9 protein. The anti-PRP9 antiserum reacts specifically with the yeast 63-kDa PRP9 protein on immunoblots of total cell lysates of *S. cerevisiae* (data not shown). Proteins from purified HeLa 17S U2 snRNPs were then tested by immunoblot analysis for the presence of proteins that are recognized by the unfractionated polyclonal rabbit anti-PRP9 antiserum. The antiserum reacts strongly with the 60-kDa protein of the 17S U2 snRNP (Fig. 1*A*, lane 1). In addition, a protein of apparent molecular mass 110 kDa is also weakly stained. Blots with a corresponding preimmune serum were blank (lane 3). Antibodies affinity-purified against the recombinant PRP9 proteins also reacted strongly with the 60-kDa protein on immunoblots but did not stain the 110-kDa protein (lane 2). The same results were obtained with a rabbit antiserum raised against the C-terminal half of PRP9 (data not shown). These data suggested a strong immunological relationship between the yeast PRP9 protein and the human 60-kDa protein from 17S U2 snRNPs. The anti-PRP9 antibodies did not react at all with proteins present in purified human U1 snRNPs or in 25S [U4/U6·U5]tri-snRNP complexes (not shown). This shows that the 60-kDa protein is associated exclusively with U2 snRNP and not present in the other purified spliceosomal snRNPs.

For the further use of the antibodies for functional inhibition studies of splicing in HeLa cell nuclear extracts (see below), it was important to assess the possible cross-reactivity of the anti-PRP9 antibodies with other proteins of HeLa nuclear extracts in addition to the U2 60-kDa protein. Fortunately, even when challenged with proteins from total HeLa nuclear extracts, unfractionated anti-PRP9 antiserum reacted predominantly with the protein in the 60-kDa region of the immunoblots (Fig. 1*B*, lane 1). Only faint signals were observed in other regions of the immunoblot. Next we affinity-purified antibodies against the 60-kDa protein in a total nuclear extract by a preparative immunoblot procedure. When these affinity-purified antibodies were allowed to react with proteins from purified 17S U2 snRNPs, they recognized specifically the 60-kDa U2 protein (Fig. 1*A*, lane 4). We conclude from these experiments that the 60-kDa protein reacting with anti-PRP9 antibodies in total HeLa nuclear extracts is identical with the 60-kDa U2 protein.

Next we investigated the reactivity of anti-PRP9 antibodies with native 17S U2 snRNPs. Attempts to immunoprecipitate intact 17S U2 snRNPs either from total nuclear extracts or from purified 17S U2 snRNPs failed. This could be due either to disruption of the 17S U2 snRNP complex during the immune precipitation procedure or to a low affinity of the anti-PRP9 antibodies for its human counterpart. Strong evidence that the anti-PRP9 antibodies do recognize the native 60-kDa 17S U2 protein was obtained by ELISA (data not shown). From these results, we conclude that the anti-PRP9 antibodies are able to react with the native 60-kDa U2 protein, albeit with low affinity.

Anti-PRP9 Antibodies Inhibit the *in Vitro* Splicing Reaction in HeLa Cell Nuclear Extracts. Given the selective reaction of the anti-PRP9 antibodies with the 60-kDa U2 protein in total nuclear extracts (see Fig. 1), we were in a position to use the antiserum for functional inhibition studies in HeLa splicing extracts. Our initial attempts to deplete splicing extracts

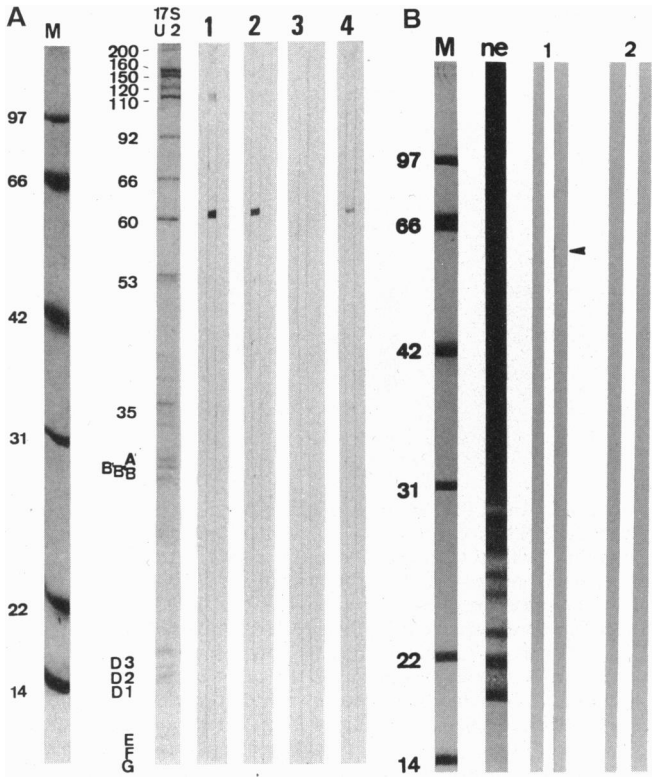

FIG. 1. Cross-reactivity of anti-PRP9 antibodies with the 60-kDa protein of human 17S U2 snRNPs. (*A*) Total proteins from purified human 17S U2 snRNPs were separated by SDS/PAGE, blotted onto nitrocellulose filters, and probed with various antibodies. Lanes 1 and 2 show the reactivity of unfractionated anti-PRP9 antiserum and anti-PRP9 antibodies affinity-purified against the recombinant PRP9 protein (for the affinity purification of antibodies, see ref. 40), respectively. Lane 3 shows a control with nonimmune serum from the same rabbit. In lane 4, U2 proteins were probed with anti-PRP9 antibodies that had been affinity-purified against the 60-kDa protein reacting with unfractionated anti-PRP9 serum on immunoblots with total extracts from HeLa nuclei (see also *B*). The two lanes on the left show marker proteins (lane M) and proteins from 17S U2 snRNPs purified from HeLa nuclear extracts (lane 17S U2) separated by SDS/PAGE and visualized by staining with Coomassie blue. Sizes are given in kDa. (*B*) Reactivity of anti-PRP9 antiserum with total proteins from HeLa cell nuclear extracts. Total proteins extracted from HeLa cell nuclear extracts were separated by SDS/PAGE and blotted onto nitrocellulose. The proteins were allowed to react with unfractionated PRP9 antiserum (lane 1) or preimmune serum (lane 2). Arrowhead indicates position of the 60-kDa protein. Lanes M and ne show marker proteins and total proteins from HeLa nuclear extracts, respectively, separated by SDS/PAGE and visualized by Coomassie blue staining. Sizes are given in kDa.

selectively of the 60-kDa U2 protein failed, which was not surprising in view of the evidence (above) that these antibodies bind comparatively weakly to the human proteins. However, in view of our finding that anti-PRP9 antibodies did react with the native 60-kDa protein, we investigated whether they would inhibit the mRNA splicing reaction in HeLa splicing extracts. For this purpose, we purified the IgG fraction of the anti-PRP9 antiserum to obtain a more concentrated RNase- and protease-free antibody preparation. As shown in Fig. 2*A*, lanes 4, 6, and 8, increasing amounts of anti-PRP9 IgG indeed completely inhibited the splicing reaction *in vitro*. The inhibition is specific since the same concentrations of IgG from preimmune serum had no inhibitory effects at all (Fig. 2*A*, lanes 3, 5, and 7).

It might be argued that the large size of the Fab part of an IgG molecule will enable any antibody against any U2 protein to inhibit the splicing reaction, simply because of steric

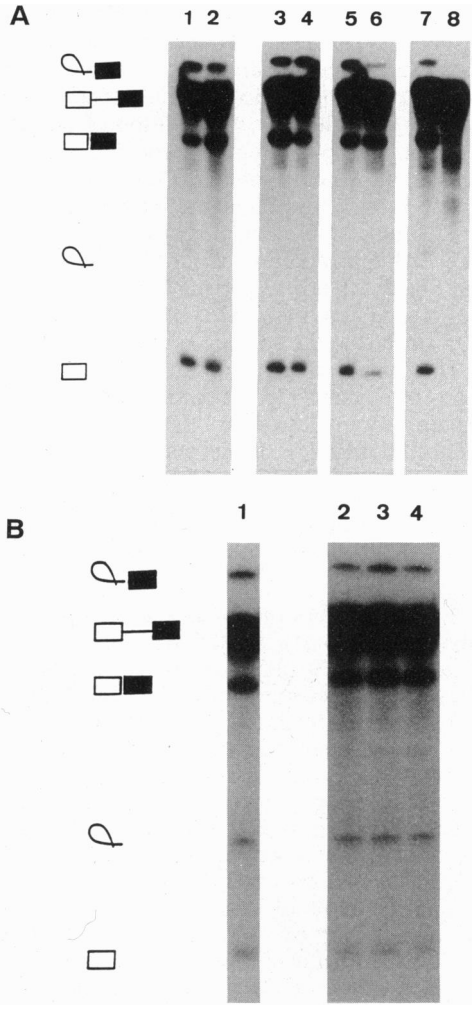

FIG. 2. Inhibition of pre-mRNA splicing in HeLa nuclear splicing extracts by anti-PRP9 antibodies. (*A*) In lanes 1 and 2, rabbit β-globin pre-mRNA was incubated under splicing conditions with nontreated nuclear extract in a total volume of 50 μl. In lane 2, 5 μl of buffer G was added to the splicing assay instead of water. In lanes 4, 6, and 8, increasing amounts of purified anti-PRP9 IgG (90, 120, and 150 μg, respectively) were incubated with nuclear extracts for 15 min at 0°C prior to the addition of pre-mRNA. In lanes 3, 5, and 7, nuclear extracts were treated with increasing amounts of purified IgG from preimmune serum (90, 120, and 150 μg, respectively). [^{32}P]RNA was detected by autoradiography. (*B*) The pre-mRNA splicing reaction was carried out essentially as described above (*A*, lanes 2, 4, 6, and 8), except that nuclear extracts were pretreated with 4, 6, and 8 μg of purified mAb 4G3 IgG specific for the U2 B″ protein (lanes 2–4, respectively) prior to the addition of the rabbit β-globin pre-mRNA (see above). Lane 1 shows the splicing reaction with untreated nuclear extracts. [^{32}P]RNA was detected by autoradiography.

hindrance. This is, however, not the case. When we added purified monoclonal antibody (mAb) 4G3, which is specific for the U2 B″ protein (47) to the HeLa splicing extract at effective concentrations comparable to that of the PRP9-specific IgG, only marginal inhibition if any of the splicing reaction was observed (Fig. 2*B*). In a separate experiment, we verified by ELISA that mAb 4G3 reacted with the B″ protein in the native 17S U2 snRNP (data not shown), which eliminates the possibility that the lack of inhibition observed with mAb 4G3 is a simple consequence of the failure of the B″ protein in the 17S U2 RNP to react with 4G3.

Purified 17S but Not 12S U2 snRNPs Restore Splicing Activity of Nuclear Extracts. The selective inhibition of splicing by anti-PRP9 antibodies but not by mAb 4G3 indicates that the 60-kDa protein makes, directly or indirectly, an

important contribution to the function of U2 snRNP in mRNA splicing. This idea was corroborated strongly by the following results. Specifically, we asked whether the inhibition of mRNA splicing observed in the presence of anti-PRP9 IgG could be abolished by the addition of purified 17S U2 snRNPs. For this purpose, we preincubated the splicing extract with an amount of anti-PRP9 IgG that was just sufficient to bring about complete inhibition of the splicing reaction (150 μg per 50-μl splicing assay; Fig. 3, lane 3). This was followed by the addition of purified 17S U2 snRNPs. As shown in Fig. 3, lanes 5 and 6, the 17S U2 snRNPs restored fully the splicing activity *in vitro*. Addition of buffer instead of 17S U2 snRNP to the splicing assay had no effect (lanes 3 and 4). Most interestingly, the addition of equivalent concentrations of purified 12S U2 snRNPs (i.e., the U2 snRNP form that completely lacks the PRP9 homolog and the other 17S U2-specific snRNP proteins) was not capable of restoring the splicing activity at all (lanes 7 and 8). In sum, these data suggest that the 17S form of the U2 snRNP is the functional entity of U2 snRNP and indicate further that the 60-kDa protein of U2 plays an important role for U2 snRNP function in splicing.

Anti-PRP9 Antibodies Inhibit an Early Step of Spliceosome Assembly. Next we investigated whether the inhibition of splicing by anti-PRP9 IgG was due to interference of the antibodies with spliceosome formation. Spliceosome assembly was investigated by native gel electrophoresis. In Fig. 4, lanes 1–4, the time dependence of spliceosome formation is shown in a spliceosome assembly assay. After 10 min of incubation at 30°C, prespliceosomal complexes (complex A, lane 2) were already formed. After 30 min, mature spliceosomes were observed (complex B, lane 3). After 1 h, almost all of the pre-mRNA was found in complexes A and B and only residual amounts of unspecific complex (complex H, lane 4) could be observed. Most interestingly, in the presence of anti-PRP9 IgG at concentrations that completely inhibited the splicing reaction (see Fig. 3), no formation of mature spliceosomes (complex B) was observed at all, even after a

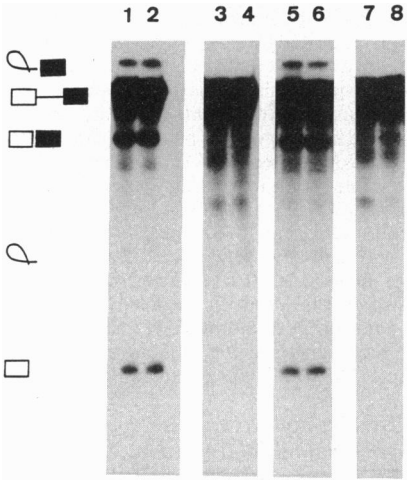

FIG. 3. Splicing reaction in nuclear extracts pretreated with anti-PRP9 IgG and complementation of this with purified 17S or 12S U2 snRNPs. In lane 1, rabbit β-globin pre-mRNA was incubated with nuclear extracts under standard splicing conditions in a total volume of 50 μl. Lane 2 was as lane 1, except that the reaction mixture was supplemented with 10 μl of purified 12S U2 snRNPs in buffer G (corresponding to 2.5 μg of total snRNP protein). Lanes 3 and 4 were as lane 1, except that the pre-mRNA was pretreated with 150 μg of purified anti-PRP9 IgG along with 10 μl (lane 3) and 5 μl (lane 4) of buffer G, respectively. Lanes 5 and 6 were as lanes 3 and 4, but the buffer G contained 17S U2 snRNPs at 0.1 μg/μl. Lanes 7 and 8 were as lanes 5 and 6, except that 12S (not 17S) U2 snRNPs were used. [^{32}P]RNA was detected by autoradiography.

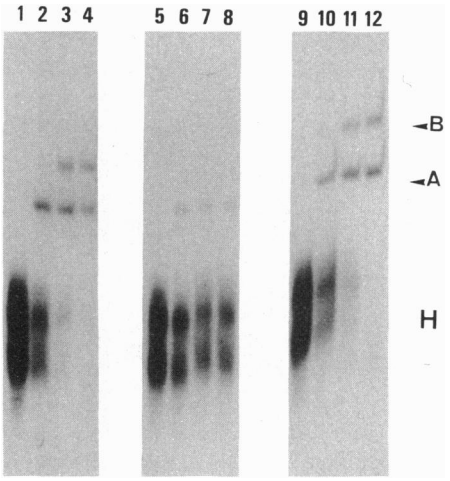

FIG. 4. Anti-PRP9 antibodies inhibit prespliceosome formation. Rabbit β-globin pre-mRNA was incubated under standard splicing conditions with nuclear extracts in the absence of antibodies (lanes 1–4), in the presence of 150 μg of purified anti-PRP9 IgG (lanes 5–8), or in the presence of 8 μg of mAb 4G3 IgG (lanes 9–12). Splicing reactions were carried out for 0 (lanes 1, 5, and 9), 10 (lanes 2, 6, and 10), 30 (lanes 3, 7, and 11), and 60 (lanes 4, 8, and 12) min. The splicing reaction was stopped by the addition of heparin at the appropriate time and spliceosomal complexes were separated by native gel electrophoresis. [^{32}P]RNA was detected by autoradiography. The positions in the gel of prespliceosomal complexes (complex A), mature spliceosomes (complex B), and unspecific pre-mRNP complexes (complex H) are indicated at the right of the figure.

1-h incubation (Fig. 4, lanes 5–8). Furthermore, the formation of prespliceosomes was inhibited strongly as compared with the control reaction. A minor band close to the prespliceosomes could be observed after a 10-min incubation, and this band did not increase with longer incubation time. Most of the pre-mRNA was found in the unspecific complex H. As expected, mAb 4G3, which failed to inhibit the splicing reaction (Fig. 2*B*), also failed to interfere with the formation of spliceosomal complexes A and B (Fig. 4, lanes 9–12). All in all, our data indicate that the binding of anti-PRP9 to the 60-kDa protein of 17S U2 snRNP strongly inhibits the addition of U2 RNP to the spliceosome. Furthermore, the minor amounts of U2 snRNPs that can bind to the pre-mRNA even in the presence of anti-PRP9 IgG—as indicated by the small amount of prespliceosome formation (Fig. 4, lanes 6–8)—associate in a nonproductive way, as they do not allow subsequent formation of mature spliceosomes.

DISCUSSION

The principal result of these experiments has been the identification of the 60-kDa protein of the 17S U2 snRNP from human (HeLa) cells as being related both immunologically and functionally to the PRP9 protein of the yeast *S. cerevisiae*. Our evidence for this is, in summary, as follows.

(*i*) Each of these proteins associates with its cognate U2 snRNP, and in each case the association is salt-sensitive. The 60-kDa HeLa protein dissociates, along with eight others, under the high salt conditions sometimes employed to isolate U2 snRNPs, but it reassociates stably at low salt concentrations used for *in vitro* splicing reactions to give active U2 particles (28). Similar behavior has been documented for the PRP9 protein that appears also to associate with U2 snRNP in yeast cellular extracts in a salt-sensitive manner (34).

(*ii*) Antibodies against PRP9 recognize the 60-kDa protein (Fig. 1). This recognition is not strong, as immunoprecipitation did not occur, but the specificity of the reaction is clear (Fig. 1). This is confirmed by the fact that anti-PRP9 could be

affinity-purified on the 60-kDa protein with as good a result as on the PRP9 against which it had been raised.

(*iii*) In yeast, PRP9 is an essential splicing factor and appears to be crucial for the binding of U2 snRNP to pre-mRNA; in its absence the formation of stable prespliceosomes is abolished. As shown in this report, binding of anti-PRP9 IgG to the human 60-kDa protein prevents the addition of U2 snRNP to the spliceosome, resulting in a complete inhibition of mRNA splicing (Figs. 2 and 3). Thus, both in yeast and humans, PRP9 and its human homolog appear to act early in the spliceosome assembly pathway.

The observation that prespliceosome formation is inhibited when anti-PRP9 binds to the 60-kDa protein does not, of course, imply that the 60-kDa protein takes direct part in the binding of U2 snRNPs to pre-mRNA. However, such a direct participation has not yet been shown for PRP9 either. The possibility could well be envisaged that the 60-kDa protein helps to bring other 17S U2 snRNP proteins to a region of U2 snRNP critical for the integration of U2 snRNP into prespliceosomes. Along these lines, our data would indicate that the broader domain of the U2 snRNP particle, encompassing the 60-kDa protein and its neighboring proteins, is important for the binding of U2 snRNP to pre-mRNA. One thing at least is clear: the size of the antibody's Fab fragment is not alone sufficient to inhibit prespliceosome formation, as the control experiment with anti-B″ protein shows (Fig. 2).

The importance for U2 snRNP function of the 60-kDa protein and probably the additional 17S U2-specific proteins as well is further underlined by our finding that purified 17S U2 snRNPs but not 12S U2 snRNPs were capable of restoring the splicing reaction in nuclear extracts where the endogeneous U2 snRNP had been functionally neutralized by anti-PRP9 antibodies (Fig. 3). This result is all the more important as it provides initial experimental evidence that the 17S form of U2 snRNP with its ≈20 proteins represents the functional form of U2 snRNP in the spliceosome. The availability of purified functionally active 17S U2 snRNP should ease considerably subsequent investigation in controlled preparations *in vitro* of the requirements of prespliceosomal formation.

The observation of immunological and functional similarity between yeast PRP9 and the human 60-kDa protein lends further weight to the assertion that the small nuclear RNAs and the proteins of the snRNPs have been phylogenetically highly conserved between yeast and humans. This was already indicated by the finding that the U5 protein PRP8 was the yeast counterpart to the human 200-kDa U5 protein (48). Once additional antibodies raised against yeast PRP proteins or human snRNP proteins will be available, the list of snRNP protein homologs between yeast and humans is expected to grow rapidly. Thus, besides PRP9, additional candidates for yeast counterparts of some of the proteins in the human 17S U2 snRNP could be PRP5, PRP11, or PRP21, which have been shown genetically to be functionally related to each other and to PRP9 (J. Abelson and S. Ruby, personal communication). Recently, by *in vivo* binding assays, interactions of PRP9, PRP11, and SPP91 (identical to PRP21; J. Abelson, personal communication) proteins have been identified and characterized (P.L. and C. Chapon, unpublished results). It is clear that the combination of yeast and human splicing systems should improve considerably our understanding of the biochemistry of the mRNA splicing reaction.

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