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Specific protein–protein interactions between the essential mammalian spliceosome-associated proteins SAP 61 and SAP 114

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ABSTRACT **Spliceosome-associated proteins (SAPs) 61, 62, and 114 can be UV-crosslinked to pre-mRNA in purified spliceosomal complexes and are associated with U2 small nuclear ribonucleoproteins (snRNP). These proteins also compose the essential heterotrimeric splicing factor SF3a, and products of yeast pre-mRNA processing genes *PRP9*, *PRP11*, and *PRP21* are their likely yeast counterparts. We report the isolation of a cDNA encoding SAP 61 and find that it is 30% identical in amino acid sequence to PRP9. A C-terminal Cys_2His_2 zinc-finger-like motif, which could be involved in the pre-mRNA binding, is the most highly conserved region of the protein. We also demonstrate specific protein–protein interactions between SAPs 61 and 114 and show that the N terminus of SAP 61 is required for this interaction. Significantly, the corresponding proteins are also known to interact in yeast: PRP9 interacts with PRP21, and the N-terminal portion of PRP9 is required. Previous work showed that direct interactions also occur between SAPs 62 and 114 and between the corresponding PRPs 11 and 21. These observations indicate that the specific protein–protein interactions that occur between the three prespliceosomal factors have been conserved between yeast and mammals.**

During spliceosome assembly *in vitro*, U1, U2, U4, U5, and U6 small nuclear RNAs (snRNAs) and a large number of proteins bind to pre-mRNA in a stepwise pathway. A series of discrete functional intermediate complexes in this pathway have been identified that assemble in the order E → A → B → C (for reviews, see refs. 1–3). Individual factors shown to be essential for assembly of the first ATP-dependent prespliceosomal complex A include U1 and U2 snRNAs and the protein splicing factors $U2AF^{65}$ (65-kDa U2 snRNP auxiliary factor), the serine–arginine (SR) family of splicing factors, and SF1 and SF3a (splicing factors 1 and 3a; for review, see refs. 1–3). The requirement for U1 snRNA and $U2AF^{65}$ is likely to be due to a prior requirement for these factors in complex E assembly, whereas U2 snRNA and SF3a are required for the E-to-A complex transition (4–8).

Purified complex A contains at least seven spliceosome-associated proteins (SAPs) that are associated with 17S U2 snRNP (5, 9, 10). Of these, three (SAPs 61, 62, and 114) also comprise the essential splicing factor SF3a (7, 8, 11). The other four (SAPs 49, 130, 145, and 155) are likely to be subunits of the essential splicing activity SF3b, which associates with SF3a and 12S U2 snRNP to generate a particle similar, if not identical, to 17S U2 snRNP (7, 8, 10). All three of the SF3a subunits, as well as SAPs 49, 145, and 155, UV-crosslink to pre-mRNA in purified prespliceosomes and spliceosomes, indicating that these factors directly contact pre-mRNA in these complexes (10).

In yeast, the pre-mRNA processing gene *PRP9* encodes a 60-kDa protein essential for complex A assembly (12). Antibodies to this protein specifically detect the 60-kDa component of mammalian 17S U2 snRNP, and mammalian extracts inactivated by this antibody can be reconstituted by the addition of purified 17S U2 snRNP (13). PRP9 antibodies also detect $SF3a^{60}$ and SAP 61, consistent with the fact that these proteins correspond to one another and to the 60-kDa 17S U2 snRNP protein (8, 11). Here we report the isolation of a cDNA encoding SAP 61.‖ Comparison of the deduced amino acid sequence of SAP 61 with the PRP9 sequence revealed that these proteins are 30% identical. We also show that SAP 61 participates in specific protein–protein interactions with SAP 114 and map a SAP 61–SAP 114 interaction domain on SAP 61 to the N terminus. Significantly, the N terminus of yeast PRP9 is required for specific protein–protein interactions with PRP21 (14, 15), the putative yeast homolog of SAP 114 (15–17). These observations and previous work (5, 7, 8–18) indicate that the functional activities of PRP9 and PRP11 are conserved in SAPs 61 and 114.

MATERIALS AND METHODS

SAP 61 cDNA Cloning. The sequence of two tryptic peptides from SAP 61 was obtained from affinity-purified spliceosomes fractionated on two-dimensional (2-D) gels (11). Antibodies to PRP9 were used for initial library screening. A plasmid encoding the glutathione *S*-transferase (GST)–PRP9 fusion protein was constructed by inserting an *Eco*RI restriction fragment containing amino acids 25–530 of the PRP9 protein into the *Eco*RI site of the bacterial expression vector pGEX-2T. For antibody production, the GST-PRP9 fusion protein was used to immunize rabbits (19). The PRP9 antiserum detects the GST-PRP9 fusion protein and a single band of 60 kDa in HeLa cell nuclear extracts (data not shown) (13). For library screening, the IgG fraction of PRP9 antisera (purified on protein A-Sepharose) was diluted 1:10 in TNT buffer (10 mM Tris·HCl, pH 8.0/150 mM NaCl/0.05% Tween-20) containing 5% nonfat dry milk and incubated with Y1090 bacterial lysate for 4 hr at room temperature. A λgt11 HeLa cDNA library (Clontech) was screened with the PRP9 antibodies absorbed with Y1090 bacterial lysate (20). Of 50 different positives 1 (clone C-40) contained a SAP 61 peptide. The C-40 insert was used to screen a Lambda ZAP human brain library (20), and clone C-B encoding the entire SAP 61 amino acid sequence except for the N-terminal 2 amino acids was isolated. The N-terminal sequence was obtained by PCR

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Abbreviations: SAP, spliceosome-associated protein; snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein; SF, splicing factor; *PRP* genes, pre-mRNA processing genes of yeast; GST, glutathione *S*-transferase; 2-D, two dimensional.

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‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. U08815).

amplification of a λGT10 fetal cardiac library using phage primers and primers that hybridize near the 5′ end of the C-B clone. A full-length SAP 61 cDNA was then constructed that contained the C-B clone and the 2 amino-terminal residues. Sequencing was carried out manually and on an Applied Biosystems model 370A automated DNA sequencer. Sequences were analyzed by using the University of Wisconsin Genetics Computer Group (GCG) software and the BLAST program (21).

Immunofluorescence, *in Vitro* Translation, RNA Blots, and Protein Interaction Blots. PRP9 IgG or SC35 monoclonal antibodies (diluted 1:100) were used for coimmunofluorescent labeling of HeLa cells. Detection was with fluorescein- or rhodamine-conjugated secondary antibodies (22). *In vitro* translation was carried out by incubating the SAP 61 cDNA clone in reticulocyte lysates (Promega TNT). Northern blots of poly(A)$^+$ RNA were probed with ^{32}P-labeled random-primed C-40 cDNA (20). Plasmids encoding the GST-SAP 61 fusion proteins were constructed by subcloning the protein coding sequence from the SAP 61 cDNA clone (nucleotides 15–1520) into the bacterial expression vector pGEX-2TK. ΔNT and ΔZF GST-SAP 61 mutants were obtained by deletion of N-terminal residues 1–92 or zinc finger (ZF) residues 398–501 of the SAP 61 protein, respectively. The ZF GST-SAP 61 mutant contained amino acids 399–501 of SAP 61. For protein–protein interaction blots ("Far-Western" blots), the fusion proteins were ^{32}P-labeled by treatment with kinase and used to probe either a blot containing total HeLa nuclear extract or a 2-D electrophoresis blot containing spliceosomes (11).

RESULTS

To isolate a cDNA clone encoding the apparent mammalian homolog of yeast PRP9, we prepared polyclonal antibodies to a bacterially synthesized GST-PRP9 fusion protein and used these antibodies to screen a λGT11 expression library. Of 50 distinct cDNA clones isolated, 1 (clone C-40) bore significant homology to PRP9. To verify that the C-40 cDNA clone encoded SAP 61, we isolated SAP 61 from 2-D gels of purified spliceosomes and obtained sequences for two SAP 61 peptides (11). One of the two peptides was identified in the predicted amino acid sequence encoded by the C-40 cDNA. This cDNA was then used to isolate a full-length cDNA (SAP

A

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  1 M E T I L E Q Q R R Y H E E K E R L M D V M A K E M L T K K S T L R D Q I N S D
 41 H R T R A M Q D R Y M E V S G N L R D L Y D D K D G L R K E E L N A I S G P N E
 81 F A E F Y N R L K Q I K E F H R K H P N E I C V P M S V E F E E L L K A R E N P
121 S E E A Q N L V E F T D E E G Y G R Y L D L H D C Y L K Y I N L K A S E K L D Y
161 I T Y L S I F D Q L F D I P K E R K N A E Y K R Y L E M L L E Y L Q D Y T D R V
201 K P L Q D Q N E L F G K I Q A E F E K K W E N G T F P G W P K E T S S A L T H A
241 G A H L D L S A F S S W E E L A S L G L D R L K S A L L A L G L K C G G T L E E
281 R A Q R L F S T K G K S L E S L D T S L F A K N P K S K G T K R D T E R N K D I
321 A F L E A Q I Y E Y V E I L G E Q R H L T H E N V Q R K Q A R T G E E R E E E E
361 E E Q I S E S E S E D E E N E I I Y N P K N L P L G W D G K P I P Y W L Y K L H
401 G L N I N Y N C E I C G N Y T Y R G P K A F Q R H F A E W R H A H G M R C L G I
441 P N T A H F A N V T Q I E D A V S L W A K L K L Q K A S E R W Q P D T E E E Y E
481 D S S G N V V N K K T Y E D L K R Q G L L
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B

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SAP 61   METILEQQRRYHEEKERLMD.....VMAKEMLTKKSTLRD.....QINSDHRTRAMQDRYMEVSGNLRDLYDDKDGLRKEE   71
PRP9     MN.LLETRRSLLEEMEIIENAIAERIQRNPELYYHYIQESSKVFPDTKLPRSSLIAENKIYKFKKVKRKRKQIILQQH..EINIFLRDYQEKQQTFNKIN   97

SAP 61   ..LNAISGPNEFAEFYNRLKQI.KEFHRKHPN.EICVPMSVEFEELLKARENPSEEAQNLVE.....FTDEEGYGRYLDLHDCYLKYINLKASEK  157
PRP9     RPEETQEDDKDLPNFERKLQQLEKELKNEDENFELDINSKKDKYALFSSSSDPSRRTNILSDRARDLDLNEIFTRDEQYGEYMELEQFHSLWLNVIKRGD  197

SAP 61   LDYITYLSIFDQLFD.....IPKERKNAEYKRYLEMLLEYLQDYTDRVKPLQDQNELFGKIQAEFEKKWENGTFPGWPKETSSALTHAGAHLDLSAFS  250
PRP9     CSLLQFLDILELFLDDEKYLLTPPMDRKNDRYMAFLLKLSKYVETFFFKSYALLDAAAVENLIKSDFEHSYCRGSLRSEAKGI.....YCPFC  285

SAP 61   SWEELASLGLDRLKSALLALGLKCGGTLEERAQRLFSTKGKSLESLDTSLFAKNPKSKGTKRDTERNKDIAFLEAQIYEYVEILGEQRHLTHENVQRKQA  350
PRP9     SR.....WFKTS.....SVFESHLVGKIHKKNESKRRNFVYSEYKLHRYLKYLNDEFSRTRSFVERKLA  344

SAP 61   RTGEER.....EEEEEEQISESESEDEENEIIYNPK..NLPLGWDGKPIPYWLYKLHGLNINYNCEICGNYTYRGPKAFQRHFAEW  429
PRP9     FTANERMAEMDILTQKYEAPAYDSTEKEGAEQVDGEQRDGQLQEEHLSGKSFDMPLGPDGLPMPYWLYKLHGLDREYRCEICSNKVYNGRRTFERHFNEE  444

SAP 61   RHAHGMRCLGIPNTAHFANVTQIEDAVSLWAKLKLQ.....KASERWQP.DTEEEYEDSSGNVVNKKTYEDLKRQGLL  501
PRP9     RHIYHLRCLGIEPSSVFKGITKIKEAQELWKNMQGQSQLTSIAAVPPKPNPSQLKVPTELELEEEDEEGNVMSKKVYDELKKQGLV  530
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FIG. 1. Deduced amino acid sequence of SAP 61 and comparison with PRP9. (*A*) The zinc finger motif is boxed, and the cysteine and histidine residues in this motif are shown in boldface letters. The two peptides obtained from SAP 61 tryptic digestion are underlined. (*B*) The BESTFIT program was used to find the optimal alignment between the two proteins. Identities are indicated by a solid line, and the similarities, by a colon. The leucine zipper (residues 109–130) and zinc finger (residues 282–304) motifs that are present in PRP9 but not in SAP 61 are underlined. The zinc finger motif is boxed and the cysteines and histidines in this motif are shown in boldface letters. The asterisks indicate residues that are altered in temperature-sensitive alleles of PRP9; these residues are conserved according to the SAP 61 alignment. Amino acids are grouped as follows: (Y F), (L I V), (S T), (K R), (D E N Q), (A G).

61 cDNA) that contained both of the SAP 61 peptides. The deduced amino acid sequence of the SAP 61 cDNA is 501 residues and is predicted to encode a protein of ≈60 kDa (shown in Fig. 1*A*). We note that characterization of several partial cDNAs that hybridized to the SAP 61 cDNA revealed one that contained a number of N-terminal amino acid differences from SAP 61 and may represent a variant of SAP 61.

Evidence that we had isolated the full-length cDNA encoding SAP 61 was obtained by translating the cDNA clone *in vitro* and then fractionating the translation product on a 2-D gel. The translation product precisely cofractionated with native SAP 61 present in purified spliceosomes (Fig. 2*A*). Moreover, a translation initiation consensus sequence is located immediately upstream of the methionine at position 1 (Fig. 1*A*), indicating that we had identified the correct initiator methionine (data not shown). Northern blot analysis of HeLa cell mRNA using the C-40 cDNA clone as a probe detected two RNAs of 3.2 and 2.1 kb (Fig. 2*B*); it is possible that these RNAs result from alternative polyadenylylation, as two polyadenylylation signals separated by about 1 kb are present in independent cDNA clones containing the same open reading frames (data not shown). An alignment of the predicted amino acid sequence generated from the SAP 61 cDNA clone and the yeast PRP9 sequence is shown in Fig. 1*B*. Comparison of the two proteins shows that they are 30% identical and 56% similar. The most conserved regions are confined to the C terminus of the proteins, where a zinc-finger-like motif is present (see *Discussion*). Both SAP 61 and PRP9 are acidic with estimated isoelectric points of 5.27 and 6.41, respectively. In addition, the two proteins are highly hydrophilic (23) and contain a large number of charged residues (33% in SAP 61, 33% in PRP9).

PRP9 antibodies were used to determine the intranuclear localization of SAP 61 (Fig. 3). This analysis revealed that SAP 61 is present in 20–50 discrete regions (Fig. 3*A*), and colocalization studies using antibodies to the essential splicing factor SC35 (ref. 24; Fig. 3*B*) revealed that these regions correspond to the nuclear speckles that are enriched in splicing components (ref. 24; see ref. 25 for a review). The U2 snRNP-associated protein, B″, also colocalizes with SAP 61 and SC35 (ref. 26; also data not shown). SAP 61 lacks an arginine–serine (RS) domain which is present on many of the proteins present in the nuclear speckles (see ref. 25 for a review). Thus, either SAP 61 associates with proteins containing an RS domain or there are other signals for nuclear speckle localization that are present in SAP 61 or in proteins associated with SAP 61.

Previous work has shown that antibodies to PRP9 inhibit splicing in HeLa cell nuclear extracts (13), consistent with the fact that SF3a^{60} (SAP 61) is essential for complex A assembly (7). We also found that PRP9 antibodies affinity-purified against a GST-SAP 61 fusion protein but not preimmune sera block splicing and spliceosome assembly in a dose-dependent manner (data not shown). However, we were unable to reconstitute spliceosome assembly by the addition of purified SAP 61 fusion protein. This is most likely because SAP 61 is tightly associated in SF3a. Thus, reconstitution studies may require the depletion and addition of all three subunits of SF3a or possibly the entire 17S U2 snRNP (13).

SAPs 61, 62, and 114, together with three other U2 snRNP components, can be specifically UV crosslinked to pre-mRNA in purified A and B complexes (10). To determine whether SAP 61 alone can bind to pre-mRNA, we carried out UV-crosslinking studies using GST-SAP 61 or *in vitro* translated SAP 61. We were unable to obtain any UV crosslinking with these proteins. These data are consistent with the finding that purified SF3a fails to crosslink to RNA (7). Thus, it is possible that SAP 61 only binds to RNA in a crosslinkable manner in the context of the spliceosome.

To identify SAP 61 protein–protein interactions, we used ^{32}P-labeled GST-SAP 61 to probe total spliceosomal proteins immobilized on a filter after 2-D gel electrophoresis (Fig. 4*A*). In this analysis, the SAP 61 probe detected only SAP 114 (Fig. 4*A*, prespliceosome). Previous work showed that SAP 62 also detects SAP 114 immobilized on a filter (11). Moreover, SAP 114 was not detected with other probes, such as SAP 49, U2AF^{65}, or SC35 (11). We conclude that SAPs 61 and 62 each interact independently with SAP 114 via protein–protein interactions. This interaction is specific because the 114-kDa band was the only major band detected in total nuclear extracts by the SAP 61 probe (Fig. 4*A*, lanes NE). When greater amounts of nuclear extract were loaded on the gel, several minor bands around 30 kDa could be detected with the SAP 61 probe (Fig. 4*A*, lane NE, 8 μl). These proteins were not detected in the purified spliceosome, and further work is needed to determine their significance.

To identify the domain in SAP 61 that interacts with SAP 114, deletion mutants of SAP 61 were used as probes in analysis of SAP 61–SAP 114 interaction in total nuclear extract. The structures of the mutant SAP 61 fusion proteins are shown in Fig. 4*B*, and the ^{32}P-labeled proteins are shown in Fig. 4*C*. With all but the ZF probe, the 30-kDa bands that were faintly detected by full-length SAP 61 in Fig. 4*A* were efficiently detected (Fig. 4*C Lower*). Our data indicate that these bands were more prominent in this experiment because

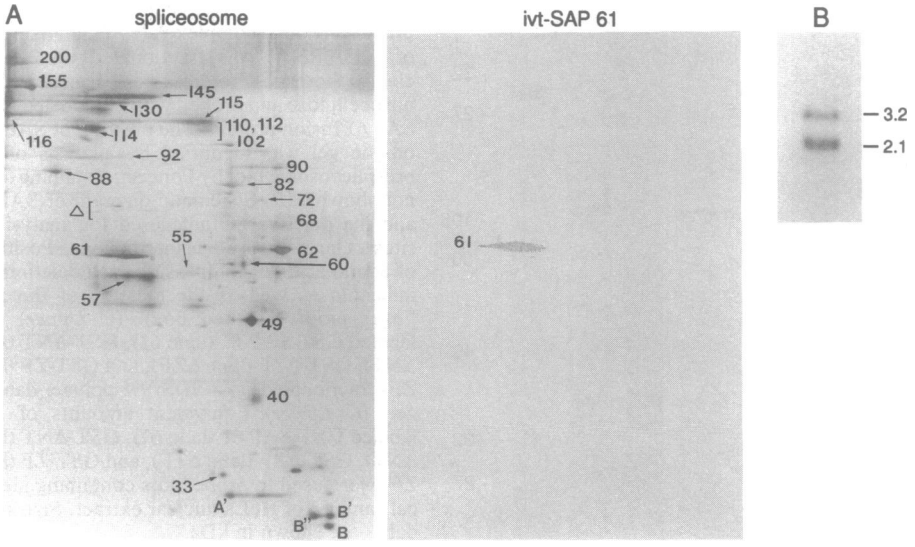

FIG. 2. Cofractionation of native and *in vitro* translated SAP 61 and Northern blot analysis. (*A*) Affinity-purified spliceosomes or the *in vitro* translation product generated from the SAP 61 cDNA was fractionated on 2-D gels. The abundant spliceosomal proteins are designated. (*B*) Poly(A)$^+$ RNA (10 μg) was isolated from HeLa cells, transferred to a membrane, and probed with ^{32}P-labeled SAP 61 cDNA. The sizes of the two bands in kb (estimated from molecular markers on another gel) are indicated.

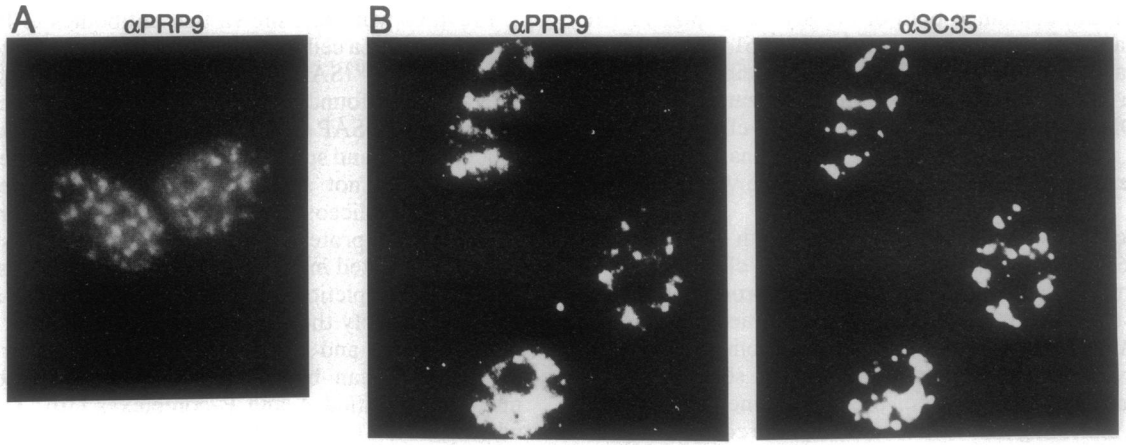

FIG. 3. SAP 61 colocalizes with the essential splicing factor SC35 in nuclear speckles. (*A*) Indirect immunofluorescent staining of HeLa cells was carried out with PRP9 IgG and detected with fluorescein-conjugated anti-rabbit IgG. (*B*) Coimmunolabeling of Hela cells was carried out with PRP9 polyclonal antibodies (α-PRP9) and SC35 monoclonal antibodies (αSC35). Detection was with fluorescein-(PRP9)- and rhodamine (SC35)-conjugated secondary antibodies. Color photographs were processed by using PHOTOSHOP software (Adobe Systems, Mountain View, CA) and then converted to black and white.

the high molecular weight proteins were less efficiently transferred to the nitrocellulose. In any case, deletion of the C terminus of SAP 61, including the zinc finger region, has no effect on the interaction of SAP 61 with SAP 114 (Fig. 4*C*, lane ΔZF). Consistent with this observation, the C terminus of SAP 61 containing the zinc finger region alone does not detect SAP 114 (Fig. 4*C*, lane ZF). Thus, the SAP 61 C terminus containing the zinc finger motif is not required for interaction with SAP 114. In contrast, deletion of the N-terminal 92 amino acids from SAP 61 abolishes the SAP 61–SAP 114 interaction (Fig. 4*C*, lane ΔNT). We conclude that the N terminus of SAP 61 contains a region required for the SAP 61–SAP 114 interaction.

DISCUSSION

We report the isolation of a cDNA encoding SAP 61, a component of the mammalian prespliceosomal complex A. This protein bears significant similarity at the amino acid level to a yeast prespliceosomal component PRP9 (30% identity, 56% similarity). These data provide additional support for the proposal that the mammalian prespliceosome components SAPs 61, 62, and 114 are the functional homologs of the yeast prespliceosome components PRP9, PRP11, and PRP21, respectively (8, 11, 13, 17, 18). This proposal originated from the observations that SAP 62 bears significant similarity at the amino acid level to PRP11 (11, 27), that PRP9, PRP11, and PRP21 interact with one another both genetically and physically (15, 17, 18), and that SAPs 61, 62, and 114 interact with one another in the heterotrimeric splicing factor SF3a (7, 8, 11). Finally, PRP9, PRP11, and PRP21 are essential for complex A assembly in yeast (12, 15–18), and SF3a is essential for complex A assembly in mammals (7).

Our data show that there are specific protein–protein interactions between SAPs 61 and 114, and previous work showed that SAP 62 interacts directly with SAP 114 (11). In yeast, the corresponding protein–protein interactions have

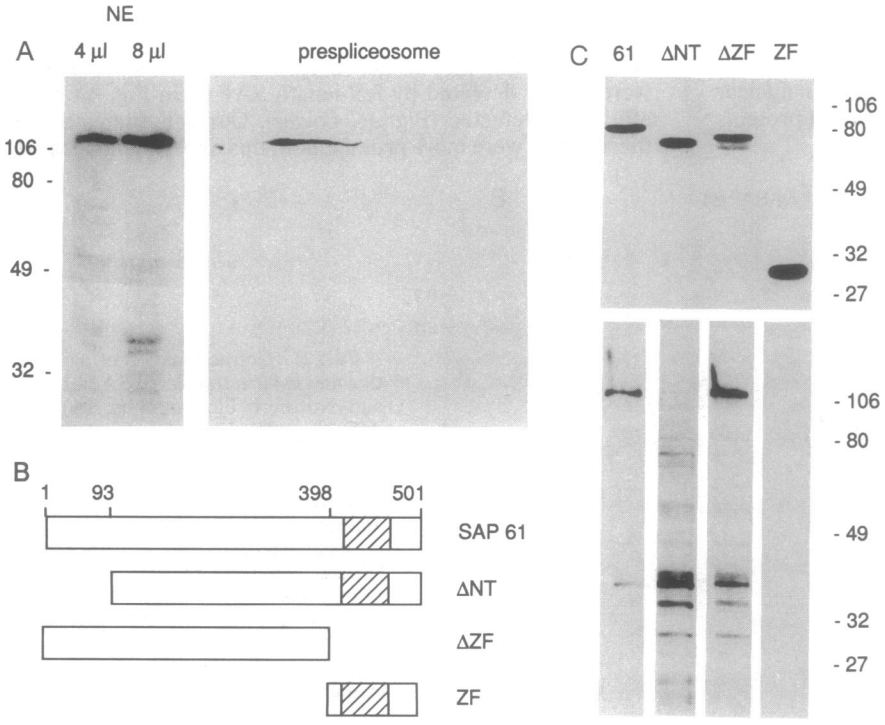

FIG. 4. Protein–protein interactions between SAPs 61 and 114. (*A*) Total nuclear extract (4 and 8 μl, as indicated) was fractionated on a SDS/9% polyacrylamide gel, and total prespliceosomal complex A (assembled on 1 μg of Ad3′ RNA) was fractionated by 2-D gel electrophoresis. Proteins were transferred to nitrocellulose and probed with ^{32}P-labeled GST-SAP 61 fusion protein. The position of SAP 114 on the gel was identified by analysis of the prespliceosome blot by Ponceau S staining (data not shown). (*B*) Schematic diagram of SAP 61 and the deletion mutants used for analysis of sites of protein–protein interactions. Positions of amino acids encompassing each deletion are indicated as well as the location of the zinc finger motif (hatched box). (*C Upper*) ^{32}P-labeled GST-SAP 61 (lane 61), GST-ΔNT (lane ΔNT), GST-ΔZF (lane ΔZF), and GST-ZF (lane ZF) fractionated on a SDS/9% polyacrylamide gel. (*C Lower*) Equivalent amounts of ^{32}P-labeled GST-SAP 61 (lane 61), GST-ΔNT (lane ΔNT), GST-ΔZF (lane ΔZF), and GST-ZF (lane ZF) were used to probe blots containing identical samples of HeLa nuclear extract. Size standards are shown in kDa.

been detected; PRP9 interacts with PRP21 (15) and PRP11 interacts with PRP21 (18). The PRP9–PRP21 interaction involves N-terminal glutamic acid and glycine residues in PRP9 (ref. 15; positions 78 and 177), and these residues are conserved in SAP 61 according to our alignment (see Fig. 1*B*). Significantly, our data indicate that, as in yeast, the N-terminal region of SAP 61 (amino acids 1–92) is required for the interaction with SAP 114. Although the interaction domain between SAP 62 and 114 remains to be determined, the PRP11–PRP21 interaction involves a residue in the C-terminal region of PRP11 that is conserved in SAP 62 (11, 18). The observations that the corresponding SAP and PRP homologs interact with one another and that the SAP 61–SAP 114 interaction requires the SAP 61 N terminus (as in the PRP9–PRP21 interaction) indicates that the protein–protein interactions of the yeast and mammalian heterotrimers are conserved.

In addition to interacting with PRP21, data from the yeast two-hybrid system indicate that PRP9 forms a homodimer (15). A region of about 45 amino acids which contains an essential C_2H_2 zinc-finger-like motif is required for the homodimerization (amino acids 409–455 in PRP9; Fig. 1*B*) (15). This zinc finger motif is conserved between PRP9 and SAP 61, including the spacing between the cysteine and histidine residues (see Fig. 1*B*). However, we have not detected homodimer formation with SAP 61 by analysis of protein–protein interactions (Fig. 4*A*) or in solution (data not shown). Thus, it is possible that this interaction is only detectable by assays such as the yeast two-hybrid system. PRP9 contains a leucine zipper domain and an additional zinc finger motif that are not present in SAP 61 (underlined in Fig. 1*B*). The role of the leucine zipper is not known, and only the first histidine in the zinc finger motif is critical for PRP9 function (14). The latter observation is consistent with the lack of conservation of the zinc finger domain in SAP 61.

Zinc finger-like motifs similar to those in PRP9/SAP61 are found in other proteins involved in RNA splicing, including PRP6, PRP11, and the mammalian U1 snRNP protein U1C (14, 27, 28). The zinc finger motif in PRP11 is conserved in its apparent mammalian counterpart SAP 62 (11, 27). The role of these motifs is not yet known. Although one possible function is protein–protein interactions, it is not clear whether the zinc finger motif itself or the region surrounding the zinc finger motif is required for the homodimerization of PRP9 (15). Both SAPs 61 and 62 can be UV crosslinked to pre-mRNA in purified prespliceosomes and spliceosomes (10). As zinc finger domains are known to be involved in nucleic acid binding, it is possible that the zinc-finger-like motifs in these proteins mediate the RNA binding. Indeed, genetic studies in yeast show that the 45-amino acid region containing the zinc finger motif in PRP9 interacts with another factor (designated "X"), in addition to being required for homodimerization (15). It is possible that this factor is a pre-mRNA sequence.

SAP 61 can be UV-crosslinked to pre-mRNA in purified spliceosomal complexes (10), but no UV crosslinking is detected with a GST-SAP 61 fusion protein with *in vitro* translated SAP 61 or with purified SF3a (7). These observations indicate that SAP 61 must associate with components other than SF3a in the spliceosome to contact pre-mRNA in a UV-crosslinkable manner.

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