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A new cyclophilin and the human homologues of yeast Prp3 and Prp4 form a complex associated with U4/U6 snRNPs*

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ABSTRACT

We have purified three new human U4/U6-snRNP proteins from HeLa cells. The three proteins formed a tightly bound complex and behaved as a single species throughout the purification. All three proteins have been identified by peptide sequencing, and full-length cDNA sequences have been obtained for all of them. Two of the proteins are homologues of the *Saccharomyces cerevisiae* splicing factors Prp3 and Prp4, and the third protein is a cyclophilin. Both the human and *S. cerevisiae* Prp4 proteins have seven repeats of the WD motif and likely fold into structures very similar to those of the beta subunits of G proteins. The human Prp3 protein is highly basic and is closely related to *S. cerevisiae* Prp3 only in its carboxyl-terminal half. The human homologues of Prp3 and Prp4 are part of a stable complex in the absence of RNA. The third protein in the complex is a new cyclophilin. Cyclophilins have been proposed to act as chaperones in a variety of cellular processes, and we discuss some possible roles of this U4/U6 snRNP-associated cyclophilin.

Keywords: pre-mRNA splicing; USA-CyP; WD motif

INTRODUCTION

The U6 snRNA is thought to play a central role in splicing catalysis (reviewed in Madhani & Guthrie, 1994; Nilsen, 1994; Ares & Weiser, 1995). The U6 snRNP associates strongly with the U4 snRNP via base pairing of the RNAs (Bringmann et al., 1984; Hashimoto & Steitz, 1984). In turn, the U4/U6 snRNP binds to the U5 snRNP (Lossky et al., 1987; Black & Pinto, 1989), and it is the U4/U6·U5 particle that binds to the pre-spliceosome (Cheng & Abelson, 1987; Konarska & Sharp, 1987). The stable association of U4 and U6 is disrupted prior to the first catalytic step of splicing, and U4 appears to leave the spliceosome (Blencowe et al., 1989; Yean & Lin, 1991).

Although some of the proteins specific to the U1, U2, and U5 snRNPs in humans have been identified and characterized, identification of proteins associated specifically with the U4, U6, or U4/U6 snRNP has lagged (reviewed in Will & Lührmann, 1997). Given the cen-

tral role of the U4/U6 snRNP in splicing, the identification and characterization of these proteins is critical for unraveling the mechanism of splicing. A patient antiserum was used to identify a 150-kDa protein that is present in a small fraction of U4/U6 snRNPs and is also associated with U2 (Okano & Medsger, 1991; Blencowe et al., 1993); further characterization of the 150-kDa protein has not been reported. Two other proteins with molecular weights of 60 kDa and 90 kDa were found associated with the U4/U6 snRNP (using the antiserum against the 150-kDa protein) and also in assembled spliceosomes (Gozani et al., 1994); these may be the same proteins seen in U4/U6·U5 snRNPs (Behrens & Lührmann, 1991). Another patient antiserum reacts against a 120-kDa protein apparently specific to U4/U6 (Fujii et al., 1992). Several proteins have been shown to be part of the purified U4/U6·U5 snRNP (Behrens & Lührmann, 1991; Lauber et al., 1996), and some of these may also be part of the U4/U6 snRNP.

Previously, we identified a 55-kDa protein that is part of both the U4/U6 and U4/U6·U5 snRNPs (Horowitz & Krainer, 1997). Antibodies raised against hPrp18, a protein required for the second step of pre-mRNA splicing, crossreacted with a human 55-kDa protein. The 55-kDa protein was present in purified snRNPs

*This paper is dedicated to the memory of Tadaatsu Goto.

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and co-sedimented with the U4/U6 and U4/U6·U5 snRNPs in glycerol gradients. Whether this protein is the same as the 60-kDa U4/U6 (Gozani et al., 1994) or 60-kDa U4/U6·U5 (Behrens & Lührmann, 1991) proteins was not known.

In *Saccharomyces cerevisiae*, five U4/U6-specific proteins are known (Banroques & Abelson, 1989; Petersen-Björn et al., 1989; Abovich et al., 1990; Shannon & Guthrie, 1991; Cooper et al., 1995). Two of these proteins, Prp4 and Prp3, are germane to this study. Prp4 is a component of the U4/U6 and U4/U6·U5 snRNPs (Banroques & Abelson, 1989; Petersen-Björn et al., 1989) and interacts primarily with the U4 snRNP (Fabrizio et al., 1989; Bordonné et al., 1990; Xu et al., 1990). Prp4 has a WD-repeat motif, suggesting that it may be involved in protein-protein interactions (Dalrymple et al., 1989; Neer et al., 1994). Prp4 is required for the first step of splicing in vivo (Rosbash et al., 1981; Hu et al., 1994; Maddock et al., 1994) and in vitro (Lustig et al., 1986; Banroques & Abelson, 1989; Petersen-Björn et al., 1989). Antibodies against Prp4 halt spliceosome formation at complex B, before the entry of the U4/U6·U5 snRNP into the spliceosome (Banroques & Abelson, 1989). Prp4 is part of the A2-1 complex (which includes the U4/U6 and U5 snRNPs), but not the A1 complex (which lacks U4) (Tarn et al., 1993). Prp4 may play a role in the conformational changes in the spliceosome that accompany the A2-1 to A1 transition (Ayadi et al., 1997).

Prp3, like Prp4, is a component of the U4/U6 and U4/U6·U5 snRNPs (Anthony et al., 1997). Prp3 is required for the first step of splicing (Rosbash et al., 1981; Lustig et al., 1986), and, in heat-inactivated extracts from *prp3* strains, the transition from complex B (pre-spliceosome) to complex A1 (spliceosome) is blocked (Anthony et al., 1997). Overexpression of Prp3 protein suppresses some mutant alleles of *PRP4*, hinting that Prp3 and Prp4 may interact (Last et al., 1987; Hu et al., 1994).

Cyclophilins are a highly conserved family of proteins found in abundance in every organism (reviewed in Galat & Metcalfe, 1995; Luban, 1996; Marks, 1996). The first cyclophilin was identified based on its high affinity for the immunosuppressive drug cyclosporin A (Handschemacher et al., 1984). It was found subsequently that cyclophilins catalyze the *cis-trans* isomerization of proline residues within peptides (Fischer et al., 1989; Takahashi et al., 1989). All eukaryotes possess multiple cyclophilins; there are 7 in *S. cerevisiae* (Duina et al., 1996), at least 11 in *Caenorhabditis elegans* (Page et al., 1996), and at least 10 in humans (Galat & Metcalfe, 1995; Wu et al., 1995; Yokoyama et al., 1995; Marks, 1996; Mi et al., 1996; Nestel et al., 1996). The first cyclophilins found were 18-kDa proteins, each with a conserved central domain of ~110 amino acids (Trandinh et al., 1992), but, more recently, larger proteins that contain cyclophilin domains have been identified

(Kieffer et al., 1992; Anderson et al., 1993; Wu et al., 1995; Yokoyama et al., 1995; Duina et al., 1996; Nestel et al., 1996).

The cellular functions of the cyclophilins have been difficult to establish (Luban, 1996; Marks, 1996). Isomerization of X-Pro bonds is often a rate-limiting step in protein folding; proline isomerases (rotamases) facilitate the folding of proteins in vitro and have therefore been thought to play an important role in protein folding in vivo (Fischer et al., 1989; Gething & Sambrook, 1992; Rutherford & Zuker, 1994). A different view of the role of cyclophilins, in which they serve as chaperones and the rotamase activity is incidental, has emerged more recently (Schreiber & Crabtree, 1992).

We have used the crossreactivity of the 55-kDa protein as the assay in a purification of the 55-kDa protein. We find that the protein is part of a stable complex that includes proteins of 90 kDa and 18 kDa. By peptide sequencing, we have identified all three proteins, and we have determined their cDNA sequences. The 55-kDa protein is the human homologue of the *S. cerevisiae* Prp4 protein, and the 90-kDa protein is the homologue of the Prp3 protein. The 18-kDa protein is a new human cyclophilin.

RESULTS

Purification of the 55-kDa protein

Antibodies raised against hPrp18 crossreact with a 55-kDa protein from HeLa-cell extracts on western blots (Horowitz & Krainer, 1997) (see also Fig. 1B). The crossreactivity was the assay for the 55-kDa protein during the purification. Purification was begun from HeLa-cell nuclear extract, which contained the vast majority of the 55-kDa protein (data not shown). Nucleic acid was removed from the extract by precipitation with polyethyleneimine; the 55-kDa protein remained in the supernatant. The extract was fractionated by ammonium sulfate precipitation, and the 55-kDa protein precipitated between 25 and 35%. This fraction was loaded on a Poros HS column (sulfonic acid) and eluted with a salt gradient. A silver-stained gel and a western blot of the column fractions are shown in Figure 1. The western blot (Fig. 1B) shows that the 55-kDa protein eluted in a single, sharp peak around 650 mM NaCl. The 55-kDa protein, purified 150-fold on this column, can be identified readily in the silver-stained gel (Fig. 1A, fractions 28 and 29). The mobility of the protein was measured as 58,000, although we will continue to refer to the protein as the 55-kDa protein to be consistent with our previous work (Horowitz & Krainer, 1997). Three proteins co-chromatographed precisely with the 55-kDa protein on the HS column: a doublet of bands that migrate near 90 kDa (Fig. 1A), which could be either two distinct proteins or proteolyzed or modified forms of a single protein, and a third protein

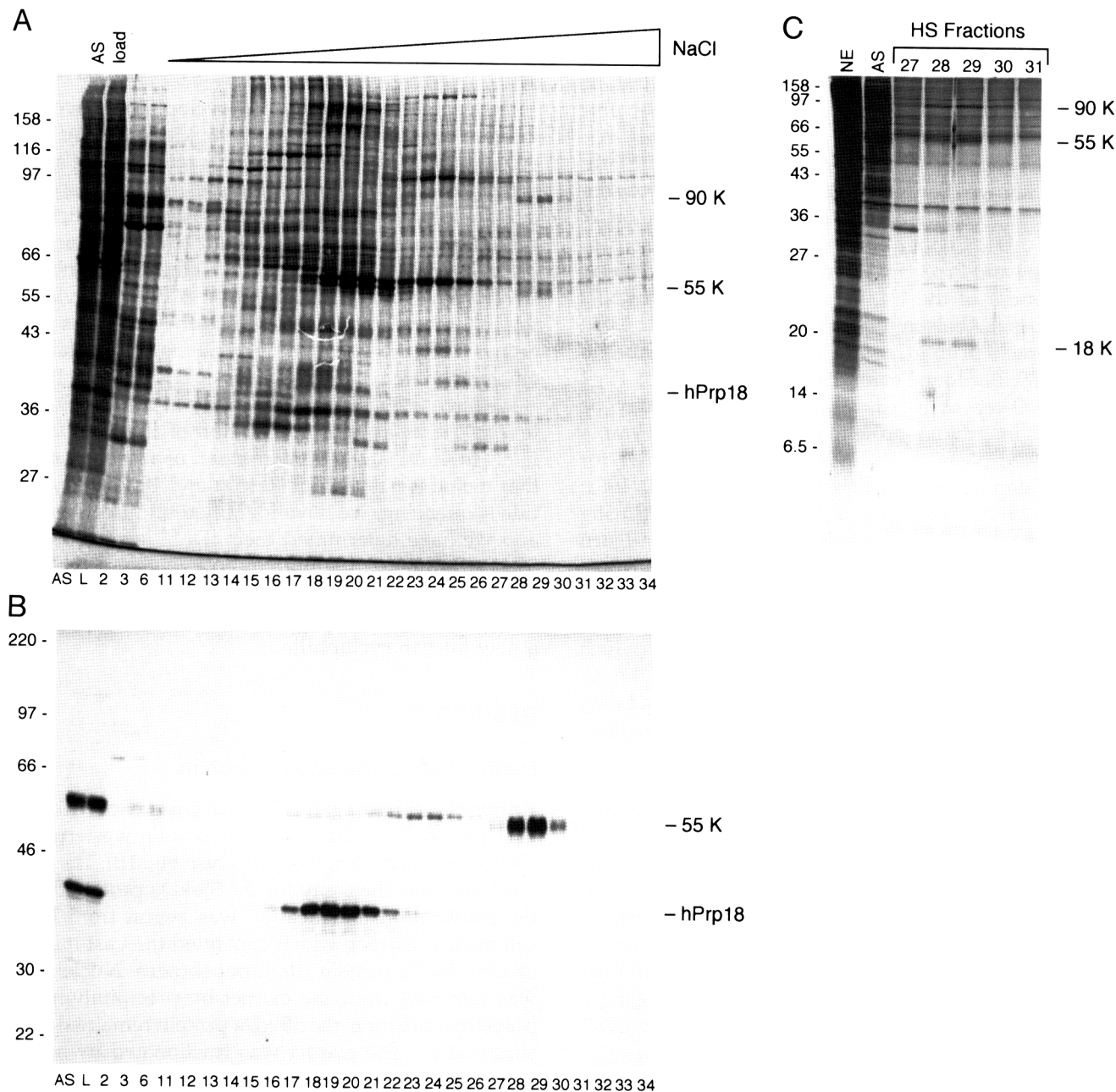


FIGURE 1. Silver-stained gels and western blot of HS column fractions. **A:** Fractions from a Poros HS (sulfonic acid) column were run on an 8% SDS-polyacrylamide gel, which was silver-stained. Positions of the 55-kDa protein, the 90-kDa protein(s), and hPrp18 are shown along the right; positions of the molecular weight markers are indicated along the left. The two lanes at the left show the ammonium sulfate fraction (before and after dialysis) that was loaded on the column. Fraction numbers for the gradient are indicated along the bottom of the gel, and the NaCl gradient is drawn schematically at the top. Fractions 2 and 3 were in the flow-through. **B:** Western blot of a gel run in parallel with that of panel A probed with antibodies raised against hPrp18. Superposition of panel B on panel A allowed clear identification of the 55-kDa protein on the silver-stained gel. Positions of the 55-kDa protein and hPrp18 are indicated along the right, and the positions of the markers are indicated along the left. Two proteins of about 60 kDa and 62 kDa crossreact with the antibody (fractions 17–19 and 22–24); this crossreaction is weak, because relatively large amounts of both of these proteins are seen in panel A. **C:** Fractions 27–31 from the HS column run on a 12.5% SDS-polyacrylamide gel to show the 18-kDa protein clearly. Nuclear extract and the ammonium sulfate pool are also shown. Positions of the 90-kDa, 55-kDa, and 18-kDa proteins, as well as those of the markers, are indicated.

of 18 kDa seen in the higher percentage gel shown in Figure 1C.

The peak fractions from the HS column were applied to a Poros heparin column, and the bound pro-

teins were eluted with a salt gradient (Fig. 2). The 55-kDa protein eluted sharply at 700 mM NaCl; most of the contaminating proteins were removed. As on the HS column, the 90-kDa and 18-kDa proteins co-

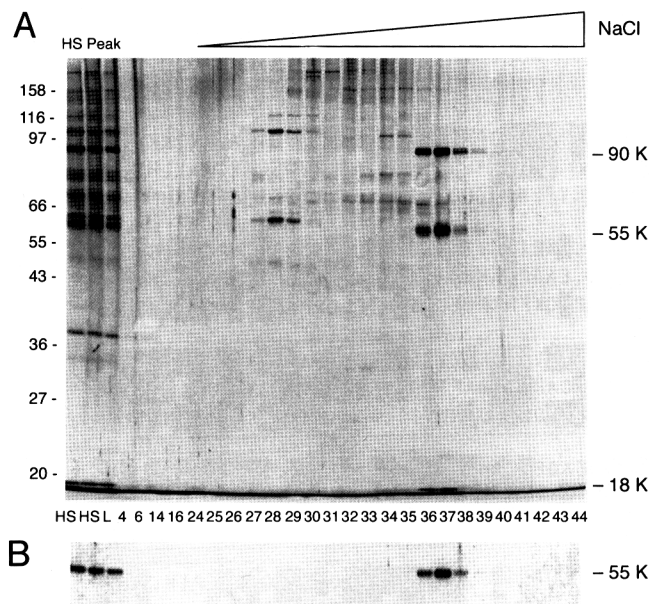


FIGURE 2. Silver-stained gel and western blot of heparin column fractions. **A:** Fractions from a Poros HE1 (heparin) column were run on an 8% SDS-polyacrylamide gel, which was silver-stained. Positions of the 55-kDa protein, the 90-kDa protein(s), and the 18-kDa protein are shown along the right. Positions of the markers are indicated along the left. Three lanes at the left show the HS peak pool before (lane 1) and after dialysis (lanes 2 and 3). Fraction numbers for the gradient are indicated along the bottom of the gel, and the NaCl gradient is drawn schematically at the top. Fractions 4, 6, 14, and 16 were all in the flow-through. **B:** A gel run in parallel with the gel in panel A was blotted and probed with antibody raised against hPrp18. The part of this blot that included the 55-kDa protein is shown, and the 55-kDa band is indicated. The remainder of the blot was blank. Fraction numbers are indicated at the top of the blot.

eluted exactly with the 55-kDa protein (Fig. 2A). The absorbances at 280 and 260 nm of the peak fractions showed that the purified proteins were essentially free of nucleic acid.

Two arguments suggest strongly that the 18-kDa, 55-kDa, and 90-kDa proteins are part of a single complex. First, they purify together on two different columns; on both columns, the elutions are sharp, and the ratios of the intensities of the bands in both Figures 1 and 2 show that the three proteins co-chromatograph exactly. The intensities of the bands are consistent with the complex containing equimolar amounts of each protein. Second, the elutions are at relatively high salt concentrations (600–750 mM), reducing the likelihood that nonspecific interactions account for their co-elution. Only a single peak of the 55-kDa protein was seen on either column (Figs. 1B, 2), implying that all the 55-kDa protein was bound in the complex.

Identification of the proteins by peptide sequencing

The peak fractions from the heparin column were concentrated by TCA precipitation (Hwang & Chu, 1996)

and separated by preparative SDS-PAGE. The two 90-kDa bands were not resolved on the preparative gel. The proteins were cleaved with *Achromobacter* protease I; the resulting peptides were separated by HPLC and sequenced. We sequenced three peptides from the 18-kDa protein, five peptides from the 55-kDa protein, and five peptides from the 90-kDa protein(s).

We used the peptide sequences for searches of the databases to identify the proteins' cDNAs. None of the proteins was found in GenBank. Searches of dbEST revealed some partial cDNA sequence for each of the proteins. For all the proteins, at least one sequence in the EST database appeared to represent a full-length clone. These full-length clones were obtained commercially and sequenced in full. The results of these searches and of the cDNA sequencing are described for each protein below.

The 55-kDa protein and Prp4

Searches of the EST databases with peptides from the 55-kDa protein yielded two, nonoverlapping sets of EST sequences. One set of three overlapping sequences (GenBank accession numbers AA179559, R19664, and N89150) was used to generate a provisional cDNA sequence. The translation of this sequence was strongly homologous to *S. cerevisiae* Prp4 (Banroques & Abelson, 1989; Petersen-Björn et al., 1989) and the *C. elegans* homologue of Prp4 (from clone CEC36B1, gene product C36B1.5). Translation of the second set of two EST sequences (AA315321 and AA347427) gave a peptide that was homologous to the amino-terminal portion of *C. elegans* Prp4, suggesting that these EST clones might contain the full-length cDNA for the 55-kDa protein. Both clones were obtained and clone AA315321 was sequenced entirely; the sequence includes all the sequence from the first set of ESTs.

The 2,187-bp sequence of AA315321 (deposited in GenBank with accession number AF016369) appears to contain the entire coding region of the 55-kDa protein, which we have renamed hPrp4. The clone encodes a 522-amino acid protein with a molecular weight of 58,500, in excellent agreement with the size measured by SDS-PAGE; the protein is nearly neutral, with a calculated pI of 7.5. The translated sequence of hPrp4 includes nearly perfect matches (72 of 76 amino acids) to the sequences of all five peptides that we had obtained from the purified protein (detailed in Materials and Methods), showing that the 55-kDa protein is indeed hPrp4.

The sequences of hPrp4, cPrp4 (*C. elegans*), and *S. cerevisiae* Prp4 are homologous throughout their lengths. An alignment is shown in Figure 3; percents of identity and similarity are listed in Table 1. The Prp4 protein sequences are dominated by seven repeats of the WD motif (Dalrymple et al., 1989; Neer et al., 1994); each repeating unit is boxed in Figure 3, and we refer

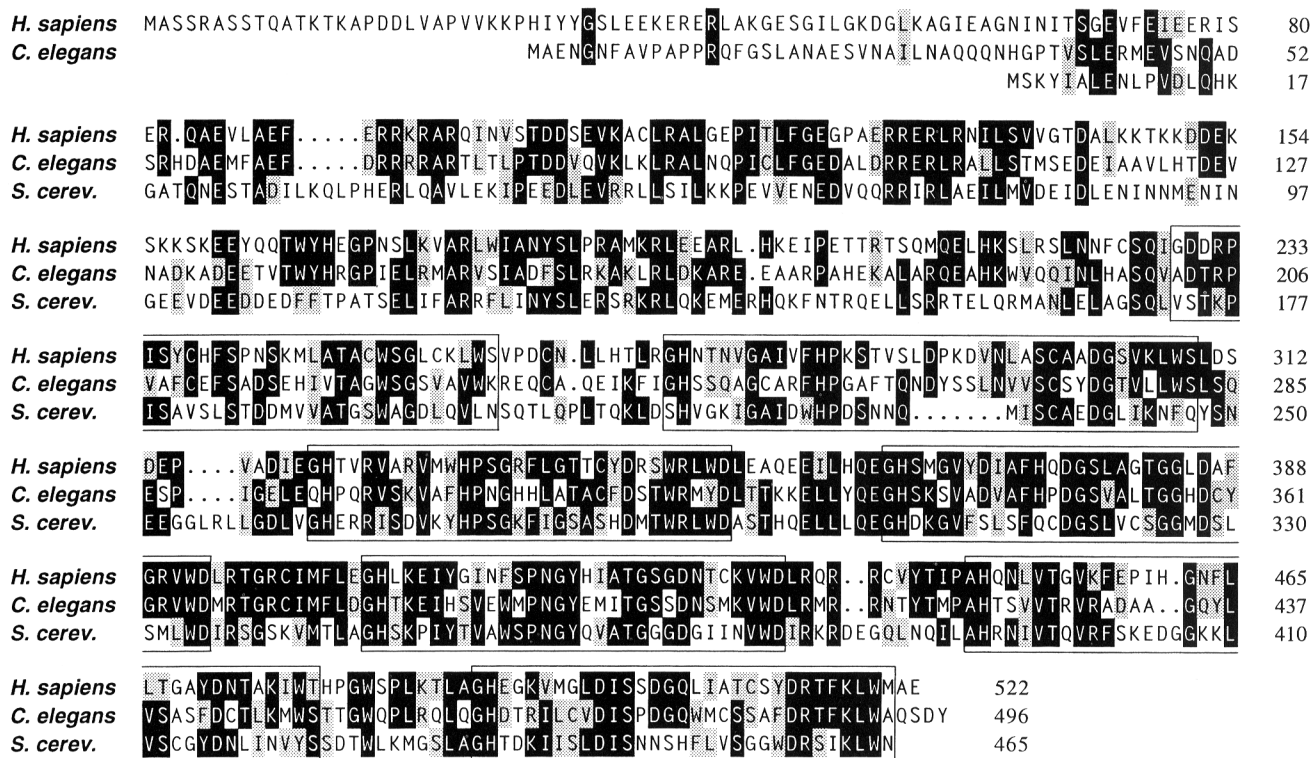


FIGURE 3. Alignment of human, nematode, and yeast Prp4 proteins. Protein sequences of Prp4 from *H. sapiens*, *C. elegans*, and *S. cerevisiae* were aligned using the PILEUP program. Sequences are described in the text. Positions at which the sequences are identical are shown white-on-black; if two of three are the same, then those two are white-on-black. Positions of similarity are shown black-on-gray; similarities used were E ~ D, R ~ K, V ~ L ~ I, Q ~ N, F ~ Y ~ W, and C ~ S ~ T. The seven repeats of the WD motif present in each protein (as defined by sequence criteria (Neer et al., 1994)) are boxed. Each sequence was numbered from its amino terminus.

TABLE 1. Percent identity and similarity (in parentheses) between pairs of Prp4, Prp3, and USA-CyP proteins.^a

Prp4			
	<i>C. elegans</i>	<i>S. cerevisiae</i>	
<i>H. sapiens</i>	42 (52)	34 (44)	
<i>C. elegans</i>		32 (43)	
Prp3			
	<i>C. elegans</i>	<i>S. pombe</i>	<i>S. cerevisiae</i>
<i>H. sapiens</i>	36 (45)	33 (41)	23 (33)
<i>C. elegans</i>		31 (41)	26 (36)
<i>S. pombe</i>			21 (31)
Prp3 (C-term.)			
	<i>C. elegans</i>	<i>S. pombe</i>	<i>S. cerevisiae</i>
<i>H. sapiens</i>	50 (61)	41 (50)	26 (38)
<i>C. elegans</i>		36 (48)	26 (37)
<i>S. pombe</i>			24 (35)
USA-CyP			
	<i>C. elegans</i>		
<i>H. sapiens</i>	68 (77)		

^a Amino acids from 322 forward (hPrp3 numbering) were used for the calculation of carboxyl terminal identities for Prp3. All the sequences are described in the text.

to them as repeats 1–7, beginning with the repeat at amino acid 229 of hPrp4 and continuing sequentially toward the C-terminus. The length and spacing of the repeats are almost constant in all the proteins; each repeat is 31 amino acids, and consecutive repeats are separated by 11 amino acids (except for repeat 2, which has an 8-amino acid insertion in hPrp4 and cPrp4, and repeat 2 in yeast, which is separated from repeat 3 by 15 amino acids).

In yeast Prp4, only five repeats had been identified (Dalrymple et al., 1989); however, in hPrp4 and cPrp4, two repeats N-terminal to the five identified in yeast can be found. Repeat 2 is evident from its homology to the consensus sequence (Neer et al., 1994). Repeat 1 is less apparent, and we identified it based on three criteria: (1) it is identified in all three Prp4 sequences by the BLOCKS program (Henikoff & Henikoff, 1994); (2) it is homologous to the other Prp4 repeats and conforms to the consensus (Fig. 4); and (3) its spacing and length are identical to the other Prp4 repeats. The high level of homology throughout the proteins strongly suggests that the sequences we have identified in yeast

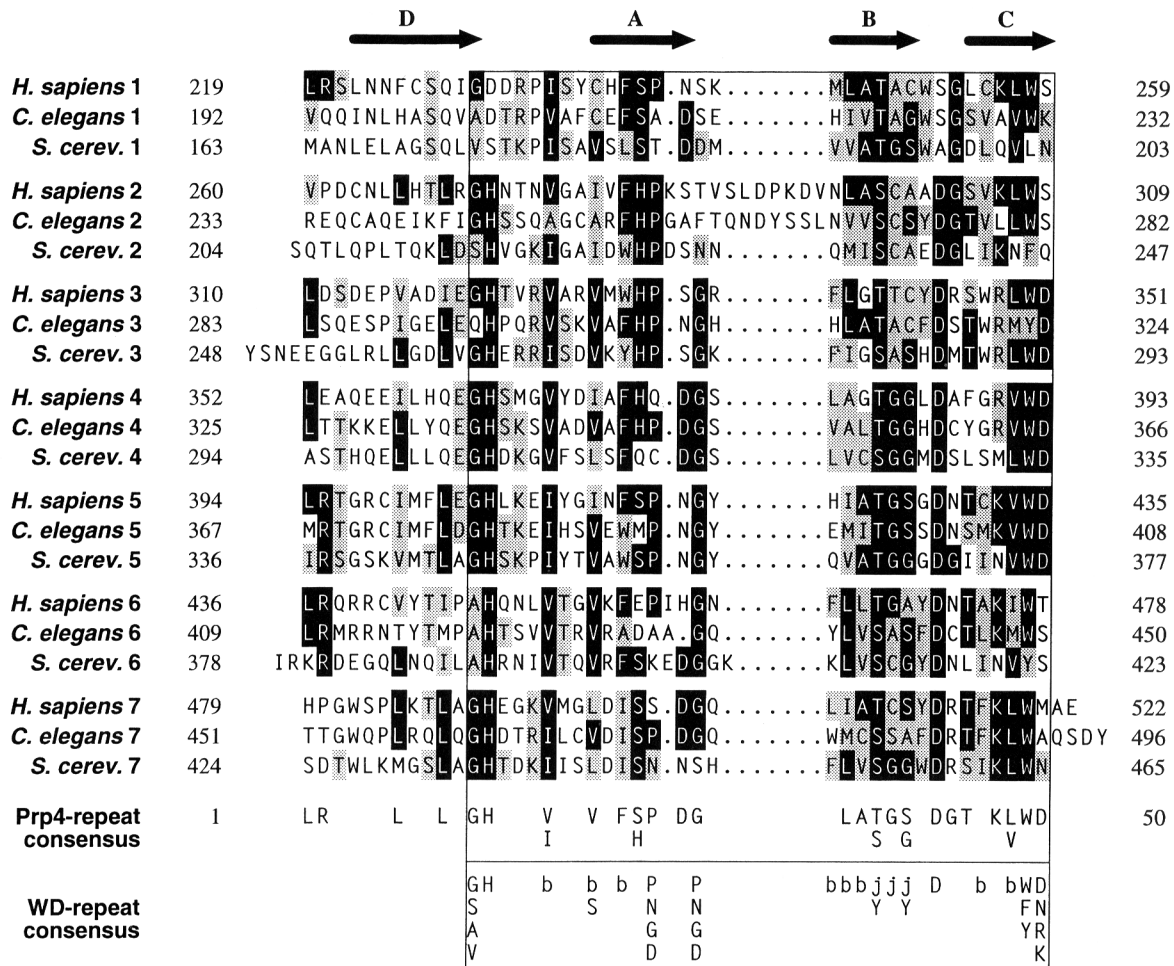


FIGURE 4. Alignment of the WD repeats from hPrp4, cPrp4, and Prp4. The seven WD repeats in the three Prp4 proteins were aligned using the PILEUP program. Repeats are displayed in order of their appearance in Prp4; each repeat is more closely related to the corresponding repeats in other Prp4's than to other repeats. Expected positions of the four β strands (D, A, B, and C) within each repeat unit are shown at the top, patterned after the structure-based alignments of Wall et al. (1995) and Sondek et al. (1996). Two consensus sequences are shown below the alignment. In the Prp4-repeat consensus, positions at which seven or more of the 21 Prp4 repeats are identical are shown; if two different amino acids are both present at least seven times at one position, then both are included in the consensus. For convenience, we have numbered the consensus from L1 to D50. A consensus sequence derived from a variety of WD-repeat proteins (Neer et al., 1994) is shown at the bottom; in the consensus b = L, I, V, F, M, A, or C and j = C, A, T, S, or G. The following scheme was used for shading the repeats: (1) Residues that occur seven or more times at one position are shaded black; if two such residues are present, both are shaded black, even if they are not structurally related. (2) Residues that are structurally related to black-shaded residues are shaded gray. (3) At positions represented in the WD-repeat consensus sequence, all residues that match the consensus are shaded gray. (4) If at least seven residues are related structurally, then they are shaded gray, where similarity was defined as in Figure 3. Amino acids were numbered relative to the amino terminus of the protein.

Prp4 represent true WD-repeat elements. In other WD-repeat proteins, some repeats do not conform well to the consensus (Neer et al., 1994).

The X-ray crystal structures of a trimeric G protein and of the dimeric $\beta\gamma$ subunits of a G protein have been solved (Wall et al., 1995; Lambwright et al., 1996; Sondek et al., 1996). The G β protein, which contains seven repeats of the WD motif, folds into a sevenfold β propeller. Each of the seven propeller blades is formed of four β strands, and the structures of the seven propeller blades are nearly superimposable. Three of the

four β strands are composed of amino acids within the conserved WD-repeat sequences; however, the fourth strand is composed of amino acids from the sequences between the conserved repeats. Several amino acid residues that are conserved among WD proteins make key contacts within each blade.

We have aligned the sequences of the seven WD repeats from three Prp4 proteins (Fig. 4). The sequences of all the repeats conform to the consensus sequence for WD-repeats (Neer et al., 1994; Neer & Smith, 1996). The consensus sequence of the seven Prp4 repeats is

shown below the alignment, and the consensus sequence derived from a wide variety of WD-repeat proteins is shown at the bottom of the figure (Neer et al., 1994; Neer & Smith, 1996). Most of the repeats match the consensus closely, although wide variation from the consensus is known in other proteins (Neer et al., 1994). The repeats are closely related to each other and display some additional homologies generally not found in WD-repeat motifs. For example, at position 23 of the consensus sequence (Fig. 4), H and S predominate in the Prp4 sequences, although the residue generally is not conserved in other WD repeats. Likewise, at position 47, basic residues predominate in the Prp4's. Despite the similarities among different repeats, each repeat (with or without its spacer sequence) is more closely related to corresponding repeats in other species than to any of the other repeats; for example, repeat 3 in cPrp4 is more closely related to repeat 3 in hPrp4 and Prp4 than it is to any of the other 18 repeats.

The structure of the $G\beta$ subunit provides a number of insights into the WD-repeat sequences of the Prp4's (Wall et al., 1995; Sondek et al., 1996). The expected positions of the four β strands within each WD-repeat unit are shown above the alignment in Figure 4. Each propeller blade is composed of the four antiparallel β strands, with the A strand innermost in the propeller, followed outward by B, C, and D. In our alignment, the D strand for each set is offset one line from A, B, and C. In the Prp4's, as in $G\beta$, the carboxyl terminus of the protein occurs immediately after the C strand in the seventh repeat; by analogy with $G\beta$, we expect that the D strand for the last repeat is the D before the first repeat (amino acids 219–229 in hPrp4), completing the propeller structure (Wall et al., 1995; Sondek et al., 1996). The positions of the β strands show that the large insertions in the second WD repeat of hPrp4 (284–295) and cPrp4 (257–268) are in the loop between strands A and B. In the other six repeats, the A-B loop sequence, (H/S)P[x]DG, is much more conserved among the Prp4's than among other WD-repeat proteins (Neer et al., 1994; Neer & Smith, 1996). The network of hydrogen bonds among Asp 43, Trp 49, Thr/Ser 39, and His 13 (numbered as in the Prp4 consensus) is central to the structure of each propeller blade (Wall et al., 1995; Sondek et al., 1996), although some of these amino acids are absent from some WD repeats. In the Prp4's, all four residues are present in repeats 2–7 (Fig. 4); however, in repeat 1, both Asp 43 and one of its proposed hydrogen bonding partners, His 13, are absent.

hPrp4 crossreacted strongly with antibodies raised against hPrp18. The intensities of the western signals and the amounts of the proteins seen by silver staining suggest that the antibody reacts almost equally against the two proteins (Fig. 1). We found a potential common epitope in the two proteins. The 30-amino acid sequence EVIRRLRERGEPIRLFGETDYDAFQRLRKI in

hPrp18 is 53% identical to amino acids 107–136 in hPrp4 (homologous amino acids are underlined). Eight of the nine amino acids in the center of this sequence are identical in the two proteins. This sequence occurs between positions 83 and 112 in the hPrp18 sequence. This region is not conserved between yeast and human Prp18 and is unlikely to be important for the function of hPrp18 (Horowitz & Krainer, 1997); we do not, therefore, believe that the homology suggests any functional similarity between hPrp18 and hPrp4.

Although the hPrp4 clone that we sequenced contains the entire protein-coding region (GenBank AF01639), it lacks 700 bases of 3' untranslated region present in the mRNA. The sequence of the 1,100 bases at the 3' end of the hPrp4 cDNA was assembled from 13 EST sequences (data not shown). The sequence of our hPrp4 clone ends at a stretch of 15 consecutive A's that apparently served as the site for priming.

The 90-kDa protein and Prp3

Searches of the EST database with two peptide sequences from the 90-kDa protein yielded one EST sequence (AA192598) that encoded both peptides. Overlapping EST sequences that stretch to the 3' end of the mRNA, a run of A's where 10 EST sequences end, were found in dbEST. The 5'-end sequences of 3 of these 10 clones (AA205466, AA223602, and AA233415) suggested that they were full-length clones. All three started at the same base and had an open reading frame that began 70 bases into the sequence. The clone AA205466 was obtained commercially and has been sequenced completely. The sequence includes all the ESTs identified initially.

The 2,344-bp sequence of AA205466 (deposited in GenBank with Accession Number AF016370) encodes a 683-amino acid protein with a calculated molecular weight of 77,500. The protein is homologous to the yeast Prp3 protein (Fig. 5; GenBank accession number U33050; gene name *YDR473C*), and we will refer to the 90-kDa protein as hPrp3. hPrp3 is strongly basic with a calculated pI of 10.3 and a net charge of +24 at neutral pH.

Five peptides from the 90-kDa doublet were sequenced. The sequences of all five peptides from the doublet of 90-kDa bands are encoded by our hPrp3 sequence (detailed in Materials and Methods), showing, first, that the hPrp3 clone does encode the 90-kDa protein, and, second, that the two bands in doublet are most likely modified or proteolyzed forms of hPrp3. If the two bands in the doublet were different proteins, then we would expect to have peptide sequences that are not encoded by our clone, because the two bands are present in roughly equal amounts in the stained gels (Figs. 1A, 2A). Therefore, we conclude that the two bands in the doublet are different forms of hPrp3.

The translated hPrp3 protein is 78 kDa, although the gel mobility of the purified protein suggests a size of

<i>H. sapiens</i>	MALSKRELDELKPWIEKTVKRVLGFSEPTVVTAALNCVGKGMDDKKAADHLKPFLLDDSTLRFVDKLFEEAVEEGRSSRHSK	80
<i>C. elegans</i>		MGKLEDI 7
<i>H. sapiens</i>	SSSDRSRKRELKEVFGDDSEISKES...SGVKKRRIIPRFEEVEEEPEVIPGPPSESPGMLTKLQIKQMMEAATR...QTE	154
<i>C. elegans</i>	CDQYLSRKKEQDKVIEAVERCLKKGYDYELKDRVQEAISDPQKARKVI SAVCDHYPLQKRRTRTRDRDGEDRDRKDRRTD	87
<i>S. pombe</i>		MDRNKRRE 9
<i>H. sapiens</i>	ERKKQLSFTSPPTPQPKTPSSSQPERLPIGNTIQPSQAATFMNDAIEKARKAAELQARIQAQLALKPGLIGNANMVGLAN	234
<i>C. elegans</i>	DRRKPDAASVPAVAEPPKPAEQSIEERLRAKEMMYKAQQEIEETKRKMAIAAGTLQPKAALQAQAKTQMVAPGGKSLSLGR	167
<i>S. pombe</i>	SSQLRESSTPNSQNKPNAMEEIKRRRLQLEQRLAQAQVPEKRSENGNNAAGMETIQNRISLEKKTAKRFNANIPKSEG	89
<i>S. cerev.</i>		MPPRNTYEKGNPKRQNSPYYK 22
<i>H. sapiens</i>	LHMGTAAPPVELKDQTKPTPLILDEQG...RTVDATGKEIELTHRMPITLKANIRAVKREQFKQQ...LKEKPSSEDMESNTF	310
<i>C. elegans</i>	DEATMLMTHSMDDKSRMEELKARLARTTVMAKIDAMVGNAGVMAPEAVVQAEKKVQ...QQ...ITKESKEPEKLEIY	243
<i>S. pombe</i>	LFRRDSSNGAKGGLKVGTHPVLLDGNINQNTILTPENRKRRTASFSTKGVSLSQHQLLKPPAITEQNPFLDTAPTRELDSPF	169
<i>S. cerev.</i>	SFLRREETTNDDEKFGHGLKTELSALKSSNLNLRRTYQTGENPYLSDPHDRGSSSRFNRRYERGLKIFYQKGEISKRI	102
<i>H. sapiens</i>	FDPRV...SIAPSQRQRRTFKFHDKGFKEIAQRLRRTKAQLEKLAQAEISQAARKTGHTSTRLLALAPKKEKLEGDIPETI	387
<i>C. elegans</i>	LDPRI...QARTADRRRRGFNFHEKGEFEKLANQRAMAKLERLQNEVSSAAQSTGISSAVKLAMVPTPTGTAKMENGVPDI	321
<i>S. pombe</i>	YDPR...KESRKTGRSRNLHLNESGKFIIEANQARQARLEDLKKRIALHSHKAGIEDELDI...TSKSIGRDTIPNI	242
<i>S. cerev.</i>	AQERTLQKQEEELKRLKQEEDEKDKRKLIESGDLNLELHEDKFLDLDSKFKIYYDNNHGYEWDYAYLDEKGL ELM	181
<i>H. sapiens</i>	EWWDYSIIP...NGFDLTEENPKREDYFGITNLVEHPAQLNPPVDNDTPVTLGVYLTKEKQKLRROTRREAQKELQEKVVR	465
<i>C. elegans</i>	EWWDMLVLDKVNIDEIPAEENDMERYSQTYSELVEHPTSMRAPTEPLTQYLVYLTKEKKTTRQRNRKELVKEKTEKIR	401
<i>S. pombe</i>	EWWDLPFIKDYNDYGDENNWLIDGPOSINSATQHPIPLPPYAKNPPSSHSVLTKEKQKLRROTRAEARKEKQDRQL	322
<i>S. cerev.</i>	EKYDMNGTSPAEEKLAEDIDEVDDDDDEHPSIRYVAHPLPEKINEAKVSIKAYLTQHERKRLRRNRKMARAEAREIKIK	261
<i>H. sapiens</i>	LGLMPPPEPKVRIISNLMRVLGTEA...VDPTKVEAHVRAQMAKRQKAHEEANAARKLTAEQRKVKKIKKLEDISQGVHTS	544
<i>C. elegans</i>	LGLEKAPEPKVKISNLMRVLGNEA...IQDPTKMEAQVRKQMAERLKKHETLNAERKLTEDQKRAKTKKLSSETSTAVNVIS	480
<i>S. pombe</i>	LGTEPPEPKVKLSNLMHVLGDDA...IKDPTKVEAEVRKQVEERLRHERENEERKLTPEERKEKAFKDKEDSAAGERCL	401
<i>S. cerev.</i>	LGLLPKPEPKVKLSNMMSVFENDQNTIDPTAWKVVVDQVLDLRRKHLLEENERRHEDAIRK...KEAVNMNVEKPTVYHCK	340
<i>H. sapiens</i>	VYVRNLSNPAKFKIEANAGQLYLTGVVVLHKDV...NVVVVEGGPKAQKFKRLMLHRIKWDE...OTSNTKGGDDDEE	618
<i>C. elegans</i>	VYRVKSLAHPKSKFKVETNAKQLQMSGAIMMHAQ...NVVVVEGGPKQKFKYKLMINRIKWS...EITIGQKKD...	550
<i>S. pombe</i>	VFRKYLARPHRLKIDLNKQWGATGVCLNANF...NVVIFEAGQKAIKKLKRMLERIDWIDTSRNSITIAQGNKLVDT	479
<i>S. cerev.</i>	VFQFKNLQNPKIRFKLKMNSKELSLKGLCLRTRDGGPGIIVVVGNEKSCKFEYENLVMKRIKWNEDEFELHT.....NT	412
<i>H. sapiens</i>	SDEEAVKKTNKCVLVWEGTAKDRSFGEMKFKQCPTEENMAREHFKKH...GAEHYWDLALSESVL ESTD	683
<i>C. elegans</i>	AEKDAPGERNLCEMIWEGOVKRRNFRDFTVHTATLEKQAREFFEKH...GVAQYWDLCYSTVLLLEGQDILPTA	621
<i>S. pombe</i>	EGRELNYTEINTCNLVWEGEIGRRAFRYWSFRSCPSENDAKSYLEEQGGAEH...WMLAKSWSENV	542
<i>S. cerev.</i>	GDIKMDMHNNSISKTWEGYLQDCKFKGWFMKVQNDQDSSLRRTLGLQDFSEHFVSPVQT	469

FIGURE 5. Alignment of the Prp3 proteins from human, nematode, and two yeasts. Protein sequences of *H. sapiens*, *C. elegans*, *S. pombe*, and *S. cerevisiae* Prp3 proteins were aligned using the PILEUP program. Sequences are described in the text. Positions at which the sequences are identical are shown white-on-black; if at least two of four are the same, then those two are white-on-black. Positions of similarity are shown black-on-gray; similarities used were the same as in Figure 3. At positions where there are two different amino acids, each represented in two sequences, hPrp3 and its matching sequence have been shaded black and the others gray. Each sequence was numbered from its amino terminus.

90 kDa. Several lines of evidence suggest that the hPrp3 clone is full-length. The cDNA sequence has stop codons in all three frames upstream of the proposed ATG translation start, suggesting that our clone includes the correct 5' end. In addition, partial cDNA sequences from other EST clones agree with ours, and there are no EST clones that extend upstream of the 5' end of our clone. We analyzed another EST clone (AA223602) by PCR and restriction mapping, and it is colinear with the clone we sequenced, suggesting that our clone does not have any internal deletions. As described above, the sequences of many EST clones show that our clone has the correct 3' end. We con-

clude, therefore, that our sequence is full-length. The aberrant mobility of hPrp3 may be caused by its positive charge, by modifications, or by an unusual peptide structure.

An alignment of the sequences of hPrp3 and *S. cerevisiae* Prp3, together with the Prp3 homologues from *C. elegans* (from clone CEM03C11, gene product M03C11.7) and *Schizosaccharomyces pombe* (GenBank accession number Z66525, gene SPAC29E6.02), is shown in Figure 5. Homology among the proteins is good in the carboxyl-terminal 350 amino acids. However, in the amino-terminal 300 amino acids, there is little homology among the proteins, even between the human

and *C. elegans* Prp3's, whose carboxyl-terminal portions are very closely related (Fig. 5). Table 1 shows the percents of identity and similarity both throughout the proteins and within the carboxyl-terminal portion. The lengths of the proteins are not conserved, and *S. cerevisiae* Prp3 is 212 amino acids shorter than hPrp3. The human, *C. elegans*, and *S. pombe* Prp3's are all highly basic; *S. cerevisiae* Prp3 is less basic, with a net charge of +6 and a pI of 9.0. The basic residues in the Prp3's do not appear to be clustered and are distributed throughout the protein. None of the Prp3 proteins has any known protein motifs.

The 18-kDa protein and cyclophilin

Searches of dbEST with the peptide sequences from the 18-kDa protein yielded a number of virtually identical EST sequences. The complete sequence of the 800-base mRNA encoding the 18-kDa protein could be found in dbEST from some EST clones for which both the 5' and 3' sequence was reported. We obtained the clone T53949 and sequenced it entirely (deposited in GenBank with accession number AF016371). All three peptides that we had sequenced from the purified 18-kDa protein are encoded by this cDNA.

The 18-kDa protein is a new human cyclophilin, which we have termed USA-CyP for U-snRNP associated cyclophilin. The cDNA encodes a 177-amino acid protein with a calculated molecular weight of 19,200 and an isoelectric point of 8.1 (Fig. 6). Although the cyclophilins are a highly conserved family of proteins (Stamnes et al., 1992; Trandinh et al., 1992; Galat & Metcalfe, 1995), the three peptide sequences that we obtained identify USA-CyP unambiguously. Two of the peptides, from amino acids 54 to 61 and from 154 to 164, are from variable regions in cyclophilins, and the peptides' sequences match the expected sequences perfectly.

An alignment of USA-CyP to human cyclophilin A (CyP-A) and *C. elegans* CyP-11 is shown in Figure 6. CyP-11 (Page et al., 1996) is likely a true homologue of USA-CyP; the proteins are 68% identical and are well-

conserved in two variable regions from positions 51 to 58 and in the carboxyl-terminal 20 amino acids. Searches of GenBank with BLAST did not reveal any other exceptionally closely related proteins, and there does not appear to be an *S. cerevisiae* homologue. Human CyP-A is 55% identical to USA-CyP, about average within this family.

Two cyclophilins with RS domains have been found recently and both have been proposed to function in splicing (Anderson et al., 1993; Nestel et al., 1996; Bourquin et al., 1997). One of the cyclophilins has been localized to the speckles and interacts with both RNA polymerase II and Clk/Sty kinase in the two-hybrid system. The two RS cyclophilins are closely related to each other, but neither cyclophilin is strongly related to USA-CyP.

DISCUSSION

We have purified and identified three new human proteins that are components of the U4/U6 and U4/U6·U5 snRNPs. In the course of work on hPrp18 protein, we found that antibodies raised against hPrp18 crossreacted with a 55-kDa protein present in U4/U6 and U4/U6·U5 snRNPs (Horowitz & Krainer, 1997). We used this crossreactivity as the assay for the purification of the 55-kDa protein. The 55-kDa protein copurifies with two other proteins. We identified all three proteins by peptide sequencing and determined the cDNA sequences of all of them. Two of the proteins are human homologues of *S. cerevisiae* splicing factors, and the third is a new human cyclophilin. The 55-kDa protein is homologous to yeast Prp4, a U4/U6-snRNP protein required for the first step of splicing (Lustig et al., 1986; Banroques & Abelson, 1989; Petersen-Bjorn et al., 1989). The second protein is homologous to yeast Prp3, which is also a component of the U4/U6-snRNP (Anthony et al., 1997). We refer to the new proteins as hPrp3 and hPrp4. The third component of the protein complex is a new cyclophilin, USA-CyP (U-snRNP-associated cyclophilin).

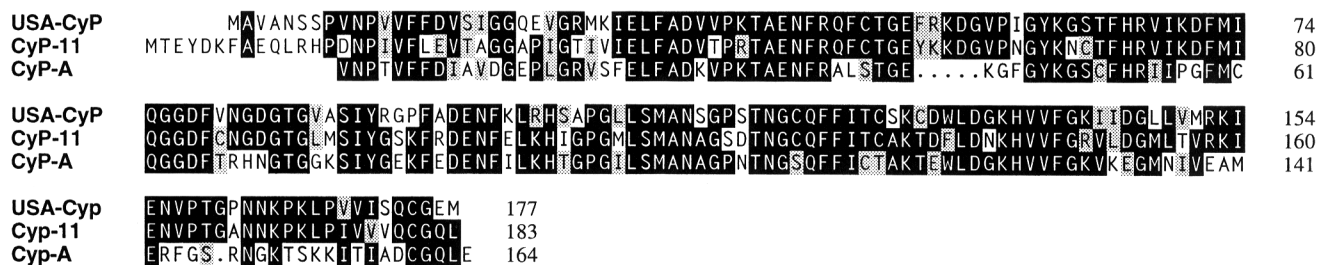


FIGURE 6. Alignment of USA-CyP, CyP-11, and CyP-A. Protein sequences of *H. sapiens* USA-CyP (U-snRNP-associated cyclophilin) and CyP-A (cyclophilin A) together with *C. elegans* CyP-11 were aligned using the PILEUP program. Sequences are described in the text. Positions at which the sequences are identical are shown white-on-black; if two of three are the same, then those two are white-on-black. Positions of similarity are shown black-on-gray; similarities used were the same as in Figure 3. Each sequence was numbered from its amino terminus.

hPrp3, hPrp4, and USA-CyP form a tightly bound complex, which we have purified to near homogeneity. In two columns where the elution pattern of the three proteins could be seen clearly, they co-chromatographed precisely (Figs. 1, 2). The complex eluted near 700 mM salt from both columns, implying that the interactions among the proteins are strong and specific. The calculated isoelectric points and charges of the proteins show that only hPrp3 is highly positively charged, suggesting that binding of hPrp3 to the negatively charged heparin and sulfonic acid columns was the basis of the fractionation. The purified complex is free of RNA and is held together by interactions among the three proteins. Previous methods used to identify human U4/U6-snRNP proteins have relied on purification of intact snRNPs (Hinterberger et al., 1983; Behrens & Lührmann, 1991; Okano & Medsger, 1991; Gozani et al., 1994). Using the serendipitous antibody crossreaction, we were able to purify proteins in the absence of the snRNAs, facilitating the identification of the interactions among the proteins.

Previous work established that hPrp4 is a component of the U4/U6 and U4/U6·U5 snRNPs (Horowitz & Krainer, 1997). Glycerol gradients and immunoprecipitations showed that the primary interaction of hPrp4 is with the U4/U6 snRNP. The strength of the interactions among the subunits of the hPrp3-hPrp4-USA-CyP complex argues that all three proteins are associated with the snRNPs. The purification data show that all the hPrp4 is complexed to the other proteins, implying that it is within this complex that hPrp4 binds the U4/U6 snRNP. Yeast Prp3 and Prp4 are components of the U4/U6 and U4/U6·U5 snRNPs, and their behaviors are similar to that of hPrp4 (Banroques & Abelson, 1989; Petersen-Björn et al., 1989; Anthony et al., 1997).

U4/U6-snRNP proteins of 60, 90, and 150 kDa had been reported previously (Blencowe et al., 1993; Gozani et al., 1994), whereas proteins of 15.5, 20, 27, 60, and 90 kDa had been identified in the U4/U6·U5 snRNP (Behrens & Lührmann, 1991). After the submission of our work, Lauber et al. (1997) reported further characterization of the U4/U6·U5 proteins. The 60-kDa protein is hPrp4, and the 90-kDa protein is hPrp3. Further, the 20-kDa U4/U6·U5 protein is the same as USA-CyP (R. Lührmann, pers. comm.). Lauber et al. (1997) showed that both the 60-kDa (hPrp4) and 90-kDa (hPrp3) proteins are components of the U4/U6 snRNP, as well as of the U4/U6·U5 snRNP; the results for hPrp4 are consistent with our previous work (Horowitz & Krainer, 1997). Lauber et al. (1997) and our study reach similar conclusions about hPrp3 and hPrp4; we show, in addition, that hPrp3 and hPrp4, together with USA-CyP, form a stable complex in the absence of RNA. The 60K U4/U6-snRNP protein identified by Gozani et al. (1994) is the same as hPrp4, based on its cross-reactivity with antibodies against hPrp18 (K. Chua and R. Reed, pers. comm.). The 90K

protein (Gozani et al., 1994) seems very likely to be hPrp3.

hPrp4 is a member of the family of WD-repeat proteins (Neer et al., 1994). By comparison with the WD-repeat consensus sequence, we have identified seven repeats of the WD motif in the hPrp4 sequence. Previously, five repeats had been found in yeast Prp4 (Dalrymple et al., 1989; Neer et al., 1994); we have found two additional repeats in yeast Prp4 that do not conform to the consensus. The hPrp4 WD repeats are spaced regularly, with a periodicity of 42 amino acids, and the seven repeats are more closely related to one another than to the consensus sequence (see Fig. 4). However, the homology between corresponding repeats in hPrp4, cPrp4, and Prp4 suggests that the ancestral Prp4 protein had seven repeats.

The X-ray structure of the archetypal WD-repeat protein, *Gβ*, has been solved (Wall et al., 1995; Sondek et al., 1996). It shows that the seven WD repeats of *Gβ* form a nearly symmetrical, sevenfold β -propeller. This propeller acts as an inflexible scaffold to which the *Gα* and *Gγ* subunits bind; both *Gα* and *Gγ* make extensive contacts with the *Gβ* propeller. The structure of *Gβ* remains essentially unchanged upon activation of the G protein (Wall et al., 1995; Lambwright et al., 1996).

All WD-motif domains are likely to fold into propeller structures quite similar to that of *Gβ* (Wall et al., 1995; Neer & Smith, 1996; Sondek et al., 1996), and we expect that hPrp4 folds in this way. Amino acids identified in the *Gβ* structure as especially important for stability of the propeller structure are present in six of the seven WD repeats in hPrp4, allowing us to predict the positions of the β strands that make up the propeller (Fig. 4). The question of whether all such propeller structures are involved in protein-protein interactions appears to be open. In *Gβ*, the residues on the surface of the WD-propeller determine the interactions, whereas the most conserved residues have essential structural roles in the propeller. Because we have identified hPrp4 within a stable complex of three proteins, it seems quite plausible that its WD-propeller is in fact involved in protein-protein interactions.

hPrp4 appears likely to contact hPrp3 within the complex with USA-CyP. Interaction between hPrp3 and hPrp4 could be bridged by USA-CyP. Cyclophilins can bind to single short peptide sequences through their active sites (Gamble et al., 1996; Luban, 1996; Zhao et al., 1997), but can also be involved in ternary complexes, such as the cyclophilin A-cyclosporin A-calci-neurin complex (Schreiber & Crabtree, 1992; Marks, 1996). Earlier work in yeast showed that overexpression of Prp3 in many *prp4* strains suppresses the mutant phenotype (Last et al., 1987; Hu et al., 1994), and more recent work shows that yeast Prp3 and Prp4 interact in the two-hybrid system (J. Banroques, pers. comm.). These studies support the notion that yeast Prp3 and Prp4 interact, although there is no biochem-

ical evidence for a direct interaction. hPrp3 and hPrp4 do not interact in the two-hybrid system (Laubert et al., 1997); USA-CyP may be needed for their interaction.

Unlike the sequences of hPrp4 and USA-CyP, the sequence of hPrp3 is not instructive at present. Homology among the Prp3 proteins from four organisms is concentrated in the carboxyl terminus (Fig. 5), but we did not find any motifs in the sequences. hPrp3 is a highly basic protein with a pI of 10.3, suggesting that it might bind RNA. The binding of the complex of hPrp4, hPrp3, and USA-CyP to the U4/U6 snRNP could be mediated through another protein (perhaps one that binds to the hPrp4 WD-domain), although no other U4/U6-snRNP proteins are known in humans (Laubert et al., 1997). If, alternatively, the complex binds directly to the RNA, then hPrp3 is a good candidate to mediate the binding. Laubert et al. (1997) noted recently that hPrp3 has some sequence similarity to RNAase III, a double-stranded RNA binding protein; our alignment of Prp3 homologues from *Homo sapiens*, *C. elegans*, *S. pombe*, and *S. cerevisiae* (Fig. 5) does not show phylogenetic conservation of the relevant sequences (not shown).

Our data show that hPrp3 and hPrp4 form a complex and might act together during splicing. The roles of Prp4 and Prp3 in splicing in yeast have been studied extensively, and the data are consistent with the notion that the proteins have allied functions. Both are part of the U4/U6 snRNP (Banroques & Abelson, 1989; Petersen-Björn et al., 1989; Anthony et al., 1997); Prp4 binds principally to the U4 snRNP (Fabrizio et al., 1989; Bordonné et al., 1990; Xu et al., 1990). In several studies with extracts from temperature-sensitive strains, inactivation of *prp3* or *prp4* affects the snRNP associations or destabilizes free U6 snRNP (Bordonné et al., 1990; Galisson & Legrain, 1993; Anthony et al., 1997), but other studies report no effect (Ayadi et al., 1997). The results may depend on the particular mutants and methods used and may imply that the proteins are necessary at several different stages in the splicing pathway. Both Prp3 and Prp4 are required for the first step of splicing (Lustig et al., 1986; Hu et al., 1994). Inactivated *prp3* extracts are blocked at the transition from complex B to complex A2-1 (the first complex containing U4/U6·U5) (Anthony et al., 1997), and antibodies against Prp4 block splicing at the same point (Banroques & Abelson, 1989). However, in extracts from some *prp4* strains, the transition from complex B to A2-1 proceeds normally, but the mutant *prp4* protein blocks splicing by preventing the dissociation of U4 from the spliceosome (Ayadi et al., 1997). Prp4 is only stably bound to spliceosomes that contain the U4 snRNP (Tarn et al., 1993).

The role of the USA-CyP protein in splicing is intriguing. USA-CyP is a canonical cyclophilin: an 18-kDa protein with high homology to other cyclophilins (Stamnes et al., 1992; Trandinh et al., 1992; Galat &

Metcalfe, 1995). Only one cyclophilin, CyP-11 from *C. elegans* (Page et al., 1996), is significantly more closely related to USA-CyP than are other cyclophilins. CyP-11 is not an abundant cyclophilin; it exhibits weak proline isomerase (rotamase) activity with a standard substrate, suggesting that it might have specific substrates (Page et al., 1996). No clear homologue of USA-CyP exists in *S. cerevisiae*.

Cyclophilins have been studied intensively because of their medical importance as the targets of the immunosuppressive drug cyclosporin A. However, the normal cellular roles of the cyclophilins have been elusive (Luban, 1996; Marks, 1996). The rotamase activity has been thought to play an important part in protein folding (Fischer et al., 1989; Gething & Sambrook, 1992; Rutherford & Zuker, 1994). An alternative view is that cyclophilins act principally as chaperones in complex macromolecular processes, binding to sequences containing proline, and that the rotamase activity is a consequence of the hydrophobicity of the binding pocket (Schreiber & Crabtree, 1992).

One of the best characterized examples of cyclophilin action is that of cyclophilin A (CyP-A) and HIV-1. CyP-A is essential for HIV-1 virion infectivity (Luban, 1996); it interacts with HIV-1 capsid protein (CA) and is incorporated into the virion (Franke et al., 1994; Thali et al., 1994). NMR data suggest that the isomerization of a proline could trigger the disassembly of the capsid (Gitti et al., 1996). However, the crystal structure of CyP-A bound to CA suggests strongly that the role of CyP-A is to facilitate disassembly of the CA core by destabilizing interactions between CA proteins by binding specifically to CA sequences (Braaten et al., 1996; Gamble et al., 1996; Zhao et al., 1997). In this view, the cyclophilin acts as a chaperone in the disassembly process. A chaperone function has also been suggested for the *Drosophila* cyclophilin NinaA, which is required for proper function of rhodopsin (Baker et al., 1994).

USA-CyP could be involved in splicing in a number of different ways. It may facilitate the folding of hPrp3 or hPrp4 or be needed for them to associate properly. USA-CyP could act as a chaperone in assembly steps that involve complicated rearrangements of protein interactions. USA-CyP could facilitate the assembly of the U4, U4/U6, or U4/U6·U5 snRNP. Alternatively, USA-CyP could act within the spliceosome, either during the entry of the U4/U6·U5 snRNP or during the rearrangement of complex B to complex C, which involves the dissociation of the U4/U6 snRNP and the departure of the U4 snRNP. Analogy with HIV as well as the results for yeast Prp4 (Ayadi et al., 1997) suggest that USA-CyP might function during the disassembly of the U4/U6 snRNP within the spliceosome. USA-CyP might not be required routinely for splicing; its function could be to assist in the repair or disassembly of spliceosomes that are in improper conformations or have halted in the splicing pathway. In heat-shocked

mammalian cells splicing is shut down, and the transition from complex B to C is blocked (Utans et al., 1992). The U4/U6-U5-snRNP specific proteins, which include the 20-kDa protein that is likely to be USA-CyP, restore splicing activity to heat-shocked extracts (Utans et al., 1992). Because cyclophilins have been shown to be chaperones in the refolding of proteins after heat shock (Bose et al., 1996; Freeman et al., 1996), USA-CyP could be responsible for the restoration of splicing activity.

Our results suggest that a new cyclophilin, USA-CyP, is involved in splicing, perhaps acting in concert with hPrp3 and hPrp4. The precise role of this cyclophilin in the splicing pathway remains to be defined.

MATERIALS AND METHODS

Purification of the 55-kDa protein

The 55-kDa protein was assayed by western blot with antibodies raised against hPrp18 (Horowitz & Krainer, 1997). Nuclear extract (36 mL; 10 mg protein/mL) was prepared from 40 L HeLa cells by the method of Dignam et al. (1983) except that the final dialysis was omitted. Protease inhibitors PMSF (0.4 mM), leupeptin (10 μ g/mL), pepstatin (2 μ g/mL), aprotinin (4 μ g/mL), chymostatin (5 μ g/mL), and E-64 (4 μ g/mL) were used throughout the purification. The KCl concentration of the extract was adjusted to 0.2 M, and polyethyleneimine, pH 7.9, was added to 0.1% (w/v) final concentration (Jendrisak & Burgess, 1975). The supernatant, which was free of nucleic acid (<1% of the starting amount by A_{260}), contained most of the 55-kDa protein. Solid ammonium sulfate was added sequentially to give concentrations of 20, 25, 30, 35, and 40%. Ninety-five percent of the 55-kDa protein was present in the 25–30% and 30–35% fractions, which were pooled and dialyzed against 50 mM MES, pH 6.5, 150 mM NaCl, 10% glycerol, 1 mM DTT, 0.5 mM EDTA, and 0.5 mM EGTA (Buffer M). The pooled fractions were loaded on a 1.7-mL Poros 20 HS column (Perseptive Biosystems) and eluted with a 150–1,000 mM NaCl gradient in Buffer M. The 55-kDa protein eluted at 650 mM NaCl. The peak fractions were pooled, dialyzed against 50 mM Tris, pH 8.0 at 25°C, 150 mM NaCl, 10% glycerol, 1 mM DTT, 0.5 mM EDTA, and 0.5 mM EGTA (Buffer T), and loaded on a 1.7-mL Poros 20 HE1 column (Perseptive Biosystems). The bound proteins were eluted with a 150–2,000 mM NaCl gradient in Buffer T. The 55-kDa protein, together with the 90-kDa and 18-kDa proteins, eluted at 700 mM NaCl. The peak fractions contained about 5 μ g of protein in a volume of 2 mL; the concentration was estimated from the A_{280} of the eluate. The A_{260} of the same fractions was about half the A_{280} , giving a molar ratio of protein complex to a 100-base RNA of at least 40:1. All columns were run at room temperature using a Perseptive Biosystems Sprint chromatography system equipped with a dual wavelength detector.

Peptide sequencing

For protein sequencing, the peak fractions were concentrated by TCA precipitation (Hwang & Chu, 1996), separated by SDS-PAGE, and digested in the gel with *Achromobacter* protease I. The resulting peptides were separated by reversed-

phase HPLC, and sequenced using an automated protein sequencer (ABI Procise 494). The sequences obtained were: for the 18-kDa protein, (1) IENVPTGPNNK (11/11); (2) DGVPIGYK (8/8); and (3) dSTFHRVIK (9/10); for the 55-kDa protein, (1) AGIEAGNINITSGEVFEIEEhISERQAE (27/28); (2) RARQINVSTDDS (12/12); (3) FEPIHGNFLLTsAYDN (15/16); (4) PHIYYkSLEIEK (10/12); and (5) LEEARLHK (8/8); for the 90-kDa protein, (1) LQAEISQAARrK (11/12); (2) EDYFGITNLVEePAaLNPV (17/19); (3) EVFGDDSEI (9/9); (4) PTPLILDEQGRIVDA (15/15); and (5) IEANAGQLY LTGVVVLH (17/17), where the fraction of amino acids that match the sequence inferred from the cDNA sequence is indicated in parentheses after the peptide and the disagreeing amino acids are in lower case within the sequence. Most of the disagreements are at positions where there was some uncertainty in the peptide sequence.

EST clones and DNA sequencing

EST cDNAs that encode the peptides were found using the BLAST search program and the EST database at the National Center for Biotechnology Information. Human EST clones encoding the proteins were obtained from the ATCC. The EST clone of the 55-kDa protein, EST187107 (GenBank accession number AA315321), is from a colon carcinoma cell line (HCC II). Lauber et al. (1997) recently reported the sequence of a HeLa cDNA encoding the same protein. Our sequence and theirs disagree at nine positions in the protein sequence (Q10, R78, V86, R134, G141, G405, A447, C510, and A521), likely reflecting polymorphisms in this gene. Some polymorphisms are also suggested by the peptide sequences. The EST clone of the 90-kDa protein, EST zq66a04 (GenBank accession numbers AA205466 and AA205409), is from Ntera-2/RA neuroepithelial cells. Our sequence agrees with the sequence of the same EST clone reported recently (Lauber et al., 1997). The EST clone of the 18-kDa protein, EST yb85b12 (GenBank accession number T53949), is from a liver library. The clones were sequenced using an ABI Prism sequencing kit and an ABI Prism 377 DNA sequencer. Both strands of all the clones reported were sequenced completely. Sequence analysis was performed with GCG version 9.0 (GCG, 1996). The parameters used for the alignments (the GCG defaults) tend to disfavor the inclusion of gaps.

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REFERENCES

- Abovich N, Legrain P, Rosbash M. 1990. The yeast *PRP6* gene encodes a U4/U6 small nuclear ribonucleoprotein particle (snRNP) protein, and the *PRP9* gene encodes a protein required for U2 snRNP binding. *Mol Cell Biol* 10:6417-6425.
- Anderson SK, Gallinger S, Roder J, Frey J, Young HA, Ortaldo JR. 1993. A cyclophilin-related protein involved in the function of natural killer cells. *Proc Natl Acad Sci USA* 90:542-546.
- Anthony JG, Weidenhammer EM, Woolford JL Jr. 1997. The yeast Prp3 protein is a U4/U6 snRNP protein necessary for integrity of the U4/U6 snRNP and the U4/U6·U5 tri-snRNP. *RNA* 3:1143-1152.
- Ares M Jr, Weiser B. 1995. Rearrangement of snRNA structure during assembly and function of the spliceosome. *Prog Nucleic Acid Res Mol Biol* 50:131-159.
- Ayadi L, Miller M, Banroques J. 1997. Mutations within the yeast U4/U6 snRNP protein Prp4 affect a late stage of spliceosome assembly. *RNA* 3:197-209.
- Baker EK, Colley NJ, Zuker CS. 1994. The cyclophilin homolog NinaA functions as a chaperone, forming a stable complex in vivo with its protein target rhodopsin. *EMBO J* 13:4886-4895.
- Banroques J, Abelson JN. 1989. PRP4: A protein of the yeast U4/U6 small nuclear ribonucleoprotein particle. *Mol Cell Biol* 9:3710-3719.
- Behrens SE, Lührmann R. 1991. Immunoaffinity purification of a [U4/U6.U5] tri-snRNP for human cells. *Genes & Dev* 5:1439-1452.
- Black DL, Pinto AL. 1989. U5 small nuclear ribonucleoprotein: RNA structure analysis and ATP-dependent interaction with U4/U6. *Mol Cell Biol* 9:3350-3359.
- Blencowe BJ, Carmo-Fonseca M, Behrens SE, Lührmann R, Lamond AI. 1993. Interaction of the human autoantigen p150 with splicing snRNPs. *J Cell Sci* 105:685-697.
- Blencowe BJ, Sproat BS, Ryder U, Barabino S, Lamond AI. 1989. Antisense probing of the human U4/U6 snRNP with biotinylated 2'-OMe RNA oligonucleotides. *Cell* 59:531-539.
- Bordonné R, Banroques J, Abelson J, Guthrie C. 1990. Domains of yeast U4 spliceosomal RNA required for PRP4 protein binding, snRNP-snRNP interactions, and pre-mRNA splicing in vivo. *Genes & Dev* 4:1185-1196.
- Bose S, Weiki T, Bügl H, Buchner J. 1996. Chaperone function of Hsp90-associated proteins. *Science* 274:1715-1717.
- Bourquin JP, Stagljar I, Meier P, Moosmann P, Silke J, Baechl T, Georgiev O, Schaffner W. 1997. A serine/arginine-rich nuclear matrix cyclophilin interacts with the C-terminal domain of RNA polymerase II. *Nucleic Acids Res* 25:2055-2061.
- Braaten D, Franke EK, Luban J. 1996. Cyclophilin A is required for an early step in the life cycle of human immunodeficiency virus type 1 before the initiation of reverse transcription. *J Virol* 70:3551-3560.
- Bringmann P, Appel B, Rinke J, Reuter R, Theissen H, Lührmann R. 1984. Evidence for the existence of snRNAs U4 and U6 in a single ribonucleoprotein complex and for their association by intermolecular base pairing. *EMBO J* 3:1357-1363.
- Cheng SC, Abelson J. 1987. Spliceosome assembly in yeast. *Genes & Dev* 1:1014-1027.
- Cooper M, Johnston LH, Beggs JD. 1995. Identification and characterization of Uss1p (Sdb23p): A novel U6 snRNA-associated protein with significant similarity to core proteins of small nuclear ribonucleoproteins. *EMBO J* 14:2066-2075.
- Dalrymple MA, Petersen-Björn S, Friesen JD, Beggs JD. 1989. The product of the *PRP4* gene of *S. cerevisiae* shows homology to β subunits of G proteins. *Cell* 58:811-812.
- Dignam JD, Lebovitz RM, Roeder RG. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res* 11:1475-1489.
- Duina AA, Marsh JA, Green RF. 1996. Identification of two CyP-40-like cyclophilins in *Saccharomyces cerevisiae*, one of which is required for normal growth. *Yeast* 12:943-952.
- Fabrizio P, McPheeters DS, Abelson J. 1989. In vitro assembly of yeast U6 snRNP: A functional assay. *Genes & Dev* 3:2137-2150.
- Fischer G, Wittman-Liebold B, Lang K, Kiefhaber T, Schmid FX. 1989. Cyclophilin and peptidyl-prolyl *cis-trans* isomerase are probably identical proteins. *Nature* 337:476-478.
- Franke EK, Yuan HEH, Luban J. 1994. Specific incorporation of cyclophilin A into HIV-1 virions. *Nature* 374:359-362.
- Freeman BC, Toft DO, Morimoto RI. 1996. Molecular chaperone machines: Chaperone activities of the cyclophilin Cyp-40 and the steroid aporeceptor-associated protein p23. *Science* 274:1718-1720.
- Fujii T, Mimori T, Hama N, Suwa A, Akizuki M, Tojo T, Homma M. 1992. Characterization of autoantibodies that recognize U4/U6 small ribonucleoprotein particles in serum from a patient with primary Sjögren's syndrome. *J Biol Chem* 267:16412-16416.
- Galat A, Metcalfe SM. 1995. Peptidylproline *cis/trans* isomerases. *Prog Biophys Mol Biol* 63:67-118.
- Galisson F, Legrain P. 1993. The biochemical defects of *prp4-1* and *prp6-1* yeast splicing mutants reveal that the PRP6 protein is required for the accumulation of the [U4/U6.U5] tri-snRNP. *Nucleic Acids Res* 21:1555-1562.
- Gamble TR, Vajdos FF, Yoo S, Worthylake DK, Houseweart M, Sundquist WI, Hill CP. 1996. Crystal structure of human cyclophilin A bound to the amino-terminal domain of HIV-1 capsid. *Cell* 87:1285-1294.
- GCG. 1996. *Wisconsin Package Version 9.0*. Madison, Wisconsin, USA: Genetics Computer Group.
- Gething MJ, Sambrook J. 1992. Protein folding in the cell. *Nature* 355:33-45.
- Gitti RK, Lee BM, Walker J, Summers MF, Yoo S, Sundquist WI. 1996. Structure of the amino-terminal core domain of the HIV-1 capsid protein. *Science* 273:231-235.
- Gozani O, Patton JG, Reed R. 1994. A novel set of spliceosome-associated proteins and the essential splicing factor PSF bind stably to pre-mRNA prior to catalytic step II of the splicing reaction. *EMBO J* 13:3356-3367.
- Handschumacher RE, Harding MW, Rice J, Drugge RJ, Speicher DW. 1984. Cyclophilin: A specific cytosolic binding protein for cyclosporin A. *Science* 226:544-547.
- Hashimoto C, Steitz JA. 1984. U4 and U6 RNAs coexist in a single small nuclear ribonucleoprotein particle. *Nucleic Acids Res* 9:3283-3293.
- Henikoff S, Henikoff JG. 1994. Protein family classification based on searching a database of blocks. *Genomics* 19:97-107.
- Hinterberger M, Pettersson I, Steitz JA. 1983. Isolation of small nuclear ribonucleoproteins containing U1, U2, U4, U5, and U6 RNAs. *J Biol Chem* 258:2604-2613.
- Horowitz DS, Krainer AR. 1997. A human protein required for the second step of pre-mRNA splicing is functionally related to a yeast splicing factor. *Genes & Dev* 11:139-151.
- Hu J, Xu Y, Schappert K, Harrington T, Wang A, Braga R, Mogridge J, Friesen JD. 1994. Mutational analysis of the PRP4 protein of *Saccharomyces cerevisiae* suggests domain structure and snRNP interactions. *Nucleic Acids Res* 22:1724-1734.
- Hwang BJ, Chu G. 1996. Trichloroacetic acid precipitation by ultracentrifugation to concentrate dilute protein in viscous solution. *Biotechniques* 20:982-984.
- Jendrisak JJ, Burgess RR. 1975. A new method for the large-scale purification of wheat germ DNA-dependent RNA polymerase III. *Biochemistry* 14:4639-4645.
- Kieffer LJ, Thalhammer T, Handschumacher RE. 1992. Isolation and characterization of a 40-kDa cyclophilin-related protein. *J Biol Chem* 267:5503-5507.
- Konarska MM, Sharp PA. 1987. Interactions between small nuclear ribonucleoprotein particles in formation of spliceosomes. *Cell* 49:763-774.
- Lambwright DG, Sondck J, Bohm A, Skiba NP, Hamm HE, Sigler PB. 1996. The 2.0 Å crystal structure of a heterotrimeric G protein. *Nature* 379:311-319.
- Last RL, Maddock JR, Woolford JL Jr. 1987. Evidence for related functions of the RNA genes of *Saccharomyces cerevisiae*. *Genetics* 117:619-631.
- Laubner J, Fabrizio P, Teigelkamp S, Lane WS, Hartmann E, Lührmann R. 1996. The HeLa 200 kDa U5 snRNP-specific protein and its homologue in *Saccharomyces cerevisiae* are members of the DEXH-box protein family of putative RNA helicases. *EMBO J* 15:4001-4015.
- Laubner J, Plessel G, Prehn S, Will CL, Fabrizio P, Gröning K, Lane WS, Lührmann R. 1997. The human U4/U6 snRNP contains 60 and 90 kD proteins that are structurally homologous to the yeast splicing factors Prp4p and Prp3p. *RNA* 3:926-941.

- Lossky M, Anderson GJ, Jackson SP, Beggs J. 1987. Identification of a yeast snRNP protein and detection of snRNP-snRNP interactions. *Cell* 51:1019-1026.
- Luban J. 1996. Absconding with the chaperone: Essential cyclophilin-Gag interaction in HIV-1 virions. *Cell* 87:1157-1159.
- Lustig AJ, Lin RJ, Abelson J. 1986. The yeast RNA gene products are essential for mRNA splicing in vitro. *Cell* 47:953-963.
- Maddock JR, Weidenhammer EM, Adams CC, Lunz RL, Woolford JL Jr. 1994. Extragenic suppressors of *Saccharomyces cerevisiae prp4* mutations identify a negative regulator of PRP genes. *Genetics* 136:833-847.
- Madhani HD, Guthrie C. 1994. Dynamic RNA-RNA interactions in the spliceosome. *Annu Rev Genet* 28:1-26.
- Marks AR. 1996. Cellular functions of immunophilins. *Physiol Rev* 76:631-649.
- Mi H, Kops O, Zimmermann E, Jäschke A, Tropschug M. 1996. A nuclear RNA-binding cyclophilin in human T cells. *FEBS Lett* 398:201-205.
- Neer EJ, Schmidt CJ, Nambudripad R, Smith TF. 1994. The ancient regulatory-protein family of WD-repeat proteins. *Nature* 371:297-300.
- Neer EJ, Smith TF. 1996. G protein heterodimers: New structures propel new questions. *Cell* 84:175-178.
- Nestel FP, Colwill K, Harper S, Pawson T, Anderson SK. 1996. RS cyclophilins: Identification of an NK-TR1 related cyclophilin. *Gene* 180:151-155.
- Nilsen TW. 1994. RNA-RNA interactions in the spliceosome: Unraveling the ties that bind. *Cell* 78:1-4.
- Okano Y, Medsger TA Jr. 1991. Newly identified U4/U6 snRNP-binding proteins by serum autoantibodies from a patient with systemic sclerosis. *J Immunol* 146:535-542.
- Page AP, MacNiven K, Hengartner MO. 1996. Cloning and biochemical characterization of the cyclophilin homologues from the free-living nematode *Caenorhabditis elegans*. *Biochem J* 317:179-185.
- Petersen-Björn S, Soltyk A, Beggs JD, Friesen JD. 1989. PRP4 (RNA4) from *Saccharomyces cerevisiae*: Its gene product is associated with the U4/U6 small nuclear ribonucleoprotein particle. *Mol Cell Biol* 9:3698-3709.
- Rosbash M, Harris PK, Woolford JL, Teem JL. 1981. The effect of temperature-sensitive *rna* mutants on the transcription products from cloned ribosomal genes of yeast. *Cell* 24:679-686.
- Rutherford SL, Zuker CS. 1994. Protein folding and the regulation of signaling pathways. *Cell* 79:1129-1132.
- Schreiber SL, Crabtree GR. 1992. The mechanism of action of cyclosporin A and FK506. *Immunol Today* 13:136-142.
- Shannon KW, Guthrie C. 1991. Suppressors of a U4 snRNA mutation define a novel U6 snRNP protein with RNA-binding motifs. *Genes & Dev* 5:773-785.
- Sondek J, Böhm A, Lambwright DG, Hamm HE, Sigler PB. 1996. Crystal structure of a G_A protein $\beta\gamma$ dimer at 2.1 Å resolution. *Nature* 379:369-374.
- Stamnes MA, Rutherford SL, Zuker CS. 1992. Cyclophilins: A new family of proteins involved in intracellular folding. *Trends Cell Biol* 2:272-276.
- Takahashi N, Hayano T, Suzuki M. 1989. Peptidyl-prolyl *cis-trans* isomerase is the cyclosporin A-binding protein cyclophilin. *Nature* 337:473-475.
- Tarn YC, Lee KR, Cheng SC. 1993. Yeast precursor mRNA processing protein PRP19 associates with the spliceosome concomitant with or just after the dissociation of U4 small nuclear RNA. *Proc Natl Acad Sci USA* 90:10821-10825.
- Thali M, Bukovsky A, Kondo E, Rosenwirth B, Walsh CT, Sodroski J, Göttlinger HG. 1994. Functional association of cyclophilin A with HIV-1 virions. *Nature* 372:363-365.
- Trandinh CC, Pao GM, Saier MH Jr. 1992. Structural and evolutionary relationships among the immunophilins: Two ubiquitous families of peptidyl-prolyl *cis-trans* isomerases. *FASEB J* 6:3410-3420.
- Utans U, Behrens SE, Lührmann R, Kole R, Krämer A. 1992. A splicing factor that is inactivated during in vivo heat shock is functionally equivalent to the [U4/U6.U5] triple snRNP-specific proteins. *Genes & Dev* 6:631-641.
- Wall MA, Coleman DE, Lee E, Iñiguez-Lluhi JA, Posner BA, Gilman AG, Sprang SR. 1995. The structure of the G protein heterotrimer G_{iat1} $\beta_1\gamma_2$. *Cell* 83:1047-1058.
- Will CL, Lührmann R. 1997. snRNP structure and function. In: Krainer AR, ed. *Eukaryotic mRNA processing*. Oxford: IRL Press. pp 130-173.
- Wu J, Matunis MJ, Kraemer D, Blobel G, Coutavas E. 1995. Nup358, a cytoplasmically exposed nucleoporin with peptide repeats, Ran-GTP binding sites, zinc fingers, a cyclophilin A homologous domain, and a leucine-rich region. *J Biol Chem* 270:14209-14213.
- Xu Y, Peterson-Björn S, Friesen JD. 1990. The PRP4 (RNA4) protein of *Saccharomyces cerevisiae* is associated with the 5' portion of the U4 small nuclear RNA. *Mol Cell Biol* 10:1217-1225.
- Yean SL, Lin RJ. 1991. U4 small nuclear RNA dissociates from a yeast spliceosome and does not participate in the subsequent splicing reaction. *Mol Cell Biol* 11:5571-5577.
- Yokoyama N, Hayashi N, Seki T, Panté N, Ohba T, Nishii K, Kuma K, Hayashida T, Miyata T, Aebi U, Fukui M, Nishimoto T. 1995. A giant nucleopore protein that binds Ran/TC4. *Nature* 376:184-188.
- Zhao Y, Chen Y, Schutkowski M, Fischer G, Ke H. 1997. Cyclophilin A complexed with a fragment of HIV-1 gag protein: Insights into HIV-1 infectious activity. *Structure* 5:139-146.