Functional Conservation of the Human Homolog of the Yeast Pre-mRNA Splicing Factor Prp17p*

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Splicing of pre-mRNAs involves two sequential transesterification reactions commonly referred to as the first and second steps. In *Saccharomyces cerevisiae*, four proteins, Prp16p, Prp17p, Prp18p, and Slu7p are exclusively required for the second step of splicing. The human homologs of Prp16p, Prp17p, and Prp18p have been identified, and the human proteins hPrp16 and hPrp18 have been shown to be required for the second step of splicing in *vitro*. Here we provide further evidence for the functional conservation of the second step factors between yeast and humans. Human hPrp17, which is 35% identical to the *S. cerevisiae* protein, is able to partially rescue the temperature-sensitive phenotype in a yeast strain where PRP17 has been knocked out, suggesting that the human and yeast proteins are functionally conserved. Overexpression of hPrp17 in the knock-out yeast strain partially rescues the splicing defect seen in *vitro* and *in vivo*. In HeLa cells, hPrp17 is highly concentrated in the nuclear speckles, as is SC35 and many other splicing factors, thus providing further support that this protein also functions as a splicing factor in humans.

Splicing is the process by which introns are removed from pre-mRNAs, and it occurs via two phosphoryl transfer reactions (reviewed in Ref. 1). In the first step, the 5′-exon is cleaved concomitantly with the production of a lariat intermediate. In the second step, the 3′-splice site is cleaved, the exons are ligated, and the intron is released as a lariat product. This process is catalyzed by a multi-component enzyme called the spliceosome. The conventional spliceosome is formed on the pre-mRNA by the assembly of U1, U2, U4/U6, and U5 snRNPs, as well as a large number of non-snRNP splicing factors.

Genetic screens in *Saccharomyces cerevisiae* have lead to the identification of four proteins (Prp16p, Prp17p, Prp18p, and Slu7p) that are specifically required for the second step of splicing in yeast (reviewed in Ref. 2). Mutations in PRP16, PRP17, and PRP18 cause accumulation of splicing reaction intermediates at nonpermissive temperature (3, 4). The genes that encode Prp17p and Prp18p are not essential for cell survival, and absence of either protein causes only a partial block to the second step of splicing in *vitro* (5, 6). This may be because of overlapping functions in some of the second step splicing factors. In fact, the overexpression of Prp16p can suppress a PRP17 mutation, and overexpression of Slu7p suppresses a PRP18 mutation (6).

Mutations in SLU7 (slu7-1) and PRP17 (slu4-1) are synthetically lethal with mutations in the invariant loop 1 of U5 snRNA (7). U5 snRNA interacts with exon sequences at both the 5′- and 3′-splice sites and most likely plays a significant role in aligning the two exons during the second step of splicing (reviewed in Ref. 1). Prp6p, a highly conserved U5 snRNP protein, is required for specificity and fidelity of 3′-splice site utilization (8, 9). Alleles of PRP16, PRP17, PRP18, and SLU7 are all synthetically lethal with an allele of PRP8, prp8–101, which impairs recognition of the 3′-splice site and inhibits the second step (8). These and other genetic studies indicate that there may be a network of physical interactions between Prp16p, Prp17p, Prp18p, Slu7p, and Prp6p (reviewed in Ref. 2).

The requirements for Prp16p, Prp17p, Prp18p, and Slu7p have been temporally ordered with respect to each other and to the requirement for ATP hydrolysis during the second step of splicing (reviewed in Ref. 2). Prp16p and Prp17p act before or concomitant with an ATP-dependent step of splicing, whereas Slu7p and Prp18p act subsequent to this ATP-dependent step (6, 10–12). After the first step of splicing, Prp16p can be efficiently cross-linked to the 3′-splice site, and this binding is Prp17p independent (13). After ATP hydrolysis by Prp16p, Slu7p and Prp18p can be efficiently cross-linked to the 3′-splice site in a manner that is dependent on Prp16p, Prp17p, and Prp18p (13). Thus the Prp17p-dependent step is after Prp16p binds the 3′-splice site but before or concomitant with ATP hydrolysis.

The second step of splicing is understood less in mammals. As in yeast, there is an ATP requirement during the second step, and partially purified second step activities have been functionally ordered with respect to this requirement (14, 15). Recently, the mammalian structural homologs of Prp18p (hPrp18), Prp16p (hPrp16), and Prp17p (hPrp17) were identified (16, 17). hPrp18 and hPrp16 were both shown to be required for the second step of splicing in *vitro* (16, 17). A functional role for hPrp17 has not been shown, although both hPrp17 and hPrp16 associate with the spliceosome late in the splicing pathway (17).

It appears that many of the second step factors are conserved between yeast and humans. In fact, here we show that the human homolog of Prp17p can partially complement the yeast knockout strain. The human protein specifically increases the

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† The abbreviations used are: snRNPs, small ribonucleoprotein(s); EST, expressed sequence tag; PCR, polymerase chain reaction; WT, wild type; LI, lariat intermediate; LP, lariat product; WD, tryptophan and aspartic acid.

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rate of the second step in vitro suggesting that this protein is not only structurally homologous to the yeast protein but also functionally related. hpPrp17 localizes to the nuclear speckles in human cells, as do many splicing factors (reviewed in Ref. 18), thus providing further support that this protein also functions as a splicing factor in humans.

EXPERIMENTAL PROCEDURES

Data Base Search and cDNA Cloning—A data base search was performed using the Genetics Computer Group Wisconsin Sequence Analysis Package TBLASTN program to identify human EST translations with high homology to S. cerevisiae Prp17p. Alignment of two human ESTs (AA057404 and Z19190) with the yeast sequence was the basis for designing two oligonucleotides, 5'-GGC CGA ATT CGA CCT TAG and 5'-GTG TTA CAT GAG G-3', which were used for PCR amplification of DNA from oligo(dT)-primed HeLa cell cDNA library (Stratagene). The resulting 960-base pair PCR product was gel purified and subcloned into a TA cloning vector (Invitrogen). Both strands of the cDNA were sequenced, and the 5'-end of the cDNA was obtained using 5'-RACE-Ready cDNA (CLONTECH) for nested PCR with the supplied anchor primer and oligonucleotides 5'-CTG TCC TCT CAG GTC CCA-3' and 5'-AAC AAT CTG ACT GCA CTG ACG CCC-3'. The 670-base pair PCR product was purified, cloned into a TA vector, and sequenced. The 3'-end of the cDNA was also obtained by nested PCR using a T7 primer (5'-GTA ATA CGA CTC ACT ATA GGG C-3') specific for the library vector and oligonucleotides 5'-GGC CGG ATC CGA CTA and 5'-AGG GCC ATA TGG TAG CAG GC-3'. The hpPrp17 cDNA sequence that we obtained is identical to that recently published by Zhou and Reed (17). spPrp17 and cePrp17 sequences were aligned with S. pombe homologs (data not shown). Probable human (hPrp17), C. elegans (cePrp17), and S. pombe (spPrp17) sequences were aligned with S. cerevisiae (scPrp17) using PILEUP and shaded with BOXSHADE where 50% identity at a position is shaded. The seven WD repeats are indicated with a bar above the sequence.

Antibody Production and Immunofluorescence—Rabbit polyclonal antibodies were generated against a synthetic peptide corresponding to amino acids 129–144 of hpPrp17 (Research Genetics). The antibodies were affinity-purified on columns with the peptide cross-linked to CNBr-activated Sepharose beads (Amersham Pharmacia Biotech) as described previously (22). Immunofluorescence was performed essentially as described previously (23). HeLa cells were grown on coverslips, fixed and stained with primary antibodies: 2 μg/ml antigen-purified hPrp17 antibodies and 1:3000 dilution of mouse anti-SC35 monoclonal antibody (Sigma); and secondary antibodies: anti-rabbit IgG coupled to rhodamine and anti-mouse IgG coupled to fluorescein. Images were photographed using an Axioskop (Carl Zeiss, Inc.), and color photographs were processed using PHOTOSHOP software (Adobe Systems, Mountain View, CA).

RESULTS AND DISCUSSION

A search of GenBankTM was performed to identify a putative human homolog of S. cerevisiae Prp17p (scPrp17p), and PCR was used to isolate a full-length cDNA. The open reading frame encodes a protein of 579 amino acids, and sequence alignment of hpPrp17 and yeast scPrp17p revealed an identity of 35% and similarity of 44% (Fig. 1). Probable Schizosaccharomyces pombe (sp) and Caenorhabditis elegans (ce) homologs of Prp17p were also identified in GenBankTM (Fig. 1). spPrp17 and cePrp17 are 41 and 51% identical to hPrp17, respectively, and 37 and 36% identical to scPrp17p. We also found EST sequences for portions of probable mouse, rat, drosophila, rice, and pine Prp17 homologs (data not shown).

The highest conservation between the Prp17 proteins lies in the carboxyl-terminal half, which contains seven WD repeats (Fig. 1). These domains are thought to adopt a propeller-like structure, as was previously demonstrated for the prototypical member of the WD-protein family, β-transducin (24–26).
members of the large family of WD repeat proteins are involved in diverse functions such as signal transduction, vesicular trafficking, cytoskeletal assembly, and cell cycle control. Many proteins with WD repeats form multi-protein complexes, and thus it has been proposed that these repeats promote protein-protein interactions (27). It is interesting to note that another yeast splicing factor, Prp4, and its mammalian homolog, U4/U6–60 kDa protein, also contain seven WD repeats (28). Prp4 promotes the interaction between the U4/U6 and U5 snRNPs, and has been proposed to be required for the conformational change that occurs in the spliceosome before the first step of splicing (29).

To determine whether hPrp17 is functionally conserved, we used a yeast strain in which PRP17 had been deleted (Δprp17) to examine whether expressing the human gene could rescue the observed temperature-sensitive phenotype. We expressed the vector alone (pG1) full-length yeast PRP17, Δprp17 strain containing pG1 and the Δprp17 strain containing the vector alone or the truncated yeast gene (pG1-ΔC), or the full-length human sequence (pG1-hPRP17) in the WT and Δprp17 strains. We compared growth of these yeast at four different temperatures (Fig. 2 and data not shown). As expected, the WT strain containing pG1 and the Δprp17 strain containing pG1-yPRP17 grow well at all the temperatures tested (Fig. 2). However, the Δprp17 strain containing the vector alone or the truncated yeast gene (pG1-yPRP17ΔC) grows slowly at 24 and 30 °C and...
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Fig. 4. hPrp17 colocalizes with the splicing factor SC35 in nuclear speckles. A, nuclei of HeLa cells stained with Hoechst; B, the same nuclei stained with anti-hPrp17 antibodies and a secondary anti-rabbit IgG coupled to rhodamine; C, anti-SC35 antibodies and a secondary anti-mouse IgG coupled to fluorescein; and D, overlay of hPrp17 and SC35 staining.

does not grow at higher temperatures (Fig. 2). Significantly, the Δprp17 strain containing pG1-hPRP17 does grow at 24, 30, and 34 °C but not at 37 °C (Fig. 2). This indicates that hPrp17 can partially rescue the temperature-sensitive phenotype in the Δprp17 strain.

We next wanted to test whether hPrp17 could rescue the splicing defects observed in vitro and in vivo in the Δprp17 strain. We made extracts from the yeast strains used above for complementation. A time course was performed of in vitro splicing of actin pre-mRNA with these extracts at 23 °C (Fig. 3A). The efficiency of the second step is represented as the amount of second step products (LP + mRNA) divided by the total amount of splicing intermediates and products (LI + LP + mRNA + 5′-exon) (Fig. 3B). Extract from the knockout strain expressing yPrp17 splices similarly to the WT extract (Fig. 3A, lanes 1–8). Splicing with the knockout strain containing the vector alone results in the accumulation of splicing intermediates (LI + 5′-exon) relative to splicing products (LP + mRNA), indicating a partial block to the second step (Fig. 3A, lanes 9–12; note that lane 12 is underloaded on this gel). Significantly, expressing hPrp17 in the knockout strain increases the efficiency of the second step (Fig. 3, A, lanes 13–16 and B); therefore, hPrp17 can partially rescue the in vitro splicing defect seen in the knockout strain, which correlates with its ability to partially complement the temperature-sensitive phenotype in vivo.

When we looked at the in vivo splicing of RP51A and actin, the primary defect of the prp17 knockout was the reduction of mRNA, and no preferential accumulation of unspliced precursor or lariat intermediates (data not shown). This phenotype was previously reported for the ts allele prp17-100 (30), and may result if inhibition of the second step of splicing destabilizes the splicing intermediates. Another possibility is that mutations in PRP17 or deletion of this gene affects the stability of mature RNA. Expression of yPrp17 in the knockout strain does rescue this phenotype, and expression of hPrp17 partially rescues it (data not shown). These results are again consistent with the partial complementation of the knockout strain.

To investigate the role of hPrp17 in mammalian cells, we raised antibodies to peptides corresponding to amino acids 129–144 of hPrp17. After affinity purification, these antibodies specifically detected a 65-kDa protein, the predicted molecular mass of hPrp17, on Western blots of HeLa nuclear extracts (data not shown). These antibodies were used to determine the cellular localization of hPrp17 in HeLa cells (Fig. 4). Immunostaining analysis revealed that hPrp17 is present in discrete regions in the nucleus (Fig. 4, A and B). Colocalization studies using antibodies to the splicing factor SC35 (23) (Fig. 4, C and D) revealed that these regions correspond to the nuclear speckles that are enriched in splicing components (18). hPrp17 probably does not shuttle between the nucleus and cytoplasm because both hPrp17 and SC35 remained localized to the nuclear speckles after treatment of the cells with actinomycin D (data not shown). The nuclear shuttling protein hnRNP A1 (31) was localized to the nucleus before actinomycin D treatment and had both nuclear and cytoplasmic localization after treatment (data not shown). Localization of hPrp17 in the nuclear speckles suggests that, as in yeast, hPrp17 may also function as a splicing factor in humans.

Together with previous complementation experiments, these results suggest that the mechanism of the second step is conserved between yeast and humans. Zhou and Reed (17) have shown that a yeast-human chimeric Prp16 protein could rescue a PRP16 knockout strain, but the full-length hPrp16 could not. Expression of hPrp18 does not rescue the yeast PRP18 knockout, but yeast Prp18p can partially complement Hprp18-depleted splicing extract in vitro (16). The yeast protein Ppr22p has been shown to play a role in the second step as well as later in spliceosome disassembly (32, 33), and the human homolog of Ppr22p, HRH1, can partially rescue the temperature-sensitive
phenotype caused by the prp22-1 allele (34). Therefore, judging by a strict requirement of complementation of a yeast knockout strain, hPrp17 may be the most functionally conserved second step factor. The partial rescue is highly significant because the only human homolog of a splicing factor that has been reported to completely rescue a yeast knockout is the snRNP core protein D1 (35).

In summary, the results presented here provide strong evidence that hPrp17 is indeed a functional homolog of the yeast splicing factor, Prp17p. Our results, showing that hPrp17 is concentrated in nuclear speckles in HeLa cells, together with previously reported results that hPrp17 associates with purified spliceosomes (17), suggest that hPrp17 functions as a splicing factor in humans.

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