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Identification and functional analysis of hPRP17, the human homologue of the *PRP17/CDC40* yeast gene involved in splicing and cell cycle control

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ABSTRACT

The *PRP17* gene of the yeast *Saccharomyces cerevisiae* encodes a protein that participates in the second step of the splicing reaction. It was found recently that the yeast *PRP17* gene is identical to the cell division cycle *CDC40* gene. The *PRP17/CDC40* gene codes for a protein with several copies of the WD repeat, a motif found in a large family of proteins that play important roles in signal transduction, cell cycle progression, splicing, transcription, and development.

In this report, we describe the identification of human, nematode, and fission yeast homologues of the *PRP17/CDC40* gene of *S. cerevisiae*. The newly identified proteins share homology with the budding yeast protein throughout their entire sequence, with the similarity being greatest in the C-terminal two thirds that includes the conserved WD repeats.

We show that a yeast–human chimera, carrying the C-terminal two thirds of the hPRP17 protein, is able to complement the cell cycle and splicing defects of a yeast *prp17* mutant. Moreover, the yeast and yeast–human chimeric proteins co-precipitate the intron–exon 2 lariat intermediate and the intron lariat product, providing evidence that these proteins are spliceosome-associated. These results show the functional conservation of the Prp17 proteins in evolution and suggest that the second step of splicing takes place by a similar mechanism throughout eukaryotes.

Keywords: Prp17; spliceosome; splicing factor; WD motif

INTRODUCTION

Pre-mRNA splicing takes place by two consecutive trans-esterification reactions. In the first step, the border between the 5′ exon and the intron is cleaved, yielding the 5′ exon and lariat intron–exon intermediates. In the second step, the 3′ splice site is cleaved and the two exons are joined, creating a mature RNA and the lariat intron (Madhani & Guthrie 1994). Pre-mRNA splicing requires the activity of a large number of *trans*-acting factors (Kramer, 1996; Wang & Manley, 1997; Will & Lührmann, 1997). Many of the proteins involved have been identified by genetic screens for conditional mutations in yeast (Vijayraghavan et al., 1989; Woolford & Peebles 1992; Beggs, 1995). These *prp* (pre-mRNA processing) mutants are partially or completely defective in the removal of intervening sequences from pre-mRNAs. Biochemical and genetic evidence suggests that Prp proteins interact with each other in the process of splicing. In addition to proteins, a number of small nuclear RNA molecules (U1, U2, U4, U5, and U6 snRNAs) form small nuclear ribonucleoprotein particles (snRNPs) that play a pivotal role in splicing, assembling onto the pre-mRNA substrate in a stepwise fashion (Madhani & Guthrie, 1994).

The *PRP17* gene of the yeast *Saccharomyces cerevisiae* encodes a protein that participates in the second step of the splicing reaction; although not essential for splicing, intermediates accumulate even at the permissive temperature in *prp17* mutants (Vijayraghavan et al., 1989). Prp17p seems to act together with the *PRP16*, *PRP18*, and *SLU7* gene products; Prp16p and Prp17p act in an ATP-dependent stage that precedes the one performed by Slu7p and Prp18p, which is ATP-independent (Jones et al., 1995). Functional

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interactions between Prp17p and the U2 and U5 snRNAs are suggested by the synergistic lethality of alleles of *PRP17* in combination with specific U2 or U5 snRNA mutations (Frank et al., 1992; Xu et al., 1998). However, physical association of Prp17p with U2 or U5 snRNA has not been reported.

It was recently found that the yeast *PRP17* gene is identical to the cell division cycle *CDC40* gene. The *PRP17/CDC40* gene codes for a protein with several copies of the WD repeat (Vaisman et al., 1995). This repeated motif is found in a large family of proteins that play important roles in signal transduction, cell cycle progression, splicing, transcription, and development (for review, see Neer et al., 1994). It has been postulated that the repeated motif can serve in protein–protein interactions, either with other members of the WD family, or with proteins carrying different motifs, such as the 34-amino acid TPR motif (Sikorski et al., 1990; Williams et al., 1991).

The *PRP17/CDC40* gene was first identified through the temperature-sensitive mutation *cdc40-1*, which affects both the mitotic and meiotic cell cycles. At the restrictive temperature, *cdc40-1* cells arrest with a large bud and one undivided nucleus (Kassir & Simchen, 1978). A full deletion allele of the *PRP17/CDC40* gene also shows a temperature-sensitive phenotype (Jones et al., 1995; Vaisman et al., 1995; Seshadri et al., 1996). The null mutant arrests in the cell cycle with a phenotype identical to that of the original *cdc40-1* allele: DNA replication seems to be completed (as measured by FACS analysis), but the cells are sensitive to hydroxyurea and to treatments that cause DNA damage (Kassir & Simchen, 1978; Kassir et al., 1985; Kupiec & Simchen, 1986; Vaisman et al., 1995). In addition, the Prp17/Cdc40 protein is needed for the maintenance and/or establishment of the mitotic spindle: in *cdc40*Δ cells held at the restrictive temperature, the spindle, detected by anti-tubulin antibodies, disappears (Vaisman et al., 1995). The identity between a gene known to participate in splicing and one isolated as a cell cycle regulator suggests that the *PRP17/CDC40* gene plays a role in regulating the cell cycle through splicing.

Here we report the identification of three apparent homologues of Prp17p from *Homo sapiens*, *Caenorhabditis elegans*, and *Schizosaccharomyces pombe*, the isolation of cDNA encoding the human homologue (hPRP17), and its functional characterization in budding yeast. The high level of similarity between budding yeast Prp17p and the predicted protein sequences from the three other organisms does not extend to their N termini. Indeed, the full-length human protein was unable to function in yeast, but a chimeric protein composed of the yeast N terminus fused to the more closely related C-terminal two thirds of the human Prp17 protein complemented the temperature-sensitive growth and second step splicing defects caused by deletion of the yeast gene.

RESULTS

Identification of putative homologues of the *PRP17/CDC40* yeast gene and isolation of human cDNA

While searching the EMBL/GenBank/DDBJ databanks, we identified genomic sequences from *S. pombe* and *C. elegans* and, in the dbEST database, a short EST sequence of human origin whose virtual translations showed similarity to the yeast Prp17/Cdc40 protein (Fig. 1). The *C. elegans* and *S. pombe* sequences are closely related to the *S. cerevisiae* protein mainly in the C-terminal two thirds (39% and 42% identity, respectively; Table 1), which includes the WD repeats. The polypeptide predicted from the human EST clone was also homologous to the C terminus of the yeast Prp17 protein (39% identity), but the EST clone appeared to be incomplete at the 5′ end, which aligned with codon 110 of the yeast sequence. A nested PCR strategy was used to isolate the 5′ region from a human breast cDNA library and a PCR product of 1,200 bp was cloned that contains an ORF whose 3′ region overlaps, as expected, the previously sequenced EST clone. The complete hPRP17 ORF was reconstructed, and encodes a single open reading frame of 578 codons (Fig. 1), with no obvious 5′ untranslated sequences (no stop codons at the 5′ end). Only the DNA around the third methionine codon (codon 103) shows a strong Kozak consensus sequence (CAAatgG; Kozak, 1996). Initiating translation at position 103 gives a human protein of 476 amino acids in length, comparable to the yeast 455 amino acid protein. However, a comparison of the putative human ORF with the other sequences suggests that the 5′ end is part of the ORF and that it may lack an initiating methionine codon at the extreme 5′ end. In order to look for cDNAs carrying extended 5′ regions of the human gene, a RACE reaction (Frohman, 1990) was performed using human placenta RNA. This produced several PCR products, the largest of which had a 5′ end identical to that previously isolated. No mRNAs carrying longer stretches of 5′ sequence were detected by either method.

Four WD repeats were originally identified in yeast Prp17p (Vaisman et al., 1995; Seshadri et al., 1996). The alignment of the sequences from all four organisms allows a better assessment of the number of likely WD repeats in the Prp17 proteins. PROSITE identifies five WD repeats that are well conserved. Another two weaker matches to the WD consensus can be identified (numbers 3 and 5 in the series, Fig. 1) that are suitably spaced and contain the highly conserved aromatic residue at the penultimate position, the structurally important aspartate or asparagine seven residues from the carboxy end, and other conserved hydrophic and aromatic residues. Thus, the Prp17 proteins appear to contain up to seven WD motifs. A search in the

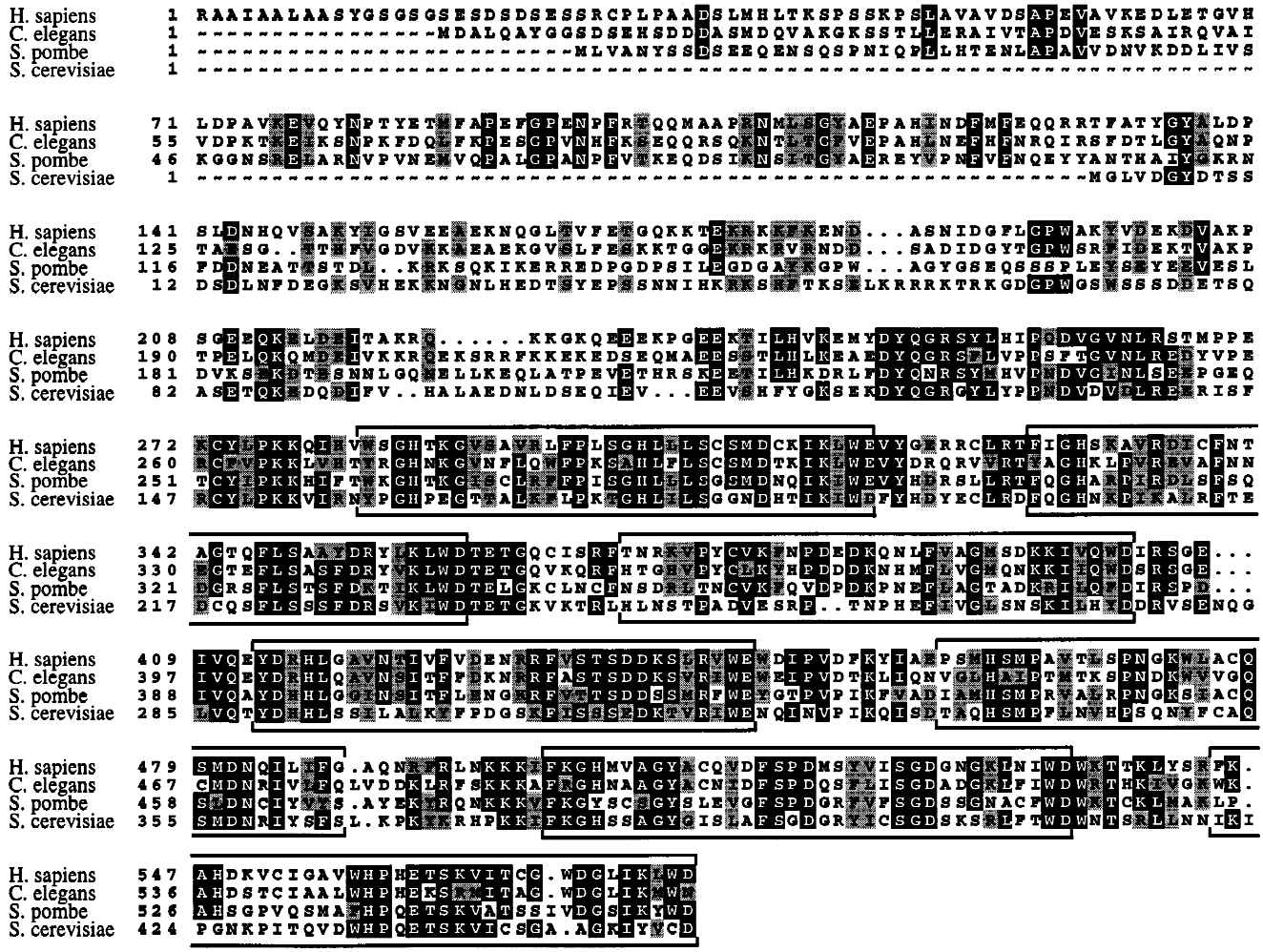

FIGURE 1. Amino acid alignment of the human, *C. elegans*, *S. pombe*, and *S. cerevisiae* Prp17 protein sequences. These sequences were identified in a BLAST search of databases through the NCBI at NIH. The alignment was made using the PILEUP program of the Wisconsin GCG package. Identical residues and chemically related residues that occur at the same position in at least three of the four sequences are shown white on black and shaded grey, respectively. WD (G-beta) repeats were identified using the PROSITE program and searching by eye for the consensus residues (Neer et al., 1994). The human sequence was derived from a skeletal muscle EST (GeneBank accession no. Z19190), with the upstream sequence produced by PCR of a human breast cDNA library or 5′ RACE of human placenta RNA (see Materials and Methods). The *C. elegans* sequence was derived from cosmid CELF49011.1 (Entrez accession no. AF039711) starting at nt 30,705. This assumes that nt 31,390–32,260 represent a previously unidentified intron, based on the existence of splice site consensus sequences and the production of an in-frame junction in codon 230. The *S. pombe* sequence is from locus SPBC6B1.10 (Entrez accession no. AL021838). The cDNA sequence of hPRP17 has been deposited in DDBJ/EMBL/GenBank (accession no. AF061241).

databases using the N-terminal half of hPRP17 showed homologous ESTs from several other organisms, including animals (mouse, rat, *Drosophila*) and plants (*O. sativa* and *A. thaliana*). These results imply that the *PRP17* gene, like other splicing factors, is conserved throughout the evolutionary scale.

Complementation of the yeast *prp17/cdc40*Δ mutant by the *hPRP17* gene

For functional analyses, we tested the expression in yeast of the full-length 578-codon ORF as well as the shorter 476-codon sequence starting at codon 103. Yeast cells bearing the deletion allele of the *PRP17/CDC40* gene show a temperature-sensitive cell cycle arrest phenotype. To explore whether the extensive homology between the yeast and human proteins is indicative of a conserved function, we tested whether *hPRP17* expression in vivo could complement the temperature sensitivity of a *prp17*Δ yeast strain (IM2). The hPRP17 full-length and the short hPrp17 version (coding from aa 103 to the C terminus) were cloned into the yeast expression vector YCpIF16 (Foreman & Davis, 1994) such that their expression was controlled by the galactose-inducible *GAL1* promoter. The YCpIF16-hPRP17 constructs (pSBY69 and pSBY77,

TABLE 1. Percent identity between pairs of sequences from *H. sapiens*, *C. elegans*, *S. pombe*, and *S. cerevisiae*.[a]

	C. elegans	*S. pombe*	*S. cerevisiae*
Overall			
H. sapiens	50	41	29
C. elegans	—	34	31
S. pombe		—	33
N-term			
H. sapiens	40	30	19
C. elegans	—	25	22
S. pombe		—	24
C-term			
H. sapiens	60	53	39
C. elegans	—	43	39
S. pombe		—	42

[a]In each case, the beginning of the first WD repeat was taken as the boundary between the N-terminal and C-terminal regions. Identities were calculated using Genestream ALIGN, CRBM.

respectively) were introduced into *prp17*Δ yeast cells and the transformants were tested for their ability to grow at the restrictive temperature either on galactose or on glucose-containing media. Neither human construct complemented the temperature-sensitive growth defect (Fig. 2 and data not shown).

It is possible that the human protein is nonfunctional because it is not homologous enough to be recognized by yeast proteins that normally interact with Prp17p. Because the yeast and human proteins differ mainly at their N termini, a yeast–human chimeric construct was made. This hybrid protein consists of the first 156 amino acids of the *S. cerevisiae* Prp17 protein followed by 297 amino acids of hPRP17p, and includes the human WD motifs. The resulting chimeric sequence was cloned into a *GAL1* expression vector (pSBY53) and introduced into yeast *prp17*Δ cells. As shown in Figure 2, the chimeric protein was able to complement the temperature-sensitive phenotype of *prp17*Δ cells in galactose-containing medium. No growth was seen at the restrictive temperature (34 °C) in glucose-based medium (data not shown). As controls, the yeast N-terminal domain (155 amino acids) and the human C-terminal domain (319 amino acids) were cloned separately into YCpIF16 and introduced into *prp17*) cells. These constructs were unable to complement the temperature-sensitive phenotype (Fig. 2). As an additional control, we created a fusion between the N terminus domain of Prp17 and a well-characterized (but functionally unrelated) WD-containing protein, Tup1. *TUP1* is a yeast gene involved in transcription regulation; its product contains, as Prp17p, seven WD repeats located at the C terminus (Komachi et al., 1994). We created a chimeric gene coding for the first 156 aa of Prp17 followed by the last 386 aa of the Tup1 protein. The chimeric gene, when cloned into YCpIF16 and introduced into yeast *prp17*Δ cells, failed to complement the temperature-sensitive phenotype (data not shown). These results rule out the possibility that the yeast N terminus, when fused to any WD-containing protein domain, is able to complement the yeast *prp17*Δ mutation. The positive results obtained with pSBY53 indicate that hPRP17 is a true functional homologue of the yeast *PRP17* gene. We also conclude that the yeast N terminus of Prp17p plays an important role, which cannot be replaced by the divergent human N terminus.

In addition, we have performed a screen for *trans*-acting mutants that show synthetic lethality with *prp17*Δ (to be published elsewhere). These mutants fall into 10 complementation groups and are unable to grow in the absence of Prp17p. Using a plasmid-shuffling procedure, we have shown that, in all the mutants, YCpIF16 carrying the chimeric yeast–human sequence is able to replace the function of the yeast *PRP17* gene on galactose-based medium. By this criterion, too, the human C terminus can replace its yeast counterpart, stressing the functional conservation between the two proteins.

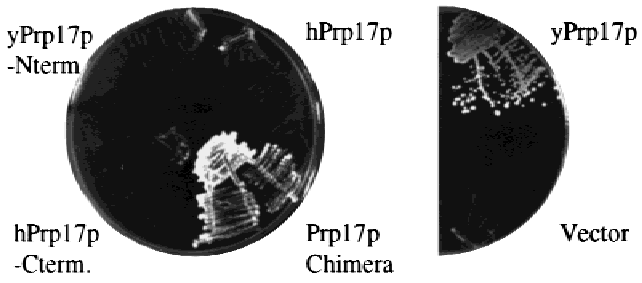

FIGURE 2. Complementation of the yeast *prp17::LEU2* strain by a yeast–human hPRP17 chimera. All the plasmids were derivatives of YCpIF16, a centromeric vector that allows expression from the *GAL1* promoter. Selective galactose plates are shown four days after streaking of the yeast transformants bearing the different plasmids: YCpIF16 (vector), and YCpIF16 carrying: the yeast *PRP17* gene (yPrp17p); the N terminus of the yeast *PRP17* gene (yPrp17-Nterm); the hPRP17 gene (hPrp17p); the C terminus of hPRP17 (hPrp17p-Cterm); the yeast–human chimeric PRP17 (Prp17p Chimera).

Effects on splicing in vitro and interaction with spliceosomes

prp17 mutant strains display a step 2 splicing defect at the restrictive temperature (Frank et al., 1992; Jones et al., 1995), although the physical association of yeast Prp17p with spliceosomes or with any splicing factor has not been demonstrated. To investigate the direct involvement of Prp17p and the chimeric yeast–human protein in the pre-mRNA splicing process, their effects on splicing in vitro were tested. IM2 (*prp17*Δ) cells bearing the YCpIF16 vector alone, or YCpIF16 carrying either the yeast *PRP17* gene (p1896) or the chimeric *PRP17* (pSBY53) were grown on galactose medium at 23 °C and then spheroplasted. The spheroplasts were heat-treated at 34 °C to inactivate the mutant Prp17 protein and splicing extracts were prepared. Splicing

assays were performed using an actin pre-mRNA substrate, and the products were analyzed at 10-min intervals (Fig. 3). Extract lacking Prp17p (IM2/YCpIF16) showed a strong splicing defect: the reaction kinetics of both steps were reduced, step 2 more so than step 1 (Fig. 3, lanes 4–6). In contrast, extract containing the yeast Prp17 protein spliced the actin substrate efficiently (Fig. 3, lanes 1–3) and the splicing products were clearly seen at the earliest time point. Extract containing the chimeric Prp17p spliced the actin substrate much better than the *prp17*Δ extract. The splicing kinetics with this extract (Fig. 3, lanes 7–9) were only slightly slower than those of extract with yeast Prp17p. Thus, the chimeric protein substantially reversed the splicing defect caused by lack of the yeast Prp17p.

To determine whether yeast Prp17p or the chimeric protein are spliceosome components, similar splicing reactions were incubated with anti-HA antibodies (that detect the HA-tagged Prp17p and HA-tagged chimera) or, as a control, with anti-Prp8 antibodies. Prp8p (a U5 snRNP protein) is a component of spliceosomes throughout the splicing reaction (Teigelkamp et al., 1995b), as indicated by the presence of radiolabeled reaction intermediates and spliced product RNA species in the precipitates (Fig. 4, lanes 1, 4, 7; the mRNA end product is not shown because it was not detected in any immunoprecipitation). The decreased signals in lane 4 reflect the poor spliceosome formation and low level of splicing in extracts lacking Prp17p. Anti-HA antibodies precipitated the intermediates and products of splicing from extracts containing either yeast or chimeric Prp17p (Fig. 4, lanes 3 and 9), although the amounts were lower in the case of the chimeric protein. Control incubations without antibodies (Fig. 4, lanes 2 and 8), or with extract lacking Prp17p (Fig. 4, lanes 4–6) did not result in precipitation of these RNA species. These results demonstrate the physical association of the HA-tagged yeast and chimeric Prp17 proteins with spliceosomes. Further, the precipitation of the excised intron as well as the splicing intermediates indicates the continued association of the Prp17 proteins with a post-splicing complex after completion of the splicing reaction. Exon 1 was observed in the Prp8 and yPrp17p immunoprecipitations, but not in the chimeric Prp17p immunoprecipitation. This reproducible absence of exon 1 may be due to the sensitivity of the assay system because the splicing efficiency of the chimeric strain was lower than that of the wild type. Alternatively, it may indicate that exon 1 is more loosely associated with the spliceosome in the presence of the chimeric protein. Figure 4 also shows that pre-mRNA is co-precipitated with Prp17p (lanes 3 and 9), significantly more than the variable background level, indicating that Prp17p associates with the spliceosome prior to the first catalytic step.

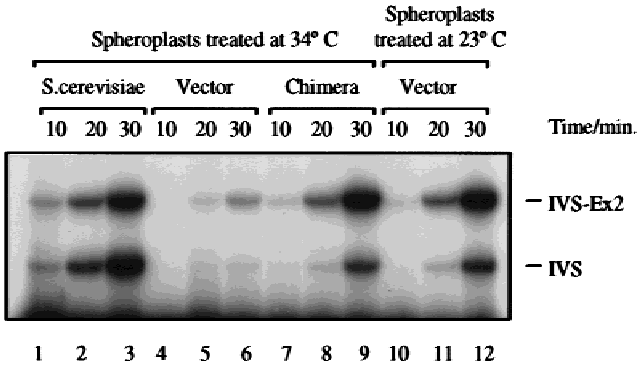

FIGURE 3. Complementation of the splicing defect as assayed by in vitro splicing. Extracts prepared from heat-treated (34 °C/45 min) IM2 (*prp17::LEU2*) spheroplasts carrying p1896 (yeast HA:Prp17p; lanes 1–3), YCpIF16 (vector; lanes 4–6), or pSBY53 (chimeric HA:Prp17p; lanes 7–9), and nonheat-treated (23 °C/45 min) spheroplasts of IM2/YCpIF16 were incubated with actin pre-mRNA under splicing conditions for 10, 20, or 30 min, as indicated. Reaction products were then analyzed by SDS-PAGE and autoradiography. The lariat intron–exon intermediate (IVS-Ex2) and lariat intron product (IVS) are shown.

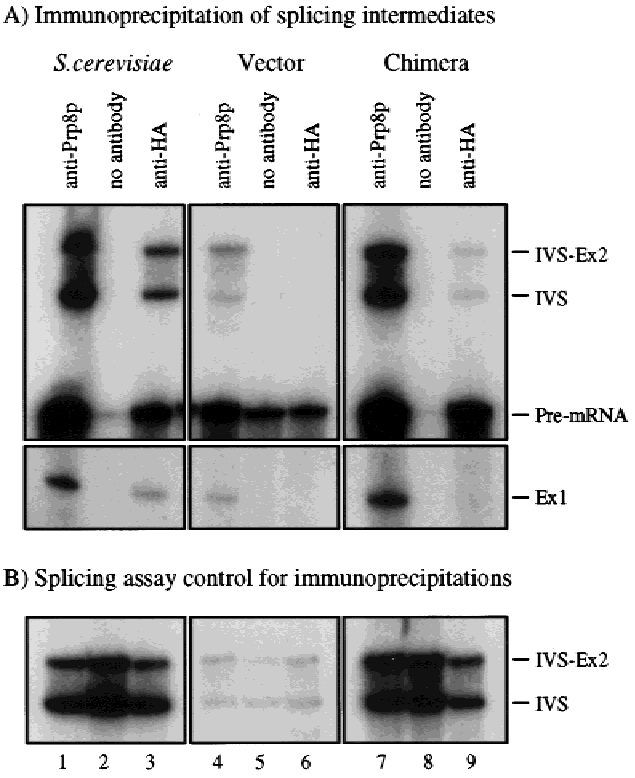

FIGURE 4. Co-immunopecipitation of yeast spliceosomes with yeast and yeast–human chimeric Prp17p. Splicing reactions (50 mL) prepared as for Figure 3 were incubated for 20 min, then 5 mL aliquots were analyzed for splicing activity (**B**) and the remainder were incubated with anti-Prp8p antibodies (lanes 1, 4, 7), protein A-Sepharose beads without antibodies (lanes 2, 5, 8), or anti-HA antibodies (lanes 3, 6, 9) and the RNAs in the precipitates were analyzed (**A**). Only the lariat intron–exon intermediate (IVS-Ex2), lariat intron product (IVS), and pre-mRNA substrate (Pre-mRNA) are shown.

Expression of the hPRP17 gene

The yeast *PRP17* gene is expressed at very low levels. Northern blot analysis shows barely detectable levels of yeast mRNA, which stay constant throughout the cell cycle (data not shown). Accordingly, very low amounts of the Prp17 protein are needed in order to complement the yeast mutant: a centromeric plasmid carrying the *PRP17* gene under the *GAL1* promoter is able to complement the temperature-sensitivity of a *prp17/cdc40*Δ mutant even on glucose-based medium, in which the gene is expressed at very low, basal levels (Boger-Nadjar et al., 1998).

RT-PCR performed to determine whether the gene is expressed in different human tissues detected hPRP17 in placenta, breast, B-cells, and testis (data not shown). In addition, a search of the databases reveals that ESTs carrying hPRP17 sequence have already been isolated from libraries made from the following tissues: leg muscle, fetal liver, spleen, uterus, colon, and fetal brain whole tissue. Taken together, these results show that, like some other human splicing factors (Gee et al., 1997), hPRP17 is expressed at low levels in all human tissues.

DISCUSSION

In this report we describe the identification of human, nematode, and fission yeast homologues of the *PRP17/CDC40* gene of *S. cerevisiae*, which plays important roles in both splicing and cell cycle progression. The greatest similarity between all these proteins is in the WD domains, and these regions are more similar to each other than to other WD sequences in the databases (not shown). In addition, the N-terminal regions of the human, nematode, and fission yeast sequences are clearly related, and the extreme N termini of all four sequences are rich in serine and acidic residues. Alignment of these four sequences allowed the detection of seven WD repeats, with each repeat unit highly conserved between the four organisms. Two of the repeat units only weakly match the consensus; however, considerable variations from the consensus have been noted in other WD proteins (Neer et al., 1994; Neer & Smith, 1996). WD repeats are also present in β-subunits of G proteins and are thought to function as protein–protein interactive surfaces. Accordingly, most of the known proteins that carry this repeat are members of multiprotein complexes (Neer et al., 1994). The crystal structure of the β-subunit of a heterotrimeric G protein shows that the WD repeats fold to form propeller-like structures (Wall et al., 1995; Lambright et al., 1996). Three other WD-containing proteins are known to participate in RNA processing: Prp4p, a factor that seems to be essential for the association of the U4/U6 snRNP with the U5 snRNP (Bordonne et al., 1990; Ayadi et al., 1997), CstF, a polyadenylation factor (Takagaki & Manley, 1992), and Sof1, a U3 snoRNP protein that participates in ribosomal RNA processing (Jansen et al., 1993). The human homologue of Prp4p has been identified and, again, seven WD repeats are recognizable in the yeast and human proteins (Horowitz et al., 1997; Lauber et al., 1997). It is therefore likely that both Prp4p and Prp17p have a seven-blade propeller structure that mediates their interaction with other splicing factors or cofactors.

We show that a yeast–human chimera, carrying the C-terminal two thirds of the hPRP17 protein, was able to complement the growth and splicing defects of a yeast *prp17* mutant, whereas the full-length hPRP17 protein, with its divergent N terminus, was unable to do so, nor was a shorter form that is a similar size to yeast Prp17p. Because the region carrying the WD repeats most likely facilitates protein–protein interactions, the complementation probably required interaction of the C terminus of hPRP17p with yeast proteins. This interaction must be specific, because a chimeric protein carrying the yeast N terminus and the WD-containing C terminus domain of an unrelated protein (Tup1) failed to complement the yeast mutant. On the other hand, the divergent human N terminus was unable to replace the N terminus of the yeast protein, suggesting that the interactions with this region of Prp17 may differ in these organisms. Significantly, three different alleles of *PRP17*, which show synthetic lethality with mutations in the U5 snRNA, in *PRP16*, and in *PRP18*, all map to the N terminus of the yeast protein (Seshadri et al., 1996).

Recently, the cloning of an almost identical human cDNA was reported (Zhou & Reed, 1998). The coding potential of that cDNA differs from those reported here by only two amino acids at the N terminus (Met, Ser instead of Arg). Because the sequence of the cDNAs differ, there might be alternative transcription start sites that may result in proteins that differ at the N terminus. The protein produced by translation of the entire ORF or by internal initiation at Met 103 of our cDNAs failed to function in yeast. Zhou and Reed (1998) presented evidence that their hPrp17 protein associates with human late spliceosomal complexes in vitro, although no functional activity was demonstrated. Here we show functional complementation by a yeast–human chimeric Prp17p of the splicing defect of a yeast *prp17* knock out mutation. In addition, data presented here demonstrate for the first time that yeast Prp17p (as well as chimeric Prp17p) directly associates with spliceosomes rather than performing a more indirect role, a distinct possibility in view of its cell cycle link and the fact that the PRP17 gene is only essential at high growth temperatures. In contrast to previous reports that *prp17* mutants are defective only in step 2, we observed reduced step 1 kinetics and reduced levels of spliceosomes in addition to a (stronger) step 2 defect with the complete absence of Prp17p (*prp17*Δ extract). Thus, Prp17p may influence the efficiency of spliceosome formation.

Prp17 is partially required for the second step of splicing. This step is performed in yeast by a group of proteins that includes the *PRP16*, *PRP17*, *PRP18*, and *SLU7* gene products. The second step can be divided into an ATP-dependent stage in which Prp16p and Prp17p participate, and an ATP-independent stage that requires Prp18p and Slu7p (Jones et al., 1995). Data presented here show that both the yeast and the yeast–human chimeric Prp17 proteins remain associated with post-splicing complexes that contain the products of the second step of splicing. This is unlike the behavior of Prp16p, which seems to interact more transiently with spliceosomes immediately prior to the second step of splicing (Schwer & Guthrie, 1991; Zhou & Reed, 1998).

The identification of a human homologue of the yeast *PRP18* gene was reported (Horowitz & Krainer, 1997). In HeLa nuclear extracts immunodepleted of hPRP18, the second step of the splicing reaction was abolished, but could be restored upon addition of recombinant hPRP18 protein. Although the yeast Prp18p also could restore splicing ability to immunodepleted HeLa cells, the complementary reaction (in vitro complementation of a yeast *prp18* mutant by hPRP18) was not successful. Neither could the hPRP18 cDNA complement the temperature sensitivity of a *prp18* yeast mutant in vivo (Horowitz & Krainer, 1997). The human homologue of the yeast *PRP16* gene was identified recently also. Immunodepletion of hPRP16 in human extracts caused a severe deficiency in the second step of the splicing reaction that could be restored by the addition of recombinant hPRP16 (Zhou & Reed, 1998). The identification of human homologues of the *PRP16*, *PRP17*, *PRP18*, and *SLU7* yeast genes (Horowitz & Krainer, 1997; Zhou & Reed, 1998; this report) suggests that the second step of splicing in humans and yeast takes place by a similar mechanism. As for the *PRP17* gene, the N termini of both the hPRP18 and hPRP16 proteins differ significantly from their yeast homologues and may account for the lack of complementation of the yeast mutants by the human cDNA. The divergent domain may prevent the correct interactions between human and yeast spliceosomal components. In the case of Prp16p (Zhou & Reed, 1998), as for Prp17 (this report), yeast–human chimeric proteins carrying the yeast N termini were able to complement the yeast mutants.

The yeast *PRP17* gene was isolated originally as a cell cycle gene (Kassir & Simchen, 1978; Kassir et al., 1985). What may be the relationship between splicing and cell cycle control? Most yeast *PRP* genes were identified originally through conditional mutants that failed to grow at the restrictive temperature. Most of these mutants do not arrest at a defined point of the cell cycle (Shea et al., 1994). However, some splicing mutants do show cell cycle-specific defects. These include *prp17/cdc40*, *prp8/dbf3*, and *prp3/dbf5* mutants of *S. cerevisiae* (Johnston & Thomas, 1982; Shea et al., 1994; Vaisman et al., 1995), and *prp1* and *prp8/cdc28* mutants of *S. pombe* (Lundgren et al., 1996; Urushiyama et al., 1997), all of which cause cell cycle arrest at the G_2 stage.

Two possible general models may explain this effect. (1) The products of these genes may be needed for the splicing of a specific transcript or transcripts required for progression through the G_2 stage of the cell cycle. By this hypothesis, splicing serves as a regulatory mechanism of the cell cycle, in a way similar to the regulation exerted at the level of transcription, translation, posttranscriptional modification, or protein degradation (Nurse, 1990; Surana et al., 1991; Irniger et al., 1995). The cell cycle-specific transcripts may be especially sensitive to mutations in these splicing genes; their splicing becomes rate-limiting and causes cell cycle arrest. This may be due to some unique feature of their introns that makes their splicing inefficient and requires either higher levels of general splicing factors or the recruitment of some specific splicing factor(s). (2) Alternatively, mutations in these genes disrupt the normal process of pre-mRNA splicing, and elicit a checkpoint response that arrests the cell cycle, similar to the one observed when the integrity of other cell components, such as the spindle or the DNA, is compromised (Elledge, 1996). It is not clear whether all disturbances to the splicing process should cause cell cycle arrest; most temperature-sensitive *prp* mutants have not been analyzed for cell cycle defects.

We have recently uncovered genetic and biochemical interactions between cell cycle control genes and splicing factors (manuscript in prep.). These include *PRP17/CDC40* and *PRP8/DBF3*. Our results suggest that they may be components of a complex of proteins with a role in both processes. The Prp8 protein is highly conserved throughout evolution (Hodges et al., 1995). In yeast, it has been shown that it participates both in the first and the second splicing reaction, interacting with both the 5′ and 3′ exons (Teigelkamp et al., 1995a). Because at least some of the components of this splicing–cell cycle interaction are present in human cells, it is possible that a similar cell cycle control through splicing exists in humans.

MATERIALS AND METHODS

Yeast strains

IM2: *MATa prp17/cdc40::LEU2 ura3-Nco*Δ *trp1-1 leu2-3, -112 his3-11, 15 ade2-1(oc) can1-100 (oc)*.

Media, growth conditions, and general procedures

Standard molecular biology procedures, such as restriction enzyme analysis and Southern blot analysis, were performed

as described in Sambrook et al. (1989). Yeast media and molecular biology procedures (transformations, DNA preparations, etc.) were as described in Sherman et al. (1986).

Nested PCR cloning

A PCR reaction was performed on a human breast cDNA library cloned in the pACT2 vector (Durfee et al., 1993) using primers Z1 and L2. Z1 is homologous to the *GAL4* AD sequence located upstream of all the cDNA inserts in the library. Primer L2 (5′-TCCTGTCTCAGTGTCCCAGAGC-3′) is homologous to bp 375–397 of the 1.3-kb human EST (Z19190). This PCR reaction produced a ladder of bands of different sizes. A second round of PCR amplification was performed using the same vector-specific primer (Z1) and a second hPRP17-specific oligonucleotide derived from an internal position (L1, bp 293-315 of the EST: CCCTAACAGC CTTACTGTGACC-3′). This second PCR reaction produced an overlapping 1.2-kb band that was cloned into pBluescript and sequenced. In order to reconstruct a full-length cDNA molecule, the 5′ fragment of this cDNA (up to the *Msc* I restriction site) was introduced into the 1.3-kb original EST cDNA clone, also digested with *Msc* I. The final construct (pSBY51) carries a 1,953-bp cDNA insert.

RACE

Marathon RACE-ready cDNA kit (Clonetech) was used, according to the manufacturer's specifications.

RT-PCR

RT-PCR was performed as described in Farrell (1993).

Plasmids

The human clone containing the hPRP17 cDNA (HSB03C101, GeneBank accession no. Z19190, gi 29009) was obtained from Genome Systems Inc. (St. Louis, Missouri) and fully sequenced.

YCpIF15 and YCpIF16 are *TRP1-CEN* vectors that allow the HA-tagged expression of genes from the galactose-inducible *GAL1* promoter (Foreman & Davis, 1994). They differ in the register of the multiple cloning site, which leads to protein fusions in different reading frames.

p1896 is YCpIF16 carrying the whole yeast *PRP17/CDC40* gene (Vaisman et al., 1995).

pSBY77 is YCpIF16 carrying the whole hPRP17 cDNA (1953 bp) of pSBY51.

pSBY69 is YCpIF16 carrying the human ORF from bp 307 to 1749 of pSBY51 (i.e., encoding amino acids 103 to the C terminus).

pSBY53, the yeast–human chimeric construct, was made by a PCR-based method that will be described elsewhere. It carries the first 468 bp of the yeast *PRP17/CDC40* gene fused to 905 bp of hPRP17, cloned in YCpIF16.

pSBY63, carrying the 3′ end of the hPRP17 gene, was created by PCR, using primer U1 (5′-CTACGGTCAACTAT GCCACCTGAG-3′: bp 790–813 of pSBY51) and the universal primer T7. The 1.2-kb PCR product was then digested with *Xho* I, filled-in, and cloned in frame into YCpIF16 digested with *Sma*I.

pM152 was created by partial *SnaB* I and total *Not* I digestion of p1896, and self ligation after filling in the 5′ protruding ends. It carries the first 464 bp of the yeast *PRP17* gene in frame in YCpIF16.

All the constructs based on YCpIF16 were subjected to DNA sequencing to confirm that they represent true in-frame fusions. Several different clones were tested when PCR fragments were used for cloning.

pSBY81, the *PRP17-TUP1* chimeric gene, was constructed as follows. A PCR product containing the 3′ region of the *TUP1* gene (bp 978–2314) was cloned in frame into pM152 digested with *Sac* I and *Eag* I.

Splicing extract preparation and in vitro splicing reactions

Preparation of yeast whole-cell extracts and in vitro splicing reactions were performed as described (Lin et al., 1985), except that the cells were spheroplasted at 34 °C for 45 min. Plasmid p283, which contains an *Alu* I fragment of the yeast actin gene, was transcribed in vitro with T7 RNA polymerase and [α-^{32}P]UTP to produce uniformly labeled splicing substrate RNA (O'Keefe et al., 1996). Splicing reactions (5 mL for splicing activity or 50 mL for immunoprecipitation) were incubated at 20 °C for the times indicated and the reaction products were fractionated on 6% (w/v) polyacrylamide–8 M urea gels and visualized by autoradiography.

Immunoprecipitation

Immunoprecipitations were performed as described by Teigelkamp et al. (1995a), using anti-Prp8 polyclonal antibodies or anti-HA 12CA5 monoclonal antibodies, and washes containing 150 mM NaCl.

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