Genetic Studies of the *PRP17* Gene of *Saccharomyces cerevisiae*: A Domain Essential for Function Maps to a Nonconserved Region of the Protein

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ABSTRACT

The *PRP17* gene product is required for the second step of pre-mRNA splicing reactions. The C-terminal half of this protein bears four repeat units with homology to the β transducin repeat. Missense mutations in three temperature-sensitive *prp17* mutants map to a region in the N-terminal half of the protein. We have generated, *in vitro*, 11 missense alleles at the β transducin repeat units and find that only one affects function *in vivo*. A phenotypically silent missense allele at the fourth repeat unit enhances the slow-growing phenotype conferred by an allele at the third repeat, suggesting an interaction between these domains. Although many missense mutations in highly conserved amino acids lack phenotypic effects, deletion analysis suggests an essential role for these units. Only mutations in the N-terminal nonconserved domain of *PRP17* are synthetically lethal in combination with mutations in *PRP16* and *PRP18*, two other gene products required for the second splicing reaction. A mutually allele-specific interaction between *prp17* and *snr7*, with mutations in U5 snRNA, was observed. We therefore suggest that the functional region of Prp17p that interacts with Prp18p, Prp16p, and U5 snRNA is in the N terminal region of the protein.

REMOVAL of intervening sequences (IVS or introns) from pre-mRNA is an important step in regulation of expression of many eukaryotic genes. The introns in pre-mRNA are removed by two *trans*-esterification reactions. In the first step, cleavage at the 5′ splice site occurs, yielding exon 1 and a branched lariat intermediate. In the second step, the 3′ splice site is cleaved and the exons are ligated, resulting in the mature RNA and the lariat intron. Pre-mRNA splicing requires a large number of *trans*-acting factors that constitute the spliceosome (BRODY and ABELSON 1985; FRENDEWAY and KELLER 1985; GRABOWSKI *et al.* 1985). The spliceosome is comprised of small-nuclear ribonucleoproteins (snRNPs) (GUTHRIE and PATTERSON 1988) and a large, as yet unknown, number of accessory proteins, which are either transiently associated with or are integral components of the spliceosome. Many protein factors required for the splicing reaction have been identified by genetic screens for conditional mutations in the yeast *Saccharomyces cerevisiae* (VIJAYRAGHAVAN *et al.* 1989; BLANTON *et al.* 1992; reviewed in RUBY and ABELSON 1991; WOOLFORD and PEEBLES 1992). Interactions between the Prp gene products have been demonstrated by characterization of extragenic suppressors (CHAPON and LEGRAIN 1992), analysis of synthetic lethal interactions (RUBY *et al.* 1993), or by the characterization of physical interactions using the two-hybrid system (LEGRAIN and CHAPON 1993). The predicted protein sequence encoded by many of the *PRP* genes allows them to be grouped into three classes with the following known functional domains: those with an ATP-dependent RNA helicase motif (*e.g.*, Prp16p and Prp2p), those with a zinc finger motif (*e.g.*, Prp9p and Prp11p) and those with a similarity to the Gβ motif present in the β subunit of the trimeric G proteins (*e.g.*, Prp4p and Prp17p). The significance of these motifs in other nonspliceosomal proteins has been tested in some cases; their presence in Prp gene products suggests that they might have a similar function in pre-mRNA splicing. For example, by analogy to transcriptional activators, one might speculate that the Zn finger motif found in Prp9p and Prp11p might mediate interaction with RNA. While the similarities that allowed the identification of these motifs suggest a function, a direct test is necessary. In the case of spliceosomal proteins, direct tests for function of conserved motifs have been done in few cases, *e.g.*, Prp2p and Prp16p, each with a conserved RNA helicase domain, possess an RNA-dependent ATPase activity that is required for the second step of the splicing reaction (SCHWER and GUTHRIE 1991; KIM *et al.* 1992; KIM and LIN 1993). Structure-function analysis of Prp4p, a spliceosomal component containing the Gβ motif, suggests that these repeat units are essential for function, although they can be altered to a great extent (HU *et al.* 1994).

PRP17 is a gene required for the second step of the splicing reaction, for a *prp17* mutant accumulates splicing reaction intermediates at nonpermissive temperatures (VIJAYRAGHAVAN *et al.* 1989). The predicted protein sequence of Prp17p was found to share homology

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with other Gβ motif containing proteins such as bovine transducin, Ste4p, Cdc4p, and Prp4p (M. COMPANY and J. ABELSON, unpublished results). An allele of *prp17* (*slu4-1* or *prp17-3*) was found to be synthetically lethal with a specific U5 snRNA mutation (FRANK *et al.* 1992). Prp17p is therefore an important player in the second step of the splicing reaction. To define the functional domains in the *PRP17* gene product, we have mapped the mutations in three temperature-sensitive alleles of *prp17*. We have assessed the functional significance of the Gβ motif in Prp17p by generating missense mutations in conserved amino acids at the third and fourth repeats of this motif, and deletion mutations in three of these units. As a second approach, we have constructed doubly mutant strains bearing a specific *prp17* allele and a mutation in another locus (*prp18, prp22, prp23, prp24* or *snr7*) that affect various steps in the splicing pathway. Our results indicate that one functional domain of Prp17p lies in the N-terminal region of the protein and therefore outside of the conserved Gβ motif. We also report that while the conserved Gβ motif appears necessary for function, it is relatively resistant to mutationally induced functional alteration.

MATERIALS AND METHODS

Strains and transformations: *Escherichia coli* DH5α cells were used for cloning and plasmid amplifications, and *E. coli* transformations were done by electroporation using a Biorad electroporator. The *E. coli* strain CJ236 (*dut1 ung1 thi-1 rela1*) was used in experiments designed to introduce mutations by a primer extension strategy. Yeast strains *SS328* (*MATα ade2-101 his3Δ200 lys2-801 ura3-52*) and *SS330* (*MATa ade2-101 his3Δ200 tyr1 ura3-52*) were used for replacement transformation, the *prp* mutants used have been described previously (VIJAYRAGHAVAN *et al.* 1989). The *prp17* ts allele, *slu4-1* (*MATa ura3-52 his3Δ200 ade2-101 trp1Δ63 leu2-1 lys2-801*) was kindly provided by Dr. CHRISTINE GUTHRIE's laboratory. Yeast strains were maintained on standard media as described by SHERMAN *et al.* (1986). The ts strains were maintained at 23°; 37° was used as the nonpermissive temperature. Yeast transformations were done by lithium acetate procedure (ITO *et al.* 1983); the transformants were selected on appropriate dropout media at 23° (SHERMAN *et al.* 1986). The colony-purified individual transformants were checked for growth at 18, 23, 30, and 37° on YPD plates.

Plasmid-rescue method for mapping mutations in *prp17* ts alleles: A wild-type copy of *PRP17* was integrated into the ts *prp17* mutants. Integrative transformation of the plasmid p17RS303 was done after linearizing 10 μg of the plasmid DNA with the restriction enzyme *Bst*EII, which cleaves a single site present in the *PRP17* genomic DNA. The transformants were selected on C-His plates. The integration should create a gene duplication at the *PRP17* genomic locus, with one wild-type and one mutant copy of *prp17* separated by the plasmid sequences. Genomic DNA from single copy integrants of each of the three ts *prp17* mutants were digested with various restriction enzymes (*Bam*HI, *Acc*I, *Sna*BI) and then allowed to self-ligate. The individual recircularized plasmids obtained were rescued by transformation in *E. coli*. Rescued plasmid DNAs were used in a complementation assay in which they were re-integrated into ts mutant strains. The integrants obtained were checked for complementation of the ts phenotype. The mapped genomic segments containing the ts mutations for the three alleles *prp17-1, prp17-2,* or *prp17-3* were sequenced.

Tagging of the *PRP17* genomic locus: The complementing 4.5-kb *Kpn*I-*Cla*I genomic fragment containing *PRP17* cloned in the yeast shuttle vector pPHY18 was subcloned into Bluescript (Stratagene) to generate the plasmid p17KB (Figure 2A). A 1.8-kb *Bam*HI fragment containing the *HIS3* gene was isolated from pYac3 and cloned into the *Xba*I site of p17KB after suitable modifications to generate p17KBH (Figure 2B). This plasmid was the parent plasmid into which mutations at the Gβ motif were introduced. For replacement transformation, the p17KBH Gβ mutant plasmids were digested with *Kpn*I and *Bam*HI and 10 μg of this released 6-kb fragment was used.

The plasmids rescued after *Bam*HI digests from three temperature-sensitive alleles of *prp17* were digested with *Kpn*I and *Bst*EII to release a 2.6-kb fragment. This 2.6-kb *prp17* fragment, containing the ts mutation, was cloned between the corresponding *Kpn*I and *Bst*EII sites of p17KBH to generate clones in which *prp17* ts mutant alleles are tagged with the *HIS3* marker (p17-1KBH, p17-2KBH and p17-3KBH). For the replacement transformation of the ts mutation, a 6-kb *Kpn*I-*Bam*HI fragment from these clones was used.

Mutagenesis of the Gβ motif repeat: Mutagenesis was done by a PCR-based strategy as described by MIKAELIAN and SERGEANT (1992). Wild-type *PRP17* was PCR amplified (94° 45 sec, 45° 1 min, 72° 1 min for 25 cycles) using Taq polymerase (Boehringer Manheim) with the oligonucleotides A1 and 43UR in one reaction, and oligonucleotides 42UR (degenerate) and Sx1 in another reaction (see below for the primer sequences). The products of the PCR reactions that share a common overlapping sequence were mixed in a 1:1 molar ratio and re-amplified with oligonucleotides A1 and Sx1. The final PCR product, containing a pool of Gβ motif mutations, was digested with *Sna*BI and *Bst*EII, and the 1-kb fragment released was ligated into *Sna*BI- and *Bst*EII-digested p17KBH to generate plasmids carrying *prp17Gβ* motif mutations. This ligation mix was transformed into *E. coli,* and the plasmids from individual transformants were sequenced using the oligonucleotide GBS (see below for the oligonucleotide sequence) and the Sequenase kit from USB. The mutant plasmids were digested with *Kpn*I and *Bam*HI and the released 6-kb fragment containing the *prp17* Gβ mutation was used for gene replacement.

KUNKEL's procedure was adopted for mutagenesis done by primer extension strategy. Mutant primer (5 pmol) was annealed to 5 μg of single stranded plasmid DNA p17KB, prepared from the strain CJ236.

Oligonucleotides, 5′-3′: A1, TTCTCCAGATGCCATGGGTTTAGT; 42UR, CAGAGGNTAAGACGGTAAGAATTNGGGNAAATCAGA; 43UR, TTGATAGGCAAGTAACATTCCGAGTGA; Sx1, ATTGACACATGGGCCAATGG; GBS, AGTTCTGAGACGGATGTAC; GBΔ3, ACGTATGATCATGGATCCAATCAGATCAAT; and GB4-1, TCAGGAGTTTCAAAAAGCAGCCTAACTGGGGCTTGGAATAC.

Preparation of extracts and western blot analysis for detection of Prp17p: *prp17* strains were grown at 23° to an OD_{600} of 2 and then a portion of each culture was shifted to 37° for 2 hr. Splicing extracts were prepared from cultures grown at 23° and from those shifted to 37° by the method described in LIN *et al.* (1985). The dialyzed crude extract was concentrated by ammonium sulfate precipitation (350 mg/ml) and dialyzed again, against 50 mM KCl in 20 mM potassium phosphate buffer (pH 7) and 20% glycerol. Protein was estimated by the Bradford assay and 200 μg of protein from each extract was electrophoresed and blotted. Prp17p levels were measured using 1:1000 dilution of anti-Prp17p antibodies directed

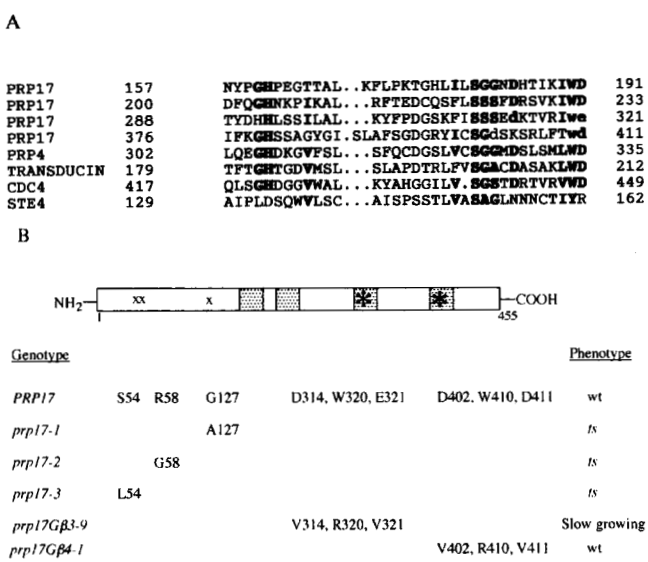


FIGURE 1.—Domain architecture of Prp17p. (A) Protein sequence alignment of the four Gβ repeat units in Prp17p with repeat units present in other proteins. The second Gβ repeat units of bovine transducin, Prp4p, Cdc4p and Ste4p are given for comparison. The amino acids indicated in bold are the highly conserved amino acids in this repeat unit. The period (.), when present, was introduced for alignment between conserved residues. The amino acids in Prp17p that have been targeted for mutagenesis are indicated in lower case. (B) Schematic representation of Prp17p summarizing the location of missense mutations that are associated with discernible phenotypes. The ts (×) mutations in three alleles of *prp17* are shown in the N-terminal region of the protein. ▒, Gβ repeats; *, missense mutations in two of the repeat units. The amino acid changes in the above mentioned missense mutants are given in the single letter code.

against either the N-terminal or C-terminal domains of Prp17p. The antibodies were kindly provided by Dr. MICHELLE JONES and Dr. CHRISTINE GUTHRIE (University of California San Francisco). The detection was by ECL (Amersham) detection kit according to the protocol provided by the manufacturer.

RESULTS

ts mutations map to the N-terminal region of the predicted Prp17p: *prp17-1* and *prp17-2* were isolated in a screen for temperature-sensitive (ts) mutations that affect pre-mRNA splicing; *prp17-3* (*slu4-1*) was isolated in a screen for components of the U5 snRNP (FRANK *et al.* 1992). As a first step to determine regions of the protein that are essential for function, we mapped the mutations in these three temperature-sensitive *prp17* mutants. The mutation in *prp17-1* (ts*365*) is a G to C transversion that results in a change of G(127) to A; in *prp17-2* (ts*487*), an A to G transition results in a change of R(58) to G, and in *prp17-3* (*slu4-1*), a C to T change results in a change of S(54) to L (Figure 1B). All three substitutions are in the N-terminal half of the protein, none in the region of Prp17p that contains four repeat units (M. COMPANY and J. ABELSON, unpublished observation; and see Figure 1A) with homology to the conserved Gβ motif found in other Gβ motif containing proteins such as bovine transducin, Ste4p, Cdc4p, and Prp4p.

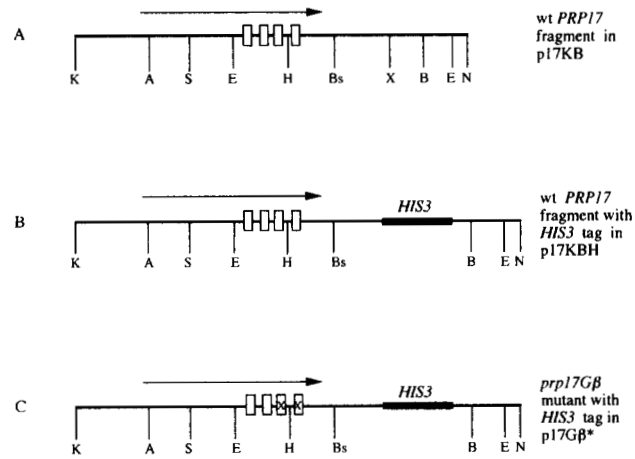


FIGURE 2.—Restriction maps of inserts containing the wild-type *PRP17* and *prp17Gβ** cloned in various plasmids. →, open reading frame; □, Gβ repeat units. (A) Wild-type genomic fragment in plasmid p17KB. (B) *HIS3* tagged wild-type *PRP17* in the plasmid pKBH (the *HIS3* tag had no discernible effect on the function of Prp17p as determined by growth profiles of strains bearing this modification). (C) Map of the insert in plasmid p17Gβ* that contains the Gβ mutations (shown as *). Restriction enzymes are indicated as follows; A, *Acc*I; B, *Bam*HI; Bs, *Bst*EII; E, *Eco*RI; H, *Hin*dIII; N, *Not*I; S, *Sna*BI; and X, *Xba*I.

Analysis of the functional significance for the presence of Gβ motif in Prp17p; generation of missense mutations in the third and fourth repeat units: Because none of the ts alleles mapped to the Gβ domain, we generated 11 missense mutations in highly conserved residues of two of these repeat units. The effects of these mutations were analyzed *in vivo*. A degenerate oligonucleotide was designed so as to change the absolutely conserved aspartic acid (D314) and the highly conserved tryptophan (W320) and glutamic acid (E321) residues of the third repeat unit. D314 was changed to V, A or G; W320 to G or R and E321 to V, A or G. By an overlapping PCR strategy, the DNA sequence encoding the entire *PRP17* ORF was amplified to give a 2-kb product that would contain in it a mixed pool of the above mentioned mutations. This 2-kb PCR product was digested with *Sna*BI and *Bst*EII to release a 1-kb pool of fragments that was cloned into p17KBH to replace the corresponding wild-type *Sna*BI-*Bst*EII fragment (see Figure 2B for the wild-type configuration and Figure 2C for the mutant configuration). Plasmids from 24 independent *E. coli* transformants were sequenced. Of the 24 clones, 12 were mutant at the third repeat and 12 represented wild-type sequences. Two were encountered twice (Table 1, mutant numbers 3 and 5). The mutant plasmids were digested with *Kpn*I and *Bam*HI to give a 6-kb fragment (see Figure 2C) that was used for replacement transformations into the wild-type haploid strains (*SS328* and *SS330*) as

TABLE 1

Missense mutations in *PRP17*

Allele	Amino acid change	Phenotype growth 18°	23°	30°	37°
PRP17		++	++	++	++
prp17-1	G127A	++	++	+/−	−−
prp17-2	R58G	++	++	+/−	−−
prp17-3	S45L	++	++	+/−	−−
Missense mutations in prp17Gβ[a]					
Third repeat					
prpGβ3-1	D314V	++	++	++	++
prpGβ3-2	D314G	++	++	++	++
prpGβ3-3	E321G	++	++	++	++
prpGβ3-4	D314G, W320G	++	++	++	++
prpGβ3-5	D314G, W320R	++	++	++	++
prpGβ3-6	D314A, W320G, E21V	++	++	++	++
prpGβ3-7	D314V, W320G, E321A	++	++	++	++
prpGβ3-8	D314G, W320G, E321A	++	++	++	++
prpGβ3-9	D314V, W320R, E321V	++	++	+	+
prpGβ3-10	D314A, W320G, E321A	++	++	++	++
Fourth repeat					
prpGβ4-1	D402V, W410R, D411V	++	++	++	++

[a] Chromosomal transplacements of *PRP17* in a haploid strain with the *prp17Gβ* mutants listed below were constructed. The amino acid in Prp17p is given first in single-letter code followed by the mutant amino acid introduced at that position.

well as into a wild-type diploid strain (*SS328* × *SS330*). Transplacements were confirmed by DNA blot analysis. The number of transformants obtained at 23° in the wild-type haploid and diploid strains were similar, suggesting that none of the Gβ motif mutations generated were lethal. The growth of the transformants were examined at 18, 30, and 37° on YPD plates. Only one of the Gβ motif mutants, *prp17Gβ3-9*, showed a slower growth rate at 30° and at 37° compared with the wild type (Table 1, mutant 9 in the third repeat) and was therefore not strictly temperature sensitive. Thus unlike the single amino acid changes in the N-terminal region of the protein (*i.e.*, ts alleles) that give a strong temperature-sensitive phenotype (Table 1), all but one of the mutations generated in the third repeat unit of Gβ motif do not cause a discernible growth phenotype.

The effect of making similar changes in the fourth Gβ repeat unit were analyzed. Two of the amino acids mutated were the highly conserved W410 and D411 at the end of the fourth repeat. These residues were changed to R410 and V411, respectively, corresponding to the two altered residues present in the slow growing *prp17Gβ3-9* mutant. In addition, D402 in the fourth repeat was altered to valine (V402). These changes were introduced by a primer extension strategy (as detailed in METHODS) and the mutant allele cloned into the plasmid p17KBH. Transplacement of this missense mutation at the fourth repeat unit (*prp17Gβ4-1*) into the haploid strain *SS328* was done. The transplaced allele did not confer any phenotype, and the cells were wild type in their growth pattern at both 30 and 37° (Table 1).

To ascertain that the phenotypes conferred by the direct transplacement of the mutant allele in haploid strains represent true phenotypes, we analyzed the effects of some of these mutations in haploid spore clones generated by dissection of diploids heterozygous for the transplaced allele. The diploids bearing a single copy of the transplaced allele were taken for analysis. The diploids of the genotype *prp17Gβ3-9/PRP17*, *prp17Gβ3-2/PRP17*, *prp17Gβ4-1/PRP17* and *prp17::LEU2/PRP17* were sporulated and the spore clones from tetrads were germinated at 23, 30 or 37°. The germination was done at different temperatures to study the effects of selective pressure that may operate in the event of a slow growing or leaky phenotype conferred by the transplaced allele. In all of the tetrads analyzed, the *HIS3* gene was used as a marker for the missense *prp17* allele. In spore clones obtained from the diploid *prp17Gβ3-9/PRP17*, two were slow growing at 30 and 37° whereas all four spores grew at similar rate at 23° (Figure 3). In all cases, we observed a 2:2 segregation of the His^+:His^- phenotype, where the His^+ phenotype cosegregated with the slow growing spore clone. All the four spores in each tetrad from *prp17Gβ4-1/PRP17* grew at similar rates at 23, 30 and 37° (Figure 3). Similar analysis of the *prp17Gβ3-2* allele showed that it does not confer any detectable growth phenotype in spore clones germinated at 23, 30 or 37° (Figure 3). Two spores in each tetrad obtained from

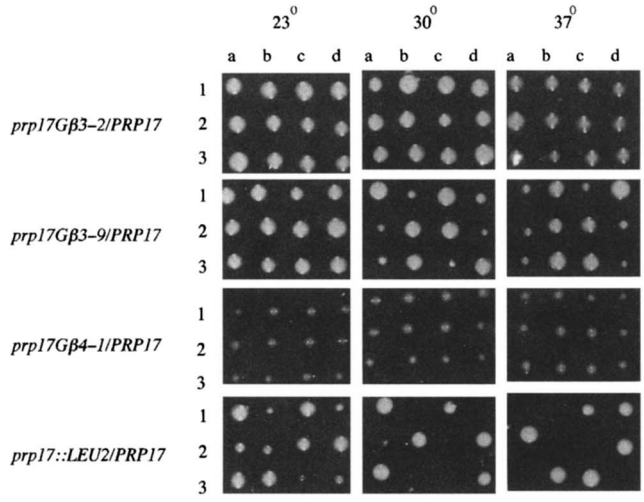


FIGURE 3.—Growth phenotypes of Gβ motif missense mutations. Spore clones with Gβ missense mutations were allowed to germinate at 23, 30 or 37° on YPD medium and their viability and growth rates compared. The diploids of the genotype *prp17Gβ3-2/PRP17*; *prp17Gβ3-9/PRP17*; *prp17Gβ4-1/PRP17* and *prp17::LEU2/PRP17* were sporulated and the tetrad products were allowed to germinate. The growth of three complete tetrads (rows 1, 2 and 3) germinated at various temperature are shown here. The slower growth at rate at 30 and 37° of *prp17Gβ3-9* bearing spore clones is observed, whereas the *prp17Gβ3-2* and *prp17Gβ4-1* spore clones did not exhibit any growth phenotype. The null mutant of *prp17* grows slowly at 23° and is inviable at 30 and 37°.

the diploid with a null allele *prp17::LEU2/PRP17* were slow growing at 23° (Figure 3) and the Leu$^+$ phenotype cosegregated with the slow growing spores. The null allele conferred a lethal phenotype at 30 and 37° (Figure 3). Thus the detailed analysis of the phenotypic effects of these Gβ mutations in haploid strains generated through sporulation of heterozygous diploids concurs with the data obtained from the analysis of direct transplacement of the *prp17* mutations into wild-type haploid strains.

Analysis of deletion mutations in *PRP17*: To determine whether the different Gβ repeat units were functionally important, we constructed individual deletions of three of these repeat units and also of the region near the N-terminus of the protein to which two *ts* alleles map. *prp17* alleles that deleted codons for amino acids 26 to 68 of the N-terminus, or the 28 amino acids of the second Gβ repeat, or the 67 amino acids that include the fourth repeat and 43 amino acids C-terminal to it were constructed (*prp17GβΔNts*, *prp17GβΔ2* and *prp17GβΔ4*, respectively) and transformed into wild-type haploids and diploids. All three mutations resulted in slow growth at 23° and no growth at 30 or 37° (Table 2). A precise deletion of the third Gβ repeat unit (amino acids 292–321) was also constructed, and the transplacement of this allele showed that it conferred a growth pattern similar to the null mutant (Table 2).

To explore the possibility that these deletion constructs give null phenotypes at 30 and 37° by exacerbating the instability of mutant proteins at higher temperatures, we have determined the levels of Prp17p in these strains with deletion alleles of *prp17*. Prp17p is present in low amounts in each cell and can be reproducibly detected only in splicing extracts that are enriched for spliceosomal components. The Prp17p levels were assayed using an antibody raised against either the C-terminal portion of the protein or the N-terminal portion of the protein. Splicing extracts were prepared from cultures grown at 23° and also from cultures that were shifted to 37° for 2 hr. Prp17p levels in wild-type extracts (Figure 4A, lanes 6, 7) are comparable with the levels present in extracts from *prp17GβΔ2* and *prp17GβΔ3* deletion strains after growth at 37° for 2 hr (Figure 4A, lanes 1, 2). Protein levels in *prp17ΔNts* strain grown at 23 and 37° are shown in lanes 3 and 4, and lane 8 shows the levels of protein in a strain with a deletion of the fourth repeat unit (*prp17GβΔ4*). Thus, the levels of Prp17p detected in splicing extracts prepared from mutant strains were similar to those in the wild-type strain, and the null phenotypes of the deletion alleles are not due to the instability of Prp17p.

Mutations at the Gβ motif of Prp17p do not enhance or suppress ts mutations in Prp17p or mutations in other splicing proteins: Since only one of the missense mutations at the conserved Gβ repeat of Prp17p resulted in a weak phenotype, we assessed the cumulative effects of the presence of this mutation with other mutations in splicing proteins. An enhancement or suppression of the conditional phenotype of mutations at any given *PRP* locus by *prp17* alleles would be indicative of interaction between these gene products. First, we constructed strains that are doubly mutant at *PRP17*, *i.e.*, containing both the temperature-sensitive mutation at the N-terminal half of the protein and the Gβ motif mutation in the third repeat unit (Table 1, mutant number 9 in third repeat) in the C-terminal half of the protein. This was done by one-step transplacement of a 3.8-kb *Eco*RI fragment from the plasmid p17Gβ3-9 (see Figure 2C for the map of this fragment) that bears

TABLE 2

Growth rates of deletion mutants of *PRP17*

Alleles	Phenotype growth 23°[a]	30°	37°
PRP17	++	++	++
prp17-1	++	+	−
prp17::LEU2	+	−	−
prp17ΔNts	+	−	−
prp17ΔGβ2	+	−	−
prp17ΔGβ3	+	−	−
prp17ΔGβ4	+	−	−

[a] Growth was monitored by colony size as well as by measuring doubling time in liquid cultures.

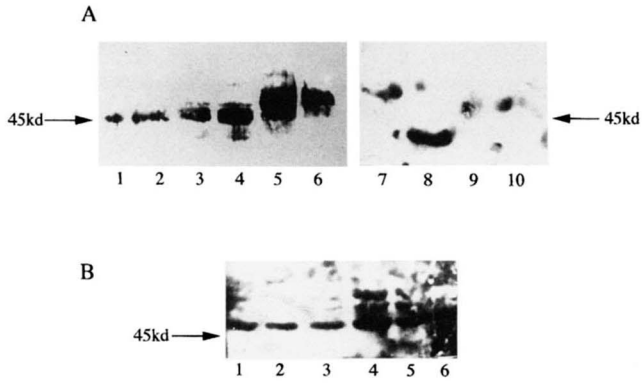


FIGURE 4.—Prp17p content and stability in wild-type and mutant *prp17* strains. Western blot analysis was done to assess the stability of mutant Prp17 protein produced in various *prp17* mutants. Protein (200 μg) present in splicing extracts, prepared as described in MATERIALS AND METHODS, was subjected to electrophoresis and blotted on to Hybond ECL nitrocellulose membrane. Antibodies raised against the C-terminal half of Prp17p were used to detect Prp17p in A, lanes 1–6, and in B, lanes 1–6. The mutant Prp17p in A, lanes 7–10, were detected using antibodies raised against the N-terminal half of the protein. (A) Prp17p levels in various *prp17* deletion strains grown at 23° and those shifted to 37° for 2 hr. The protein extracts in different lanes are; lane 1, *prp17GβΔ2* at 37°; lane 2, *prp17GβΔ3* at 37°; lanes 3 and 4, *prp17GβΔNts* at 23 and 37°, respectively; lane 5, *prp17Gβ3-9* at 37°; lane 6, wild-type *PRP17* at 23°; lane 7, is again wild-type *PRP17* at 23°; lane 8, *prp17GβΔ4* at 37°; lane 9 and 10 are extracts from the null mutant *prp17::LEU2* at 23 and 37°, respectively. (B) Prp17p levels in strains with missense mutations. Lanes 1 and 2, *prp17Gβ3-9* at 23 and 37°, respectively; lanes 3 and 4, *prp17-1* at 23 and 37°, respectively; lane 5, *prp17-3* at 37°; and lane 6, wild-type *PRP17* at 23°. The arrow indicates the migration position of a molecular weight size standard, ovalbumin of 45 kD.

the Gβ mutation into the chromosomal locus in the *prp17* ts mutants (*prp17-1, prp17-2,* and *prp17-3*). The transplacement of this fragment into the ts mutants should create a double mutation in two regions of *PRP17* at the chromosomal locus. The haploid transformants were again analyzed for their growth rates at various temperatures (18, 23, 30 and 37°). None of the double mutants showed any difference in growth when compared with the parent *ts prp17* mutation (data not shown). This implies that acquiring a Gβ motif mutation does not enhance or suppress the temperature-sensitive phenotype in *prp17-1, prp17-2* or *prp17-3* (Table 3). A double mutant strain was also constructed that contains missense mutations in the third and the fourth Gβ repeat units to assess interaction between these two repeat units. This was done by cloning the fourth repeat containing the missense mutations as a *Hin*dIII-*Not*I fragment (see Figure 2C for map), into the plasmid bearing the *Gβ3-9* mutation. After transplacement of this double mutant into haploid wild-type strains, we found that it conferred a null phenotype (Table 3), thereby showing an enhancement of the *prp17Gβ3-9* phenotype.

We have also screened for genetic interaction between the Gβ domain of Prp17p and other splicing factors involved in different steps of the splicing pathway by constructing double mutants. *prp17Gβ3-9* and *prp17Gβ4-1* were each combined with temperature-sensitive *prp16-2, prp18-1, prp22-1,* or *prp24-1.* By one-step gene replacement, the 6-kb *Kpn*I-*Bam*HI fragment from the plasmid p17Gβ3-9 or p17Gβ4-1 (see Figure 2C for map of the fragment) was introduced into each of the above mentioned haploid ts strains. Since transplacements were obtained at *PRP17* in the haploid *prp* strains, we infer that the double mutants of *prp17Gβ3-9* or *prp17Gβ4-1* when combined with the *prp16-2, prp18-1, prp22-1,* or *prp24-1* loci are not lethal (Table 3). The growth rates of these double mutant transformants were analyzed at various temperatures and suppression or enhancement of the ts *prp* phenotypes was not observed.

Since the above tests for interaction between Prp proteins is relevant only if the missense Prp17p is stable at high temperatures, we have determined levels of Prp17p in splicing extracts prepared from cells grown at permissive temperature and those shifted to nonpermissive temperature for 2 hr. We found that the protein with a missense mutation in the third repeat unit was stable, and the levels are comparable with those present in a haploid wild-type strain (see Figure 4B; lanes 1, 2 show mutant protein levels).

ts alleles of *prp17* are synthetically lethal in combination with *prp16-2* and *prp18-1* alleles: Interactions between ts alleles of *PRP17* and other *PRP* gene products involved in the second-step of splicing *PRP16* and *PRP18* were tested. In addition, we chose to screen for interaction with a gene product required for the first step of the splicing reaction: *PRP24* and one required for late events in the splicing reaction, *PRP22.* It has been previously reported that *prp17-3* shows a synthetic lethal interaction with *prp18-1* and with *prp16-2* (FRANK *et al.* 1992). We have investigated the allele specificity of this interaction. The cloned genomic DNA from the ts alleles of *prp17-1, prp17-2,* and *prp17-3* were used for this experiment. A 2.6-kb *Kpn*I to *Bst*EII fragment containing the entire *PRP17* ORF with the ts mutation (see Figure 1B for a map of this genomic fragment) was isolated from all three alleles. This fragment was cloned into the corresponding sites of the plasmid p17KBH (see Figure 2B for map of insert in p17KBH). The 6-kb *Kpn*I-*Bam*HI fragment from each of the resulting plasmids p17-1KBH, p17-2KBH and p17-3KBH, was then used for the homologous replacement of the wild-type *PRP17* locus in ts *prp16-2, prp18-1, prp22-1,* and *prp24-1* haploid and diploid strains. Homologous replacement transformants were obtained in the diploids *prp18-1/prp18-1, prp22-1/prp22-1, prp16-2/prp16-2* and *prp24-1/prp24-1.* Viable haploid transformants were obtained only in *prp22-1* and *prp24-1* strains (Table 3), indicating that double mutants *prp17 prp22-1* or *prp17 prp24-1* are not lethal. The diploids *prp18-1/prp18-1;*

TABLE 3

Growth of haploid double mutants

prp17 alleles tagged with *HIS3*	*prp* mutants				
	prp18-1[a]	*prp22-1*[b]	*prp16-2*[a]	*prp24-1*[b]	*prp17Gβ3-9*[b]
prp17-1	−	+	−	+	+
prp17-2	−	+	−	+	+
prp17-3	−	+	−	+	+
prp17Gβ3-9[b]	+	+	+	+	
prp17Gβ4-1[b]	+	ND	+	ND	+/−[c]

+, the double mutant is viable at 23°; −, inviability at 23°; ND, not done.

[a] *prp17-HIS3* transplacements generated in diploid *prp18-1/prp18-1* or *prp16-2/prp16-2* strains were sporulated. At least 15 tetrads were screened for the recovery of His^+ spores at 23°.

[b] Growth of *prp17-HIS3* transplacements in haploid *prp* mutants at 23°.

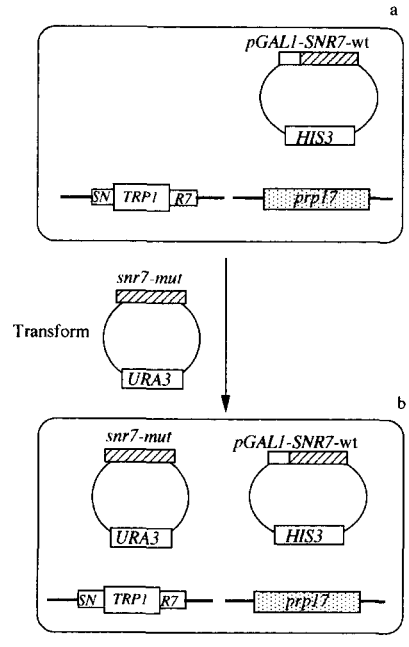
[c] Growth of the double mutant at 23° was poor, and similar to that seen with the null mutant of *prp17*.

PRP17/ts prp17-HIS3 and *prp16-2/prp16-2*; *PRP17/ts prp17-HIS3* were sporulated. At least 15 tetrads were analyzed for each diploid. In all of the tetrads, only two spores germinated, while the other two did not germinate at 23°. The surviving spore in each case was found to be temperature sensitive and His^-, indicating that all the double mutants *prp18-1 prp17-HIS3* and *prp16-2 prp17-HIS3* are inviable (Table 3). Therefore, a synthetic lethal interaction was observed between each *prp17* ts allele and both *prp18-1* or *prp16-2*. This is in contrast to the lack of enhancement or suppression of *prp18-1* or *prp16-2* seen in double mutants constructed with the missense mutations at the third or the fourth Gβ repeat units of *PRP17*. The levels of the mutant Prp17p in the ts mutants were comparable with those present in wild-type cells and did not vary significantly with temperature shift (Figure 4B). Therefore the differences in synthetic lethality observed with various *prp17* alleles, and lack of suppression of *prp16-2* and *prp18-1* observed with the mutants in the Gβ motif cannot be attributed to differences in protein stability. The synthetic lethal interaction between the gene products required at the second splicing reaction could either be due to physical interaction or because of functional overlap between the gene products. Recent high-copy suppression experiments suggest functional interaction between *PRP16* and *PRP17*, and *SLU7* and *PRP18* (JONES *et al.* 1995).

Interaction of *PRP17* with U5 snRNA: *prp17-3* (*slu4-1*) was isolated as a temperature-sensitive mutation that was synthetically lethal with a specific U98A mutation in loop I of the U5 snRNA. This allele did not show an interaction with other mutations in Loop I of U5 snRNA (FRANK *et al.* 1992). We have combined two other ts alleles *prp17-1* and *prp17-2*, with three U5 snRNA mutations to assess the allele specificity of this interaction. The strategy adopted was to cross the ts *prp17* alleles to a haploid strain with a chromosomal null allele for the *SNR7* gene that codes for the U5 snRNA (Figure 5A). This haploid was viable only with galactose as a carbon source because of the presence of the plasmid borne *SNR7* gene under the control of the *GAL1* promoter. The diploids were sporulated and haploid spores that were ts (*prp17-1* or *prp17-2*), Trp^+, and His^+ were isolated. These spores were transformed with plasmids, bearing mutant *snr7* alleles (U98A, U98C or UU97, 99CC) under the control of their endogenous promoter. These transformants were grown in glucose to repress the transcription from the wild-type *SNR7* gene and the interaction between *prp17* alleles and mutant U5 snRNA studied. These experiments showed that the *prp17-3* allele was extremely allele specific in its interaction with U5 and that other alleles of *prp17* did not show this interaction (Figure 5B). This is in contrast to the relatively nonallele specific interaction of *prp17* with other components of the spliceosome that are required for the second step of the splicing reaction (Table 3). We also have assessed whether the null or ts phenotypes caused by the various alleles of *prp17* could be due to destabilized association with U5 snRNA. Northern analysis of U5 levels in all of the *prp17* mutants showed the levels to be near wild type in all these strains (V. SESHADRI and U. VIJAYRAGHAVAN, unpublished observation).

In a reciprocal test for interaction between U5 snRNA and the mutant alleles of *prp17*, we have overproduced wild-type U5 snRNA in all of the *prp17* mutants to determine if overexpression of U5 snRNA, both the long and the short form, can alleviate the ts or null phenotype caused by any of the *prp17* alleles. The wild-type *SNR7* gene under the control of the *GAL1* promoter was transformed into the haploid mutant strains. The transformants were tested for the rescue of the growth phenotype after induction of the plasmid borne wild-type *SNR7*. Overexpression of the U5 snRNA by two- to threefold was observed by northern analysis of U5 snRNA levels in haploid mutants grown in galactose (data not shown). The induction pattern and the levels obtained were similar to those observed by PATTERSON and GUTHRIE (1987). The results from these experiments are summarized in Table 4. We found no evidence for suppression of the *prp17* phenotype, by overexpression of U5 snRNA.

A



B

	Growth on YPglucose plates		
	snr7 allele		
	U98A	*U98C*	*UU97,99,CC*
PRP17	+	+	+
prp17-1	+	+	+
prp17-2	+	+	+
prp17-3	-	+	+

FIGURE 5.—Interaction between mutant U5 snRNAs and alleles of *prp17*. (A) Generation of haploid *prp17* strains with a chromosomal disruption of *snr7* and plasmid borne mutant *snr7*. The spore clone (a) was generated after crossing the *SNR7* null strain to *prp17* ts strains, and sporulation of the diploids. The random spore populations were screened for Trp^+, His^+, $glucose^-$ and ts spore clones; these correspond to the spore clone depicted as (a). This strain was transformed with a plasmid carrying the mutant *snr7* under the control of its endogenous promoter. The resulting transformants (b) were checked for their ability to grow in glucose containing medium. (B) Viability of *prp17, snr7* double mutants. The growth phenotypes of double mutants, in which various temperature-sensitive *prp17* alleles are combined with various *snr7* alleles, are shown here. The growth, if observed, at 23° in glucose containing medium is indicated as +. The − sign indicates the inability of the strain to grow in glucose containing medium. All double mutants grow on galactose containing medium due to the expression of the wild-type *SNR7* gene.

DISCUSSION

Prp17p has been shown to be required for the second step of the splicing reaction (VIJAYRAGHAVAN *et al.* 1989). The predicted amino acid sequence (M. COMPANY and J, ABELSON, unpublished results) of the wild-type Prp17p shows homology with a class of proteins containing the Gβ repeat units (also called WD-40 repeats) present in the β subunit of the trimeric G proteins (WEIZSACKER *et al.* 1992; M. COMPANY and J. ABELSON, unpublished results, and see Figure 1A). Prp17p either interacts transiently with U5 snRNA or is a component of U5 snRNP, since the *prp17-3* is synthetically lethal with a specific U5 snRNA mutation (FRANK *et al.* 1992). We have conducted a structure-function analysis of the domains of Prp17p that are required for its function in splicing. In the present study, we have shown that one functional domain in Prp17p is in the N-terminal region of the protein that interacts with other splicing factors that act at the second step of pre-mRNA splicing, including U5 snRNA.

TABLE 4

Overexpression of U5 snRNA does not suppress *prp17* alleles

	Growth of *SNR7* transformants in YP galactose		
Alleles	23°	32°	37°
prp17-1	++	+/−[a]	
prp17-2	++	+/−[a]	
prp17-3	++	+/−[a]	
prp17Gβ3-9	++	+[a]	+/−[a]
prp17Gβ4-1	++	++[a]	+[a]
prp17::LEU2	+	−	−
prp17ΔNts	+	−	−
prp17ΔGβ2	+	−	−
prp17ΔGβ3	+	−	−
prp17ΔGβ4	+	−	−

[a] The growth of the transformants at 32° and 37° was comparable to the untransformed strains.

We have analyzed the region in *PRP17*, which, when mutated, gives a temperature-sensitive phenotype by mapping the mutations in three ts alleles of *prp17*. We found that the mutations in all three alleles map to a small region in the N-terminal half of the protein, outside the four Gβ repeat units. Prp4p, another splicing factor that contains five Gβ repeat units, was subjected to structure-function analysis (HU *et al.* 1994); in this case, some of the ts mutations mapped to the general region of the gene containing the conserved Gβ repeat

units. In *prp17-2,* isolated from an EMS mutagenized population, the ts phenotype resulted from a R to G change at position 58. This mutation was only four amino acids away from the mutation in the *prp17-3* allele (S54L) that was isolated in an independent screen for mutations that are synthetically lethal with specific U5 snRNA mutations. The synthetic lethal interaction between *prp17-3* and U5 snRNA that was reported earlier was also shown to be allele-specific with respect to alleles of U5 snRNA (FRANK *et al.* 1992). Only one mutation (U98A) in the conserved loop I of U5 snRNA showed this interaction, while the other U5 snRNA mutations (U98C or UU97,99CC) in the same loop did not confer any synthetic lethality with *prp17-3* (FRANK *et al.* 1992). Our analysis of interaction between *prp17-1, prp17-2,* or *prp17-3* and U5 snRNA showed this interaction to be extremely allele specific. *prp17-2,* in which the mutation maps only four amino acids away from *prp17-3,* is not synthetically lethal with any of the U5 mutations tested. These results indicate a specific, and probably direct, interaction between Prp17p and U5 snRNA.

Gβ repeat units have been found in a number of proteins with diverse functions, like Cdc4p (FONG *et al.* 1986), Prp4p (DALRYMPLE *et al.* 1989; HU *et al.* 1994), Ste4p (WHITEWAY *et al.* 1989), e-spl (HARTLEY *et al.* 1988), Cop1p (DENG *et al.* 1992), Rack1p (RON *et al.* 1994), and P85 TFIID subunit (KOKUBO *et al.* 1993). These proteins are involved in a wide array of cellular processes, including signal transduction, gene repression, secretion, and RNA splicing. The presumed function of these repeat units is to mediate protein-protein recognition in multiprotein complexes, where dynamic association and dissociation of proteins occur (DALRYMPLE *et al.* 1989). Recent evidence to support this idea has been obtained, for the interaction between the α and β subunits of the yeast G protein was shown to be disrupted with a mutation in the second WD repeat unit of Ste4p (WHITEWAY *et al.* 1994), and the WD repeats of Tup1p mediate an interaction between Tup1p and α2 repressor (KOMACHI *et al.* 1994).

The occurrence of two WD repeat unit containing proteins (Prp4p and Prp17p) among the ~40 identified gene products involved in RNA splicing provides ways to test the requirement of these repeat units for protein-protein interactions that must occur in spliceosome assembly. In a study on the structure-function analysis of Prp4p, HU *et al.* (1994) conducted random and a site-directed mutagenesis of *PRP4.* Their analysis showed that a large 100 amino acid N-terminal domain was nonessential for function and that a central arginine rich domain and the five WD repeat units present, C terminal to this central domain were essential for function. The mutations in two EMS induced temperature-sensitive *prp4* alleles were found to be in the general region of the gene corresponding to the WD repeats. However, these changes were not in any of the highly conserved residues of the repeat units. Site-directed mutagenesis of three highly conserved residues in the second unit showed that most of the changes did not produce any phenotypic effect. However, since ts and null phenotypes resulted from amino acid changes in the region of the repeat units, they point to the general importance of these units for Prp4p function. Additional support for their functional importance comes from their demonstration that deletions that include these repeat units result in null phenotypes.

We have tested the functional significance of the presence of these repeat units in Prp17p by mutating three conserved amino acids in the third of the four repeat units found in Prp17p. The mutation generated in each case was a change of the conserved amino acids to an amino acid not found in any of the variants of the Gβ repeat unit. Our analysis of 11 different missense mutations at Gβ repeat units show that these changes in the Gβ repeat units do not have any appreciable effect on the growth rate of haploid transformants. Only one allele, *prp17Gβ3-9,* having three mutations at the third Gβ repeat unit caused a slower growth rate at both 30 and 37° when compared with the wild-type strain. Four other alleles with triple amino acid changes in this repeat unit did not cause any growth phenotype. This is in contrast to the tight temperature-sensitive phenotype conferred by single amino acid changes in the N-terminal region of the protein. Two of the residues targeted for mutagenesis in *PRP4* (HU *et al.* 1994) were also those that were mutagenized in this study. Changing D328 in the second Gβ repeat unit of Prp4p (see Figure 1A) to a similarly charged residue had no effect as did changing it to a neutral small amino acid, but introduction of a positively charged residue resulted in a temperature-sensitive phenotype. Our data from the single amino acid substitutions at the similarly positioned conserved residue in the third repeat of *PRP17* (D314) (see Figure 1A) shows that changes to neutral or nonaromatic amino acids do not have any phenotypic effect. While the consequence of the presence of a positively charged residue at this position of *PRP17* has not been assessed, we find that combining a phenotypically wild-type change in this residue (D314V) with a change to a charged residue at a nearby conserved amino acid (W320R) can produce a weak temperature-sensitive phenotype. Thus it appears that introduction of positively charged residues in the D—WD region of the second repeat unit of Prp4p or the third repeat unit of Prp17p can result in partial loss of function. However, the effects of such changes on different repeat units are variable, since in *PRP17* the similar amino acid changes made in the conserved residues of the fourth repeat unit of *PRP17* did not cause any new phenotype. The overall conclusions about the effects of missense mutations in Gβ repeat units of Prp4p or Prp17p are similar, *i.e.,* even the most highly conserved residues can be changed drastically without

affecting function but each of these repeat unit is likely to serve a unique function, since deletion in any of these units results in null phenotypes.

We have assayed for genetic interactions between *PRP17* and other factors involved in the second step of splicing: *PRP18, PRP16,* and U5 snRNP. The double mutants of the *prp17-1, prp17-2,* or *prp17-3* alleles together with *prp18-1* or *prp16-2* are inviable. This type of genetic interaction was not observed with *prp24,* a component of the U6 snRNP that is required for the first step of the splicing pathway or with *prp22,* a factor required for the release of the mRNA from the spliceosome. An interaction between *prp17-3* and *prp18-1* or *prp16-2* was previously reported (FRANK *et al.* 1992). Our analysis shows that while these interactions are relatively allele nonspecific with regard to alleles of *prp17* having mutations in the N-terminal region of the protein, alleles of *prp17* with missense mutations in the C-terminal Gβ repeat units of *prp17* do not show any genetic interactions. We also find it unlikely that the differences in the synthetic lethal interactions observed for various alleles of *prp17* are due to the varying stabilities of the mutant protein. While one possible explanation for the synthetic lethality between *prp17* and *prp18,* and *prp17* and *prp16* is a physical interaction between the mutant gene products, a different interpretation could be that the lethality in the double mutants is due to overlap of function between *PRP17, PRP18,* and *PRP16,* all of which act at the second step of the splicing reaction.

In addition to the analysis of missense alleles of *prp17,* our analysis of deletion mutations in the repeat units of *PRP17* shows each of them to be essential for function and they cannot substitute for each other. Similar results have been reported for *PRP4.* A probable secondary structure for Gβ motif containing proteins was predicted by NEER *et al.* (1994). This prediction, based on hydrophobicity patterns, suggests that each repeat unit may fold into a βstrand-turn-βstrand-turn-βstrand that ends at the WD residues of each repeat. The authors propose that these small β-strands are unlikely to be stable in the absence of a ligand or in the absence of interaction between repeat units. Our analysis shows an enhanced, lethal phenotype upon combining phenotypically silent mutations in the fourth repeat with changes in conserved residues of the third repeat. These results suggests that interaction between the repeat units could be important for function. In the case of Tup1p, interaction between the repeat units most likely confers specificity of Tup1p binding to wild-type α2 repressor (KOMACHI *et al.* 1994). Similar genetic interaction between the amino terminal and carboxy terminal region of the β-transducin domain in Prp4p has been reported (HU *et al.* 1994).

We find that a region corresponding to 40 amino acids near the N terminus of Prp17p is essential for function. This result is in contrast to nonessential nature of a large 100 amino acid N-terminal domain in Prp4p. This 40 amino acid N-terminal region in Prp17p is one where two independently isolated ts mutations map and one that is a potential interaction site with the U5 snRNA, Prp16p and Prp18p. Because Prp17p is likely to associate with U5 snRNA in either space or time, we have assessed if in any of the *prp17* alleles the ts or null phenotype is the result of poor association with U5 RNA. We found that U5 RNA levels are close to that of wild type in the missense and the deletion mutations and that overexpression of wild-type U5 does nor rescue the ts or null phenotype caused by any of the alleles. Therefore, destablized assembly/association of the protein with U5 snRNA is unlikely to be the cause for the null phenotypes conferred by the deletion alleles of *prp17.*

In summary, we find that the N-terminal region of the Prp17 protein is functionally significant for its interactions with both U5 snRNA and with other Prp proteins involved in the second step of the splicing reaction.

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LITERATURE CITED

BLANTON, S., A. SRINIVASAN and B. C. RYMOND, 1992 *PRP38* encodes a yeast protein required for pre-mRNA splicing and maintenance of stable U6 small nuclear RNA levels. Mol. Cell. Biol. **12:** 3939–3947.

BROACH, J., 1983 Construction of high copy yeast vectors using 2 micron circle sequences. Methods Enzymol. **101:** 307–324.

BRODY, E., and J. ABELSON, 1985 The spliceosome: yeast pre-mRNA associates with a 40S complex in a splicing dependent reaction. Science **228:** 963–967.

CHAPON, C., and P. LEGRAIN, 1992 A novel gene *spp91-1,* suppresses the splicing defect and the pre-mRNA nuclear export in the *prp9-1* mutant. EMBO J. **11:** 3279–3288.

DALRYMPLE, M. A., S. P. BJORN, J. D. FRIESEN and J. D. BEGGS, 1989 The product of *PRP4* gene of *Saccharomyces cerevisiae* shows homology to beta subunit of G proteins. Cell **58:** 811–812.

DENG, X. W., M. MATSUI, N. WEI, D. WAGNER, A. M. CHU *et al.,* 1994 *COP1,* an Arabidopsis regulatory gene encodes a protein with both Zinc binding motif and a G beta homology domain. Cell **71:** 791–801.

FONG, K. W., J. B. HURLEY, R. S. HOPKINS, R. MIAKE-LYE, M. S. JOHNSON *et al.,* 1986 Repetitive segmental structure of the transducin subunit: homology with *CDC4* gene and identification of related mRNA. Proc. Natl. Acad. Sci. USA **83:** 2162–2166.

FRANK, D., B. PATTERSON and C. GUTHRIE, 1992 Synthetic lethal mutation suggest interaction between U5 small nuclear RNA and four proteins required for splicing. Mol. Cell. Biol. **12:** 5197–5205.

FRENDEWEY, D., and W. KELLER, 1985 Stepwise assembly of pre-mRNA splicing complex requires U snRNP and specific intron sequences. Cell **42:** 355–367.

GRABOWSKI, P. J., S. R. SEILER and P. A. SHARP, 1985 A multicompo-

nent complex is involved in splicing of mRNA precursors. Cell **42:** 345–353.

GUTHRIE, C., 1991 mRNA splicing in yeast: clues to why the spliceosome is a ribonucleoprotein. Science **253:** 157–163.

GUTHRIE, C., and B. PATTERSON, 1988 Splicesomal snRNAs. Annu. Rev. Genet. **22:** 387–419.

HARTLEY, D. A., A. PREISS, and T. S. ARTAVANIS, 1988 A deduced gene product from the Drosophila neurogenic locus, *enhancer of split* shows homology to mammalian G protein beta subunit. Cell **55:** 785–795.

HU, J., Y. XU, K. SCHAPPERT, T. HARRINGTON, A. WANG *et al.*, 1994 Mutational analysis of the PRP4 protein of *Saccharomyces cerevisiae* suggests domain structure and snRNP interaction. Nucleic Acids Res. **22:** 1724–1734.

ITO, H., FUKUDA, K. MURATA and A. KIMURA, 1983 Transformation of intact yeast cells treated with alkali cation. J. Bacteriol. **153:** 163–168.

JONES, M. H., D. FRANK and C. GUTHRIE, 1995 Characterization and functional ordering of Slu7p and Prp17p during the second step of pre-mRNA splicing in yeast. Proc. Natl. Acad. Sci. USA **92:** 9687–9691.

KIM, S. H., and R. J. LIN, 1993 pre-mRNA splicing with in an assembled yeast spliceosome requires an RNA dependent ATPase and ATP hydrolysis. Proc. Natl. Acad. Sci. USA **90:** 888–892.

KIM, S. H., J. SMITH, A. CLAUDE and R. J. LIN, 1992 PRP2 is an RNA dependent NTPase. EMBO J. **11:** 2319–2326.

KOMACHI, K., M. J. REDD and A. D. JOHNSON, 1994 The WD repeats of TUP1 interact with the homeodomain protein α2. Genes Dev. **8:** 2857–2867.

KOKUBO, T., D. W. GONG, S. YAMASHITA, R. TAKEDA, R. G. ROEDER *et al.*, 1993 Molecular cloning, expression, and characterization of the Drosophila 85 KD, TFIID protein. Mol. Cell. Biol. **13:** 7859–7863.

KUNKEL, T. A, J. D. ROBERTS and R. A. ZAKOUR, 1987 Rapid and efficient site specific mutagenesis without phenotypic selection. Methods Enzymol. **154:** 367–382.

LEBRAR, E., D. DIGNARD, L. HOUGAN, D. THOMAS and M. WHITEWAY, 1992 Dominant negative mutant of a yeast G protein β subunit identify two functional region involved in pheromone signaling. EMBO J. **11:** 4805–4813.

LEGRAIN, P., and C. CHAPON, 1993 Interaction between PRP11 and SPP91 yeast splicing factors and characterization of a PRP9-PRP11-SPP91 complex. Science **262:** 108–111.

LIN, R. J., A. J. NEWMAN, S. C. CHENG and J. ABELSON, 1985 Yeast mRNA splicing *in vitro*. J. Biol. Chem. **260:** 14780–14792.

MIKAELIAN, I., and A. SERGEANT, 1992 A general and fast method to generate multiple site directed mutations. Nucleic Acids Res. **20:** 376.

NEER, E. J., C. J. SCHMIDT, R. NAMBUDRIPAD and T. F. SMITH, 1994 The ancient regulatory protein family of WD repeat proteins. Nature **371:** 297–300.

PATTERSON, B., and C. GUTHRIE, 1987 An essential yeast snRNA with U5-like domain is required for splicing *in vivo*. Cell **49:** 613–624.

POLIANA, J., and A. ADAM, 1991 A fast procedure for yeast DNA purification. Nucleic Acids Res. **19:** 5443.

RON, D., C. H. CHEN, J. CALDWELL, L. JAMIESSON, E. ORR *et al.*, 1994 Cloning of intracellular receptor for protein kinase C: a homolog of the beta subunit. Proc. Natl. Acad. Sci. USA **91:** 839–843.

RUBY, S. W., and J. ABELSON, 1991 pre-mRNA splicing in yeast. Trends Genet. **7:** 79–85.

RUBY, S. W., T. H. CHANG and J. ABELSON, 1993 Four yeast spliceosomal proteins (PRP5, PRP9, PRP11, and PRP21) interact to promote U2 snRNP binding to pre-mRNA. Genes Dev. **7:** 1909–1925.

SCHWER, B., and C. GUTHRIE, 1991 PRP16 is an RNA dependent ATPase that interacts transiently with the spliceosome. Nature **349:** 494–499.

SHARP, P. A., 1987 Splicing of messenger RNA precursors. Science **235:** 766–771.

SHERMAN, F., G. R. FINK and J. B. HICKS, 1986 *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SIKORSKI, R. S., and P. HIETER, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19–27.

VIJAYRAGHAVAN, U., and J. ABELSON, 1989 pre-mRNA splicing in yeast. Nucleic Acids Mol. Biol. **3:** 197–215.

VIJAYRAGHAVAN, U., M. COMPANY and J. ABELSON, 1989 Isolation and characterization of pre-mRNA splicing mutants of *Saccharomyces cerevisiae*. Genes Dev. **3:** 1206–1216.

WEIZSACKER, V. E., M. P. STRATHMANN and M. I. SIMON, 1992 Diversity among the beta subunits of heterotrimeric GTP binding proteins: characterization of a novel beta-subunit cDNA. Biochem. Biophys. Res. Commun. **183:** 350–356.

WHITEWAY, M., L. HOUGAN, D. DIGNARD, D. Y. THOMAS, L. BELL *et al.*, 1989 The *STE4* and *STE18* genes of yeast encode potential beta and gama subunit of the mating factor receptor coupled G protein. Cell **56:** 467–477.

WHITEWAY, M., K. L. CLARK, E. LEBRAR, D. DIGNARD and D. Y. THOMAS, 1994 Genetic identification of residues involved in association of alpha and beta G protein subunits. Mol. Cell. Biol. **14:** 3223–3229.

WOOLFORD, J. L., JR., and C. L. PEEBLES, 1992 RNA splicing in lower eukaryotes. CURT. OPIN. GENET. DEV. **2:** 712–719.

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