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 repeats. 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 Abel 1985; Frendewey 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 Abel 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...
with other Gβ motif containing proteins such as bovine transducin, Ste4p, Cdc4p, and Prp4p (M. COMPANY and J. ABELOSON, 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 electroplator. The *E. coli* strain CJ236 (Δulin1 thi-1 relA1) was used in experiments designed to introduce mutations by a primer extension strategy. Yeast strains S328 (MATα ade2-101 his3Δ200 lys2-801 ura3-52) and S330 (MATα ade2-101 his3Δ200 trpl 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 (MATα ura3-52 his3Δ200 ade2-101 trplΔ63 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 (IT0 et al. 1987) and 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 p17S803 was done after linearizing 10 μg of the plasmid DNA with the restriction enzyme BstEII, which cleaves a single main 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.

**Tagging of the PRP17 genomic locus:** The complementing 4.5 kb to KpnI-Gfd genomic fragment containing PRP17 cloned in the yeast shuttle vector pHY18 was subcloned into Bluescript (Stratagene) to generate the plasmid p17KB (Figure 2A). A 1.8-kb BamHI fragment containing the HIS3 gene was isolated from pYac3 and cloned into the XbaI 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 KpnI and BamHI and 10 μg of this released 6-kb fragment was used.

The plasmids rescued after BamHI digests from three temperature-sensitive alleles of prp17 were digested with KpnI and BstEII to release a 2.6-kb fragment. This 2.6 kb prp17 fragment, containing the ts mutation, was cloned between the corresponding KpnI and BstEII 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 KpnI-BamHI fragment from these clones was used.

**Mutagenesis of the Gβ motif repeat:** Mutagenesis was done by a PCR-based strategy as described by MIKELIAN and SERGENT (1992). Wild-type PRP17 was PCR amplified (94° 45 sec, 45° 1 min, 72° 1 min for 25 cycles) using Taq polymerase (Boehringer Mannheim) 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 SnaBI and BstEII, and the 1-kb fragment released was ligated into SnaBI- and BstEII-digested p17KBH to generate plasmids carrying prp17Gβ motif mutations. This ligation mix was transferred 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 KpnI and BamHI 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, TTTTCCAGATCCTGAGGTTT-AGT; 42UR, CAAGGCCTAAGCCTCAATTGGGAAA-TCCAG; 43UR, TTTTATAGCAGTAAATTGGAATC; Sx1, ATTTGCACATGCGCAATG; GBS, AGTTCTGAGCGGATG-TAC; GB3A, ACCTGTATCATGCCTGCATACAT; and GB4+1, TACAGGGTTTCAAAAAAGCACGCTAAGTGGG-CITGTGAATAC.

**Preparation of extracts and western blot analysis for detection of Prp17p:** prp17 strains were grown at 23° to an OD600 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
Functional Domains in Prpl7p

FIGURE 1.—Domain architecture of Prpl7p. (A) Protein sequence alignment of the four Gβ repeat units in Prpl7p 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 acid 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 Prpl7p that have been targeted for mutagenesis are indicated in lower case. (B) Schematic representation of Prpl7p summarizing the location of missense mutations that are associated with discernible phenotypes. The ts (X) 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 mutations are given in the single letter code.

against either the N-terminal or C-terminal domains of Prpl7p. 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 (stu4-1) 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 (ts365) is a G to C transversion that results in a change of G(127) to A; in prp17-2 (ts487), an A to G transition results in a change of R(58) to G, and in prp17-3 (stu4-1), 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. ABELOM, 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.

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 SnaBI and BstEII to release a 1-kb pool of fragments that was cloned into p17KBH to replace the corresponding wild-type SnaBI-BstEII 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 KpnI and BamHI 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

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<tbody>
<tr>
<td>prp17-3</td>
<td>L54</td>
<td>G127</td>
<td>D314, W320, E321</td>
<td>D402, W410, D411</td>
<td>ts</td>
<td></td>
<td></td>
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<tr>
<td>prp17GB-1</td>
<td>V314, R320, V321</td>
<td>SnaBI-BstEII fragment with HIS3 tag in p17KBH</td>
<td>Slow growing</td>
<td>wt</td>
<td>prp17GB-1</td>
<td>V402, R410, V411</td>
<td>wt</td>
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wild-type haploid and diploid strains were similar, suggesting that none of the mutations generated were lethal. The growth of the transformants were examined at the fourth repeat unit (SS328) showing that none of the Gp motif mutants, prpl7Gp3-9, 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., is 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 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 prp17Gp4-1/PRP17, prpl7Gp4-1/PRP17, prp17Gβ3-2/PRP17, prp17Gβ3-1/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

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**TABLE 1**

<table>
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<tr>
<th>Missense mutations in PRP17</th>
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<tr>
<td>Allele</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>prp17-1</td>
</tr>
<tr>
<td>prp17-2</td>
</tr>
<tr>
<td>prp17-3</td>
</tr>
</tbody>
</table>

*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.
whereas the viability and growth rates compared. The diploids of the constructed, and the transplacement of this allele showed at 30 or 37°C (Table 2).

To explore the possibility that these deletion constructs give null phenotypes at 30 and 37°C 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°C and also from cultures that were shifted to 37°C for 2 hr. Prp17p levels in wild-type extracts (Figure 4A, lanes 6, 7) are comparable with the levels present in extracts from prp17ΔGβ2 and prp17ΔGβ3 deletion strains after growth at 37°C for 2 hr (Figure 4A, lanes 1, 2). Protein levels in prp17ΔNts strain grown at 23 and 37°C 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 (prp17ΔGβ4). 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 Prp17 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 EcoRI fragment from the plasmid p17Gβ3-9 (see Figure 2C for the map of this fragment) that bears

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**TABLE 2**

Growth rates of deletion mutants of PRP17

<table>
<thead>
<tr>
<th>Alleles</th>
<th>23°C</th>
<th>30°C</th>
<th>37°C</th>
</tr>
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<tbody>
<tr>
<td>PRP17</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>prp17-1</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>prp17::LEU2</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>prp17ΔNts</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>prp17ΔGβ2</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>prp17ΔGβ3</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>prp17ΔGβ4</td>
<td>+</td>
<td>-</td>
<td>-</td>
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</table>

* Growth was monitored by colony size as well as by measuring doubling time in liquid cultures.
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°C). 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 HindIII-NdeI 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 KpnI-BamHI 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 KpnI to BstEII 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 KpnI-BamHI 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 prp16-1/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

<table>
<thead>
<tr>
<th>prpl7 alleles tagged with HIS3</th>
<th>prpl8-1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>prpl22-1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>prpl6-2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>prpl24-1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>prpl7β-3&lt;sup&gt;a, b&lt;/sup&gt;</th>
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<tr>
<td>prpl7-1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>prpl7-2</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>prpl7-3</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>prpl7β-3&lt;sup&gt;a, b&lt;/sup&gt;</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>+/-</td>
</tr>
<tr>
<td>prpl7β-4&lt;sup&gt;a, b&lt;/sup&gt;</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>+/-</td>
</tr>
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</table>

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

<sup>a</sup> prpl7-HIS3 transplacements generated in diploid prpl8-1/prpl8-1 or prpl6-2/prpl6-2 strains were sporulated. At least 15 tetrads were screened for the recovery of His<sup>+</sup> spores at 23°.

<sup>b</sup> Growth of prpl7-HIS3 transplacements in haploid prp mutants at 23°.

<sup>c</sup> Growth of the double mutant at 23° was poor, and similar to that seen with the null mutant of prpl7.

**PRP17/ts prpl7-HIS3 and prpl6-2/prpl6-2, PRP17/ts prpl7-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<sup>+</sup>, indicating that all the double mutants prpl18-1 prpl7-HIS3 and prpl6-2 prpl7-HIS3 are inviable (Table 3). Therefore, a synthetic lethal interaction was observed between each prpl7 ts allele and both prpl8-1 or prpl6-2. This is in contrast to the lack of enhancement or suppression of prpl8-1 or prpl6-2 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 Prpl7p 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 prpl7 alleles, and lack of suppression of prpl6-2 and prpl8-1 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:** prpl7-3 (sλ4-1) 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 prpl7-1 and prpl7-2, with three U5 snRNA mutations to assess the allele specificity of this interaction. The strategy adopted was to cross the ts prpl7 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 (prpl7-1 or prpl7-2), Trp<sup>+</sup>, and His<sup>+</sup> 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 prpl7 alleles and mutant U5 snRNA studied. These experiments showed that the prpl7-3 allele was extremely allele specific in its interaction with U5 and that other alleles of prpl7 did not show this interaction (Figure 5B). This is in contrast to the relatively nonallelic specific interaction of prpl7 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 prpl7 could be due to destabilized association with U5 snRNA. Northern analysis of U5 levels in all of the prpl7 mutants showed the levels to be near wild type in all these strains (V. Seshadri and U. Vijayaraghavan, unpublished observation).

In a reciprocal test for interaction between U5 snRNA and the mutant alleles of prpl7, we have overproduced wild-type U5 snRNA in all of the prpl7 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 prpl7 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 prpl7 phenotype, by overexpression of U5 snRNA.
Aa

B

compare growth on YP Glucose and YP Galactose media

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 (Weitzsacker 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.

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

TABLE 4

Overexpression of U5 snRNA does not suppress prp17 alleles

<table>
<thead>
<tr>
<th>Alleles</th>
<th>23°</th>
<th>32°</th>
<th>37°</th>
</tr>
</thead>
<tbody>
<tr>
<td>prp17-1</td>
<td>+ +</td>
<td>/-</td>
<td>/-</td>
</tr>
<tr>
<td>prp17-2</td>
<td>+ +</td>
<td>/-</td>
<td>/-</td>
</tr>
<tr>
<td>prp17-3</td>
<td>+ +</td>
<td>/-</td>
<td>/-</td>
</tr>
<tr>
<td>prp17Gβ3-9</td>
<td>+ +</td>
<td>/-</td>
<td>/-</td>
</tr>
<tr>
<td>prp17Gβ4-1</td>
<td>+ +</td>
<td>/-</td>
<td>/-</td>
</tr>
<tr>
<td>prp17::LEU2</td>
<td>+ +</td>
<td>/-</td>
<td>/-</td>
</tr>
<tr>
<td>prp17ΔNts</td>
<td>+ +</td>
<td>/-</td>
<td>/-</td>
</tr>
<tr>
<td>prp17ΔGβ2</td>
<td>+ +</td>
<td>/-</td>
<td>/-</td>
</tr>
<tr>
<td>prp17ΔGβ3</td>
<td>+ +</td>
<td>/-</td>
<td>/-</td>
</tr>
<tr>
<td>prp17ΔGβ4</td>
<td>+ +</td>
<td>/-</td>
<td>/-</td>
</tr>
</tbody>
</table>

* The growth of the transformants at 32° and 37° was comparable to the untransformed strains.
units. In prpl17-2, 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 prpl17-3 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 prpl17-3 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 prpl17-3 (Frank et al. 1992). Our analysis of interaction between prpl17-1, prpl17-2, or prpl17-3 and U5 snRNA showed this interaction to be extremely allele specific. prpl17-2, in which the mutation maps only four amino acids away from prpl17-3, 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. 1989), Cop1p (Deng et al. 1992), Rack1p (Ron et al. 1994), and P85 TFIID subunit (Korubo 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 spliceosomes 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 β-strand-turn-β-strand-turn-β-strand 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 suggest 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, destabilized 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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