MOLECULAR AND CELLULAR BIOLOGY, Mar. 1996, p. 960–967 Vol. 16, No. 3
0270-7306/96/$04.00+0

Identification of Prp40, a Novel Essential Yeast Splicing Factor Associated with the U1 Small Nuclear Ribonucleoprotein Particle

HUNG-YING KAO AND PAUL G. SILICIANO*

Department of Biochemistry and Institute of Human Genetics, University of Minnesota, Minneapolis, Minnesota 55455

Received 14 September 1995/Returned for modification 23 October 1995/Accepted 18 December 1995

We have used suppressor genetics to identify factors that interact with *Saccharomyces cerevisiae* U1 small nuclear RNA (snRNA). In this way, we isolated *PRP40-1*, a suppressor that restores growth at 18°C to a strain bearing a cold-sensitive mutation in U1 RNA. A gene disruption experiment shows that *PRP40* is an essential gene. To study the role of *PRP40* in splicing, we created a pool of temperature-sensitive *prp40* strains. Primer extension analysis of intron-containing transcripts in *prp40* temperature-sensitive strains reveals a splicing defect, indicating that Prp40 is a general splicing factor. In vitro heat inactivation and complementation assays demonstrate that Prp40 plays a direct role in pre-mRNA splicing. In addition, U1 RNA coimmunoprecipitates with Prp40, indicating that Prp40 is bound to the U1 small nuclear ribonucleoprotein particle in vivo. Therefore, we conclude that PRP40 encodes a novel, essential splicing component that associates with the yeast U1 small nuclear ribonucleoprotein particle.

Pre-mRNA splicing takes place in a multicomponent complex called the spliceosome (5, 14, 15). Spliceosomes assemble in a highly ordered pathway that is mediated by the association of small nuclear ribonucleoprotein particles (snRNPs) with one another and with conserved sequences in the intron (7, 35, 43). snRNPs are stable, abundant, and highly conserved particles consisting of small nuclear RNA molecules (snRNAs) complexed with sets of proteins. snRNP-snRNP and snRNP-intron interactions are thought to identify the ends of the intron and bring them into a catalytically active configuration (35).

Early in splicing, the U1 snRNP binds to the 5′ splice site (24, 33, 34, 46, 50). In both *Saccharomyces cerevisiae* and mammalian systems, this binding occurs in the absence of ATP and any other snRNP. Recognition of the 5′ splice site by the U1 snRNP relies in part on RNA-RNA base pairing between U1 RNA and the intron (49, 54, 58). This recognition event also requires the U1 snRNP proteins (36). An intact 5′ splice site and an intact branch site are necessary for the formation of this complex, which is committed to splicing and is a functional precursor to the mature spliceosome (51). After U1 snRNP binding, the U2 snRNP binds to the branch site in an ATP-dependent manner. Subsequently, the U5 and U4/U6 snRNPs associate as a tri-snRNP to form an snRNP complete splicing complex, which undergoes conformational rearrangements and the addition of non-snRNP proteins to become a mature spliceosome.

Metazoan U1 snRNPs contain a single 164-nucleotide U1 RNA complexed with at least 11 proteins. A set of common proteins, called the core or Sm proteins, binds near the 3′ end of U1 RNA. These proteins are also present in the U2, U5, and U4/U6 snRNPs (29) and are thought to be involved in snRNP stability (21) and nuclear localization (32). In addition to the Sm proteins, the metazoan U1 snRNPs contain three U1-specific proteins called A, C, and 70K.

Phylogenetic comparison of U1 RNAs from diverse species indicates that the U1 RNA folds into a conserved cruciform structure with four prominent stem-loop structures (37). The 70K protein binds directly to stem-loop 1 of U1 RNA (17, 44, 57), while the A protein binds directly to stem-loop 2 (30). The C protein binds stem-loop 1 as well and requires the 70K protein in order to associate with the U1 snRNP (39).

Despite only limited sequence homology, *S. cerevisiae* U1 RNA (568 nucleotides) is fundamentally analogous to human U1 RNA. The first 10 nucleotides are precisely conserved between *S. cerevisiae* and humans: this is the sequence that base pairs with the 5′ splice site (49, 54). Also conserved are 9 of 10 residues in stem-loop 1, the binding site for 70K. Furthermore, yeast U1 RNA forms an equivalent cruciform structure that places the conserved 5′ end, stem-loop 1, and Sm-binding site in the same configuration (23).

Yeast U1 snRNPs contain the core Sm proteins (53) and homologs of the 70K (55) and A (26) proteins. The yeast 70K homolog, encoded by the *SNP1* gene, has 30% overall amino acid identity with human 70K (55). We have shown that Snp1 binds to stem-loop 1 of U1 RNA (22).

The *MUD1* gene product has sequence homology with the human U1A protein and, like Snp1, associates with the U1 snRNP in vivo. Unexpectedly, neither *SNP1* (19) nor *MUD1* (26) is required for yeast viability. Prp39, the third yeast U1 snRNP-specific protein identified to date, differs from Snp1 and Mud1 in that it is required for viability and has no known mammalian homolog (28). Prp39 functions early in spliceosome assembly to facilitate the U1 snRNP–5′ splice site interaction (28). The yeast U1 snRNP contains additional proteins as well: biochemical purification of yeast U1 snRNPs reveals the presence of at least nine U1-specific proteins (12).

To extend our understanding of the functions of the U1 snRNP, we have employed suppressor genetics to identify new U1 snRNP proteins. Starting with a cold-sensitive point mutation in the 5′ end of U1 RNA, U1-4U, we isolated suppressor mutations in the *PRP40* gene. Here, we describe *PRP40*, a novel essential gene encoding a splicing factor that interacts genetically and physically with the yeast U1 snRNP.

MATERIALS AND METHODS

Yeast strains. Strain 19SS [*MAT***a** Δ*TRP1* Δ*HIS3 ura3-52 lys2-801 ade2-101 snr19*::*LYS2* (YCp50-*SNR19*)] has a disruption of the chromosomal U1 RNA gene and a wild-type U1 gene carried on the *URA3-CEN* plasmid. Strain

* Corresponding author. Mailing address: Department of Biochemistry, University of Minnesota, 4-225 Millard Hall, 435 Delaware St. SE, Minneapolis, MN 55455. Phone: (612) 625-4928. Fax: (612) 625-2163. Electronic mail address: pauls@lenti.med.umn.edu.

19SS-4U is derived from strain 19SS by replacing YCp50-*SNR19* with pSE358-*U1-4U* via plasmid shuffle. Strain 19SSW differs from 19SS by the mating type and deletion of the *LEU2* gene. 19SSW-4U is derived from 19SSW by plasmid shuffle as described above. The *PRP40* gene disruption was carried out in a homozygous diploid strain, TR1 (*MAT***a**/*MAT*α *ura3-52*/*ura3-52 trp1-289*/*trp1-289 his3-352*/*his3-352 lys2-801*/*lys2-801 ade2-101*/*ade2-101*). A pSE360-*PRP40* plasmid was used to transform the *PRP40* heterozygous strain TR1-40ΔD (the same as TR1 except *prp40*::*HIS3*/*PRP40*). Stable transformants were isolated and sporulated, and tetrads were dissected to create the haploid plasmid-shuffling strain PRP40-SS [*MAT***a** *ura3-52 trp1-289 his3-352 lys2-801 ade2-101 prp40*::*HIS3* (pSE360-*PRP40*)]. Strain PRP40-001 with a low-copy-number PRP40 plasmid (pSE358-*PRP40*) and strain PRP40-002 carrying a high-copy-number overexpression plasmid (pG1-*PRP40*) were derived from strain PRP40-SS by plasmid shuffle. Temperature-sensitive strains *prp40-10*, *prp40-12b*, and *prp40-23* were also derived from PRP40-001 by transformation of a bank of mutagenized *PRP40* plasmids prior to plasmid shuffling.

UV mutagenesis. Strain 19SS-4U was grown to mid-log phase, collected, and resuspended in sterile deionized water. A total of 10^7 cells were exposed to 25,000 μJ on yeast extract-peptone-dextrose plates, causing 65% killing. Immediately after UV-irradiation, the plates were wrapped with tinfoil to prevent exposure to visible light. The plates were then incubated at 18°C, and viable colonies were isolated after 5 days.

Deoxyoligonucleotides. Oligonucleotides were synthesized by Karin Musier-Forsyth at the University of Minnesota, by National Bioscience (Hopkins, Minn.) or by Amitof (Boston, Mass.). The oligonucleotides used were as follows. The mutagenic oligonucleotides used for U1 mutagenesis were U13G4U, 5′-GATATCTTAAGGTAAACATGAGGATTTTA-3′; U13G5A, 5′-GATATCTTAAGGTATGCATGAGGATTTTA-3′; U14U5A, 5′-GATATCTTAAGGTATATATGAGGATTTTA-3′; and U15′D, 5′-CTCTGATATCTTAAATGAGGATTTTAA-3′; and that used for *PRP40* mutagenesis was 64(HA)$_3$/*Not*I, 5′-TCAGAATCAATAGCGGCCGCCGTCCAATTCCA-3′. The PCR oligonucleotides were 64AUG, 5′-TCTGGATCCCATATGTCTATTTGGAAGGAA-3′; and 64C, 5′-TTCTGAGTGTCGATGAGGGT-3′. The primer extension oligonucleotides were U1, 5′-CAAGGGAATGGAAACGTCAGC-3′; U4, 5′-AGGTATTCCAAAAATTCCCTAC-3′; U5, 5′-AAGCGCATAGTAAGAC-3′; U6, 5′-TGCTGATCATCTCTGTATTG-3′; RPL32, 5′-GTGGACTTGTAACCTAAGGTG-3′; and SAR1461, 5′-TACCATGTTTGTTCCACAGA-3′.

Library construction. Genomic DNA was prepared from the suppressor strain as previously described (45), except that instead of CsCl gradient purification, the DNA was gel purified and electroeluted from a 0.8% agarose gel. After phenol extraction and ethanol precipitation, the DNA was partially digested with *Sau*3A. Inserts with sizes between 10 and 15 kb were gel purified, electroeluted, and ligated to a *Bam*HI-linearized, *CEN*-based *URA3* plasmid, pSE360. The ligation mixes were transformed into *Escherichia coli* MC1066 to generate a library representing six genomes. To clone *PRP40-1*, 10 genomes were screened.

Plasmid construction. A 3.2-kb *Spe*I fragment containing *PRP40* was subcloned into the Bluescript KS+ vector (Stratagene), generating plasmid BS-*PRP40Spe*. This plasmid was digested with *Pst*I and *Nsi*I and self ligated, to yield plasmid BS-*PRP40*, which contains 440 bp upstream and 194 bp downstream of the *PRP40* coding sequence in addition to the *PRP40* coding region. Derivatives of plasmids pSE358, pSE360 (11), and pRS306 (52) that lack an *Sst*I site were constructed by digestion of these plasmids with *Sst*I, Klenow treatment, and self ligation. A 2.3-kb *Eco*RI fragment was released from BS-*PRP40* and subcloned into plasmid pSE358Δ*Sst*I, pSE360Δ*Sst*I, and pSE306Δ*Sst*I, generating plasmids pSE358-*PRP40*, pSE360-*PRP40*, and pSE306-*PRP40*, respectively. BSΔ*Bam*HI was constructed from Bluescript KS+ (Stratagene) by *Bam*HI digestion, Klenow treatment, and self ligation. A PCR fragment, generated by primers 64AUG (which creates a *Bam*HI site 3 nucleotides upstream of the start codon) and 64C, was digested with *Bam*HI and *Afl*II and swapped into *Bam*HI-*Afl*II-linearized pSE358-*PRP40*, generating plasmid pSE358-*PRP40AUG*. A 1.75-kb *Bam*HI-*Sal*I fragment containing the *PRP40* coding sequence from pSE358-*PRP40AUG* was inserted into *Bam*HI-*Sal*I-linearized pG1 (47), a high-expression, multicopy plasmid, to create plasmid pG1-*PRP40*. This 1.75-kb *Bam*HI-*Sal*I fragment was also inserted into the *Bam*HI-*Sal*I-cut plasmid pQ30 (Qiagen) to create a $(His)_6$-Prp40 fusion construct.

Primer 64(HA)$_3$/*Not*I was used to introduce a *Not*I site by Transformer mutagenesis (Clontech) at the penultimate codon of *PRP40* in plasmid pSE358-*PRP40*. A 111-bp *Not*I fragment encoding three copies of the hemagglutinin (HA) epitope, a kind gift from Jim Umen and Christine Guthrie, was inserted to generate plasmid pSE358-*PRP40*$(HA)_3$.

Genetic manipulations. All genetic experiments including medium preparation, diploid selection, sporulation, dissection, *cis* and *trans* tests, and the plasmid shuffle were performed as previously described (16). Yeast transformation was carried out by the method of Schiestl and Gietz (48).

Integration and pop-out. Plasmid pRS306-*PRP40* was linearized with *Sst*I and transformed into parental strain 19SS-4U (diagrammed in Fig. 2). Stable integrants were selected on −Ura minimal medium (minimal medium lacking uracil) and confirmed by Southern blot analyses. The pop-out experiment was performed on 5-fluoro-orotic acid (5-FOA) plates and confirmed by Ura$^-$ phenotype and Southern blot analyses. The pop-out integration strain was used for all phenotypic analyses of *prp40-1*, because we can be certain that its genome contains no other UV-induced mutations.

Genomic sequencing. Genomic DNA prepared from parental strain 19SS-4U was digested with *Acc*I, and 1- to 2-kb fragments were gel purified and electroeluted. Cycle sequencing was performed according to the protocol of Epicentre Technologies except that regular dGTP, 10× PCR buffer, and *Taq* polymerase (Perkin-Elmer) were used. For one reaction, 5 ng of ^{32}P-labeled primer 64C (5×10^6 cpm) and 3 μg of genomic DNA were used. Reaction mixtures were denatured at 92°C for 15 s, annealed at 60°C for 15 s, and extended at 71°C for 1 min for 30 cycles.

Gap repair. Plasmid pSE360-*PRP40* was cut with *Afl*II and *Sst*I within *PRP40*, creating an internal 457-bp deletion which contains the suppressor mutation C1421-T (a C-to-T mutation at position 1421; diagrammed in Fig. 2). The linearized plasmid was transformed into parental strain 19SS-4U. Ura$^+$ colonies were selected on a −Ura plate, and *URA3* plasmids were recovered for sequencing.

Gene disruption. Plasmid BSΔ*BamHI-PRP40* was digested with *Sty*I and *Age*I and treated with Klenow fragment prior to the addition of *Bam*HI linkers and self ligation. A 1.8-kb *Bam*HI fragment containing the *HIS3* gene was inserted. The resulting plasmid was cut with *Eco*RI, releasing a *PRP40* disruption allele fragment (diagrammed in Fig. 2). This fragment was used for transformation into a homozygous strain, TR1. Stable His$^+$ colonies were selected and sporulated, and tetrads were dissected.

Hydroxyamine mutagenesis. Twenty micrograms of plasmid pSE358-*PRP40* was incubated with 1 M hydroxyamine–50 mM $NaPO_4$ (pH 7.0)–2 mM EDTA–100 mM NaCl at 75°C for 30 min. The reaction was stopped by a 10-min incubation at 0°C and followed by the use of two G-25 spin columns to remove hydroxyamine. The mutagenized plasmid was phenol extracted, ethanol precipitated, and used to transform *E. coli* MC1066. The mutation rate was determined by screening Ap^r colonies for the Trp phenotype. Approximately 1% of the transformants failed to grow on *E. coli* −Trp minimum plates. A total of 15,000 *E. coli* transformants were collected. This library was transformed into strain PRP40-SS, and 34,000 transformants were screened for a temperature-sensitive phenotype.

Primer extension. Yeast total RNA was isolated at a cell optical density at 600 nm of 0.5 to 1.0 as previously described (10). Primer extension was carried out according to the method of Frank and Guthrie (13) with the oligonucleotides described above.

In vitro splicing, heat inactivation, and complementation. Splicing extracts were prepared and fractionated as described by Cheng and Abelson (6). Splicing reactions were carried out according to the method of Lin et al. (27). For heat inactivation, 4 μl of extract was preincubated at 37°C for 35 min. Complementation was performed by adding 1 μl of the 40W fraction [supernatant fraction of 40% $(NH_4)_2SO_4$ precipitation] to heat-treated extracts prior to a 20-min splicing assay at 23°C.

Preparation of antisera to the $(His)_6$-PRP40 fusion protein. The $(His)_6$-PRP40 fusion protein was overexpressed and purified according to the manufacturer's instructions (Qiagen). The column-purified protein was further purified as previously described (19). Anti-$(His)_6$-PRP40 antibodies were prepared by the Berkeley Antibody Company. The rabbit serum was affinity purified as previously described (19), except that a $(His)_6$-PRP40-coupled CNBr-activated Sepharose column was used.

Coimmunoprecipitation of U1 snRNA. Affinity-purified anti-$(His)_6$-PRP40 antiserum was coupled to protein A-agarose beads (GIBCO/BRL) in IPP150 buffer (10 mM Tris-HCl [pH 8.0], 150 mM NaCl, 0.1% Nonidet P-40, 0.1% sodium azide) and washed four times before use. Twenty-five microliters of antibody-coupled beads was incubated with 5 μl of splicing extract in 200 μl of IPP150 buffer on a Nutator at 25°C for 40 min. The supernatant was saved, and the pellet was washed five times with 600 μl of IPP150 buffer. RNA was recovered from the immune supernatants and immune pellets by phenol extraction and ethanol precipitation. The presence of snRNAs in the immune supernatants and immune pellets was assayed by primer extension by using a cocktail containing oligonucleotides specific for the U1, U2, U4, U5, and U6 RNAs.

RESULTS

Isolation of suppressors of U1 RNA point mutations. Early in spliceosome assembly, the 5′ end of U1 RNA base pairs with the 5′ splice site consensus sequence. Point mutations in the 5′ end of the U1 RNA cause a variety of growth phenotypes ranging from mild to severe (Fig. 1). However, the severity of the mutant phenotype does not always correlate with the number of hydrogen bonds lost between the 5′ end of U1 RNA and the 5′ splice site consensus sequence, suggesting that other factors might interact with the 5′ end of U1 RNA. We used suppressor genetics to search for such factors. We started with a yeast strain carrying a C-to-U transition mutation at the fourth nucleotide of U1 RNA, U1-4U. This mutation causes cold sensitivity, preventing growth at 18°C, yet does not greatly disturb base pairing with the 5′ splice site. Yeast strain 19SS-

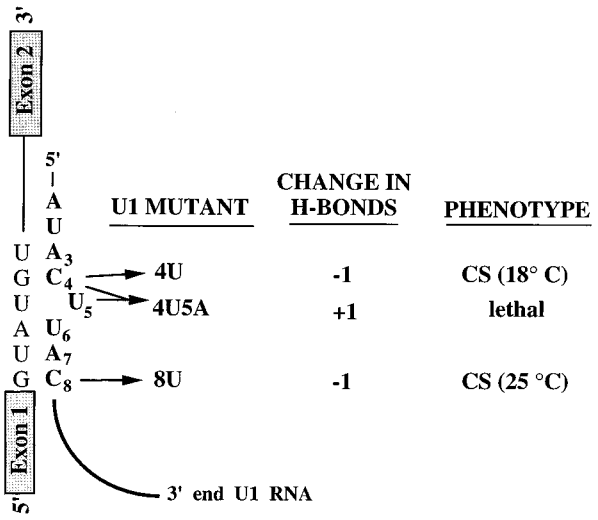

FIG. 1. The growth phenotype of a U1 mutant does not always correlate with the number of hydrogen bonds lost between the 5′ end of U1 RNA and the 5′ splice site. Shown on the left is the base pairing between the yeast 5′ splice site consensus sequence and the 5′ end of U1 RNA (boldface letters). The growth phenotypes of selected U1 mutants and the number of hydrogen bonds lost between U1 RNA and the 5′ splice site are also indicated. CS, cold sensitive.

4U, in which the gene encoding the yeast U1 RNA has been disrupted (*snr19*::*LYS2*) and complemented by the U1-4U mutant on a *CEN*-based *TRP1* plasmid, was mutagenized by UV-irradiation to 35% survival. After incubation at 18°C for 5 days, 30 viable colonies were collected. We focused on three potential candidates that grew well at 18°C.

To rule out reversion of the U1-4U mutation, we performed *cis* and *trans* tests. In the *cis* test, *TRP1* plasmids were isolated from suppressor strains and retransformed into the plasmid-shuffling strain 19SS. This strain carries the *snr19*::*LYS2* gene disruption and a wild-type U1 RNA gene on a *URA3* vector. In the plasmid shuffle assay, cells that have lost the *URA3* plasmid can be selected by growth on media containing the drug 5-FOA (3). After selection on 5-FOA plates, the retransformed strains still exhibit the U1-4U cold sensitivity. Thus, the loss of cold sensitivity was not due to reversion of U1-4U. In addition, the presence of the U1-4U mutation in the recovered plasmids was confirmed by sequence analysis.

We also used a *trans* test to verify the presence of an unlinked suppressor mutation. In this test, fresh U1-4U plasmids were transformed into candidate suppressor strains. These retransformed suppressor strains were able to grow at 18°C, and we therefore concluded that these strains contained extragenic suppressors of U1-4U. When backcrossed to the parental strain, all three suppressors showed dominant, single-gene segregation (data not shown). When individual suppressor candidates were crossed to each other and sporulated, all four spores suppressed the U1-4U cold-sensitive phenotype, indicating that the suppressors are allelic to each other.

One suppressor strain was further analyzed to determine its effects on other mutations in U1 RNA (Table 1). A total of 13 U1 mutants were tested. While the phenotypes of most U1 mutants were similar in both wild-type and suppressor strains (Table 1), the suppressor did rescue the cold sensitivity of U1-8U at 25°C (Table 1). Intriguingly, the suppressor can also partially suppress the U1-4U,5A double mutant, which is lethal in the wild-type background. Thus, from our screen we isolated a suppressor capable of relieving the growth phenotypes of several U1 RNA point mutations.

TABLE 1. Allele specificity of the *PRP40-1* suppressor[a]

U1 allele	*PRP40* allele	Growth at indicated temp (°C)[b]:				
		18	25	30	33	37
WT[c]	WT	++	++	+++	+++	++
	PRP40-1	++	++	+++	+++	++
4U	WT	−	+/−	++	++	+
	PRP40-1	+	++	+++	+++	+
8U	WT	−	−	+	+	+/−
	PRP40-1	+	++	++	++	++
4U,5A	WT	−	−	−	−	−
	PRP40-1	+/−	+	+	++	+/−

[a] Cells were grown on rich media at the temperature indicated. Also tested were U1 alleles 4A; 4G; 5A; 5C; 8A; 8G; 3G,4U; 3G,4U,5A; and 5′ end Δ. The growth phenotype of these alleles was not suppressed by *PRP40-1*.
[b] +/−, doubling time longer than 9 h; +, doubling time longer than 4.5 h; ++, doubling time longer than 2.1 h; +++, doubling time less than 2.1 h; −, inviable.
[c] WT, wild type.

Identification of the suppressor genes. To clone the suppressor gene, we constructed a library from one of the suppressor strains. Genomic DNA was isolated, partially digested with *Sau*3A, and ligated to a *CEN*-based *URA3* vector. This library was transformed into the parental strain 19SS-4U and incubated at 18°C to screen for a clone(s) that rescues the lethality at 18°C. Ten suppressor clones were recovered and retested for rescue of the cold sensitivity. On the basis of restriction mapping and Southern analyses, these clones were grouped into two classes. Interestingly, both classes were able to suppress the U1-4U mutant at 18°C and the U1-8U mutant at 25°C. The suppressor genes were localized within the suppressor clones by testing subclones for rescue at 18°C.

Sequence analysis revealed that each of these suppressor genes has been previously described. One of the suppressor genes, *SAR1*, is a single-copy essential gene that encodes a polypeptide involved in protein transport from the endoplasmic reticulum to the Golgi apparatus (2, 38). The *SAR1* transcript contains a single small (139-nucleotide) intron with an unusually short distance between branch site and 3′ splice site (18 nucleotides).

We do not believe that *SAR1* is directly involved in pre-mRNA splicing. First, our *SAR1* suppressor clone contains no sequence differences from the published sequence. Furthermore, a wild-type *SAR1* clone is able to suppress the U1-4U mutant at 18°C. Most likely, the *SAR1* clone rescues the U1-4U cold-sensitive phenotype by multicopy suppression. That is, by increasing the *SAR1* copy number, even by one to two copies, sufficient *SAR1* gene product is made to allow improved growth. These findings indicate that the *SAR1* gene product is rate limiting for growth in U1-4U cells at 18°C.

The other suppressor was originally reported by the Yeast Genome Sequencing Project as *YKL165* (41). We have renamed this gene *PRP40* for its role in pre-mRNA processing (see below). *PRP40* encodes a polypeptide of 583 amino acids with a predicted molecular mass of 69 kDa. It has no compelling sequence homology to any known protein and contains no RNA recognition motif (RRM). The original report (41) noted weak homology to the *S. cerevisiae* Myo2 protein (20), although we do not believe this homology is significant (see Discussion).

The *PRP40* suppressor clone differs from the published sequence at a single nucleotide (1421; TCC→TTC), resulting in a Ser-to-Phe change in the Prp40 protein (Fig. 2A). To show

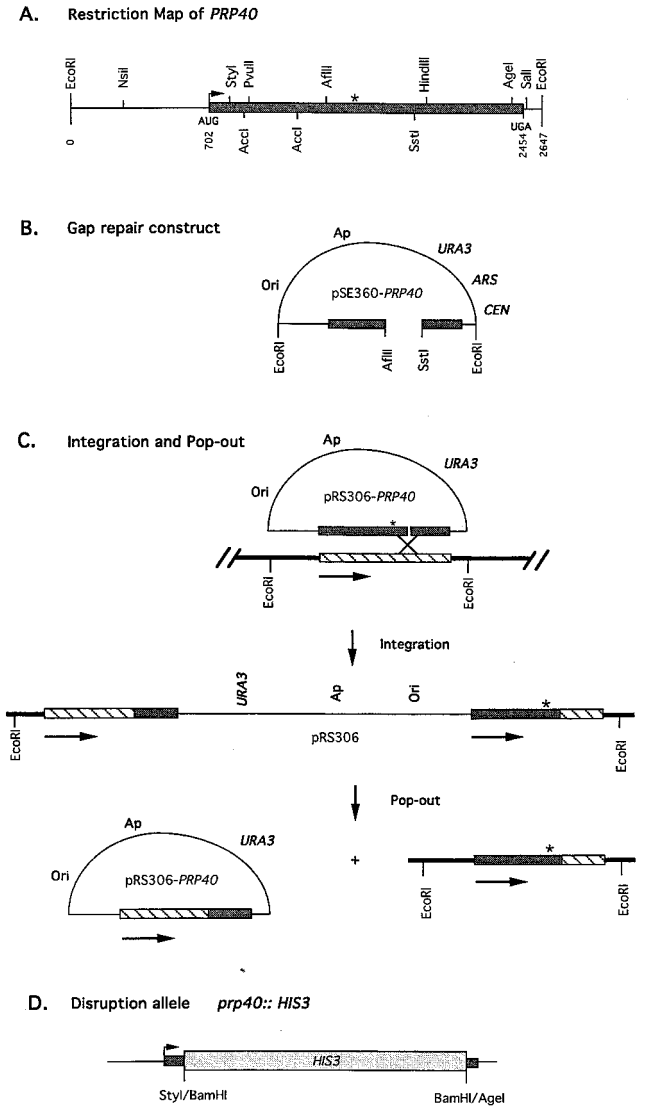

FIG. 2. Constructs for genetic analyses. (A) Restriction map of *PRP40*. The translation start codon and termination codon are indicated. The asterisk denotes the suppressor mutation C1421-T. Nucleotide positions are assigned according to the method of Pascolo et al. (41). (B) Gap repair construct. Plasmid pSE360-*PRP40* was built as described in Materials and Methods. An internal deletion within *PRP40* was created by *Afl*II-*Sst*I digestion. This deletion generated a gap that includes the position of the suppressor mutation, 1421. Note that the scale is different from that in panel A. (C) Integration and pop-out. Plasmid pRS306-*PRP40* is described in Materials and Methods. An *Sst*I-linearized plasmid was used for transformation. The arrow indicates the direction of transcription, and the asterisk denotes the suppressor mutation. The stippled and hatched boxes represent the suppressor and wild-type sequences, respectively. The thick and thin lines denote chromosomal and plasmid sequences, respectively. In the example shown here, the wild-type allele is "popped-out," leaving a suppressor allele in the chromosome. (D) *prp40* disruption allele. The *prp40* disruption allele was generated as described in Materials and Methods.

that this nucleotide change represents a UV-induced mutation, as opposed to sequence polymorphism, genomic sequencing and gap repair experiments were performed. Genomic DNA was isolated from the parental strain 19SS-4U and digested with *Acc*I. Nucleotide 1421 is contained in a 1.3-kb *Acc*I fragment. We purified *Acc*I fragments with sizes between 1 and 2 kb by gel electrophoresis and used these pooled fragments for PCR cycle sequencing. We found that the sequence of *PRP40* in the 19SS-4U parental strain is identical to the published *PRP40* sequence. We also generated a gap repair construct on a *URA3* plasmid in which the *Afl*II-*Sst*I fragment encompassing C-1421 was deleted (Fig. 2B). This plasmid was transformed into the wild-type parental strain 19SS-4U. In order for the plasmid to replicate, the gap must be repaired by using the chromosomal copy of *PRP40* as a template. Ura^+ transformants were isolated, and *URA3* plasmids were recovered for sequencing. All the recovered *PRP40* sequences are also identical to the published *YKL165* sequence. These results unambiguously demonstrate that the nucleotide change seen in the *PRP40* suppressor allele is a true mutation as opposed to a sequence polymorphism.

To determine if the cloned *PRP40* suppressor is the original suppressor gene, an integrating plasmid was created (Fig. 2C). A *URA3* plasmid containing the cloned *PRP40* suppressor allele was linearized with *Sst*I within *PRP40* and transformed into the parental strain 19SS-4U. This integration resulted in the duplication of *PRP40* with one wild-type allele and one suppressor allele separated by the *URA3* vector (Fig. 2C). All the integrants could grow at 18°C, consistent with the dominant phenotype of the suppressor strain. Pop-out experiments were carried out on 5-FOA plates to select against the *URA3* marker. In these experiments, recombination occurs between the two *PRP40* alleles, leaving a single *PRP40* allele in the chromosome. Depending on where the recombination event occurs in *PRP40*, strains that carry either a wild-type *PRP40* allele or a suppressor *PRP40* allele are generated. Precise integration and excision (pop-out) were confirmed by Southern analyses (data not shown). As expected, we recovered both Sup^+ and Sup^- pop-out strains. The pop-out strain with the *PRP40* suppressor phenotype was backcrossed to the original suppressor strain. This diploid strain was sporulated, and tetrads were dissected. Seven four-spored tetrads were recovered; all spores from these were able to grow at 18°C. These data prove that *PRP40* (C1421-T) is the bona fide suppressor. We name this allele *PRP40-1*.

***PRP40* is an essential gene.** We have identified *PRP40* as a gene that genetically interacts with yeast U1 RNA. To determine if *PRP40* is required for cell growth, a *prp40*::*HIS3* disruption allele was constructed. This allele deletes 83% of the coding region and replaces it with the *HIS3* gene (Fig. 2D). This disruption allele was used to disrupt one allele of *PRP40* in the homozygous diploid strain TR1. The resulting *PRP40*/*prp40*::*HIS3* diploids were isolated on $^-$His plates, confirmed by Southern blot analyses, and sporulated, and tetrads were dissected. In all cases, only two viable spores were recovered. Viability always cosegregated with the His^- phenotype, indicating that the *prp40*::*HIS3* allele is lethal. In a complementary experiment, a *URA3* plasmid carrying wild-type *PRP40* was transformed into the heterozygous diploid strain prior to sporulation. The resulting strain was sporulated, and tetrads were dissected. Seven tetrads in which all four spores retained the *URA3* plasmid were isolated. Selection on 5-FOA plates revealed that only two spores in each individual tetrad are able to grow in the absence of wild-type *PRP40*. Again, these viable cells were always His^-. Therefore, we conclude that *PRP40* is an essential gene.

The *PRP40-1* suppressor affects *SAR1* mRNA accumulation in the U1-4U mutant at 18°C. To test the effect of *PRP40-1* on splicing in U1-4U cells, we examined the processing of the *SAR1* transcript in U1-4U cells by primer extension. One common feature of poorly spliced introns is the short distance between branch site and 3′ splice site, such as *MAT***a***1* and *SNR17* (40). We hypothesized that the *SAR1* transcript contains a poorly spliced intron and that the production of *SAR1* mature transcript is rate limiting for growth in the U1-4U

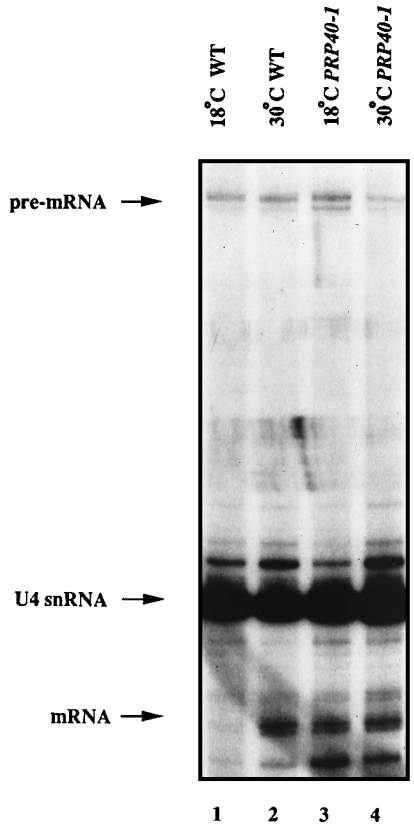

FIG. 3. The suppressor allele of *PRP40* affects *SAR1* transcript accumulation in the U1-4U mutant at 18°C. Parental and suppressor strains containing U1-4U as the only source of U1 snRNA were grown at 30°C to an optical density at 600 nm of 0.5 and shifted to 18°C for 6 h. Total yeast RNA was isolated and analyzed by primer extension. Unspliced and spliced *SAR1* transcripts are indicated by arrows. The primer extension of U4 snRNA was used as an internal control. Lane 1, strain U1-4U with wild-type (WT) *PRP40* at 18°C; lane 2, strain U1-4U with wild-type *PRP40* at 30°C; lane 3, strain U1-4U with the *PRP40-1* suppressor at 18°C; lane 4, strain U1-4U with the *PRP40-1* suppressor at 30°C.

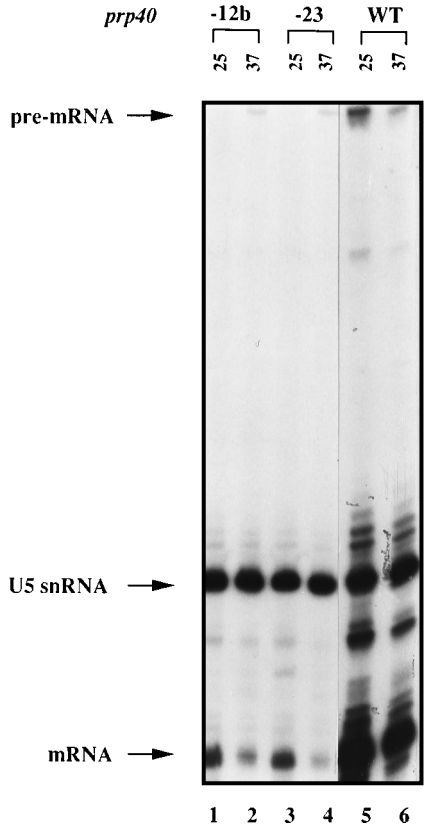

FIG. 4. In vivo splicing phenotype of *prp40* mutant alleles. *prp40* mutants were grown at 25°C to an optical density at 600 nm of 0.4 and shifted to 37°C for 4 h. Total yeast RNA was isolated, and primer extension was performed with an oligonucleotide specific for *RPL32* exon 2. *RPL32* pre-mRNA and mRNA are indicated by arrows. Also included is the primer extension product of U5 snRNA as an internal control. Lanes 1 and 2, *prp40-12b*; lanes 3 and 4, *prp40-23*; lanes 5 and 6, wild type (WT). Lanes 1, 3, and 5, cells grown at 25°C; lanes 2, 4, and 6, cells shifted to 37°C.

mutant at 18°C (1); we also hypothesized that by alleviating the *SAR1* splicing deficiency, *PRP40-1* allows production of more mature *SAR1* transcripts and thereby rescues the U1-4U cold sensitivity (2). To test our hypotheses, we analyzed *SAR1* splicing in U1-4U strains with and without the *PRP40-1* suppressor. Parental and suppressor strains were grown at 30°C to early log phase and shifted to 30°C (permissive temperature) and 18°C (nonpermissive temperature) for 6 h. Total RNA was isolated, and primer extension was performed. We found that very little *SAR1* mature mRNA is detected in the U1-4U mutant when grown at 18°C (Fig. 3, lane 1). We did not observe an accumulation of *SAR1* pre-mRNA, as might be expected if U1-4U inhibited *SAR1* splicing. However, in the *PRP40-1* suppressor strain, *SAR1* mature mRNA accumulated to normal levels at both 18 and 30°C (Fig. 3, lanes 3 and 4). Thus, the *PRP40-1* suppressor increases the steady-state level of *SAR1* mature mRNA in U1-4U cells at 18°C.

This observation is consistent with the U1-4U mutant affecting the stability or the splicing of the *SAR1* pre-mRNA. We believe that the low steady-state level of *SAR1* mature mRNA in U1-4U cells results from inefficient splicing of the *SAR1* pre-mRNA and hypothesize that the *SAR1* pre-mRNA does not accumulate because it is degraded if it is not spliced. This hypothesis is supported by observations that unspliced pre-mRNA can be degraded when splicing is inhibited (18, 42). On the basis of these arguments, we predict that Prp40 increases the level of *SAR1* mature mRNA by relieving this U1-4U splicing defect.

***PRP40* encodes a general splicing factor.** To determine if Prp40 is a general splicing factor, we created a *prp40* mutant library by hydroxyamine mutagenesis. This library was transformed into a plasmid-shuffling strain (*prp40*::*HIS3* pSE360-*PRP40*). A total of 34,000 yeast transformants were screened for plasmids bearing a temperature-sensitive *prp40* mutation. After 5-FOA selection, 17 temperature-sensitive clones were isolated and retested. Among them, three were 37°C sensitive, one was 30°C sensitive, and the rest were 34°C sensitive. We tested the in vivo splicing phenotype of these mutant strains by primer extension analysis of the *RPL32* transcript, which has an intron architecture different from that of the *SAR1* transcript and is normally efficiently spliced (8, 9). Temperature-sensitive strains were grown at 25°C to early log phase and shifted to 37°C for another 4 h. RNA was isolated and analyzed by primer extension.

In wild-type cells, *RPL32* is efficiently spliced with or without a temperature shift (Fig. 4, lanes 5 and 6). The *prp40* temperature-sensitive mutants *prp40-12b* (Fig. 4, lanes 1 and 2) and *prp40-23* (Fig. 4, lanes 3 and 4) display the phenotype expected for a strain defective in pre-mRNA splicing. That is, after

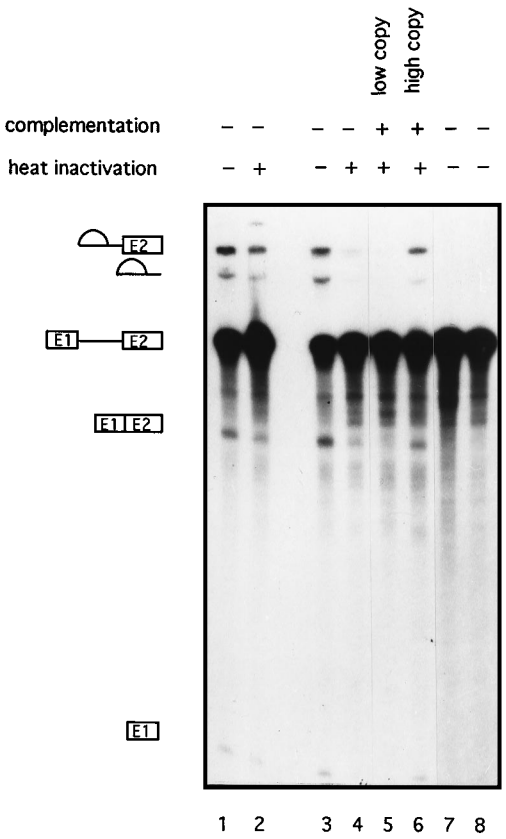

FIG. 5. In vitro splicing, heat inactivation, and complementation of a temperature-sensitive allele of *PRP40*. Splicing extracts were preincubated at 37°C for 35 min; this was followed by complementation and splicing. Lane 1, wild-type extract without heat treatment; lane 2, wild-type extract with heat treatment; lane 3, *prp40-10* extract without heat treatment; lane 4, *prp40-10* extract with heat treatment; lane 5, *prp40-10* extract with heat treatment and complemented with 40W fraction from low-copy-number *PRP40* extract; lane 6, *prp40-10* extract with heat treatment and complemented with 40W fraction from high-copy-number *PRP40* extract; lane 7, 40W fraction from low-copy-number *PRP40* extract; lane 8, 40W fraction from high-copy-number *PRP40* extract.

temperature shift, the level of mature mRNA is reduced, with a concomitant increase in the level of pre-mRNA. Although the *RPL32* 5′ splice site differs from the yeast consensus (/GU **CA**GU [the slash indicates the exon-intron boundary, and nucleotides that differ from the yeast consensus sequence are in boldface type]), primer extension experiments with the *ACT1* pre-mRNA also yielded similar results (data not shown). Accordingly, we conclude that Prp40 is a general splicing factor.

Prp40 is required for splicing in vitro. To prove that Prp40 has a direct role in pre-mRNA splicing, wild-type and temperature-sensitive strains were grown at the permissive temperature (25°C) and splicing extracts were prepared. To heat inactivate the *prp40* mutant protein, extracts were preincubated at either the permissive temperature (23°C) or a nonpermissive temperature (37°C) for 35 min. Without preincubation at the nonpermissive temperature, the wild-type and the *prp40* mutant extracts are able to splice an in vitro-transcribed *ACT1* pre-mRNA (Fig. 5, lanes 1 and 3). Upon heat treatment, the splicing activity of the extract from the *prp40* mutant strain is greatly reduced (Fig. 5, lane 4). In contrast, the wild-type extract remains active (Fig. 5, lane 2).

To determine if this inhibition of splicing activity is due to heat inactivation of the mutant protein, we performed a complementation experiment. First, we constructed the *PRP40* $(HA)_3$ allele, which contains three copies of the HA epitope at the *PRP40* carboxy terminus. This epitope is recognized by the 12CA5 monoclonal antibody. The *PRP40*$(HA)_3$ allele retains Prp40 function, as this allele fully complements the *prp40* gene disruption (data not shown). By Western blot (immunoblot) analysis, we found that Prp40$(HA)_3$ fractionates to the 40P3 fraction of yeast splicing extract [pellet fraction of 40% $(NH_4)_2SO_4$ precipitation; U1 RNA also fractionates to the 40P3 fraction]. No Prp40$(HA)_3$ is detected in the 40W fraction [40% $(NH_4)_2SO_4$ supernatant]. However, overexpression of Prp40$(HA)_3$ on a high-copy-number plasmid results in partial fractionation of Prp40$(HA)_3$ to the 40W fraction.

The 40W fraction was prepared from strains harboring either a low-copy-number *PRP40* plasmid or a high-copy-number *PRP40* plasmid that overexpressed Prp40. The 40W fraction from the strain with the high-copy-number *PRP40* plasmid is able to restore the splicing activity of a heat-inactivated *prp40-10* extract (Fig. 5, lane 6). However, the 40W fraction from the low-copy-number *PRP40* does not restore activity (Fig. 5, lane 5). These results indicate that wild-type Prp40 $(HA)_3$ can complement the heat-inactivated temperature-sensitive Prp40. Moreover, neither 40W fraction is able to support splicing activity alone (Fig. 5, lanes 7 and 8). In addition, the 40P3 fractions from both the low-copy-number and high-copy-number extracts can complement the heat-inactivated extract, indicating that the low-copy-number extract was active (data not shown). These findings demonstrate that the *PRP40* gene product is directly involved in pre-mRNA splicing.

Prp40 associates with U1 snRNA in vivo. The isolation of the *PRP40-1* allele that suppresses mutations in U1 RNA demonstrates that *PRP40* interacts genetically with U1 RNA. We next asked if the Prp40 protein is physically associated with the U1 snRNP. To answer this question, we constructed a His-tagged allele, (His_6)-Prp40, and purified the tagged protein. This protein was used to raise rabbit polyclonal antibodies specific for Prp40. We used the anti-Prp40 serum and preimmune control serum to immunoprecipitate Prp40 from splicing extract. We analyzed the immune supernatants and immune pellets for the presence of the U1, U2, U4, U5, and U6 RNAs by primer extension (Fig. 6A and B). The preimmune serum does not immunoprecipitate any snRNAs (Fig. 6A, lane 3). However, the anti-Prp40 serum specifically immunoprecipitates U1 RNA (Fig. 6A, lane 5), demonstrating that Prp40 is associated with the U1 RNA.

In the experiment whose results are shown in Fig. 6A, 70% of the U1 RNA was recovered in the immune pellet. In other experiments (Fig. 6B), the anti-Prp40 antiserum quantitatively precipitated all the U1 RNA (lanes 5 and 6). We conclude that all the U1 RNA is associated with Prp40 in vivo. In addition, we find that Prp40 coimmunoprecipitates U1 RNA just as well at 300 mM NaCl (data not shown), indicating that Prp40 is stably associated with U1 RNA. On the basis of these data, we propose that Prp40 is a component of the U1 snRNP.

DISCUSSION

Our understanding of snRNP function rests primarily on the discovery of base pairing interactions of the snRNAs with each other and with conserved sequences within the intron. This network of base pairing interactions is thought to culminate in the creation of a ribozyme that catalyzes splicing (31). In contrast to our detailed knowledge of the snRNAs, the roles of snRNP proteins in splicing remain largely unknown.

Here we identify Prp40, a novel component of the yeast splicing machinery. Prp40 is essential for yeast viability and for

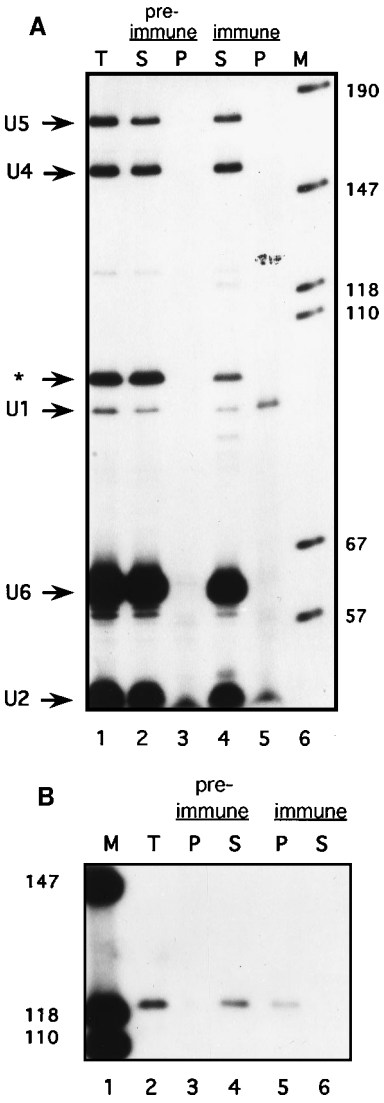

FIG. 6. (A) Prp40 associates specifically with yeast U1 snRNA in vivo. Splicing extract prepared from wild-type cells was immunoprecipitated with either preimmune serum or serum specific for $(His)_6$-Prp40. RNAs were extracted from the unprecipitated total (T), immune supernatant (S), and immune pellet (P) fractions and analyzed by primer extension by using a cocktail containing oligonucleotides specific for the U1, U2, U4, U5, and U6 RNAs. The primer extension products for these RNAs are indicated by arrows. Also marked (*) is an artifactual primer extension product caused by the U6-specific oligonucleotide. Lane 1, total RNA without immunoprecipitation; lane 2, preimmune supernatant; lane 3, preimmune pellet; lane 4, immune supernatant; lane 5, immune pellet; lane 6, molecular weight markers (M). (B) All the U1 RNA is associated with Prp40. Splicing extract was immunoprecipitated as described above and analyzed by primer extension with an oligonucleotide specific for the U1 RNA. Note that a different U1-specific oligonucleotide was used for panel A. Lane 1, molecular weight markers; lane 2, total RNA without immunoprecipitation; lane 3, preimmune pellet; lane 4, preimmune supernatant; lane 5, immune pellet; lane 6, immune supernatant. M, T, P, and S are as defined for panel A.

the first step of pre-mRNA splicing in vivo and in vitro. We show that Prp40, originally identified as a factor that suppresses mutations in the 5′ end of U1 RNA, associates with the U1 snRNA in vivo.

Prp40 has weak homology to the *S. cerevisiae* Myo2 protein; this homology consists of 31 identical amino acids in a 93-amino-acid stretch and does not include an ATP binding domain. Because of the extensive gaps needed to make this match (41), we do not believe that this Myo2 homology is significant. BLASTP searches revealed several sequences with homology scores greater than that of Myo2 but still well below a level generally considered significant. *PRP40* does have a stronger match to ZK1098.1, a *Caenorhabditis elegans* open reading frame of unknown function, but does not appear homologous to any known metazoan or yeast splicing factor.

Further domain searches revealed two copies of a loosely conserved motif called the WWP domain at the amino terminus of Prp40. The WWP domain was identified as a protein motif encompassing 38 amino acids found in various structural, regulatory, and signaling molecules in *S. cerevisiae*, nematodes, and mammals (reviewed in references 1, 4, and 56). These proteins, which have no known common functions, include human dystrophin, YAP65 (Yes-associated protein), yeast Rsp5, and ZK1098.1. The WWP domain contains four highly conserved aromatic residues, including two tryptophans. In addition, several consensus amino acids are found at conserved positions (1, 4). This structural feature is proposed to be involved in protein-protein interactions. In Prp40, the suppressor mutation Ser240-Phe is located downstream of the WWP domain.

Is Prp40 a U1 snRNP protein? Fabrizio et al. (12) have purified yeast U1 snRNPs and analyzed their protein composition. Intriguingly, they detected a protein of 69 kDa in purified U1 snRNPs; this size matches the calculated size of Prp40 (69 kDa) closely.

What is the role of Prp40 in splicing? Both in vivo and in vitro, *prp40* mutants block the first step of splicing, as expected for a factor that functions with the U1 snRNP. A more specific hypothesis for Prp40 function can be based on the U1 RNA mutations that are suppressed by the PRP40-1 mutation. Both position 4 and position 8 of U1 RNA are known to base pair with their cognate nucleotides in the 5′ splice site. At each position, the C-to-U transition is the only viable mutation; all other mutations at positions 4 and 8 are lethal (54). Intriguingly, both U1-4U and U1-8U cause cold-sensitive as well as temperature-sensitive growth. The temperature sensitivity of U1-4U and U1-8U might result from the weakening of base pairing with the 5′ splice site. However, the cold sensitivity of these mutants was not anticipated and argues in favor of these nucleotides of U1 having an additional function besides interaction with the 5′ splice site. On the basis of the allele specificity of the *PRP40-1* suppressor, we propose that interaction with Prp40 is another essential function of these nucleotides of U1 RNA. Finally, it is interesting that in vitro splicing extracts prepared from U1-4U mutants can partially bypass the requirement for ATP in U2 addition (25). Our demonstration that Prp40 interacts genetically with U1-4U could suggest a role for Prp40 in U2 snRNP association. Our *prp40* mutant extracts will allow us to test this exciting possibility.

ACKNOWLEDGMENTS

We thank Brian Rymond, Jim Umen, and Christine Guthrie for reagents and Dave LaPorte for helpful discussions. We are grateful to Stew Scherer for help with the *PRP40* homology searches and to Patricia Hilleren and Mark Murphy for comments on the manuscript.

This work was supported by a grant from the NIH (GM-44628) to P.G.S.

REFERENCES

1. **Andre, B., and J. Springael.** 1994. WWP, a new amino acid motif present in single or multiple copies in various proteins including dystrophin and the SH3-binding Yes-associated protein YAP65. Biochem. Biophys. Res. Commun. **205:**1201–1205.
2. **Barlowe, C., C. d'Enfert, and R. Schekman.** 1993. Purification and characterization of Sar1p, a small GTP-binding protein required for transport

vesicle formation from the endoplasmic reticulum. J. Biol. Chem. **268:**873–879.
3. **Boeke, J. D., J. Truehart, G. Natsoulis, and G. R. Fink.** 1987. 5-Fluoro-orotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. **154:**164–175.
4. **Bork, P., and M. Sudol.** 1994. The WW domain: a signaling site in dystrophin? Trends Biochem. Sci. **19:**531–533.
5. **Brody, E., and J. Abelson.** 1985. The "spliceosome": yeast pre-messenger RNA associates with a 40S complex in a splicing-dependent reaction. Science **228:**963–967.
6. **Cheng, S.-C., and J. Abelson.** 1986. Fractionation and characterization of a yeast mRNA splicing extract. Proc. Natl. Acad. Sci. USA **83:**2387–2391.
7. **Cheng, S.-C., and J. Abelson.** 1987. Spliceosome assembly in yeast. Genes Dev. **1:**1014–1027.
8. **Dabeva, M., M. Post-Beittenmiller, and J. Warner.** 1986. Autogenous regulation of splicing of the transcript of a yeast ribosomal protein gene. Proc. Natl. Acad. Sci. USA **83:**5854–5857.
9. **Dabeva, M., and J. Warner.** 1987. The yeast ribosomal protein L32 and its gene. J. Biol. Chem. **262:**16055–16059.
10. **Domdey, H., B. Apostol, R.-J. Lin, A. Newman, E. Brody, and J. Abelson.** 1984. Lariat structures are in vivo intermediates in yeast pre-mRNA splicing. Cell **39:**611–621.
11. **Elledge, S., and R. Davis.** 1988. A family of versatile centromeric vectors designed for use in the sectoring-shuffle assay in Saccharomyces cerevisiae. Gene **70:**303–312.
12. **Fabrizio, P., S. Esser, B. Kastner, and R. Luhrmann.** 1994. Isolation of S. cerevisiae snRNPs: comparison of U1 and U4/U6.U5 to their human counterparts. Science **264:**261–265.
13. **Frank, D., and C. Guthrie.** 1992. An essential splicing factor, SLU7, mediates 3′ splice site choice in yeast. Genes Dev. **6:**2112–2124.
14. **Frendewey, D., and W. Keller.** 1985. The stepwise assembly of a pre-mRNA splicing complex requires U-snRNPs and specific intron sequences. Cell **42:**355–367.
15. **Grabowski, P. J., S. R. Seiler, and P. A. Sharp.** 1985. A multicomponent complex is involved in the splicing of messenger RNA precursors. Cell **42:**345–353.
16. **Guthrie, C., and J. Fink.** 1991. Guide to yeast genetics and molecular biology. Academic Press, San Diego, Calif.
17. **Hamm, J., V. L. van Santen, R. Spritz, and I. W. Mattaj.** 1988. Loop I of U1 small nuclear RNA is the only essential RNA sequence for binding of specific U1 small nuclear ribonucleoprotein particle proteins. Mol. Cell. Biol. **8:**4787–4791.
18. **He, F., S. Peltz, J. Donahue, M. Rosbash, and A. Jacobson.** 1993. Stabilization and ribosome association of unspliced pre-mRNAs in a yeast upf1-mutant. Proc. Natl. Acad. Sci. USA **90:**7034–7038.
19. **Hilleren, P. J., H.-Y. Kao, and P. G. Siliciano.** 1995. The amino-terminal domain of yeast U1-70K is necessary and sufficient for function. Mol. Cell. Biol. **15:**6341–6350.
20. **Johnston, G., J. Prendergast, and R. Singer.** 1991. The Saccharomyces cerevisiae MYO2 gene encodes an essential myosin for vectoral transport of vesicles. J. Cell Biol. **113:**539–551.
21. **Jones, M., and C. Guthrie.** 1990. Unexpected flexibility in an evolutionarily conserved protein-RNA interaction: genetic analysis of the Sm binding site. EMBO J. **9:**2555–2561.
22. **Kao, H.-Y., and P. G. Siliciano.** 1992. The yeast homolog of the U1 snRNP protein 70K is encoded by the SNP1 gene. Nucleic Acids Res. **20:**4009–4013.
23. **Kretzner, L., A. Krol, and M. Rosbash.** 1990. Saccharomyces cerevisiae U1 small nuclear RNA secondary structure contains both universal and yeast-specific domains. Proc. Natl. Acad. Sci. USA **87:**851–855.
24. **Legrain, P., B. Seraphin, and M. Rosbash.** 1988. Early commitment of yeast pre-mRNA to the spliceosome pathway. Mol. Cell. Biol. **8:**3755–3760.
25. **Liao, X., H. Colot, Y. Wang, and M. Rosbash.** 1992. Requirements for U2 snRNP addition to yeast pre-mRNA. Nucleic Acids Res. **20:**4237–4245.
26. **Liao, X., J. Tang, and M. Rosbash.** 1993. An enhancer screen identifies a gene that encodes the yeast U1 snRNP A protein: implications for snRNP protein function in pre-mRNA splicing. Genes Dev. **7:**419–428.
27. **Lin, R.-J., A. J. Newman, S.-C. Cheng, and J. Abelson.** 1985. Yeast mRNA splicing in vitro. J. Biol. Chem. **260:**14780–14792.
28. **Lockhart, S., and B. Rymond.** 1994. Commitment of yeast pre-mRNA to the splicing pathway requires a novel U1 small nuclear ribonucleoprotein polypeptide, Prp39p. Mol. Cell. Biol. **14:**3623–3633.
29. **Luhrmann, R.** 1988. snRNP proteins, p. 71–99. *In* M. L. Birnstiel (ed.), Small nuclear ribonucleoprotein particles. Springer-Verlag KG, Berlin.
30. **Lutz-Reyermuth, C., and J. D. Keene.** 1989. The U1 RNA-binding site of the U1 small nuclear ribonucleoprotein (snRNP)-associated A protein suggests a similarity with U2 snRNPs. Mol. Cell. Biol. **9:**2975–2982.
31. **Madhani, H., and C. Guthrie.** 1994. Dynamic RNA-RNA interactions in the spliceosome. Annu. Rev. Genet. **28:**1–26.
32. **Mattaj, I.** 1988. U snRNP assembly and transport, p. 100–114. *In* M. L. Birnstiel (ed.), Structure and function of major and minor small nuclear ribonucleoprotein particles. Springer-Verlag, New York.
33. **Michaud, S., and R. Reed.** 1991. An ATP-independent complex commits pre-mRNA to the mammalian spliceosome assembly pathway. Genes Dev. **5:**2534–2546.
34. **Michaud, S., and R. Reed.** 1993. A functional association between the 5′ and 3′ splice sites is established in the earliest prespliceosomal complex (E) in mammals. Genes Dev. **7:**1008–1020.
35. **Moore, M., C. Query, and P. Sharp.** 1993. Splicing of precursors to messenger RNAs by the spliceosome, p. 303–357. *In* R. Gesteland and J. Atkins (ed.), The RNA world. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
36. **Mount, S. M., I. Pettersson, M. Hinterberger, A. Karmas, and J. A. Steitz.** 1983. The U1 small nuclear RNA-protein complex selectively binds a 5′ splice site in vitro. Cell **33:**509–518.
37. **Mount, S. M., and J. A. Steitz.** 1981. Sequence of U1 RNA from Drosophila melanogaster: implications for U1 secondary structure and possible involvement in splicing. Nucleic Acids Res. **9:**6351–6368.
38. **Nakano, A., and M. Muramatsu.** 1989. A novel GTP-binding protein, Sar1p, is involved in transport from the endoplasmic reticulum to the Golgi apparatus. J. Cell Biol. **109:**2677–2691.
39. **Nelissen, R., C. Will, W. Venrooij, and R. Luhrmann.** 1994. The association of the U1-specific 70K and C proteins with U1 snRNPs is mediated in part by common U snRNP proteins. EMBO J. **17:**4113–4125.
40. **Parker, R., and R. Patterson.** 1987. Architecture of fungal introns: implications for spliceosome assembly, p. 451–461. *In* M. Inouye and B. Dudock (ed.), Molecular biology of RNA: new perspectives. Academic Press, San Diego, Calif.
41. **Pascolo, S., M. Ghazvini, J. Boyer, L. Colleaux, A. Thierry, and B. Dujon.** 1992. The sequence of a 9.3 kb segment located on the left arm of the yeast chromosome XI reveals five open reading frames including the CCE1 gene and putative products related to MYO2 and to the ribosomal protein L10. Yeast **8:**987–995.
42. **Pikielny, C., and M. Rosbash.** 1985. mRNA splicing efficiency in yeast and the contribution of nonconserved sequences. Cell **41:**119–126.
43. **Pikielny, C., B. C. Rymond, and M. Rosbash.** 1986. Electrophoresis of ribonucleoproteins reveals an ordered assembly pathway of yeast splicing complexes. Nature (London) **324:**341–345.
44. **Query, C. C., R. C. Bently, and J. D. Keene.** 1989. A common RNA recognition motif identified within a defined U1 RNA binding domain of the 70K U1 snRNP protein. Cell **57:**89–101.
45. **Rose, M., and J. Broach.** 1991. Cloning genes by complementation in yeast. Methods Enzymol. **194:**195–230.
46. **Ruby, S. W., and J. Abelson.** 1988. An early hierarchic role of U1 small nuclear ribonucleoprotein in spliceosome assembly. Science **242:**1028–1035.
47. **Schena, M., D. Picard, and K. Yamamoto.** 1991. Vectors for constitutive and inducible gene expression in yeast. Methods Enzymol. **194:**389–398.
48. **Schiestl, R., and R. Gietz.** 1989. High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. Curr. Genet. **16:**339–346.
49. **Seraphin, B., L. Kretzner, and M. Rosbash.** 1988. A U1 snRNA:pre-mRNA base pairing interaction is required early in yeast spliceosome assembly but does not uniquely define the 5′ cleavage site. EMBO J. **7:**2533–2538.
50. **Seraphin, B., and M. Rosbash.** 1989. Identification of functional U1 snRNA-pre-mRNA complexes committed to spliceosome assembly and splicing. Cell **59:**349–358.
51. **Seraphin, B., and M. Rosbash.** 1991. The yeast branchpoint sequence is not required for the formation of a stable U1 snRNA-pre-mRNA complex and is recognized in the absence of U2 snRNA. EMBO J. **10:**1209–1216.
52. **Sikorski, R., 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.
53. **Siliciano, P. G., D. A. Brow, H. Roiha, and C. Guthrie.** 1987. An essential snRNA from S. cerevisiae has properties predicted for U4, including interaction with a U6-like snRNA. Cell **50:**585–592.
54. **Siliciano, P. G., and C. Guthrie.** 1988. 5′ splice site selection in yeast: genetic alterations in base-pairing with U1 reveal additional requirements. Genes Dev. **2:**1258–1267.
55. **Smith, V., and B. Barrell.** 1991. Cloning of a yeast U1 snRNP 70K protein homologue: functional conservation of an RNA-binding domain between yeast and humans. EMBO J. **10:**2627–2634.
56. **Sudol, M., P. Bork, A. Einbond, K. Kastury, T. Druck, M. Negrini, K. Huebner, and D. Lehman.** 1995. Characterization of the mammalian YAP (Yes-associated protein) gene and its role in defining a novel protein module, the WW domain. J. Biol. Chem. **270:**14733–14741.
57. **Surowy, C. S., V. L. van Santen, S. M. Scheib-Wixted, and R. A. Spritz.** 1989. Direct, sequence-specific binding of the human U1-70K ribonucleoprotein antigen protein to loop I of U1 small nuclear RNA. Mol. Cell. Biol. **9:**4179–4186.
58. **Zhuang, Y., and A. M. Weiner.** 1986. A compensatory base change in U1 snRNA suppresses a 5′ splice site mutation. Cell **46:**827–835.